510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232545

B Applicant

First Light Diagnostics, Inc.

C Proprietary and Established Names

The SensiTox *B. anthracis* Toxin Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QUU	Class II	21 CFR 866.3046 - Simple In Vitro Diagnostic Device For The Detection Of Secreted Proteins From Bacillus Species (Spp.) In Human Clinical Samples	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the SensiTox *B. anthracis* Toxin Test for the qualitative detection of lethal factor in suspected cases of inhalation anthrax.

B Measurand:

Lethal factor secreted by *Bacillus anthracis*

C Type of Test:

Automated qualitative immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The SensiTox *B. anthracis* Toxin Test for use with the MultiPath Analyzer, is a qualitative immunofluorescence assay to aid in the diagnosis of inhalation anthrax. The test is intended for the rapid, qualitative detection of lethal factor, a biomarker associated with *Bacillus anthracis* (*B. anthracis*). The test can be used with whole blood collected with dipotassium EDTA anticoagulant by venipuncture. This test is indicated for testing samples from individuals who have signs and symptoms consistent with inhalation anthrax and a likelihood of exposure *to B. anthracis*. A positive SensiTox *B. anthracis* Toxin Test result is presumptively diagnostic for *B. anthracis* infection. Diagnosis of *B. anthracis* infection must be made in conjunction with medical history, likelihood of exposure, signs, and symptoms of disease, as well as other laboratory evidence. The definitive identification of *B. anthracis* from blood samples requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reports are required. Testing should be performed and reported in accordance with current guidelines provided by the appropriate public health authorities. The level of lethal factor present in blood from individuals with early systemic infection is unknown. Negative results do not preclude infection with the biothreat microbial agents targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Laboratories performing the SensiTox *B. anthracis* Toxin Test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities, and personnel trained in the safe handling of clinical specimens potentially containing *B. anthracis*.

The SensiTox *B. anthracis* Toxin Test is for prescription use only.

This assay is not FDA-cleared or approved for testing blood or plasma donors.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with the MultiPath Analyzer

IV Device/System Characteristics:

A Device Description:

The SensiTox *B. anthracis* Toxin Test is a cartridge-based immunoassay intended to detect *B. anthracis* lethal factor (LF), an early biomarker of anthrax infection, in venous whole blood samples using an immunofluorescence assay and the MultiPath Analyzer. A whole blood specimen, collected in dipotassium EDTA from individuals with signs and symptoms consistent with inhalation anthrax and a likelihood of exposure, is used for the test.

The blood sample is added directly to the SensiTox *B. anthracis* cartridge that is a single use consumable containing all the reagents required to run a single test. The barcode-labeled cartridge is loaded onto the MultiPath Analyzer for processing and is moved to the fluidics station. There it is first heated to 35^{o}C before sample is split into three equal aliquots to separate wells within the cartridge. From these three wells, sample aliquots flow to three reagent wells containing lyophilized reagents required to perform three measurements: an LF Total Signal Measurement (Test Well), a Neutralization Measurement (Neutralization Well), and a Positive Control Measurement (Positive Control Well). After the sample rehydrates lyophilized beads containing LF-specific antibodies conjugated to fluorescent particles and to magnetic particles, the three reaction mixtures flow into imaging wells with bottoms coated with a dye cushion reagent. The dye-cushion is a dense opaque aqueous layer that separates sample and reagents from the bottom optical surface of the imaging wells. In the upper assay layer, if LF toxin is present it will bind to and tether together the magnetic and fluorescent particles (the fluorescent and magnetic particles are conjugated to complementary antibodies, so that the two types of particles bind to distinct epitopes on the LF toxin). Magnets draw the magnetic particles and any tethered fluorescent particles through the dye-cushion layer to deposit them on the bottom imaging surface, away from the upper aqueous layer. Captured fluorescent particles are then imaged and quantified using non-magnified digital imaging.

Up to 20 cartridges can be loaded onto the Analyzer in parallel, and results are interpreted using the MultiPath applications software as ‘valid’ or ‘invalid’. If ‘valid’, the results are reported as Lethal Factor ‘detected’ or ‘not detected’. Results are displayed on the instrument touch screen and can be printed.

B Principle of Operation:

The SensiTox *B. anthracis* Toxin Test uses separate antibodies that are conjugated to magnetic particles or fluorescent particles, respectively, to capture and detect antigen. Labeled antibody-antigen complexes are deposited onto an imaging surface, and the MultiPath Analyzer uses digital imaging to automatically detect and interpret the assay signal.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Active Anthrax Detect Plus Rapid Test

B Predicate 510(k) Number(s):
DEN220044

C Comparison with Predicate(s):

Device & Predicate Device(s):	K232545	DEN220044
Device Trade Name	SensiTox *B. anthracis* Toxin Test	Active Anthrax Detect Plus Rapid Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	The SensiTox *B. anthracis* Toxin Test for use with the MultiPath Analyzer, is a qualitative immunofluorescence assay to aid in the diagnosis of inhalation anthrax. The test is intended for the rapid, qualitative detection of lethal factor, a biomarker associated with *Bacillus anthracis* (*B. anthracis*). The test can be used with whole blood collected with dipotassium EDTA anticoagulant by venipuncture. This test is indicated for testing samples from individuals who have signs and symptoms consistent with inhalation anthrax and a likelihood of exposure *to B. anthracis*. A positive SensiTox *B. anthracis* Toxin Test result is presumptively diagnostic for *B. anthracis* infection. Diagnosis of *B.*	The Active Anthrax Detect Plus Rapid Test point-of-care diagnostic test for pulmonary anthrax is an in vitro immunochromatographic device for use as an aid in the diagnosis of inhalation anthrax. It provides visual and rapid qualitative detection of lethal factor of *Bacillus anthracis* (*B. anthracis*). The test can be used to test serum and venous whole blood (dipotassium EDTA, sodium citrate, and sodium heparin). The assay is indicated for testing samples from individuals who have signs and symptoms consistent with inhalation anthrax and a likelihood of exposure. This test is intended for use by military personnel, medical, and/or healthcare professionals only. The diagnosis of B. *anthracis* infection must be based on history, signs, symptoms, exposure likelihood, and additional laboratory evidence. A positive Active Anthrax Detect

