November 20, 2023

First Light Diagnostics, Inc.
Joanne Spadoro
Chief Executive Officer
2 Omni Way
Chelmsford, Massachusetts 01824

Re: K232545
Trade/Device Name: The SensiTox *B. anthracis* Toxin Test
Regulation Number: 21 CFR 866.3046
Regulation Name: Simple In Vitro Diagnostic Device For The Detection Of Secreted Proteins From Bacillus Species (Spp.) In Human Clinical Samples
Regulatory Class: Class II
Product Code: QUU
Dated: August 22, 2023
Received: August 22, 2023

Dear Joanne Spadoro:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Noel J. Gerald -S

Noel J. Gerald, Ph.D.
Branch Chief
Bacterial Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K232545

Device Name
SensiTox B. anthracis Toxin Test

Indications for Use *(Describe)*

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) Summary

August 21, 2023

The summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

Contact Details

Sponsor: First Light
Diagnostics, Inc.
2 Omni Way
Chelmsford, MA 01824

Correspondent: Joanne Spadoro, Ph.D.
President and CEO
First Light Diagnostics, Inc.
2 Omni Way
Chelmsford, MA 01824
Phone: (781) 271-0112, extension 224
Email: joanne@firstlightdx.com

Device

Device Trade Name: SensiTox® *B. anthracis* Toxin Test

Common Name: *B. anthracis* Toxin Test

Classification Name: Class II

Regulation Number: 866.3046

Product Code: QUU

Predicate Device: InBios International, Inc. Active Anthrax Detect Plus Rapid Test (DEN220044)

Device Description Summary

The SensiTox *B. anthracis* Toxin Test, run on the MultiPath Analyzer, detects lethal factor in venous whole blood samples using an immunofluorescence assay and the proprietary MultiPath detection technology.

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, a single use consumable that contains all the reagents required to run the test. The Cartridge is loaded onto the MultiPath Analyzer for processing through the steps of the assay.

Once loaded onto the Analyzer, the barcodes on the cartridge that identify the test type and associated test specific information (manufacturer installed barcode) and sample (laboratory affixed barcode) are read. The cartridge is moved to the fluidics station where it is first heated to 35°C. The sample is then split into 3 equal aliquots in 3 distribution wells within the cartridge. The sample aliquots flow from the distribution wells to the reagent wells containing target-specific antibody conjugated fluorescent and magnetic particles in the form of lyophilized beads. Upon contact with the sample, the lyophilized beads rehydrate and the reaction mixtures flow into the imaging wells, the bottoms of which are coated with a dye cushion reagent. Upon contact with the reagents, the dye-cushion dissolves forming a dense opaque aqueous layer that separates the sample and reagents from the bottom optical surface of the Imaging Well. In the upper assay layer, the toxins, if present, bind to the magnetic and fluorescent particles tethering them together. The cartridge is incubated for 12 minutes to allow the reaction to take place and then is moved to the magnetics station. At the magnetics station, the imaging well is placed over permanent magnets that draw the magnetic particles and any fluorescent particles that are tethered to them via the target molecules through the dye-cushion layer, depositing them on the bottom imaging surface. The captured fluorescent particles are imaged and quantified using non-magnified digital imaging.

The Analyzer can be run in batch mode or by random access. Up to 20 cartridges can be loaded onto the Analyzer in parallel. The first result is reported in approximately 21 minutes of loading the cartridge onto the Analyzer with subsequent results being reported in 2.5-minute increments. The results are interpreted using the MultiPath applications software as valid or invalid, and 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.

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. 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.

Special Conditions for Use Statement(s)

For Prescription Use Only. Please refer to the SensiTox *B. anthracis* Toxin Test labeling for a more complete list of warnings, precautions, and contraindications.

