510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K183462

B. Purpose for Submission:

Clearance of the Applied Biosystems *Bacillus anthracis* Detection Kit on the Applied Biosystems (AB) 7500 Fast Dx instrument and Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS).

C. Measurand:

Nucleic acid sequences of *Bacillus anthracis* pX01 and pX02 plasmids

D. Type of Test:

Real-time polymerase chain reaction

E. Applicant:

MRIGlobal

F. Proprietary and Established Names:

Applied Biosystems *Bacillus anthracis* Detection Kit
Applied Biosystems (AB) 7500 Fast Dx

G. Regulatory Information:

1. Regulation section:

   CFR 866.4000

2. Classification:

   Class II

3. Product code:

   QIF, OOI

4. Panel:

   83- Microbiology

H. Intended Use:

1. Intended use
   The Applied Biosystems *Bacillus anthracis* Detection Kit is a real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA

sequences for *Bacillus anthracis* (*B. anthracis*, or BA). The Applied Biosystems *Bacillus anthracis* Detection Kit is intended to test human whole blood (EDTA) specimens and blood culture specimens with growth detected by a continuous monitoring blood culture system. Blood culture specimens must be determined to contain gram-positive bacilli by Gram stain prior to testing. Testing of whole blood specimens must be performed concomitantly with standard of care blood culture.

The Applied Biosystems *Bacillus anthracis* Detection Kit is indicated for use in CLIA-certified high-complexity laboratories in response to a confirmed *Bacillus anthracis* event only in accordance with the guidelines provided by public health authorities prior to or during a public health emergency. Testing with the Applied Biosystems *Bacillus anthracis* Detection Kit must only be performed when public health authorities have determined the need for this test. The test must only be used with specimens from individuals with clinical signs and symptoms of *B. anthracis* infection and who have either been exposed to *B. anthracis* or may have been exposed to *B anthracis*.

The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use as an aid in the diagnosis of anthrax infection and results are for the presumptive identification of *Bacillus anthracis*. The diagnosis of *B. anthracis* infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence, in addition to the identification of *B. anthracis* from cultures or directly from clinical specimens. The definitive identification of *B. anthracis* requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required.

The Applied Biosystems *Bacillus anthracis* Detection Kit has not been clinically evaluated with specimens collected from individuals with *B. anthracis* infection or those presumed to be exposed to *B. anthracis*. '*B. anthracis* Not detected' results do not preclude infection with *Bacillus anthracis* and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Laboratories implementing this test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing *B. anthracis*. Anthrax is a nationally notifiable disease caused by a biothreat microbial agent and must be reported to public health authorities.

The distribution of *in vitro* diagnostic devices for *Bacillus* spp. detection is limited to laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.

The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use with the ABI 7500 Fast Dx Real-Time PCR Instrument with analysis using the Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS).

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   - For Prescription Use Only
   - Laboratories must maintain records of completed manufacturer required training including training related to use of the assay, instrumentation, biosafety precautions, appropriate PPE and contamination control and waste disposal.
   - Laboratories must maintain manufacturer required documentation verifying the laboratory is

certified as high-complexity, has appropriate biosafety equipment and PPE as required per assay instructions and has procedures for following public health guidelines for working with specimens containing *B. anthracis*.

- Laboratories must maintain manufacturer required documentation verifying the laboratory has procedures for following applicable state, local and federal public health regulations for reporting results and for referring specimens and or isolates to their public health reference laboratory.

4. Special instrument requirements:

   MagNA Pure LC 2.0 extraction System, Roche

   Applied Biosystems (AB) 7500 Fast Dx Real-Time PCR Instrument with Sequence Detection System (SDS), Thermo Fisher Scientific (Life Technologies)

I. Device Description:

The Applied Biosystems *Bacillus anthracis* Detection Kit is a multiplexed real-time polymerase chain reaction (PCR) test kit intended for the qualitative detection of *B. anthracis* DNA sequences. PCR reagents are lyophilized in PCR well strips and run in a 96-well plate format. The kit is designed for performing real-time PCR using the Applied Biosystems (AB) 7500 Fast Dx instrument and software, with nucleic acids extracted from clinical specimens using either a Qiagen manual extraction method using Qiagen's QIAamp DSP DNA Blood Mini Kit (DSP) or Roche's MagNA Pure LC 2.0 Robot (MagNA Pure, MNP). Roche MagNA Pure is an automated extraction method. An automated interpretative software component (BaIS) is included in the kit, but supplied separately, and operates on a separate computer from the AB 7500 Fast Dx computer.

Materials provided

Materials Provided in the Applied Biosystem *Bacillus anthracis* Detection Kit

Part Name	Quantity and Content	Storage Conditions
Applied Biosystems *Bacillus anthracis* Test Kit Lyophilized Assay Plates	10 Plates; Each plate contains a lyophilized Mastermix and primers/probes specific for the *Bacillus anthracis* 3-plex assay; each plate is individually sealed and foil-wrapped.	Ambient or room temperature (20°C – 25°C)
Applied Biosystems *Bacillus anthracis* Test Kit Controls	1 box contains 1 vial of External Positive Control (EPC) and 1 vial of External Negative Control (ENC); Each vial contains enough reagent volume for approximately 10 PCR reactions; no further dilution of the control reagent is necessary. See below for final concentrations of control targets per 20 µl PCR reaction.	Frozen (-15°C to -25°C)
Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS) Version 1.0	1 BaIS Interpretive Software Server Installer; 1 BaIS Interpretive Software Client Installer.	Not applicable

External Controls and Final Target Concentration

External Control	Final Target Concentration per Reaction
EPC	pX01 (100fg), pX02 (200fg) and TERT (750fg)
ENC	*E. coli* DNA (4.8ng)

Equipment and materials required but not supplied

Equipment

- Applied Biosystem 7500 Fast Dx Real-Time PCR Instrument with Sequence Detection System (SDS), Thermo Fisher Scientific (Life Technologies)
- Separate computer for installation of Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS) Version 1.0 (It is not recommended to install and run BaIS on Applied Biosystems 7500 Fast Dx Real-Time PCR instrument computer)
- MagNA Pure LC 2.0 Robot, Roche
- Automated blood culture monitoring system (for testing blood culture samples only)
- Biosafety cabinet (BSC) (Class II, Type A or B)
- 2-8°C Refrigerator
- ≤ -20°C Freezer
- < -70°C Freezer
- Plate centrifuge compatible with Applied Biosystems MicroAmp Fast Optical 96-Well Reaction Plate, 0.1 mL, with at least 1000 rpm centrifuging speed

- Microcentrifuge, able to hold 2 mL tubes and capable of reaching 20,800 x g
- Vortexer
- Heat block capable of 56°C with insert for 2 mL tubes
- Micropipette 1000 µL, 200 µL, 20 µL
- Caplocks for 2 mL microcentrifuge tubes

Consumables and Reagents

- QIAamp DSP DNA Blood Mini Kit (IVD), Qiagen (DSP)
- 100% Ethanol
- Nuclease-free water
- MagNA Pure LC DNA Isolation Kit – Large Volume, Roche
- Blood culture bottles, aerobic and/or anaerobic (for testing blood culture samples only)
- Aerosol-resistant filter tips for micropipettes – 1000 µL, 200 µL, 20 µL
- Serological pipets – 50 mL, 25 mL, 10 mL, 5 mL
- Optical plate film (seals) for 96-well PCR plates, compatible with AB7500 Fast Dx
- Clean RNase- and DNase-free 2.0 mL microcentrifuge tubes

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FilmArray NGDS Warrior Panel

2. Predicate 510(k) number(s):

   DEN160048

3. Comparison with predicate:

Similarities		
Item	**Applied Biosystem *Bacillus anthracis* Detection Kit (K183462)**	**FilmArray NGDS Warrior Panel (DEN160048)**
Intended Use	The Applied Biosystems *Bacillus anthracis* Detection Kit is a real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA sequences for *Bacillus anthracis* (*B. anthracis*, or BA). The Applied Biosystems *Bacillus anthracis* Detection Kit is intended to test human whole blood (EDTA) specimens and blood culture specimens with growth detected by a continuous monitoring	The FilmArray NGDS Warrior Panel is a qualitative, multiplexed, nucleic acid-based *in vitro* diagnostic test intended for use with the FilmArray® 2.0 system. The FilmArray NGDS Warrior Panel detects and identifies *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii,* Ebola virus, and Marburg virus nucleic acids directly from human whole blood (EDTA). The FilmArray NGDS

Similarities		
Item	**Applied Biosystem *Bacillus anthracis* Detection Kit (K183462)**	**FilmArray NGDS Warrior Panel (DEN160048)**
	blood culture system. Blood culture specimens must be determined to contain gram-positive bacilli by Gram stain prior to testing. Testing of whole blood specimens must be performed concomitantly with standard of care blood culture. The Applied Biosystems *Bacillus anthracis* Detection Kit is indicated for use in CLIA-certified high-complexity laboratories in response to a confirmed *Bacillus anthracis* event only in accordance with the guidelines provided by public health authorities prior to or during a public health emergency. Testing with the Applied Biosystems *Bacillus anthracis* Detection Kit must only be performed when public health authorities have determined the need for this test. The test must only be used with specimens from individuals with clinical signs and symptoms of *B. anthracis* infection and who have either been exposed to *B. anthracis* or may have been exposed to *B anthracis*. The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use as an aid in the diagnosis of anthrax infection and results are for the presumptive	Warrior Panel is also intended to be used to test for *Bacillus anthracis or Yersinia pestis* nucleic acids in blood cultures that are determined to be positive either by an automated system, by turbidity, or by daily Gram stain. In addition, the FilmArray NGDS Warrior Panel may also be used to detect and identify *Yersinia pestis and Francisella tularensis* nucleic acids directly from sputum specimens. The FilmArray NGDS Warrior Panel is intended to test individuals with signs and symptoms of infection from biothreat agents and/or individuals who are at risk for exposure or may have been exposed to these agents. The FilmArray NGDS Warrior Panel is indicated as an aid in the diagnosis of anthrax, plague, tularemia, Q fever, and the hemorrhagic fevers caused by Ebola and Marburg viruses, in response to a suspected or confirmed bioterrorism event or outbreaks. It is for diagnostic use in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the appropriate Department of Defense and public health authorities. Results are for the presumptive identification

Similarities		
Item	**Applied Biosystem *Bacillus anthracis* Detection Kit (K183462)**	**FilmArray NGDS Warrior Panel (DEN160048)**
	identification of *Bacillus anthracis*. The diagnosis of *B. anthracis* infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence, in addition to the identification of *B. anthracis* from cultures or directly from clinical specimens. The definitive identification of *B. anthracis* requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required. The Applied Biosystems *Bacillus anthracis* Detection Kit has not been clinically evaluated with specimens collected from individuals with *B. anthracis* infection or those presumed to be exposed to *B. anthracis*. '*B. anthracis* Not detected' results do not preclude infection with *Bacillus anthracis* and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Laboratories implementing this test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing *B. anthracis*. Anthrax is a	of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus. The definitive identification of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus requires additional testing and confirmation procedures in consultation with the appropriate Department of Defense and public health authorities for whom reports may be necessary.