	anthracis infection must be made in conjunction with medical history, likelihood of exposure, signs, and symptoms of disease, as well as other laboratory evidence. The definitive identification of *B. anthracis* from blood samples requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reports are required. Testing should be performed and reported in accordance with current guidelines provided by the appropriate public health authorities. The level of lethal factor present in blood from individuals with early systemic infection is unknown. Negative results do not preclude infection with the biothreat microbial agents targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Laboratories performing the	Plus Rapid Test result is presumptively diagnostic for *B. anthracis* infection. The definitive identification of *B. anthracis* from blood samples requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reports are required. Testing should be performed and reported in accordance with current guidelines provided by the appropriate public health authorities. The level of lethal factor present in blood from individuals with early systemic infection is unknown. Negative results do not preclude infection with the biothreat microbial agents targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. This assay is for prescription use. The distribution of in vitro diagnostic devices for *Bacillus* spp. detection must be performed in accordance with guidelines established by the public health authorities that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public

	SensiTox *B. anthracis* Toxin Test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities, and personnel trained in the safe handling of clinical specimens potentially containing *B. anthracis*. The SensiTox *B. anthracis* Toxin Test is for prescription use only. This assay is not FDA-cleared or approved for testing blood or plasma donors.	health authorities. This assay is not FDA-cleared or approved for testing blood or plasma donors.
Analyte	Lethal Factor	same
Conjugated antibodies	Anti-Lethal Factor antibodies	same
Prescription status	Rx only	same
General Device Characteristic Differences		
Specimen type	Whole blood collected with dipotassium EDTA anticoagulant by venipuncture	Serum and venous whole blood (dipotassium EDTA, sodium citrated, and sodium heparin)
Technology	Immunfluorescent assay	Immunochromatographic assay
Test format	Fluidic cartridge with direct digital imaging and automated interpretation	Lateral flow with visual interpretation
Automated	Yes	No
Instrument	Multipath Analyzer	None

VI Standards/Guidance Documents Referenced:

• IEC 60601-1-2:2014+AMD1:2020 – Medical electrical equipment – Part 1-2: General requirements for basic safety and essential performance – Collateral Standard: Electromagnetic disturbances – Requirements and tests.

• UL 61010-1: 2012 R4.16 - Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements

• UL 61010-2-101:2019 - Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment

• UL 61010-2-081: 2015 - Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

• UL 61010-2-010: 2015 - Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-010: Particular requirements for laboratory equipment for the heating of materials

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The reproducibility of the SensiTox *B. anthracis* Toxin Test was evaluated at three sites over the course of five non-consecutive days by two operators per site each day. Three lots of cartridges were tested in this study. Randomized and blinded samples comprised of whole blood spiked with two concentrations of recombinant Lethal Factor (LF) – a low positive sample (1.5X LoD) and a moderate positive sample (3X LoD). Negative (unspiked) samples were also prepared and provided to each participating site.

As shown in Table 1, the reproducibility was 98.9% and 97.8% for the moderate and low positives, respectively. The negative samples were 100% reproducible. There were six invalid results reported in the study for an invalid rate of 2.2% (6/270) with all six samples being valid upon retesting following labeled instructions. Of the 270 samples tested there were three incorrect results reported from three samples. Two low-positive samples and one moderate-positive sample generated the incorrect results ("not detected").

Table 1. Reproducibility Study Data Stratified by Site

Sample description	Site 1		Site 2		Site 3		Total	
	#	Agreement (%)	#	Agreement (%)	#	Agreement (%)	#	Agreement (%)
Negative	30/30	100	30/30	100	30/30	100	90/90	100
75 pg/mL LF	30/30	100	29/30	96.7	29/30	96.7	88/90	97.8
150 pg/mL LF	30/30	100	29/30	96.7	30/30	100	89/90	98.9
Total	90/90	100	88/90	97.8	89/90	98.9	267/270	98.9

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

Interfering Substances

Endogenous and exogenous substances commonly found in blood were evaluated for their potential effect on the performance of the SensiTox *B. anthracis* Toxin Test. Drugs and their metabolites were tested at approximately 3X the highest concentration reported following therapeutic dosage. Endogenous substances were tested at the highest physiological concentration expected in the population. The solvents used to dissolve exogenous substances used in the study were tested independently.

Each compound was tested in K_2EDTA anticoagulated whole blood either unspiked and spiked with 150 pg/mL (3X LoD) of recombinant Lethal Factor and run in triplicate. The list of endogenous and exogenous substances evaluated and the concentrations at which they were tested is shown in Table 2. None of the substances tested interfered with the SensiTox *B. anthracis* Toxin Test.