Special Instrument Requirements

For use with the Multipath Analyzer

Indications for Use/Technology Comparison

Table 5.1. Comparison of Subject and Predicate Device

Description	First Light Diagnostics, Inc. Subject Device SensiTox *B. anthracis* Toxin Test	InBios International, Inc. Predicate Device DEN220044 Active Anthrax Detect Plus Rapid Test
Regulation	866.3046	Same
Product Code	QUU	Same
Device Class	Class II	Same
Panel	Microbiology	Same
Intended 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	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

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

	This assay is not FDA-cleared or approved for testing blood or plasma donors.	
Similarities		
Sample Type	Whole blood collected with dipotassium EDTA anticoagulant by venipuncture	Serum and venous whole blood (dipotassium EDTA, sodium citrated, and sodium heparin)
Analyte	Lethal Factor	Same
Prescription Status	Prescription Use Only	Same
Antibodies	Anti-Lethal Factor antibodies	Same
Differences		
Technology	Immunofluorescent assay	Immunoassay
Test Format	Fluidic cartridge with direct digital Imaging and automated interpretation	Lateral flow with visual interpretation
Automated	Yes	No
Instrument	Multipath Analyzer	None

Non-Clinical and/or Clinical Tests Summary

Limit of Detection (LoD)

The limit of detection (LoD) for Lethal Factor was determined by adding recombinant Lethal Factor or native Lethal Factor contained in culture supernatants from the Ames and SK-102 strains of *B. anthracis* to K_2EDTA venous whole blood collected from healthy donors. A minimum of 5 serial dilutions of known concentrations of Lethal Factor 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) as well as Probit analysis. 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.

Reproducibility

The reproducibility of the SensiTox *B. anthracis* Toxin Test was evaluated at 3 sites over the course of 5 non-consecutive days by 2 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 – low positive (1.5X LoD), moderate positive (3X LoD), and negative (unspiked) were prepared and provided to each participating site. Each operator mixed a blinded sample with whole blood prescreened and known to be negative for Lethal Factor and tested in triplicate using the SensiTox *B. anthracis* Toxin Test.

As shown in Table 5.2, the overall reproducibility of the SensiTox *B. anthracis* Toxin Test is 98.9%. Of the 270 samples tested, there were 3 incorrect results reported from 3 samples (Table 5.3). Two low positive samples and 1 moderate positive sample generated negative results.

Table 5.2. Reproducibility study data analyzed by sample type and by site.

Sample Description	Site 1		Site 2		Site 3		Total	
	#	% Accuracy	#	% Accuracy	#	% Accuracy	#	% Accuracy
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

Table 5.3. Summary of reproducibility study analyzed by positive and negative samples.

Site	Total Samples	# Invalid	FP	FN	TP	TN	% Accuracy Positives	% Accuracy Negatives
Site 1	90	4	0	0	60	30	100	100
Site 2	90	2	0	2	58	30	96.7	100
Site 3	90	0	0	1	59	30	98.3	100
Total	270	6	0	3	177	90	98.3	100

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 5.4). 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 5.4. Clinically relevant strains of *B. anthracis* tested and detected.

***B. anthracis* Strains**	
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.

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 5.5.

With the exception of two *B. cereus* strains, G9241 and 03BB102, which contain the extrachromosomal plasmid pXO1 that harbors the gene for Lethal Factor, 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 5.5. Bacteria, viruses, fungi, and protozoa tested for cross-reactivity. 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

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	*Trypanosoma brucei*, Lister 427 VSG 221	1.0E+06
Babesia microti, R1*	3.9 %		

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 5.6 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 5.6. Bacteria, viruses, fungi, and protozoa tested for interference. 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.

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

Bacteria			
Strain	**CFU/mL**	**Strain**	**CFU/mL**
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

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 3 times 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 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 5.7. None of the substances tested interfered with the SensiTox *B. anthracis* Toxin Test.

Table 5.7 Endogenous and exogenous interferents tested.

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**	**Exogenous Substances**	**Concentration**
Acetaminophen	200 µg/mL	Heparin	90.3 U/mL
Acid-citrate-dextrose 3x	300 µL/mL	Ibuprofen	500 µg/mL
Albuterol (Salbutamol)	400 ng/mL	Malarone (Atovaquone)	180 µg/mL
Amoxicillin	75.2 µg/mL	Mefloquine	18 µg/mL
Artemisinin	9 µg/mL	N-acetylcysteine	1.7 mg/mL
Ascorbic acid	60 µg/mL	Naproxen sodium	500 µg/mL
Aspirin	652 µg/mL	Oseltamivir (Tamiflu)	4.95 µg/mL
Biothrax	0.9 µL/mL	Ribavirin	51.3 µg/mL
Cefotaxime	306 µg/mL	Rifampin	66 µg/mL
Chloroquine	36 µg/mL	Sodium citrate	9.6 mg/mL
Ciprofloxacin	10 µg/mL	Sodium polyanetholesulfonate	1.5 mg/mL
Cromolyn sodium	825 ng/mL	Streptomycin	450 µg/mL
Doxycycline	30 µg/mL	Sulfamethoxazole	400 µg/mL
EDTA	5.4 mg/mL	Tetracycline	15 µg/mL
Erythromycin	60 µg/mL	Tobramycin	24 µg/mL
Flunisolide (Flovent)	90 ng/mL	Trimethoprim	40 µg/mL
Gentamicin sulfate	10 µg/mL		