Similarities		
Item	**Applied Biosystem *Bacillus anthracis* Detection Kit (K183462)**	**FilmArray NGDS Warrior Panel (DEN160048)**
	nationally notifiable disease caused by a biothreat microbial agent and must be reported to public health authorities. The distribution of *in vitro* diagnostic devices for *Bacillus* spp. detection is limited to laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities. The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use with the ABI 7500 Fast Dx Real-Time PCR Instrument with analysis using the Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS).	
Methodology/ Technological Principle	Real-time PCR	Nested PCR followed by melt curve analysis
Specimen Types	Whole blood and blood culture specimens	Whole blood, blood culture (for *Bacillus anthracis and Yersinia pestis*), sputum (for *Yersinia pestis* and *Francisella tularensis*)
Result Output	Qualitative detection of *B. anthracis* DNA	Qualitative detection of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii,* Ebola virus, and Marburg virus nucleic acids
Test Interpretation	Automated test, interpretation, and report generation	Automated test, interpretation, and report generation

Differences		
Item	**Device**	**Predicate**
	Multiplexed assay. Two *B.*	Multiplexed assay for

Differences		
Item	**Device**	**Predicate**
Multiplex Capability	*anthracis* gene targets and internal process control run in single reaction	detection of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus
Assay Targets	DNA sequences unique to *B. anthracis*	Nucleic acid sequences unique to multiple biothreat analytes.
Sample Extraction	Qiagen QIAamp DSP DNA Blood Mini Kit (manual) and Roche MagNA Pure LC 2.0 automated nucleic acid extraction (automated)	Automated extraction in FilmArray pouch
PCR Reagents	Lyophilized reagents are in PCR plate wells and are reconstituted upon addition of the specimen or external control	Reagents contained within FilmArray pouch
Instrumentation	Applied BioSystems 7500 Fast Dx	FilmArray 2.0
Throughput	96-well format allows for analysis of up to 93 patient samples (+ controls) for detection of two *Bacillus anthracis* targets and an internal process control on a single plate	One specimen tested at a time
User Complexity End User	High Complexity, CLIA-certified, sentinel laboratories, only during an anthrax event	Moderate Complexity, for use by United States Department of Defense laboratories, and laboratories designated by the Department of Defense

K. Standard/Guidance Document Referenced (if applicable):

N/A

L. Test Principle:

Extraction of whole blood and positive blood culture specimens is performed using a manual Qiagen QIAamp DSP DNA Blood Mini Kit (DSP) extraction method or the automated MagNA Pure (MNP) extraction method. Real-time PCR of the extracted nucleic acids is accomplished with the use of an Applied Biosystems probe, which detects a specific sequence within the region amplified by the assay primers. The probe is labeled on one end with a fluorescent reporter molecule and a quencher molecule on the other end. When the probe is intact, the quencher absorbs the light emitted by the reporter molecule; when cleaved during the reaction (or extension of target sequence), the reporter fluoresces.

The characteristics of the amplification curves from the external positive control (EPC), external negative control (ENC) and internal process control (IPC) and from each unknown sample are analyzed by automated secondary interpretative software (BaIS) with data uploaded from the AB 7500 Fast Dx. When EPCs or ENCs are unacceptable, the test results for all samples included in the test run are considered Invalid and must be repeated. The IPC is a human endogenous sequence (TERT) that is also extracted and amplified for whole blood and blood culture specimens. The IPC controls for the extraction process, integrity of the reagents, equipment functionality, and the presence of inhibitors in the specimen. Interpretation of the IPC results are: (1) If the target nucleic acid is 'Detected' and the internal control is 'Not Detected' the result is Valid; (2) If the target nucleic acid and the internal control are 'Not Detected' the result is Invalid and the sample should be re-tested; and (3) If the target nucleic acid is 'Not Detected' and the internal control is 'Detected', the result is Valid.

Assay technology includes multiplexed real-time PCR for the specific detection of *B. anthracis* – specific nucleic acid sequences on the pX01 and pX02 plasmids. The internal positive control (IPC) targets the human telomerase reverse transcriptase (TERT) gene.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   A multi-user reproducibility study was conducted at one testing site using a sample panel prepared by spiking whole blood (K2 EDTA) with quantified preparations of *B. anthracis* Ames or *Bacillus cereus* (*B. cereus*) E33L strains. Each panel contained seven panel members comprised of three high-positive (HP) *B. anthracis* samples, three low-positive (LP) *B. anthracis* samples and one negative sample spiked with *B. cereus*. All organism concentrations were confirmed by colony counts.

   - HP = High Positive: *B. anthracis* Ames spiked at 10 × LoD; $1.5x10^3$ CFU/mL for DSP and $5x10^2$ CFU/mL for MNP
   - LP = Low Positive: *B. anthracis* Ames spiked at 3 × LoD; $4.5x10^2$ CFU/mL for DSP and $1.5x10^2$ CFU/mL for MNP
   - NEG = Negative: non-*B. anthracis* organism (*B. cereus*) spiked at $4.5x10^2$ CFU/mL for DSP and $1.5x10^2$ CFU/mL for MNP

   The reproducibility panel was tested twice a day by three teams of two different operators on five non-consecutive days using two Applied Biosystems 7500 Fast Dx Real-Time PCR instruments across three different reagent lots. Two teams used manual DSP extraction and one team used the automated MagNA Pure (MNP) extraction. Operators were representative of those that work in high-complexity laboratories (i.e., medical technologists) and were not involved in the design and development of the assay.

   One low positive sample extracted by one operator returned negative results when tested against three reagent lots, indicating an error during the sample preparation procedure. In a separate instance a high positive sample returned "BA suspected" results when the pX01 assay failed to amplify. The PCR for this sample was repeated and returned "BA detected" results as expected. No false positive events occurred out of the 270 PCR tests of negative samples. A total of 24 (24/270) negative whole blood samples initially generated indeterminate results. After supervisory review as per the assay instructions, all 24 samples were determined to be negative as expected. There were no invalid results observed in the study.

Reproducibility study results shown in Table 1 are stratified by testing team and extraction method. Lot-to-lot and between instrument reproducibility is presented in Table 2.

The assay was found to be reproducible across instruments and study sites.

Table 1: Results Summary by Team

		DSP Team 1						
Panel Member	**Test Level**	**Agreement with Expected Result**	**Avg. pX01 Ct**	**pX01 SD**	**Avg. pX02 Ct**	**pX02 SD**	**Avg. TERT Ct**	**TERT SD**
Bacillus anthracis Ames	HP	270/270	29.68	0.86	33.06	0.49	27.42	1.30
	LP	267/270	31.75	0.76	34.61	0.58	27.25	1.52
Bacillus cereus E33L	NEG	90/90	N/A	N/A	N/A	N/A	26.77	1.18
		DSP Team 2						
Panel Member	**Test Level**	**Agreement with Expected Result**	**Avg. pX01 Ct**	**pX01 SD**	**Avg. pX02 Ct**	**pX02 SD**	**Avg. TERT Ct**	**TERT SD**
Bacillus anthracis Ames	HP	269/270	29.58	0.91	32.80	0.47	26.13	1.13
	LP	270/270	31.13	0.73	34.58	0.55	26.96	1.24
Bacillus cereus E33L	NEG	90/90	N/A	N/A	N/A	N/A	26.71	1.07
		MNP Team						
Panel Member	**Test Level**	**Agreement with Expected Result**	**Avg. pX01 Ct**	**pX01 SD**	**Avg. pX02 Ct**	**pX02 SD**	**Avg. TERT Ct**	**TERT SD**
Bacillus anthracis Ames	HP	270/270	27.93	1.01	31.63	0.8	27.65	0.95
	LP	270/270	30.42	0.93	33.61	0.79	27.81	0.91
Bacillus cereus E33L	NEG	90/90	N/A	N.A	N/A	N/A	27.63	0.92

Panel Member	**Test Level**	**Total Agreement**	**% Agreement**
Bacillus anthracis Ames	HP	809/810	**99.88%**
	LP	807/810	**99.63%**
Bacillus cereus E33L	NEG	270/270	**100%**

Table 2: Reproducibility Results, by Lot and by Instrument

	Lot 1					
	Avg pX01 Ct		Avg pX02 Ct		Avg TERT Ct	
	Instrument 1	Instrument 2	Instrument 1	Instrument 2	Instrument 1	Instrument 2
BA Ames @ 1.5E3 CFU/mL N=90	28.64	28.67	32.29	32.07	26.99	27.46
BA Ames @ 4.5E2 CFU/mL N=90	30.76	30.94	34.10	33.96	26.85	27.29
B. cereus E33L @ 4.5E2 CFU/mL N=30	N/A	N/A	N/A	N/A	26.59	27.14

	Lot 2					
	Avg pX01 Ct		Avg pX02 Ct		Avg TERT Ct	
	Instrument 1	Instrument 2	Instrument 1	Instrument 2	Instrument 1	Instrument 2
BA Ames @ 1.5E3 CFU/mL N=90	29.68	29.45	32.55	32.40	26.43	27.13
BA Ames @ 4.5E2 CFU/mL N=90	30.70	30.74	33.43	33.74	26.39	27.24
B. cereus E33L @ 4.5E2 CFU/mL N=30	N/A	N/A	N/A	N/A	27.36	26.94

	Lot 3					
	Avg pX01 Ct		Avg pX02 Ct		Avg TERT Ct	
	Instrument 1	Instrument 2	Instrument 1	Instrument 2	Instrument 1	Instrument 2
BA Ames @ 1.5E3 CFU/mL N=90	28.96	28.82	32.15	31.94	26.73	27.04
BA Ames @ 4.5E2 CFU/mL N=90	31.17	31.00	33.96	33.72	26.61	26.98
B. cereus E33L @ 4.5E2 CFU/mL N=30	N/A	N/A	N/A	N/A	26.54	26.79

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Assay Controls: The following controls are required and/or recommended for use with the Applied Biosystems *Bacillus anthracis* Detection Kit:

1. External Positive Control (EPC): The EPC is included in the test kit and is required for each test run. The EPC contains a synthetic plasmid sequence containing gene targets for *B. anthracis* and human endogenous control (TERT). The EPC is expected to produce a "VALID" result.