Table 2. Interfering Substances Evaluated

Endogenous Substances	Concentration
Glucose	10 mg/mL
Hemoglobin	2 mg/mL
Human Immunoglobulins (IgG)	19.7 mg/mL
Triglycerides	22 mg/mL
Cholesterol	2.5 mg/mL
Human Serum Albumin	50 mg/mL
Bilirubin	200 µg/mL
Human Anti-Mouse Antibodies	1 µg/mL
Exogenous Substances	**Concentration**
Acetaminophen	200 µg/mL
Acetone	88 µL/mL
Acid-citrate-dextrose 3x	300 µL/mL
Albuterol (Salbutamol)	400 ng/mL
Amoxicillin	75.2 µg/mL
Artemisinin	9 µg/mL
Ascorbic acid	60 µg/mL
Aspirin	652 µg/mL
Biothrax	0.9 µL/mL
Cefotaxime	306 µg/mL
Chloroquine	36 µg/mL
Ciprofloxacin	10 µg/mL
DMSO	18 µL/mL
Doxycycline	30 µg/mL
EDTA	5.4 mg/mL

Erythromycin	60 µg/mL
Ethanol	10 µL/mL
Flunisolide (Flovent)	90 ng/mL
Gentamicin sulfate	10 µg/mL
Heparin	90.3 U/mL
Ibuprofen	500 µg/mL
Malarone (Atovaquone)	180 µg/mL
Mefloquine	18 µg/mL
Methanol	3 µL/mL
N-acetylcysteine	1.7 mg/mL
Naproxen sodium	500 µg/mL
Oseltamivir (Tamiflu)	4.95 µg/mL
PBS	75.2 µL/mL
Propanol	62.5 µL/mL
Ribavirin	51.3 µg/mL
Rifampin	66 µg/mL
Sodium citrate	9.6 mg/mL
Sodium polyanetholesulfonate	1.5 mg/mL
Streptomycin	450 µg/mL
Sulfamethoxazole	400 µg/mL
Tetracycline	15 µg/mL
Tobramycin	24 µg/mL
Trimethoprim	40 µg/mL

Analytical Specificity – Cross-reactivity

Analytical specificity testing was conducted to assess the potential cross-reactivity of the SensiTox *B. anthracis* Toxin Test to bacterial, viral, protozoan, and fungal pathogens potentially found in human blood. Bacterial, protozoan, and fungal pathogens were typically tested at $1x10^6$ CFU/mL and viral pathogens at $1x10^5$ PFU/mL by the addition to pre-screened K_2EDTA human venous whole blood. Each pathogen was tested in triplicate. A list of all pathogens and the concentrations tested is shown in Table 3.

With the exception of two *B. cereus* strains, G9241 and 03BB102, which contain the extrachromosomal plasmid pXO1 that harbors the gene for Lethal Factor (LF), no pathogens cross-reacted when tested in the SensiTox *B. anthracis* Toxin Test. These two cross-reactive strains of *B. cereus* were detected as expected.

Table 3. Cross-reactivity Study Summary

Bacteria Tested with No Cross-Reactivity			
Strain	**CFU/mL**	**Strain**	**CFU/mL**
Acinetobacter baumannii, 307-0294	1.0E+06	*Enterococcus faecium*, ZeptoMetrix Z265	1.0E+06
Bacillus cereus, ZeptoMetrix Z091	1.0E+06	*Escherichia coli*, ZeptoMetrix 0801517	1.0E+06
Bacillus cereus, 3A (Fri-41)	1.0E+06	*Francisella philomiragia*, O#319-036	1.0E+06
Bacillus cereus, D17	1.0E+06	*Francisella tularensis*, Schu S4	7.7E+05
Bacillus cereus, E33L	7.0E+05	*Haemophilus influenzae*, type b; Eagan	1.0E+06
Bacillus cereus, m1550	1.0E+06	*Klebsiella aerogenes*, ZeptoMetrix Z052	1.0E+06
Bacillus cereus, MSX-A1	1.0E+06	*Klebsiella oxytoca*, ZeptoMetrix Z115	1.0E+06
Bacillus cereus, VD148	6.7E+05	*Klebsiella pneumoniae*, KPC-2	1.0E+06
Bacillus cereus, 03BB108	1.0E+06	*Legionella pneumophila*, Philadelphia	1.0E+06