Hook Effect

This study was conducted to determine if a false negative result could be produced by high concentrations of analyte. Recombinant 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 5.8 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. This result was not unexpected as 50 pg/mL is the limit of detection (LoD) of the SensiTox *B. anthracis* Toxin Test. 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 5.8. Qualitative results with LF concentrations ranging from 50 pg/mL – 50 µg/mL

Dilution Name	Lethal Factor (pg/mL)	# Detected / # Tested	# Invalid
S1	50,000,000	3 / 3	0
S2	10,000,000	3 / 3	0
S3	2,000,000	3 / 3	0
S4	400,000	3 / 3	0
S5	80,000	3 / 3	0
S6	16,000	3 / 3	1
S7	3,200	3 / 3	0
S8	640	3 / 3	0
S9	128	3 / 3	0
S10	50	2 / 3	0

Clinical Performance Evaluation

The performance of the SensiTox *B. anthracis* Toxin Test was evaluated at three sites across the US. The evaluation consisted of two study arms, one with residual whole blood specimens collected in K_2EDTA for routine hematology analysis to determine negative percent agreement (NPA), and one with prospectively collected whole blood specimens from consented febrile patients that were spiked with recombinant or native Lethal Factor to determine positive percent agreement (PPA). All specimens were tested by the SensiTox *B. anthracis* Toxin Test within 72 hours of draw.

A total of 325 residual blood specimens were collected at a single clinical site. Seven specimens were withdrawn from this arm of the study, resulting in 318 specimens that were tested at two sites and included in the data analysis.

For the prospective study, a total of 126 subjects presenting with fever and flu-like symptoms were enrolled for the collection of fresh venous whole blood specimens. Twenty-one (21) specimens were withdrawn from the study resulting in 105 eligible samples.

For the prospective arm of the study, patient specimens were tested at a single site. Additionally, contrived specimens prepared from the prospectively collected blood specimens and containing either native Lethal Factor (LF) contained in cell supernatants at 75 pg/mL (1.5X LoD) and 250 pg/mL (5X LoD) or recombinant LF at concentrations of 75 pg/mL, 250 pg/mL, and 5 µg/mL were prepared and tested blinded at a second site.

Table 5.9 summarizes the data from the testing of residual specimens at two sites. All specimens tested reported not detected results for *B. anthracis* Lethal Factor for a 100% NPA.

Table 5.9. Performance summary for residual whole blood specimens tested

All Sites		**Expected**		
		Pos	**Neg**	**Total**
Residual whole blood specimens				
SensiTox *B. anthracis* Toxin Test	Pos (LF detected)	0	0	0
	Neg (LF Not detected)	0	318	318
	Total	0	318	318
All Sites		**(95% C.I.)**		
Negative Percent Agreement (NPA) (%)		100.0% (98.8% - 100.0%)		

In the prospective study, two vials of blood drawn from 105 subjects presenting with fever and flu-like symptoms were tested at two sites. Table 5.10 shows the overall data summary with a performance of 96.2% PPA and 100% NPA. Four false negative results, generated at a concentration of 75 pg/mL LF (1.5X LoD), were reported in the contrived study.

Table 5.10. Performance summary for prospective specimens tested

All Sites		**Expected**		
		Pos	**Neg**	**Total**
Specimens from the Prospective Arm				
SensiTox *B. anthracis* Toxin Test	Pos (LF detected)	101	0	101
	Neg (LF not detected)	4	105	109
	Total	105	105	210
All Sites		**(95% C.I.)**		
Positive Percent Agreement (PPA) (%)		96.2%* (90.6% - 98.5%)		
Negative Percent Agreement (NPA) (%)		100.0% (96.5% - 100.0%)		

* All samples were confirmed positive when retested.

Conclusion

The conclusions drawn from the nonclinical and clinical tests demonstrate that the device is as safe, as effective, and performs as well as or better than the legally marketed device. 807.92(b)(3).