2. External Negative Control (ENC): The ENC is included in the test kit and is required in each test run. The ENC contains DNA of a non-*B. anthracis* organism (e.g. *E. coli* DNA). The ENC is expected to produce a "VALID" result.

3. No Template Control (NTC): The NTC (Nuclease-free water added in place of sample to monitor reagent integrity) is required for each PCR run. The NTC is expected to produce a "VALID" result.

4. Extraction Control Blank (BLK): An extraction Control Blank is required for each extraction batch. The BLK is a sample free from target serving as a negative or "blank" control sample (e.g., nuclease-free water). The BLK monitors for reagent contamination as well as potential sample-to-sample cross-contamination. The extraction blank is expected to produce a "VALID" result. If the extraction blank result is "INVALID", the samples extracted in the batch with that blank should be repeated from extraction of the original samples.

5. Internal Process Control (IPC): The IPC target sequence is derived from a conserved genetic element of the human genome (TERT) and is an endogenous component of human whole blood and blood culture samples. Each whole blood and blood culture sample will be analyzed by the amplification of the Internal Process Control (IPC). If the IPC fails (for whole blood or blood culture specimens that are negative for both *B. anthracis* targets), the result is INVALID and repeat testing is indicated.

*****If the EPC, ENC, NTC or BLK fails, the entire run should be repeated. The BaIS automatically analyzes each control reaction and reports QC Passed or QC Failed. No additional analysis or interpretation by the user is required.

Table 3 includes control results observed in the clinical studies. See clinical study section below for invalid rates due to failed IPC.

Table 3: External Control Results, Clinical Study

Control Type	Percent Correct
ENC	57/57 (100%)
EPC	57/57 (100%)
NTC	57/57 (100%)
BLK	98/99 (99.0%)

Specimen Stability:
The Applied Biosystems *Bacillus anthracis* Detection Kit instructions for use recommend that specimens are tested immediately after collection; however, specimens may also be stored prior to testing, if needed. A study was conducted to assess the stability of specimens intended for testing with the Applied Biosystems *Bacillus anthracis* Detection Kit when stored and/or shipped under claimed storage conditions. Testing included whole blood specimens, blood culture specimens, and extracted DNA. The conditions assessed in the study are noted in Table 4.

Table 4: Specimen Stability Temperature and Timepoints

Specimen Matrix	Temperature(s)	Time Points
Whole Blood in K2 EDTA	2-8°C	T = 0, Day 1, Day 5, Day 6
Blood Culture	2-8°C	T = 0, Day 1, Day 4, Day 5
Blood Culture	-70°C	T = 0, Day 10, Day 30, Day 60, Day 120, Day 151, Day 240
Frozen DNA extracted from Whole Blood in K2 EDTA	-70°C	T = 0, Day 1, Day 10, Day 30, Day 60, Day 91 with no freeze-thaws. Additionally, evaluate 1 aliquot after 1, 3, and 5 freeze-thaw cycles

Whole Blood Samples*:*
Negative whole blood samples (K2 EDTA) were spiked using quantified *B. anthracis* (Ames Strain) at a concentration of 3x LoD with organism concentrations confirmed via colony

count. Negative whole blood specimens were also evaluated. Six replicates each of *B. anthracis* positive and *B. anthracis* negative samples were extracted using the manual DSP method and tested at each timepoint for the specified storage temperatures as noted in the table above. As 100% of samples generated the expected results, the study demonstrated that whole blood specimens can be stored at 2-8°C for up to five days if stored prior to testing with the Applied Biosystems *Bacillus anthracis* Detection Kit.

Positive Blood Culture Samples*:*
BACTEC Standard Aerobic and Standard Anaerobic blood culture bottles with human whole blood (K2 EDTA) added, were pre-incubated at 37°C for 18-24 hours or overnight to mimic incubation on a blood culture instrument. The blood culture bottles were then inoculated with a quantified stock of *B. anthracis* (Ames strain) at a concentration of $1x10^6$ CFU/mL as confirmed by colony count. Blood culture bottles without organism were also tested. Six replicates each of positive and negative blood culture samples were extracted using the DSP method and were tested at each storage and time point noted in the table above. As 100% of samples generated the expected results, the study demonstrated that positive blood culture specimens are stable at 2-8°C for up to five days and up to 240 hours at -70°C if stored prior to testing with the Applied Biosystems *Bacillus anthracis* Detection Kit.

Frozen DNA Extract*:*
Frozen DNA extracts obtained from spiked K2 EDTA whole blood using the DSP extraction method were tested in replicates of six to evaluate frozen stability as well as for five freeze/thaw cycles. Results from the study demonstrated that DNA extracts are stable at -70°C for up to 90 days. Although the assay instructions indicate that multiple freeze/thaw cycles should be avoided, study results demonstrated no negative impact on test performance for DNA extracts subjected to five freeze/thaw cycles.

d. Detection limit (Limit of Detection, LoD):

A study was conducted to determine the Limit of Detection (LoD) of the Applied Biosystems *Bacillus anthracis* Detection Kit for whole blood, aerobic blood culture (BACTEC Standard Aerobic) and anaerobic blood culture (BACTEC Standard Anaerobic) specimens using both the Qiagen DSP DNA Blood Mini Kit and Roche MagNA Pure LC 2.0 extraction methods.

For whole blood samples, a quantified preparation of *B. anthracis* (Ames strain) was spiked into human blood (EDTA) matrix. For blood culture specimens, blood culture bottles inoculated with 8 mL of human whole blood, were pre-incubated at 37°C for 18-24 hours to mimic incubation on a blood culture instrument. The bottles were then inoculated with a quantified stock of *B. anthracis* (Ames strain). Organism quantification was performed using a standard plate count method.

An initial range finding study was conducted to determine the estimated LoD of the assay. For each extraction method, testing was conducted with one reagent lot and included four replicates for each of four organism levels. The estimated LoD was determined to be the lowest concentration that generated 100% detection with the Applied Biosytems *Bacillus anthracis* Detection Kit.

The LoD of the Applied Biosystems *Bacillus anthracis* Detection Kit was then confirmed by testing 60 replicates at the estimated LoD (20 replicates for each of three test kit lots). The final LoD for each specimen type and extraction method was determined to be the lowest concentration of *B. anthracis* that generated ≥ 95% detection. Final LoD results are shown in Table 5.

Table 5: Limit of Detection, Whole Blood, Aerobic and Anaerobic Blood Culture, per Extraction Method

Extraction Method	Matrix	Limit of Detection (CFU/mL)
Qiagen DSP DNA Blood Mini Kit	Whole Blood	150
	Blood Culture, Aerobic*	10,000
	Blood Culture, Anaerobic*	10,000
Roche MagNA Pure	Whole Blood	50
	Blood Culture, Aerobic*	2270
	Blood Culture, Anaerobic*	3040

* Higher LoD for blood culture specimens is due to 1:100 dilution of specimen extract that is needed to minimize potential inhibition from blood culture media. Specimen extracts for whole specimens are not diluted.

e. Analytical Reactivity/Inclusivity

To evaluate the inclusivity of the Applied Biosystems *Bacillus anthracis* Detection Kit, quantified stocks of 24 different *B. anthracis* strains representing geographic, temporal, genotypic, and phenotypic diversity were prepared from culture and quantitated by enumeration on agar media. Each strain was spiked into whole blood (K2 EDTA), at 3× the established LoD for whole blood and tested in triplicate using both the Roche MagNA Pure LC 2.0 and Qiagen DSP DNA Blood Mini Kit extraction methods. For all strains that carry both plasmid targets, the study generated 100% detection of *B. anthracis* as expected, demonstrating that the Applied Biosystems *Bacillus anthracis* assay detects a diversity of *B. anthracis* strains. The strains evaluated in the study are shown in Table 6.

Table 6: Inclusivity Study, *Bacillus anthracis* Strains

***Bacillus anthracis* Strains Evaluated**	
K2129	Carbosap
Turkey No 32	S. African
K2883	Ohio ACB
PAK-1	V770-NP-R1*
K4834	Canadian Bison
K1285	SK-102
K3974	1035
K1811 (Scotland)	Sterne*
Bekasi	Ames
Zimbabwe 89	Vollum 1B
506, Heroin Ba	RA3
Smith 1013*	Pasteur**

*‘*B. anthracis* Suspected’ result generated, strain includes only pX01 plasmid target
**‘*B. anthracis* Suspected’ result generated, strain includes only pX02 plasmid target

To augment inclusivity testing, *in silico* analysis was performed using assay primers and probe sequences aligned against 144 applicable *B. anthracis* genome assemblies in the GenBank database. One strain of *B. anthracis* was identified with a single mismatch to the forward primer for the pX01 plasmid assay; however, the effect of this mismatch is not predicted to affect amplification due to the location of the mismatch within the forward primer region. No *B. anthracis* strains were identified with mismatches to the pX02 plasmid assay. In summary, results from *in silico* analysis

support the predicted detection of *B. anthracis* strains containing both pX01 and pX02 targets and also predicts a '*B. anthracis* suspected' result will be generated for all *B. anthracis* strains known to possess only one plasmid target.

f. Analytical specificity/Exclusivity:

The exclusivity of the Applied Biosystems *Bacillus anthracis* Detection Kit was evaluated by testing a panel of non-target microorganisms, including near-neighbors and other bacteria, viruses and fungi that might be present in blood specimens or may cause infections with a similar clinical presentation to those patients suspected of anthrax. Bacterial exclusivity panel members were prepared from fresh preparations that were quantified by colony count and viral exclusivity panel members were prepared from fresh or previously titered stocks. Purified nucleic acids were used to prepare samples for instances where bacteria, virus or fungi were not available as isolates. Samples were prepared at high concentrations ($\geq 10^7$ CFU/mL or genome copies/mL) and testing was performed in triplicate.

Three *B. cereus* strains (BAG1X1-1 NR28575, 03BB102, and G-9241 NR-9564) evaluated contain a pX01-like plasmid and should generate a '*Bacillus anthracis* suspected' result with the Applied Biosystems *Bacillus anthracis* Detection Kit. These strains generated the expected result of ' *anthracis* suspected' and the amplification of the pX01 plasmid was manually confirmed by manual review of PCR curves.