Bacillus circulans Jordan, 26	1.0E+06	*Leishmania donovanii*, MHOM/IN/80/DD8	1.0E+06
Bacillus coagulans Hammer, NRS 609	1.0E+06	*Listeria monocytogenes*, Serotype 1/2b	1.0E+06
Bacillus mycoides Flugge, NRS 273	1.0E+06	*Mycobacterium tuberculosis* (avirulant), H37Ra-1	1.0E+06
Bacillus subtilis Marburg	1.0E+06	*Mycoplasma pneumoniae*, M129	1.0E+06
Bacillus thuringiensis, Al Hakam	1.0E+06	*Neisseria gonorrhoeae*, ZeptoMetrix Z017	1.0E+06
Bacillus thuringiensis, Berliner, CCUG 7429T	1.0E+06	*Neisseria meningitidis*, Serogroup A	1.0E+06
Bacillus thuringiensis, NRRL B-3792	1.0E+06	*Proteus mirabilis*, ZeptoMetrix Z050	1.0E+06
Bacillus thuringiensis, USDA HD522	4.0E+05	*Pseudomonas aeruginosa*, ZeptoMetrix Z139	1.0E+06
Bacillus thuringiensis, HD1011	1.0E+06	*Pseudomonas luteola*, JCM 3352	1.0E+06
Bacillus thuringiensis, HD974	1.0E+06	*Rickettsia prowzekii*, Naples-1	1.0E+05
Bacillus thuringiensis, HD571	1.0E+06	*Rickettsia sibirica*, 246	1.0E+05
Bacillus thuringiensis, HD682	1.0E+06	*Salmonella enterica*, Typhi, ZeptoMetrix Z152	1.0E+06
Bacillus thuringiensis, ZeptoMetrix Z096	1.0E+06	*Salmonella enterica*, Typhimurium, ZeptoMetrix Z005	1.0E+06
Bacillus thuringiensis, 97-27	1.0E+06	*Serratia marcescens*, ZeptoMetrix Z053	1.0E+06
Bacteroides fragilis, ZeptoMetrix Z029	1.0E+06	*Shigella sonnei*, ZeptoMetrix Z004	1.0E+06
Bordetella bronchiseptica, ZeptoMetrix	1.0E+06	*Staphylococcus aureus*, MSSA; ΔmecA	1.0E+06
Borrelia burgdorferi, B31	1.0E+06	*Staphylococcus epidermidis*, MSSE; HER 1292	1.0E+06
Brucella melitensis, 16M (NCTC 10094)	1.0E+06	*Streptococcus agalactiae*, ZeptoMetrix Z019	1.0E+06
Burkholderia cepacia, ZeptoMetrix Z066	1.0E+06	*Streptococcus pneumoniae*, ZeptoMetrix Z022	1.0E+06
Burkholderia mallei, Ivan (NTCC 10230)	1.0E+06	*Streptococcus pyogenes*, ZeptoMetrix Z018	1.0E+06
Burkholderia pseudomallei, NCTC 4845	1.0E+06	*Vibrio cholera*, ZeptoMetrix Z133	1.0E+06
Chlamydophila pneumoniae, ZeptoMetrix Z500	1.0E+06	*Yersinia aldovae*, CNCTC Y 49/84	1.0E+06
Citrobacter koseri, ZeptoMetrix Z039	1.0E+06	*Yersinia bercovieri*, CDC 2475-87	1.0E+06
Clostridium bifermentans, ZeptoMetrix Z176	1.0E+06	*Yersinia enterocolitica*, ZeptoMetrix Z036	1.0E+06
Clostridium botulinum, IBCA 10-7060	7.6E+04	*Yersinia fredericksenii*, CDC 1462-81	1.0E+06
Clostridium perfringens, Type A	1.0E+06	*Yersinia intermedia*, CDC 881-81	1.0E+06
Clostridium sordellii, ZeptoMetrix Z077	1.0E+06	*Yersinia kristensenii*, CDC 1460-81	1.0E+06
Clostridium sporogenes, ZeptoMetrix Z355	1.0E+06	*Yersinia mollaretti*, CDC 2465-87	1.0E+06
Corynebacterium diptheriae, ZeptoMetrix Z116	1.0E+06	*Yersinia pestis*, A1122	1.0E+06
Enterococcus faecalis, ZeptoMetrix Z346	1.0E+06	*Yersinia pseudotuberculosis*, ZeptoMetrix Z222	1.0E+06
		Yersinia ruckeri, CDC 2396-61	1.0E+06

Bacteria Tested with Cross-Reactivity

Strain	CFU/mL	Strain	CFU/mL
Bacillus cereus, G9242	1.0E+06	*Bacillus cereus*, 03BB102	1.0E+06

Viruses Tested with No Cross-Reactivity

Strain	PFU/mL	Strain	PFU/mL
Adenovirus (C1), 1 (Species C)**	1.0E+05	Influenza A (H1N1), New York/18/09	2.9E+04
Chikungunya Virus, S-27	1.0E+05	Influenza A (H3N2), Kumamoto/102/02	1.2E+04
Coronavirus 229E, 229E	9.1E+04	Influenza B, B/Colorado/06/2017**	1.0E+05
Cytomegalovirus, AD169	1.0E+05	Japanese Encephalitis Virus (JEV), Nakayama	1.0E+05
Eastern Equine Encephalitis Virus, FL93-939	1.0E+05	Measles Virus, ZeptoMetrix 0810025CF	2.9E+04
Enterovirus Type 68, 2014 isolate 1	1.0E+05	Mumps Virus, 1	1.7E+04
Epstein-Barr Virus (EBV), B95-8	1.0E+05	Parainfluenza Virus 1, ZeptoMetrix 0810014CF	8.8E+04
Hantaan Virus, 76-118	2.0E+04	Respiratory Syncytial Virus Type A, 1/2015 isolate 1	8.8E+04
Hepatitis A Virus (HAV), HM 175 (clone 1)**	1.0E+05	Rhinovirus, 16**	1.0E+05
Hepatitis B Virus (HBV), ZeptoMetrix 0810031C**	1.0E+05	Rubella Virus, ZeptoMetrix 0810048CF	2.5E+04
Hepatitis C Virus (HCV), Seracare**	1.0E+05	St. Louis Encephalitis Virus, V 07457	1.0E+05
Herpes Simplex Virus 1 (HSV-1), MacIntyre	1.0E+05	Vaccinia Virus, ZeptoMetrix 0810310CF	1.0E+05
Herpes Simplex Virus 2 (HSV-2), G Strain**	1.0E+05	Varicella Zoster Virus (VZV), A	1.0E+05
Human Herpesvirus 6A (HHV-6A), GS†	1.0E+05	West Nile Virus, CO 08-13386	1.0E+05
Human Immunodeficiency Virus (HIV), BaL**	1.0E+05	Western Equine Encephalitis, CO921356	1.0E+05
Human Metapneumovirus, IA 10-2003	8.8E+04	Yellow Fever Virus, 17D	1.7E+04

Fungi and Protozoa Tested with no Cross-Reactivity

Strain	CFU/mL	Strain	CFU/mL
Aspergillus fumigatus, ZeptoMetrix Z014	1.0E+06	*Cryptococcus neoformans*, serotype A	1.0E+06
Candida albicans, ZeptoMetrix Z006	1.0E+06		

Note: The entries marked with (*) indicate that those organisms were tested in % parasitemia. Entries marked with (**) indicate that those organisms were tested in IU/mL. The entries marked with (†) indicate that those organisms were tested in copies/mL.

Analytical Specificity – Microbial Interference

Testing was conducted to assess the interference of bacterial, viral, fungal, and protozoan pathogens potentially found in human blood on the detection of *B. anthracis* Lethal Factor with the SensiTox *B. anthracis* Toxin Test. Bacterial, fungal, and protozoan pathogens were typically tested at $1x10^6$ CFU/mL and viral pathogens at $1x10^5$ PFU/mL by addition to pre-screened K_2EDTA human venous whole blood containing 150 pg/mL of recombinant Lethal Factor (3X LoD). Each pathogen was tested in triplicate.

None of the organisms shown in Table 4 interfered with the SensiTox *B. anthracis* Toxin Test at the concentrations shown and generated detected results for all replicates containing 150 pg/mL of Lethal Factor.

Table 4. Organisms Evaluated for Microbial Interference

Bacteria			
Strain	**CFU/mL**	**Strain**	**CFU/mL**
Acinetobacter baumannii, 307-0294	1.0E+06	*Enterococcus faecalis,* ZeptoMetrix Z346	1.0E+06
Bacillus cereus, ZeptoMetrix Z091	1.0E+06	*Enterococcus faecium,* ZeptoMetrix Z265	1.0E+06
Bacillus cereus, 3A (Fri-41)	1.0E+06	*Escherichia coli,* ZeptoMetrix 0801517	1.0E+06
Bacillus cereus, D17	1.0E+06	*Francisella philomiragia,* O#319-036	1.0E+06
Bacillus cereus, E33L	1.0E+06	*Haemophilus influenzae,* type b, Eagan	1.0E+06
Bacillus cereus, m1550	1.0E+06	*Klebsiella aerogenes,* ZeptoMetrix Z052	1.0E+06
Bacillus cereus, MSX-A1	1.0E+06	*Klebsiella oxytoca,* ZeptoMetrix Z115	1.0E+06
Bacillus cereus, VD148	7.0E+05	*Klebsiella pneumoniae, KPC-2*	1.0E+06
Bacillus cereus, 03BB108	1.0E+06	*Legionella pneumophila,* Philadelphia	1.0E+06
Bacillus cereus, G9241	1.0E+06	*Listeria monocytogenes,* Serotype 1/2b	1.0E+06
Bacillus cereus, 03BB102	6.7E+05	*Mycobacterium tuberculosis* (avirulant), H37Ra-1	1.0E+06
Bacillus circulans Jordan, 26	1.0E+06	*Mycoplasma pneumoniae,* M129	1.0E+06
Bacillus coagulans Hammer, NRS 609	1.0E+06	*Neisseria gonorrhoeae,* ZeptoMetrix Z017	1.0E+06
Bacillus mycoides Flugge, NRS 273	1.0E+06	*Neisseria meningitidis,* Serogroup A	1.0E+06
Bacillus subtilis, Marburg	1.0E+06	*Proteus mirabilis,* ZeptoMetrix Z050	1.0E+06
Bacillus thuringiensis, Al Hakam	1.0E+06	*Pseudomonas aeruginosa,* ZeptoMetrix Z139	1.0E+06
Bacillus thuringiensis, Berliner, CCUG 7429T	1.0E+06	*Pseudomonas luteola,* JCM 3352	1.0E+06
Bacillus thuringiensis, NRRL B-3792	1.0E+06	*Salmonella enterica,* Typhi, ZeptoMetrix Z152	1.0E+06
Bacillus thuringiensis, USDA HD522	1.0E+06	*Salmonella enterica,* Typhimurium, ZeptoMetrix Z005	1.0E+06
Bacillus thuringiensis, HD1011	1.0E+06	*Serratia marcescens,* ZeptoMetrix Z053	1.0E+06
Bacillus thuringiensis, HD974	4.0E+05	*Shigella sonnei,* ZeptoMetrix Z004	1.0E+06
Bacillus thuringiensis, HD571	1.0E+06	*Staphylococcus aureus, MSSA*; ΔmecA	1.0E+06
Bacillus thuringiensis, HD682	1.0E+06	*Staphylococcus epidermidis,* MSSE; HER 1292	1.0E+06
Bacillus thuringiensis, ZeptoMetrix Z096	1.0E+06	*Streptococcus agalactiae,* ZeptoMetrix Z019	1.0E+06
Bacillus thuringiensis, 97-27	1.0E+06	*Streptococcus pneumoniae,* ZeptoMetrix Z022	1.0E+06
Bacteroides fragilis, ZeptoMetrix Z029	1.0E+06	*Streptococcus pyogenes,* ZeptoMetrix Z018	1.0E+06
Bordetella bronchiseptica, ZeptoMetrix	1.0E+06	*Vibrio cholera,* ZeptoMetrix Z133	1.0E+06
Borrelia burgdorferi, B31	1.0E+06	*Yersinia aldovae,* CNCTC Y 49/84	1.0E+06
Burkholderia cepacia, ZeptoMetrix Z066	1.0E+06	*Yersinia bercovieri,* CDC 2475-87	1.0E+06
Chlamydophila pneumoniae, ZeptoMetrix Z500	1.0E+06	*Yersinia enterocolitica,* ZeptoMetrix Z036	1.0E+06
Citrobacter koseri, ZeptoMetrix Z039	1.0E+06	*Yersinia fredericksenii*, CDC 1462-81	1.0E+06
Clostridium bifermentans, Z176	1.0E+06	*Yersinia intermedia,* CDC 881-81	1.0E+06
Clostridium perfringens, Type A	1.0E+06	*Yersinia kristensenii,* CDC 1460-81	1.0E+06
Clostridium sordellii, ZeptoMetrix Z077	1.0E+06	*Yersinia mollaretti,* CDC 2465-87	1.0E+06
Clostridium sporogenes, ZeptoMetrix Z355	1.0E+06	*Yersinia pseudotuberculosis, ZeptoMetrix Z222*	1.0E+06
Corynebacterium diptheriae, ZeptoMetrix Z116	1.0E+06	*Yersinia ruckeri, CDC 2396-61*	1.0E+06
Viruses			
Strain	**PFU/mL**	**Strain**	**PFU/mL**
Adenovirus (C1), 1 (Species C)*	1.0E+05	Influenza A (H1N1), New York/18/09	2.9E+04