Two strains of *B. thuringiensis* unexpectedly generated '*Bacillus anthracis* suspected' results for all replicates tested. One strain of *B. atrophaeus* generated '*Bacillus anthracis* suspected' results for one of three replicates. The discordant results, due to amplification of the pX01 target, were determined to be due to operator error. Newly prepared samples for these three strains were tested in triplicate and generated a result of '*Bacillus anthracis* not detected' as expected.

Exclusivity results are shown in Tables 7, 8 and 9 for organisms evaluated with whole organism, genomic DNA or by *in silico* analysis respectively.

Table 7: Exclusivity Study Results, Samples Prepared with Whole Organism

Organisms/Nucleic Acid	Test Concentration	Initial Correct Calls	Initial Correct Call Percentage	Final Correct Calls	Final Correct Call Percentage
B. cereus 3A (Fri-41)	3.83E8 CFU/mL	3/3	100.00%		
B. cereus E33L	3.10E8 CFU/mL	3/3	100.00%		
B. cereus D17	2.33E8 CFU/mL	3/3	100.00%		
B. cereus S-28	3.07E8 CFU/mL	3/3	100.00%		
B. cereus MSX-A1	2.87E8 CFU/mL	3/3	100.00%		
B. cereus m1550	4.43E8 CFU/mL	3/3	100.00%		
B. cereus VD148 NR-22150	2.50E8 CFU/mL	3/3	100.00%		
B. cereus VD014 NR-22141	2.97E8 CFU/mL	3/3	100.00%		
B. cereus BAG60-1 NR-28593	1.67E8 CFU/mL	3/3	100.00%		
B. cereus BAG1X1-1 NR28575*	3.13E8 CFU/mL	3/3	100.00%		
B. cereus 03BB102*	4.97E8 CFU/mL	3/3	100.00%		
B. cereus G-9241 NR-9564*	1.43E8 CFU/mL	3/3	100.00%		
B. thuringiensis HD 1011[1]	3.10E8 CFU/mL	0/3	0.00%	3/3	100.00%

Organisms/Nucleic Acid	Test Concentration	Initial Correct Calls	Initial Correct Call Percentage	Final Correct Calls	Final Correct Call Percentage
B. thuringiensis HD 974[1]	4.37E8 CFU/mL	0/3	0.00%	3/3	100.00%
B. thuringiensis 97-27	8.77E8 CFU/mL	3/3	100.00%		
B. thuringiensis HD682	6.30E8 CFU/mL	3/3	100.00%		
B. thuringiensis HD571	4.67E8 CFU/mL	3/3	100.00%		
B. thuringiensis HD 4	5.57E8 CFU/mL	3/3	100.00%		
B. thuringiensis Israelensis	1.20E8 CFU/mL	3/3	100.00%		
B. thuringiensis Morrisoni	2.43E8 CFU/mL	3/3	100.00%		
B. thuringiensis Al Hakam 4/2014	4.10E8 CFU/mL	3/3	100.00%		
B. megaterium	3.17E7 CFU/mL	3/3	100.00%		
B. mycoides ATCC 23258	1.57E7 CFU/mL	3/3	100.00%		
B. licheniformis NR 2494 NCIB 9375	6.67E8 CFU/mL	3/3	100.00%		
B. licheniformis NR-2499 NRS 712	6.83E8 CFU/mL	3/3	100.00%		
B. circulans NR681 Ford 26	9.30E6 CFU/mL	3/3	100.00%		
B. subtilis NR604 NRS 231	4.00E8 CFU/mL	3/3	100.00%		
B. pumilus NR 605 NRS272	2.20E8 CFU/mL	3/3	100.00%		
B. atrophaeus NR-687 NRS 1221A[1]	3.07E8 CFU/mL	2/3	66.67%	3/3	100.00%

***** *Bacillus cereus* strains known to contain a pX01 like plasmid. Correct detection of these strains is indicated by a test result of "*Bacillus anthracis* suspected" with amplification of the correct plasmid manually confirmed by the supervisor.

[1] Initial false positive results were for pX01 only, generating a "*Bacillus anthracis* suspected" result.

Table 8: Exclusivity Study Results, Samples Prepared with Genomic DNA

Organisms/Nucleic Acid	Test Concentration	Initial Correct Calls	Initial Correct Call Percentage
Burkholderia pseudomallei	6.47E7 Genome copies/mL	3/3	100.00%
Burkholderia mallei	1.94E9 CFU/mL	3/3	100.00%
Burkholderia cepacia	7.54E7 Genome copies/mL	3/3	100.00%
Mycobacterium smegmatis	9.28E7 Genome copies/mL	3/3	100.00%
Vaccinia virus (Pox)	4.55E9 Genome copies/mL	3/3	100.00%
Yersinia pestis Colorado-92	8.99E7 Genome copies/mL	3/3	100.00%
Yersinia pestis A1122	2.17E8 CFU/mL	3/3	100.00%
Francisella tularensis subsp. tularensis Schu-S4	1.68E8 Genome copies/mL	3/3	100.00%
Rickettsia prowezekii	6.61E8 Genome copies/mL	3/3	100.00%
Brucella melitensis	1.45E8 Genome copies/mL	3/3	100.00%

Organisms/Nucleic Acid	Test Concentration	Initial Correct Calls	Initial Correct Call Percentage
Vibrio cholerae	1.43E8 Genome copies/mL	3/3	100.00%
Shigella flexneri	2.05E9 CFU/mL	3/3	100.00%
Bacteroides fragilis	1.43E8 Genome copies/mL	3/3	100.00%
Aspergillus fumigatis	3.36E7 Genome copies/mL	3/3	100.00%
Clostridium botulinum Type B	8.85E7 Genome copies/mL	3/3	100.00%
Zika virus IBH30656 ATCC VR-1839	9.60E11 Genome copies/mL	3/3	100.00%
Venezuelan Equine Encephalitis TC-83	5.50E11 Genome copies/mL	3/3	100.00%
Yellow Fever virus 17D NIAD V-525-001-522	5.60E11 Genome copies/mL	3/3	100.00%
Dengue virus	2.13E11 Genome copies/mL	3/3	100.00%
Acinetobacter lwoffii	1.70E8 CFU/mL	3/3	100.00%
Staphylococcus aureus	1.09E9 CFU/mL	3/3	100.00%
Streptococcus pneumoniae	5.00E5 CFU/mL	3/3	100.00%
Pseudomonas aeruginosa	Plate culture too numerous to count	3/3	100.00%
Cytomegalovirus	2.25E10 Genome copies/mL	3/3	100.00%
Epstein-Barr virus	8.07E10 Genome copies/mL	3/3	100.00%

In silico analysis was conducted for additional microorganisms and viruses to evaluate the primers and probes of the Applied Biosystems *Bacillus anthracis* Detection kit for potential cross-reactivity. Assay primers and probe sequences were aligned against sequences from the non-target organisms listed in Table 9. Results from the analysis demonstrated lack of significant homology to available sequences for each organism which were therefore predicted to be non-cross-reactive.

Table 9: Exclusivity, Organisms Evaluated by *In silico* Analysis

Organisms	
Mycobacterium spp (MAC, MTB)	*Trypanosoma brucei*
Bordetella spp	*Candida albicans*
Leptospira	*Clostridium perfringens*
Coccidioides immitis	*Clostridium septicum*
Histoplasma capsulatum	*Citrobacter koseri*
Bacillus halodurans	*Escherichia coli*
Bacillus luciferensis	*Enterococcus faecalis*
Hepatitis B virus (HBV)	*Enterococcus faecium*
Hepatitis C virus (HCV)	*Enterobacter cloacae*
Herpes Simplex 1 virus (HSV1)	*Klebsiella oxytoca*
Herpes Simplex 2 virus (HSV2)	*Klebsiella pneumoniae*
Human herpesvirus 6 virus (HHV6)	*Lactobacillus* species
Human Immunodeficiency virus (HIV)	*Listeria monocytogenes*

West Nile virus (WNV)	*Propionibacterium acnes*
Babesia microti	*Rothia* species
Leishmania spp	*Staphylococcus epidermidis*
Plasmodium falciparum	*Staphylococcus haemolyticus*
Plasmodium vivax	*Streptococcus viridans*
Trypanosoma cruzi	*Streptococcus pyogenes*

g. Interfering Substances Study

A study was conducted to evaluate the effect of 50 potentially interfering endogenous, exogenous and technique-specific substances on the performance of the Applied Biosystems *Bacillus anthracis* Detection Kit. For endogenous and exogenous substances, samples were prepared in a whole blood (K2 EDTA) matrix. Positive samples with *B. anthracis* Ames strain (at 3x LoD) and negative samples were each tested in triplicate using both the DSP and MagNA Pure extraction methods. Substances were spiked at concentrations typically found in human whole blood or at the highest achievable concentration representing the clinically 'worst case'. To mirror potential procedural errors, technique-specific substances were spiked at three different test concentrations directly into PCR reaction wells containing positive (*B. anthracis* at 3x LoD) or negative sample extracts. The substances evaluated are presented in Tables 10, 11 and 12 for endogenous, exogenous and technique-specific substances respectively.

Table 10: Interfering Endogenous Substances

Endogenous Substances	Concentration
Hemoglobin (hemolysate)	625 mg/dL
Glucose	20 mmol/L
Bilirubin conjugated	20 mg/dL
Bilirubin unconjugated	20 mg/dL
Cholesterol (total)	13 mmol/L
Triglycerides	1000 mg/dL
Total protein (from albumin and immunoglobulins)	2 g/dL

Table 11: Interfering Exogenous Substances

Exogenous Substance	Concentration
Acetaminophen	1324 µmol/L
Amoxicillin	206 µmol/L
Ascorbic acid	342 µmol/L
Acetylsalicylic acid	4.34 mmol/L
Cefotaxime	673 µmol/L
Ciprofloxacin	30.2 µmol/L
Doxycycline	67.5 µmol/L
Erythromycin	81.6 µmol/L
Gentamicin sulfate	21 µmol/L
Ibuprofen	2425 µmol/L
Naproxen sodium	2170 µmol/L
Rifampin	78.1 µmol/L
Streptomycin	50 mcg/ml
Sulfamethoxazole	1.58 mmol/L

Exogenous Substance	Concentration
Tetracycline	34 µmol/L
Tobramycin	51.4 µmol/L
Trimethoprim	138 µmol/L
Acid-citrate-dextrose	23mM citric acid, 65mM sodium citrate, 136mM dextrose
Ribavirin	2700 ng/mL
Oseltamivir (Tamiflu)	65.2 ng/mL
Artemisinin	70 ng/mL (artemisin) 5 µg/mL (lumefantrine)
Malarone (Proguanil/Atovaquone)	Proguanil: 363 ng/mL Atavaquone : 908 ng/mL
Chloroquine	0.04 mg/L
Mefloquine	2000 ng/mL
Anthrax vaccine (Biothrax)	0.01 % (v/v) Based on 0.5 mL dose in 5 liters of blood

Table 12: Interfering Substances, Technique-Specific Substances

Extraction Method Sample Source	Technique-Specific Substances	Concentration Tested (v/v)
Qiagen DSP DNA Blood Mini Kit	**QIAGEN Protease**	2.5%, 5%, and 10%
	QIAGEN Buffer AL	
	100% Ethanol	
	QIAGEN Buffer AW1 (with EtOH)	
	QIAGEN Buffer AW1 (w/o EtOH)	
	QIAGEN Buffer AW2 (with EtOH)	
	QIAGEN Buffer AW2 (w/o EtOH)	
Roche MagNA Pure	**MagNA Pure Wash Buffer I**	
	MagNA Pure Wash Buffer II	
	MagNA Pure Wash Buffer III	
	MagNA Pure Lysis/Binding Buffer	
	MagNA Pure Magnetic Glass Particles Suspension	
	MagNA Pure Proteinase K	
	10% Bleach	
	Isopropyl alcohol	
	Fisherbrand DNase Displace	
	RNase Away	

A total of 702 samples were evaluated in the study. No interference was observed for positive or negative samples containing the endogenous or exogenous substances listed in Tables 11 and 12. The following nine substances (bolded in Table 12) demonstrated either false negative or invalid results for the technique-specific substances evaluated in the study.