Coronavirus 229E, 229E	9.1E+04	Influenza A (H3N2), Kumamoto/102/02	1.2E+04
Cytomegalovirus (CMV), AD169	1.0E+05	Influenza B, B/Colorado/06/2017*	1.0E+05
Enterovirus Type 68, 2014 isolate 1	1.0E+05	Measles Virus, ZeptoMetrix 0810025CF	2.9E+04
Epstein-Barr Virus (EBV), B95-8	1.0E+05	Mumps Virus, 1	1.7E+04
Hepatitis A Virus (HAV), HM 175 (clone 1)*	1.0E+05	Parainfluenza Virus 1, ZeptoMetrix 0810014CF	8.8E+04
Hepatitis B Virus (HBV), ZeptoMetrix 0810031C*	1.0E+05	Respiratory Syncytial Virus Type A, 1/2015 isolate 1	8.8E+04
Hepatitis C Virus (HCV), Seracare*	1.0E+05	Rhinovirus, 16*	1.0E+05
Herpes Simplex Virus 1 (HSV-1), MacIntyre	1.0E+05	Rubella Virus, ZeptoMetrix 0810048CF	2.5E+04
Herpes Simplex Virus 2 (HSV-2), G Strain*	1.0E+05	Vaccinia Virus, ZeptoMetrix 0810310CF	1.0E+05
Human Herpesvirus 6A (HHV-6A), GS†	1.0E+05	Varicella Zoster Virus (VZV), A	1.0E+05
Human Immunodeficiency Virus (HIV), BaL*	1.0E+05	Yellow Fever Virus, 17D	1.7E+04
Human Metapneumovirus, IA 10-2003		8.8E+04	
Fungi and Protozoa			
Strain	**CFU/mL**	**Strain**	**CFU/mL**
Aspergillus fumigatus, ZeptoMetrix Z014	1.0E+06	*Cryptococcus neoformans, serotype A*	1.0E+06
Candida albicans, ZeptoMetrix Z006	1.0E+06	*Trypanosoma brucei, Lister 427 VSG 221*	1.0E+06

Note: Entries marked with (*) indicate that those organisms were tested in IU/mL. The entries marked with (†) indicate that those organisms were tested in copies/mL.

Hook Effect

This study was conducted to determine if a false negative result could be produced by high concentrations of analyte. Recombinant Lethal Factor (LF) was diluted to concentrations ranging from 50 pg/mL to 50 µg/mL in K_2EDTA anticoagulated venous whole blood and three replicates of each dilution were tested.

Table 6 summarizes the qualitative results obtained in the study. All concentrations of LF greater than 50 pg/mL yielded 3/3 detected results. At 50 pg/mL, 1/3 replicates yielded a result of not detected (false negative) that may be due to 50 pg/mL being the limit of detection (LoD) of the SensiTox *B. anthracis* Toxin Test (Table 5). No hook effect was observed in the reporting of the qualitative data between 50 pg/mL to 50 µg/mL of LF in K_2EDTA anticoagulated venous whole blood.

Table 5. Qualitative results with LF concentrations ranging from 50 pg/mL – 50 µg/mL

Dilution Name	Lethal Factor (pg/mL)	# Detected/# Tested
S1	50,000,000	3/3
S2	10,000,000	3/3
S3	2,000,000	3/3
S4	400,000	3/3
S5	80,000	3/3
S6	16,000	3/3
S7	3,200	3/3
S8	640	3/3
S9	128	3/3
S10	50	2/3

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Quality Control

The SensiTox *B. anthracis* Toxin Test uses internal positive controls (individual well with recombinant LF within the test cartridge) as well as external positive and negative controls. The SensiTox *B. anthracis* External Controls are provided as an independent verification of performance of the SensiTox *B. anthracis* Toxin Test. The Positive Control is comprised of recombinant Lethal Factor formulated in buffer and bovine plasma at a concentration of 160 pg/mL. The Negative Control is buffer diluted in bovine plasma. Both the Positive and Negative Controls are provided as single use lyophilized reagents.