MagNA Pure Substances:

- MagNA Pure Wash Buffer I: For all concentrations of MagNA Pure Wash Buffer I, all samples (with or without *B. anthracis*) generated INVALID results due to no amplification in any of the targets including the IPC.

- MagNA Pure Lysis/Binding Buffer: For all concentrations of Lysis/Binding Buffer, samples without *B. anthracis* generated INVALID results due to no amplification of any of the targets including the IPC. For all concentrations of Lysis/Binding Buffer evaluated, samples spiked with *B. anthracis* demonstrated no amplification in the IPC. In some samples spiked with *B. anthracis*, a Ct was produced for the pX01 target which led to an interpretive result of '*Bacillus anthracis* suspected'; however, upon review of the PCR curves, the result was not due to true amplification but rather to interference of fluorescence.

- MagNA Pure Proteinase K: For all concentrations of MagNA Pure Proteinase K evaluated, all samples generated INVALID results due to no amplification in any of the targets including the IPC.

QIAGEN DSP Substances:

- QIAGEN Protease: For all concentrations of QIAGEN Protease evaluated, all samples without *B. anthracis* generated INVALID results due to no amplification of the IPC. For QIAGEN Protease at 10% and 2.5%, all samples spiked with *B. anthracis* generated INVALID results due to no amplification in any of the targets including the IPC. For QIAGEN Protease at 5%, samples spiked with *B. anthracis* generated the expected '*B. anthracis* detected' result; however, QIAGEN Protease regent is considered an interferent for all concentrations $\geq$ 2.5% due to the INVALID results observed at the lower concentration.

- QIAGEN Buffer AL: For all concentrations evaluated, samples with or without *B. anthracis* generated INVALID results due to no amplification in any of the targets including the IPC.

- QIAGEN Buffer AW1 (with and without EtOH): For all concentrations of QIAGEN Buffer AW1 (with and without EtOH), samples without *B. anthracis* generated INVALID results due to no amplification of the IPC. For all concentrations of QIAGEN Buffer AW1 without EtOH, all samples with *B. anthracis* generated INVALID results due to no amplification of any targets, including the IPC. For the two highest concentrations of QIAGEN Buffer AW1 (10% and 5%) evaluated with EtOH, samples spiked with *B. anthracis* generated INVALID results. For the lowest concentration of QIAGEN Buffer AW1 with EtOH (2.5%), one sample spiked with *B. anthracis* generated an INVALID result while two replicates were reported as *B. anthracis* suspected. In summary, these results indicate interference of target amplification by QIAGEN Buffer AW1 both with and without EtOH added.

- EtOH (QIAGEN extraction): At the highest concentration evaluated (10%), samples spiked with *B. anthracis* generated an INVALID result for one replicate and '*B. anthracis* not detected' for two replicates. All other concentrations tested both in the presence and absence of *B. anthracis* produced the expected results (i.e., no interference observed at 5% or 2.5%).

Decontamination Substances:

- Bleach: For both extraction methods, INVALID results were observed for all concentrations evaluated and for all samples with and without *B. anthracis*.

- Fisherbrand DNase Displace: For both extraction methods, INVALID results were observed for all concentrations evaluated and for all samples with and without *B. anthracis.*

A limitation is included in the Applied Biosystem *Bacillus anthracis* Detection Kit package insert warning the user of the technique-specific substances that demonstrated interference with the assay. It is noted that if the extraction and decontamination procedures included in the instructions for use are carefully followed, these substances are unlikely to be present in specimen extracts and are unlikely to be introduced directly into final PCR reactions.

h. Microbial Interference Study

A study was conducted to evaluate the performance of the Applied Biosystems *Bacillus anthracis* Detection Kit to detect *B. anthracis* in samples mixed with other clinically-relevant organisms, including pathogens or skin contaminants that may be present in whole blood or positive blood culture specimens.

For whole blood, samples were prepared in a blood (K2 EDTA) matrix with high concentrations of non-targeted organisms and *B. anthracis* (Ames strain) at 1.5x the established LoD for each extraction method (75 CFU/mL for MNP and 225 CFU/mL for DSP). All organism concentrations were confirmed by colony count. Testing was performed in triplicate with 100% of samples generating the expected result of '*Bacillus anthracis* detected' for each extraction method. Results are presented in Table 13.

Table 13: Microbial Interference, Organisms Evaluated in Whole Blood

Non-Target Organism	Non-target Organism Concentration	Extraction Method	BA Detection Rate
Corynebacterium xerosis	1 X 10^6 CFU/mL	DSP	100%
		MNP	100%
Escherichia coli	1 X 10^6 CFU/mL	DSP	100%
		MNP	100%
Enterococcus faecalis	1 X 10^6 CFU/mL	DSP	100%
		MNP	100%
Staphylococcus epidermidis	1 X 10^6 CFU/mL	DSP	100%
		MNP	100%
Cytomegalovirus (CMV)	1 X 10^6 $TCID_{50}$/mL	DSP	100%
		MNP	100%
Epstein-Barr Virus (EBV)	>1.2 X 10^3 $TCID_{50}$/mL	MNP	100%
	>7.8 X 10^2 $TCID_{50}$/mL	DSP	100%
Absence of Non-target Organism (*Bacillus anthracis* Ames only at 3x LoD)	N/A	MNP	100%
		DSP	100%

For blood culture samples, aerobic (BACTEC Standard Aerobic) and anaerobic (BACTEC Standard Anaerobic) blood culture bottles inoculated with 8 mL human whole blood were pre-incubated at 37°C for 18-24 hours to mimic incubation on a blood culture instrument. Blood culture bottles were then co-inoculated with *B. anthracis* (Ames strain) and each potentially interfering non-target microorganism at concentrations of $1x10^6$ CFU/mL as confirmed by colony count. Testing was performed in triplicate for each potentially interfering microorganism and extraction method. No interference was observed as 100% of aerobic and anaerobic blood culture samples generated the expected result of '*Bacillus anthracis* detected' for each extraction method. Results are presented in Table 14.

Table 14: Microbial Interference Study, Blood Culture Specimens

Non-Target Organism	Matrix	Extraction Method	Correct Call of *B. anthracis* detected	Correct Call Percentage
Staphylococcus haemolyticus	Aerobic and Anaerobic Blood Culture Media	DSP	6/6	100%
		MNP	6/6	100%
Corynebacterium jeikeium		DSP	6/6	100%
		MNP	6/6	100%
Propionibacterium acnes		DSP	6/6	100%
		MNP	6/6	100%
Bacillus cereus		DSP	6/6	100%
		MNP	6/6	100%
Candida albicans		DSP	6/6	100%
		MNP	6/6	100%
Lactobacillus acidophilus		DSP	6/6	100%
		MNP	6/6	100%
None (*Bacillus anthracis* Ames only)		DSP	6/6	100%
		MNP	6/6	100%

i. Carryover/Cross-Contamination Study

The Applied Biosystems *Bacillus anthracis* Detection Kit workflow was evaluated for potential cross-contamination or sample carryover during extraction using the MagNA Pure automated system (MNP) and the manual method (Qiagen DSP Mini Kit, DSP).

For the automated extraction method, nine full MagNA Pure runs were performed with each run containing 31 samples placed in a checkerboard pattern (i.e., alternating high positive and negative samples). Positive samples included whole blood (K2 EDTA) spiked with *B. anthracis* (Ames strain) at 500 CFU/mL (10x LoD), and aerobic and anaerobic blood culture samples spiked at 1×10^6 CFU/mL. Negative samples included both whole blood and blood culture matrices. A total of 279 samples were extracted followed by testing with the Applied Biosystems *Bacillus anthracis* Detection Kit using three full PCR plates. The study generated false positive results for a total of 2/63 (3.2%) whole blood samples and 0/72 (0%) blood culture samples, demonstrating a carryover/cross-contamination rate of 1.5% for all specimen types combined. See results in Table 15 below.

Table 15: Carryover/Cross-Contamination Study, MagNA Pure Extraction

Sample Type	False Positives/Total Sample Number	% of False Positives
Whole Blood	2/63	**3.2%**
BC, aerobic	0/36	0%
BC, anaerobic	0/36	0%
All Combined	**2/135**	**1.5%**

For the manual Qiagen DSP extraction, 12 batches of 15-24 samples were extracted with positive and negative samples tested in a similar checkerboard pattern described above. Positive samples included whole blood (K2 EDTA) spiked with *B. anthracis* (Ames strain) at 1,500 CFU/mL (10x LoD), and aerobic and anaerobic blood culture samples spiked at 1 x 10^6 CFU/mL. A total of 279 samples were extracted using the DSP Qiagen and were then tested using three full PCR plates with the Applied Biosystems *Bacillus anthracis* Detection Kit. The study generated no false positive results as shown in Table 16.