Both the Positive and Negative Controls are processed in a manner similar to a clinical specimen, but without the presence of whole blood. Each Control is rehydrated with the Control Diluent provided in the kit and then added to the Sample Well of the Cartridge. The Cartridge is then loaded onto the MultiPath Analyzer for processing. The MultiPath Analyzer allows for the selection of External Controls upon setup of a run. External Control results are reported as pass, fail, or invalid. Any invalid result for either the Positive or Negative Control requires a repeat test. It is recommended that External Controls be tested at least once every 30 days, when a new reagent lot is received in the laboratory, and when the MultiPath Analyzer undergoes installation, repair, or maintenance procedures.

On each day an analytical study was performed, a minimum of one Positive and one Negative Control was run. If a failed or invalid result was obtained, the Control was rerun. A total of 207 Controls were run, of which 102 were Positive Controls and 105 were Negative Controls. Of the 207 controls run, 96% (198/207) generated valid results (validity rates for the Positive and Negative Controls were 97% and 94%, respectively). Of the 198 valid results obtained, the overall pass/fail rates were 99% for both the Positive and Negative Controls.

In the clinical study, a minimum of one Positive and one Negative Control was run each day prior to testing clinical specimens. A total of 106 Controls were run overall, of which 53 were Positive Controls and 53 were Negative Controls. 99% (105/106) of the control runs generated valid results (100% valid for Positive Controls and 98% valid for Negative Controls). Of the 105 valid results obtained, the overall accuracy of the Controls was 97% with the Positive Controls generating only two false negative results (96% accuracy) and the Negative Controls generating only one false positive (98% accuracy). Performance of the Controls was also assessed at each of the three sites participating in the clinical study with 100% validity at two sites and 96% validity at a third. Accuracy of valid tests at the three sites ranged from 94-100%.

Specimen Stability

A study was conducted to evaluate the stability of lethal factor in whole blood that has been stored refrigerated at 2-8^{o}C or at elevated temperatures of 15-30^{o}C. Three whole blood specimens were collected by venipuncture from normal donors in K_2EDTA vacutainer tubes, were left unspiked or spiked with 150 pg/mL of recombinant LF (3X LoD) and were stored at 5, 15, and 30^{o}C for the following time frames: 0, 4, 8, 24, 48, 72, 96, and 120 hours. At each time point, three replicates of contrived and unspiked whole blood were tested by the SensiTox *B. anthracis* Toxin Test using three different cartridge lots (one lot per sample tested) in two different Multipath Analyzers. Results were analyzed both qualitatively

("detected", "not detected", and "invalid") and quantitatively by linear regression to assess the change in signal in the test wells over time.

Qualitatively, all samples and all storage conditions gave the correct results for all time points leading up to and including 120 hours. For the qualitative results, net signal (total counts in test well minus counts from neutralization well) was determined over time for both contrived and unspiked whole blood samples stored at the three temperatures. The study data support sample storage at 2-8 or 15-30^{o}C for up to 72 hours prior to testing.

6. Detection Limit and Analytical Reactivity:

Limit of Detection

The limit of detection (LoD) for Lethal Factor (LF) was determined by spiking known concentrations of recombinant LF or native LF from culture supernatants of the Ames and SK-102 strains of *B. anthracis* into K_2EDTA venous whole blood collected from healthy donors. A minimum of five serial dilutions of known concentrations of LF were tested in replicates of 20 at concentrations ranging from 20 to 60 pg/mL.

The data were evaluated by analysis of hit rate (detected results/total number of test). The LoD was determined by combining the replicate data across the three cartridge lots and identifying the lowest LF concentration (pg/mL) with a ≥95% "detected" hit rate. The LoD established for Lethal Factor is 50 pg/mL.

Analytical Reactivity (Inclusivity)

To evaluate the inclusivity of the SensiTox *B. anthracis* Toxin Test, 29 *B. anthracis* strains containing the wildtype pXO1 plasmid and representing geographical diversity of clinically relevant strains were tested (Table 6). Supernatants from mid-log phase cultures were isolated, sterilized, and the LF content quantified by ELISA. Based on this quantitation, supernatants from each strain were spiked into venous K_2EDTA anticoagulated whole blood at 3X LoD (150 pg/mL) and tested in triplicate. All 29 *B. anthracis* strains were detected.

Table 6. Inclusivity Study Results

***B. anthracis* Strains Tested and Detected**	
Vollum 1B	Turkey 32
CDC 684	Zimbabwe 89
CDC 607	N-99
K2129	108
K3	506/ Heroin Ba
2002013094	SK-31
RA3	K1811 (Scotland)
Vollum	English Vollum
Ohio ACB	South African
G-28/ BA1035	K1285

SK-128	K4834
PAK-1	New Hampshire
Buffalo	Bekasi
Carbosap	Sterne
205	

Additionally, an *in silico* analysis of Lethal Factor amino sequences found in the National Center for Biotechnology Information (NCBI) database was conducted. Of the 303 unique *B. anthracis* strains identified, 299 (98.7%) had amino acid sequences identical to the strains tested in the inclusivity study, and 4 (1.3%) had amino acid substitutions not represented in the panel.