Table 16. Carryover, Cross-contamination Study, DSP Extraction

Sample Type	False Positives/Total Sample Number	% of False Positives
Whole Blood	0/69	0%
BC, aerobic	0/35	0%
BC, anaerobic	0/35	0%
All Combined	0/139	0%

Monitoring of test area surfaces, MagNA Pure extraction:

Control swabs from test area surfaces were collected at various procedural steps during the Carryover/Cross-Contamination study to support a detailed evaluation of sample handling and processing steps with potential to introduce contamination. A total of 162 swabs were collected during testing of nine separate MagNA Pure runs performed during the study. Swabs were processed and analyzed in singlet PCR. A total of nine swab locations were tested to include three swab locations in the biosafety cabinet (BSC) and six surfaces inside the MagNA Pure (MNP) work deck.

Results from swab testing demonstrated that contamination events occurred on one BSC surface location and five surfaces of the MNP during testing. After extraction, various locations on the MagNA Pure instrument deck were identified as having a high rate of contamination with a total of 18/54 (33.3%) swab tests generating '*B. anthracis* detected' results. The following three areas on the work deck of the MNP instrument were identified as having the highest rates of contamination.

- MNP Deck, reagent loading area
- MNP Deck, cartridge processing area
- MNP, magnetic head

Results of the study were assessed to determine areas of the instrument and steps in the testing process that are at high risk of cross-contamination and to determine appropriate mitigations. To reduce the risk for run-to-run carryover, detailed contamination control steps were included in the instructions for use. These steps include required instrument decontamination/cleaning of the

MagNA Pure test platform (i.e., deck and magnetic head) as well as running the MagNA Pure UV Decontamination Program after each extraction run.

Because of the significant contamination observed with the MagNA Pure instrument and the potential risk to laboratorians from exposure to aerosols that might be generated during extraction, it was determined that the MagNA Pure extraction procedure should be performed in BSL-3 laboratories only. This requirement was included in the procedural steps and the test limitations section of the Applied Biosystems *Bacillus anthracis* Detection Kit package insert.

A summary of the test results for all MagNA Pure runs performed in the study are presented in Table 17 below.

Table 17: MagNA Pure Test Area Surfaces, Control Swab Results Summary

MNP Swab Location	Swab Time	# Positives/Swabs Tested
BSC - left surface	Before Extraction	0/9
BSC - middle surface	Before Extraction	0/9
BSC - right surface	Before Extraction	0/9
MNP left side wall	Before Extraction	0/9
MNP deck by reagent loading	Before Extraction	0/9
MNP deck by processing cartridges	Before Extraction	0/9
MNP deck by elution cartridges	Before Extraction	0/9
MNP right side wall	Before Extraction	0/9
MNP magnetic head	Before Extraction	0/9
BSC - left surface	After Extraction	0/9
BSC - middle surface	After Extraction	1/9
BSC - right surface	After Extraction	0/9
MNP left side wall	**After Extraction**	**1/9**
MNP deck by reagent loading	**After Extraction**	**5/9**
MNP deck by processing cartridges	**After Extraction**	**5/9**
MNP deck by elution cartridges	**After Extraction**	**2/9**
MNP right side wall	After Extraction	0/9
MNP magnetic head	**After Extraction**	**5/9**
BSC Combined	**Before Extraction**	**0/27 (0%)**
	After Extraction	**1/27 (3.7%)**

MNP Swab Location	Swab Time	# Positives/Swabs Tested
MNP Combined	**Before Extraction**	**0/54 (0%)**
	After Extraction	**18/54 (33.3%)**

Monitoring of test area surfaces, DSP extraction:

For the QIAGEN DSP extraction, a total of 216 swabs were collected during 12 separate extraction batches tested in the Carryover/Cross-Contamination study. Swabs were processed and analyzed for the presence of *B. anthracis* by PCR. Results were used to determine areas of the BSC and specific process steps that are at high risk of cross-contamination. One workflow location (BSC – left and middle surfaces - after loading DSP columns) was identified as having a higher rate of contamination observed (4/12, 30.0% positive swab tests). To mitigate the potential risk of false positive results due to carryover/Cross-contamination, specific contamination control steps were included in the Applied Biosystems *Bacillus anthracis* Detection Kit package insert. A summary of results for control swab samples for all DSP batches is presented in Table 18.

Table 18: DSP Test Area Surfaces, Control Swab Results Summary

DSP Swab Location	Swab Time	# Positive/Swabs Tested
BSC - left, middle, right surfaces	Before Extraction	0/36
BSC - left, middle, right surfaces	After loading lysis tubes	0/36
BSC - left, middle, right surfaces	After ethanol addition	0/36
BSC - left surface	After loading DSP columns	3/12
BSC - middle surface	After loading DSP columns	1/12
BSC - right surface	After loading DSP columns	0/12
BSC - left, middle, right surfaces	Before Buffer AE addition	0/36
BSC - left, middle, right surfaces	After Extraction	0/36
Before Extraction		0/36 (0%)
During Extraction (Combined)		4/144 (2.8%)
After Extraction		0/36 (0%)

j. Blood Culture Bottle Media Equivalency Study

A study was conducted to assess the performance of the Applied Biosystems *Bacillus anthracis* Detection Kit for various blood culture bottle types commonly used in clinical laboratories. Blood culture media bottles, each containing five to eight mL of human whole blood (or 4 mL for BACTEC Pediatric Plus and BacT/Alert Pediatric FAN bottles), were pre-incubated at 37ºC for 18-24 hours to mimic incubation on a blood culture instrument. After incubation, samples were prepared with each blood culture/blood matrix using two *B. anthracis* strains (Ames and Carbosap strains) to create a final organism concentration of 1 x 10^6 CFU/mL. For positive samples, a total of 20 replicates were tested for each blood culture bottle type and extraction method. Additionally, negative samples containing blood without *B. anthracis* were tested in triplicate for each type of blood culture media. Table 19 outlines the study plan. Results from the study are shown in Tables 20, 21 and 22 for the BacT/Alert, BACTEC, and TREK blood culture bottles evaluated.

Table 19: Testing Scheme for Blood Culture Equivalency Study

Sample Type	Number of Strains	Replicates	Number of Blood Culture Media	Number of Extraction Methods	Total Number of Samples
Positive (samples spiked with *B. anthracis*)	2	20	12	2	960
Negative (Blank)	N/A	3	12	2	72
Total number of samples					1032

Table 20: Blood Culture Media Equivalency Results, BacT/Alert Bottles

Bottle Type	Extraction Method	Organism	Number Correct Calls	Correct Call Percentage
Standard Aerobic (SA)	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Standard Anaerobic (SN)	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Aerobic fastidious antibiotic neutralization (FAN)	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Anaerobic FAN	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Pediatric FAN	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%

Table 21: Blood Culture Equivalency Results, BACTEC Bottles

Bottle Type	Extraction Method	Organism	Number Correct Calls	Correct Call Percentage
BACTEC Standard aerobic	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
BACTEC Standard Anaerobic	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Plus Aerobic/F	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Plus Anaerobic/F	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
Peds Plus	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%

Table 22: Blood Culture Media Equivalency Results, Trek ESP Media

Bottle Type	Extraction Method	Organism	Number Correct Calls	Correct Call Percentage
REDOX 1 (aerobic)	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
REDOX 2 (anaerobic)	DSP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%
	MNP	Negative/Blank	3/3	100%
		BA Ames	20/20	100%
		BA Carbosap	20/20	100%

The detection rate for all positive blood culture specimens was 100% for all blood culture media types evaluated in the study. All blood culture specimens without organism generated the expected '*Bacillus anthracis* Not Detected' result . The study results support use of the following blood culture bottle types with the Applied Biosystems *Bacillus anthracis* Detection Kit.

- BacT/Alert Standard aerobic (SA)
- BacT/Alert Standard anaerobic (SN)
- BacT/Alert Aerobic fastidious antibiotic neutralization (FAN)
- BacT/Alert Anaerobic FAN
- BacT/Alert Pediatric FAN
- BACTEC SA Standard aerobic
- BACTEC SN Standard anaerobic
- BACTEC Plus aerobic/F Medium
- BACTEC Plus anaerobic/F Medium
- BACTEC Pediatric Plus
- Trek ESP REDOX 1 (aerobic)
- Trek ESP REDOX 2 (anaerobic)

k. Analytical Study, Summary of Indeterminate Rates:

Indeterminate results may be generated when testing samples with the Applied Biosystems *Bacillus anthracis* Detection Kit. This result is generated from a 'cross-talk' phenomenon when negative whole blood specimens produce a strong signal in the IPC (TERT) dye layer (ABY). This causes the baseline of the internal dye layer (ROX) to rise, which in turn causes the *B. anthracis* pX01 (FAM) dye layer to cross the threshold. Upon examination of the amplification curve it is evident that no amplification of pX01 has occurred. The BaIS interpretive software is designed to return an "indeterminate'

result when this occurs and instructs the user to seek 'Supervisor Review'. The 'supervisor review' process is described in the package insert and includes detailed instructions for how to view the amplification charts and determine if the Ct value generated is due to true amplification.

The rates of indeterminate calls generated during analytical testing are summarized in Table 23 below. Indeterminate results occurred only in negative whole blood (i.e., without organism) at a rate of 4.6% (51/1101 samples tested) for all analytical studies combined. Indeterminate results were not observed in whole blood specimens spiked with *Bacillus anthracis* or blood culture specimens with or without organism. A summary of indeterminate rates for each analytical study is presented in Table 23.

Table 23: Indeterminate Total and Rate by Analytical Study and Sample Matrix

Study	WB w/o BA	WB with BA	BC w/o BA	BC with BA
Reproducibility	24/270 8.9%	0/1620 0.0%	N/A	N/A
Potentially Interfering Substances	7/351 2.0%	0/351 0.0%	N/A	N/A
LoD Confirmation	5/120 4.2%	0/400 0.0%	0/280 0.0%	0/1701 0.0%
LoD Dynamic Range Finding	0/8 0.0%	0/64 0.0%	0/16 0.0%	0/128 0.0%
Inclusivity	N/A	0/138 0.0%	N/A	N/A
Exclusivity	N/A	N/A	N/A	N/A
Microbial Interference	N/A	0/42 0.0%	N/A	0/84 0.0%
Carry-Over Cross-Contamination	0/40 0.0%	0/40 0.0%	0/40 0.0%	0/40 0.0%
Specimen Stability	0/42 0.0%	0/102 0.0%	0/60 0.0%	0/60 0.0%
Blood Culture Bottle Equivalency	N/A	N/A	0/72 0.0.%	0/960 0.0%
Whole Blood Equivalency	0/18 0.0%	0/240 0.0%	N/A	N/A
Real-Time Stability	15/252 6.0%	0/252 0.0%	0/252 0.0%	0/252 0.0%
All Analytical Studies Combined	**51/1101 4.6%**	**0/3249 0.0%**	**0/720 0.0%**	**0/3225 0.0%**

N/A: Not applicable
WB: Whole blood
BC: Blood culture

l. Assay cut-off:

The BaIS software automatically interprets and displays valid results as '*Bacillus anthracis* detected', '*Bacillus anthracis* suspected' or '*Bacillus anthracis* not detected'. Ct values that are <38 for both the pX01 and pX02 targets will generate a "*Bacillus anthracis* detected" result. A sample may result as "*Bacillus anthracis* suspected" if one of the two BA targets is not detected, or if one or both BA targets results in a Ct value ≥ 38 but less than 40. Ct values for ≥40 will generate a result of "*Bacillus anthracis* not detected".