7. Assay Cut-Off:

   Assay parameter thresholds, including the neutralization ratio, were established based on the evaluation of more than 600 fresh negative venous whole blood samples. All samples were run unspiked and spiked at 150 pg/mL of recombinant LF, for a total of approximately 1200 data points.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable

2. Matrix Comparison:

   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

The performance of the SensiTox *B. anthracis* Toxin Test was evaluated at three sites across the U.S. (Table 7). The evaluation consisted of two study arms: one arm with residual whole blood specimens collected in K_2EDTA for routine hematology analysis to help determine negative percent agreement (NPA), and one arm with prospectively collected whole blood specimens from consented febrile patients what were either left unspiked (to help determine the overall NPA for the two study arms) or were spiked with Lethal Factor (LF) to determine positive percent agreement (PPA). Since there are no *B. anthracis* infections in the U.S., all unspiked residual and prospectively collected specimens were presumed to be negative and were used to demonstrate assay NPA. Prospectively collected samples used to prepare contrived specimens were spiked with either native LF from cell supernatants at 75 pg/mL or 250 pg/mL or with recombinant LF at concentrations of 75 pg/mL (1.5X LoD), 250 pg/mL (5X LoD), or 5 µg/mL (100,000X LoD), and all were expected to be positive and were used to demonstrate assay PPA. All blood specimens were tested by the SensiTox *B. anthracis* Toxin Test within 72 hours of draw.

Table 7. Clinical Study Sites

Study site	
acronym	address
LACNY	Laboratory Alliance of Central New York, LLC
MRN	Medical Research Networx, LLC
FLDx	First Light Diagnostics, Inc.

a. Residual Specimens Study Arm (Retrospective Arm)

In the residual arm of the study, 325 specimens were collected at a single clinical site as part of routine hematology analysis with left over whole blood specimens collected in K_2EDTA. Of the subjects enrolled in the retrospective arm (325), 198 (61%) were from females and 127 (39%) were from males, and subjects ranged in age from 14 to 101 years old. All 325 samples were collected at LACNY with 60 specimens being shipped to FLDx for testing while 265 remaining specimens were tested at LACNY. Seven specimens were withdrawn from the residual study due to repeat invalid test results (n=4), failure to repeat testing following an invalid result within the allotted time frame (n=1) and observed issues with specimen labeling (n=2). 6.2% of residual specimens required repeat testing with the SensiTox *B. anthracis* Toxin Test due to initial invalid results. Upon retesting, the invalid was resolved for 16/20 specimens. 1.2% specimens had a repeat invalid result and were not included in the overall performance analysis that is show in Table 8.

Table 8. Performance Summary for Residual Whole Blood Study

		Expected		
		Pos	**Neg**	**Total**
SensiTox *B. anthracis* Toxin Test	**Pos (detected)**	0	0	0
	Neg (not detected)	0	318	318
	Total	0	318	318
Negative Percent Agreement (95% CI)		100% (98.5 – 100%)		

A total of 53 controls were run corresponding to 22 days of testing at LACNY and 3 days of testing at FLDx. There were 27 EPCs and 26 ENCs. Of the 53 controls run, one miscall was observed. Additionally, the operator selected an incorrect QC type for a positive control and the run was repeated, in accordance with the protocol. Upon repeat all controls generated expected results.

b. Prospective Specimens Study Arm

In the prospective arm of the study, 126 subjects presenting with fever of unknown causes were enrolled for the collection of fresh venous whole blood specimens. Of the subjects enrolled, 57% were from females, 42% were from males, and one was unknown. Twenty-one specimens were withdrawn from the study because of improper specimen handling (n=10), failure to test within 72 hours of specimen collection (n=10), or failure to repeat testing following an invalid result within the allotted time frame (n=1) resulting in 105 eligible samples. Table 9 summarizes the age distribution of the subjects enrolled in the prospective study.

Table 9. Distribution of Subject Ages Enrolled in the Prospective Clinical Study.

Age in Years	Enrolled	Withdrawn	Eligible
6-15	31	6	25
16-21	34	10	24
22-40	36	4	32
>40 (41-48)	25	1	24
Total	126	21	105

To evaluate both the NPA and the PPA, two vials of blood were collected from each consenting subject with one vial used to prepare contrived samples that were spiked with either recombinant LF or native LF from *B. anthracis* cell supernatants at various concentrations. The second vial for each blood sample remained unspiked. Of the 105 contrived samples, 64/105 (61%) were spiked with supernatant LF and 41/105 (39%) were spiked with recombinant LF. Of the concentrations used to spike the contrived samples, 54/105 (51%) were 1-2X LoD, 41/105 (39%) were 5-6X LoD, and 10/105 (10%) were 100,000X LoD (5 µg/mL). Each of the 105 uncontrived samples was tested with the SensiTox *B. anthracis* Toxin Test at MRN, and each of the 105 contrived samples was tested at FLDx, and performance at these two sites is combined in Table 10.

Table 10. Performance summary for prospective specimens tested.

		Expected		
		Pos	**Neg**	**Total**
SensiTox *B. anthracis* Toxin Test	**Pos (detected)**	101	0	101
	Neg (not detected)	4*	105	109
	Total	105	105	210
Positive Percent Agreement (95% CI)		96.2% (90.6 – 98.5%)		
Negative Percent Agreement (95% CI)		100% (96.5 – 100%)		

* A root cause analysis of the observed 4 false negative results was performed and per the protocol repeat testing was performed. All 4 samples were positive upon repeat testing. False negatives were observed at concentrations of 1.5X LoD, indicating that testing at or close to LoD. The 4 false negative contrived samples shown in Table 10 were with *B. anthracis* culture supernatant LF. Of the 17 samples of 1.5X LoD recombinant LF tested in Table 10, all 17 tested true positive.

A total of 52 valid controls were run at MRN an FLDx, of which 26 were EPCs and 26 were ENCs. Of the 52 valid control runs there were two miscalls and runs were repeated, in accordance with the protocol. Upon repeat all controls generated expected results.

2. Clinical Specificity:

   See Section VII.C.1

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

VIII Proposed Labeling:

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

IX Conclusion:

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