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Blood Anticoagulant Equivalency Study

A matrix comparison study was conducted to evaluate the performance of the Applied Biosystems *Bacillus anthracis* Detection Kit when testing human whole blood collected in three commonly used anti-coagulants (K2 EDTA, Lithium Heparin, and Sodium Citrate). Each anticoagulant was evaluated using two strains of *B. anthracis* (Ames and RA3) to determine performance for low positive samples with organism concentrations of <3x LoD. Organism concentrations were confirmed by colony count. For each anti-coagulant and extraction method (MagNA Pure and DSP) a total of 20 low positive and three negative (without organism) sample replicates were evaluated. Study results generated 100% of expected results, demonstrating that K2 EDTA, Lithium Heparin, and Sodium Citrate are acceptable anticoagulants for collection of whole blood specimens to be tested with the Applied Biosystems *Bacillus anthracis* Detection Kit. Result are presented in Table 24.

Table 24: Whole Blood Anti-Coagulant Equivalency Results

Anti-Coagulant	Extraction Method	Organism	Correct Calls	Detection Rate
K2 EDTA	MNP	Negative/Blank	3/3	100.0%
		Ba Ames	20/20	100.0%
		Ba RA3	20/20	100.0%
	DSP	Negative/Blank	3/3	100.0%
		Ba Ames	20/20	100.0%
		Ba RA3	20/20	100.0%
Lithium Heparin	MNP	Negative/Blank	3/3	100.0%
		Ba Ames	20/20	100.0%
		Ba RA3	20/20	100.0%
	DSP	Negative/Blank	3/3	100.0%
		Ba Ames	20/20	100.0%
		Ba RA3	20/20	100.0%
Sodium Citrate	MNP	Negative/Blank	3/3	100.0%
		Ba Ames	20/20	100.0%
		Ba RA3	20/20	100.0%
	DSP	Negative/Blank	3/3	100.0%
		Ba Ames	20/20	100.0%
		Ba RA3	20/20	100.0%

3. Clinical Studies

Clinical 'Specificity' Study, Whole Blood Specimens:

A study was conducted at three U. S. clinical testing sites to evaluate the performance of the Applied Biosystems *Bacillus anthracis* Detection Kit with whole blood specimens. A total of 350 leftover human whole blood specimens collected in K2 EDTA blood tubes (e.g., leftover hematology specimens) were prospectively collected and tested at three clinical testing sites with a minimum of 100 specimens tested per site. Specimens were from patients who may or may not have had fever and the specimens were not required to have any correlation to positive blood culture specimens collected and tested in the study. All specimens were stored at 2-8°C and tested within five (5) days of receipt in the laboratory.

Additionally, a total of 97 whole blood specimens were collected from consented patients presenting with fever and flu-like symptoms at three separate collection sites including a physician office, urgent care center and hospital clinic/ER. Table 25 summarizes the number of febrile whole blood specimens collected from each age group.

Table 25: Whole Blood Sample, Febrile Patients by Age Group

Age Group (years)	Number of febrile samples collected
2 to 15	0
16 to 21	1
22 to 40	23
>40	73

All specimens collected from febrile patients were shipped to a single in-house testing site on the day of collection and were processed within 24 hours of receipt with the Applied Biosystems *Bacillus anthracis* Detection Kit.

A total of eight specimens (from the febrile population) were withdrawn either due to shipping issues or not meeting study inclusion criteria. The final sample size included 439 whole blood specimens (350 leftover whole blood specimens and 89 whole blood specimens from patients with fever).

Leftover whole blood samples were prepared for PCR using the Qiagen (DSP) extraction procedure at two test sites and the MagNA Pure extraction procedure at one test site. Specimens from patients with fever were processed at one site with approximately equal numbers of specimens extracted using each extraction method.

There were no invalid results generated in the study; however, an indeterminate rate of 6% was observed for negative whole blood specimens. 'Supervisor Review' was performed for all indeterminate results generated in the clinical study and cross-talk instances were resolved with all specimens reported as '*Bacillus anthracis* not detected'. No whole blood samples were excluded due to indeterminate results.

Results were evaluated for negative percent agreement only. Reference/comparator testing was not performed as all specimens were assumed to be negative for *B. anthracis*. Study results demonstrated 100% negative percent agreement and are presented in Table 26 for all whole blood specimens combined.

Table 26: Clinical 'Specificity', Whole Blood Samples

All Sites: Whole Blood Leftover and Febrile Patient Specimens Combined		
Applied Biosystems *B. anthracis* Detection Kit	Test Positive	0
	Test Negative (*B. anthracis* not detected)	439
	Negative Percent Agreement (%)	100.0% 95% CI (99.1%, 100%)

Clinical 'Specificity' Study, Blood Culture Specimens

The Applied Biosystems *Bacillus anthracis* Detection Test System was evaluated with 425 leftover fresh and frozen blood culture specimens flagged as positive by a continuous monitoring automated blood culture instrument and determined to contain microorganisms by Gram stain. Contrived specimens were also evaluated. Testing was comprised of the following blood culture specimen cohorts with the sample size for fresh and frozen specimens shown in Table 27 below.

- Prospectively collected and tested fresh blood culture specimens determined by Gram stain to be positive for gram-negative or gram-positive species
- Prospectively collected, retrospectively tested frozen blood culture specimens determined by Gram stain to be positive for gram-negative or gram-positive species
- Selected fresh specimens determined by Gram stain to contain gram-positive bacilli
- Selected frozen specimens determined by Gram stain to contain gram-positive bacilli
- Contrived specimens (24 specimens, various gram-positive bacilli)

Table 27: Clinical Study, Blood Culture Specimens

Sample Type (Gram Stain)	**Clinical Site**			**Sample Total**
	Site 1	*Site 2*	*Site 3*	
Blood Culture, fresh				
Gm (+) bacilli	10	10	12*	32
Any species	21	5	0	26
Total (fresh)	31	15	12	58
Blood Culture, frozen				
Gm (+) bacilli	55	43	23**	121
Any species	48	76	122	246
Total (frozen)	103	119	145	367
Total (Blood Culture)	**134**	**134**	**157**	**425**

*Blood culture sample contrived in-house (12 fresh specimens with gram-positive bacilli)
**12 of 23 Frozen blood culture samples with gram-positive bacilli were contrived

Results from standard of care organism identification were provided by each collection laboratory for all clinical blood culture specimens evaluated in the study. Table 28 includes a listing of organisms (and numbers of specimens) identified by standard of care testing (i.e., subculture of blood culture media, incubation of plate media, and isolate identification).

Table 28: Clinical Study, Blood Cultures, Organisms Identified by Standard of Care

Organisms (Numbers of specimens)		
Abiotrophia granulicatella species (1)	*Cutibacterium acnes* (1)	*Pseudomonas aeruginosa* (7)
Actinobaculum spp (1)	*Dermabacter hominis* (1)	*Rothia mucilaginosa* (1)
Actinomyces neuii (1)	*Diphtheroids (not group JK)* (29)	*Salmonella enterica subsp. enterica* (1)
Actinomyces odontolyticus (1)	*Enterobacter aerogenes* (1)	*Serratia marcescens* (3)
Actinomyces oris (1)	*Enterobacter cloacae complex* (4)	*Staphylococcus aureus* (25)
Alloicoccus otitis (1)	*Enterobacteriaceae* (1)	*Staphylococcus capitis* (5)
Bacillus cereus (4)	*Enterococcus faecalis* (14)	*Staphylococcus coagulase negative* (1)
Bacillus megaterium (1)	*Enterococcus faecalis group 1* (1)	*Staphylococcus epidermidis* (66)
Bacillus non anthracis (18)	*Enterococcus faecium* (6)	*Staphylococcus haemolyticus* (7)
Bacillus subtilis var pumilus (1)	*Enterococcus faecium group D* (2)	*Staphylococcus hominis* (11)
Bacillus thuringiensis (1)	*Escherichia coli* (30)	*Staphylococcus infantarius* (1)
Bacteroides thetaiotaomicron (2)	*Fusobacterium nucleatum* (2)	*Staphylococcus pettenkoferi* (2)
Bacteroids distasonis (Parabacteroides distasonis) (1)	*Klebsiella aerogenes* (1)	*Staphylococcus pseudintermedius* (2)
Beta hemolytic Streptococcus group A (1)	*Klebsiella oxytoca* (3)	*Staphylococcus salivarius* (1)
Beta hemolytic Streptococcus group B (2)	*Klebsiella pneumoniae* (13)	*Staphylococcus species* (17)
Brevibacterium (1)	*Klebsiella variicola* (1)	*Staphylococcus warneri* (1)
Burkholderia cepacia (1)	*Lactobacillus gasseri* (1)	*Stenotrophomonas maltophilia* (3)
Butyricimonas virosa (1)	*Lactobacillus rhamnosus* (1)	*Streptococcus agalactiae* (2)
Chryseomonas luteola (1)	*Lactobacillus species* (3)	*Streptococcus canis* (1)
Citrobacter freundii (1)	*Listeria monocytogenes* (3)	*Streptococcus constellatus* (2)
Citrobacter koseri (1)	*Methicillin-resistant Staphylococcus aureus* (4)	*Streptococcus dysgalactiae* (2)
Citrobacter spp (1)	*Microbacterium species* (1)	*Streptococcus gallolyticus* (1)
Clostridium perfingens (3)	*Micrococcus luteus* (4)	*Streptococcus gordonii* (1)
Clostridium septicum (1)	*Micrococcus species* (2)	*Streptococcus oralis* (2)
Corynebacterium afermentans (1)	*Morganella morganii* (1)	*Streptococcus pneumoniae* (3)

Organisms (Numbers of specimens)		
Corynebacterium freneyi (1)	*Nocardia abscessus* (1)	*Streptococcus salivarius* (1)
Corynebacterium imitans (1)	*Peptoniphilus asaccharolyticus* (1)	*Streptococcus sanguis I* (1)
Corynebacterium propinquum (1)	*Prevotella nigrescens* (1)	*Streptococcus species* (6)
Corynebacterium pseudodiphtheriticum (1)	*Propionibacterium* (9)	*Streptococcus vestibularis* (1)
Corynebacterium spp. (10)	*Propionibacterium acnes* (14)	*Trueperella bernardiae* (1)
Corynebacterium striatum (3)	*Proteus mirabilis* (3)	*Turicella otitidis* (2)
Corynebacterium tuberculostearicum (3)	*Providencia stuartii* (3)	

Table 29 includes the organisms, concentrations and numbers of replicates of contrived blood culture specimens tested in the clinical study. Replicates were distributed evenly between BACTEC Standard Aerobic and BACTEC Standard Anaerobic blood culture bottles.

Table 29: Contrived Blood Specimens

Spiked Organism	Spiking Concentration (CFU/mL)	# of Replicates
B. cereus E33L	1.00E+06	4
Clostridium innocuum	1.00E+05	4
Corynebacterium xerosis	1.00E+06	4
Lactobacillus acidophilus	1.00E+06	4
Listeria monocytogenes	1.00E+05	4
Propionibacterium acnes	1.00E+06	4

Testing of blood culture specimens initially generated invalid results for 10% of specimens. Upon repeat testing, the final invalid rate was 5.6% (24/425). These invalid results are generated because of weak or absent amplification of the endogenous internal positive control (IPC) (human telomerase reverse transcriptase (TERT)). The invalid results observed only in blood culture specimens were thought to be due to insufficient whole blood input into blood culture bottles and/or dilution factors which may minimize the quantities of human DNA in the specimen. Dilution factors include:

- Whole blood diluted in blood culture media when inoculated
- Dilution of extracted DNA prior to PCR because of known inhibitors in blood culture media

With the 24 invalid specimens excluded, all remaining blood culture specimens generated the expected '*Bacillus anthracis* not detected' result,

demonstrating 100% negative percent agreement. See Table 30.

Table 30: Clinical 'Specificity' Study, Blood Culture Specimens

All Sites: Fresh, Frozen and Contrived Specimens Combined		
Applied Biosystems *B. anthracis* Detection Kit	Test Positive	0
	Test Negative (*B. anthracis* not detected)	401
	Negative Percent Agreement (%)	100.0% 95% CI (99.1%, 100%)

Validation with Contrived Specimens

To assess the Applied Biosystems *Bacillus anthracis* Detection Test System for detection of *B. anthracis* in positive whole blood specimens, a study was conducted using contrived specimens prepared with individual whole blood specimens collected from febrile patients presenting with fever of unknown origin and flu-like symptoms. Each specimen was inoculated with quantitated *B. anthracis* organisms into a unique whole blood specimen. Each specimen was inoculated with one of 18 *B. anthracis* strains and final specimen concentrations were near the assay LoD. A total of 25 specimens contained *B. anthracis* (Ames strain) at LoD and the remainder of contrived specimens contained one of 17 other *B. anthracis* strains at ≤3x LoD. Contrived positive specimens were randomized with negative whole blood specimens and specimen identities were blinded to test operators.

Two different operators processed specimens using either the DSP or MagNA Pure extraction method. Two Applied Biosystem 7500 Fast Dx Real-Time PCR instruments were used in the study. A total of 108 specimens were evaluated in the study (87 positive and 21 negative). Study results demonstrated 96.6% positive percent agreement for contrived positive whole blood specimens and 100% negative percent agreement for specimens without *B. anthracis*.

For four contrived positive specimens, '*B. anthracis* suspected' results were encountered during clinical sensitivity testing of spiked febrile whole blood. These specimens account for 4% of positive contrived specimens tested.

- One contrived positive sample spiked with *Bacillus anthracis* Carbosap strain at 3x LoD initially generated a '*B. anthracis* suspected" result. Repeat testing of this sample generated the expected '*B. anthracis* detected' result which was considered concordant.
- One contrived positive sample spiked with *Bacillus anthracis* Ames strain at LoD initially generated a 'BA suspected' result. Testing of this sample was repeated with a result of '*B. anthracis* suspected' and the sample was considered discordant.
- Two simulated positive samples spiked with *Bacillus anthracis* Carbosap strain at 3x LoD and initially generated "BA suspected" results. Upon repeat testing, one

sample generated a negative result and one sample generated a '*B. anthracis* suspected' result. Both samples were considered discordant.

Due to known variation in plasmid copy number among *Bacillus anthracis* strains, the discordant results were attributed to target concentration levels at or below LoD. Results for the study are shown in Table 31.

Table 31: Clinical Study, Contrived Positive Whole Blood Specimens

Simulated Positive Whole Blood		***B. anthracis* positive**	***B. anthracis* negative**
Applied Biosystems *B. anthracis* Detection Kit	Test Positive	84	0
	Test Negative	0	21
	Unresolved	3*	0
	Positive Percent Agreement (%)	96% 95% CI (90.4%, 98.8%)	
	Negative percent agreement (%)		100% 95% CI (84.5%, 100%)

*Initially reported as '*B. anthracis* suspected'. See above for details on Unresolved/Discordant whole blood specimens.

Clinical Study, Summary of Indeterminate Calls: Indeterminate results generated by the BaIS software may occur with specimens that produce a pX01 Ct value without true amplification. An indeterminate result/supervisor review interpretive result for a sample is caused by the combination of a positive pX01 with either a negative or an indeterminate call for pX02. This may be due to the 'cross-talk' phenomenon and requires review of the pX01 graph in the 7500 analysis software for resolution. The rate of indeterminate calls that occurred during clinical testing is summarized in the table below. Indeterminate results only occurred in negative or un-spiked whole blood. Indeterminate results did not occur in spiked whole blood specimens or in spiked or un-spiked blood culture specimens. A summary of Indeterminate results is shown in Table 32.

Table 32: Clinical Study, Summary of Indeterminate Results by Sample Matrix and Test Site

Clinical Study	Site	Negative WB (w/o *B. anthracis*)	WB (w/ *B. anthracis*)	BC
Specificity	1	22/100 22.0%	N/A	0/129 0.0%
Specificity	2	0/100 0.0%	N/A	0/120 0.0%
Specificity	3	7/239 2.9%	N/A	0/157 0.0%
Specificity	**Total**	29/439 6.6%	N/A	0/406 0.0%
Sensitivity	3	2/21 9.5%	0/87 0.0%	N/A
All Combined	**Total**	31/460 6.7%	0/87 0.0%	0/406 0.0%

N/A: Not applicable; WB: Whole blood; BC: Blood culture

Clinical Specificity Testing, Summary of Invalid Results, Failed Runs:

There were no invalid results observed for whole blood specimens during the clinical study. A total of 43 blood culture specimens were initially reported as invalid and required repeat testing; 33 of those were invalid due to Sample TERT failure, and 10 were invalid due to QC BLK TERT failure. This yielded an initial error rate of 10.1% [43/425].

Repeat testing was performed on the 43 invalid blood culture samples. A total of 19/43 [44.2%] generated valid results upon repeat testing, while 24/43 remained invalid due to Sample TERT failure. The final invalid rate for blood culture specimens was 24/425 [5.6%] after repeat testing.

A total of 32 instrument runs were required to test the 864 samples included in the specificity study. 1/32 [3.1%] runs performed was invalid due to QC failure [BLK TERT Invalid]. The invalid QC was resolved upon repeat testing of the entire run.

Tables 33 and 34 include the numbers and percentage of invalid results for initial and repeat testing. Table 35 includes a summary of test runs.

Table 33: Initial Invalid Rate During Clinical Specificity Testing

Initial Results									
	Samples Tested			VALID			BC Samples INVALID		
Site	**WB**	**BC**	**Total**	**WB**	**BC**	**Total**	**Sample TERT Failure**	**QC BLK Tert Failure**	**Total Invalid**
1	100	134	234	100	119	219	5	10	15
2	100	134	234	100	113	213	21	0	21
3	239	157	396	239	150	389	7	0	7
Total	**439**	**425**	**864**	**439/439 100%**	**382/425 89.9%**	**821/864 95.0%**	**33/425 7.8%**	**10/425 5.5%**	**43/425 10.1%**

Table 34: Repeat Invalid Rate During Clinical Specificity Testing

	Repeat Samples Tested	Repeat Results			
		VALID	INVALID		
Site			**Sample TERT Failure**	**QC BLK Tert Failure**	**Total Invalid**
1	15	10	5	0	5
2	21	7	14	0	14
3	7	2	5	0	5
Total	**43**	**19/43 44.2%**	**24/43 55.8%**	**0/43 0.0%**	**24/43 55.8%**

Instrument Runs during Clinical Testing

Table 35: Instrument Runs During Clinical Specificity Testing

	Instrument Runs Performed	Instrument Run Results	
Site		**Valid**	**Invalid**
1	7	6	1
2	11	11	0
3	14	14	0
Total	**32**	**31/32 96.9% [84.3-99.4%]**	**1/32 3.1% [0.6-15.7%]**

5. Clinical cut-off:

   N/A

6. Expected values/Reference range:

N/A

N. Instrument Name:

Applied Biosystems (AB) 7500 Fast Dx

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes _____X___ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No __X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Users must fill in Batch Information including patient information, number of samples, and sample IDs. Prior to initiating the experiment, the user must prepare a plate layout to assign a location for samples and controls on the plate. For each extracted specimen, a prefix MUST be added to the sample ID to indicate the sample type for recognition by the automated BaIS. In addition to the prefix, for extraction blanks, the task must be changed to NTC in the Well Inspector Screen.

4. Specimen Sampling and Handling:

   DNA is extracted manually using the Qiagen DSP DNA Mini Kit or the automated MagNA Pure LC 2.0 extraction system. Specimen extracts and controls are then manually pipetted into the Applied Biosystems *Bacillus anthracis* Test Kit Lyophilized Assay Plate for analysis prior to loading onto the AB 7500 Fast Dx Real-Time PCR Instrument for amplification and detection.

5. Calibration:

   Prior to testing with the Applied Biosystems *Bacillus anthracis* Detection Kit, the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument must be calibrated by the manufacturer to read the following dyes: FAM, VIC, ABY, and ROX. It is noted that ABY is outside of the routine calibration dyes and must be specifically requested when scheduling calibration.

   A background calibration plate should be periodically run as recommended by ThermoFisher or alternatively, the user can perform a background calibration run as per the instructions in the AB 7500 Fast Dx Real Time PCR Instrument Reference Guide.

6. Quality Control:

   See Section M.1.c. Section above.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.