September 15, 2023

T2 Biosystems, Inc.
Rachel Gilbert
Manager, Regulatory Affairs
101 Hartwell Avenue
Lexington, Massachusetts 02421

Re: K231336
Trade/Device Name: T2Biothreat Panel
Regulation Number: 21 CFR 866.4000
Regulation Name: Device To Detect And Identify Biothreat Microbial Agents In Human Clinical Specimens
Regulatory Class: Class II
Product Code: QVR, QBX, NSU
Dated: May 8, 2023
Received: May 8, 2023

Dear Rachel Gilbert:

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 (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 located 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.

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 803) for devices or postmarketing safety reporting (21 CFR 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 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 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)*
K231336

Device Name
T2Biothreat Panel

Indications for Use *(Describe)*
The T2Biothreat Panel is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with the T2Dx Instrument. The T2Biothreat Panel detects nucleic acids from the following organisms directly from K2EDTA whole blood samples:

1. Bacillus anthracis (plasmids pXO1 and pXO2)
2. Francisella tularensis
3. Burkholderia spp. (B. mallei/B. pseudomallei)
4. Yersinia pestis
5. Rickettsia prowazekii

The T2Biothreat Panel will not distinguish between detection of Burkholderia mallei and Burkholderia pseudomallei but will present valid detections as a positive detection of Burkholderia species.

The T2Biothreat 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 T2Biothreat Panel is indicated as an aid in the diagnosis of anthrax, tularemia, melioidosis, glanders, typhus fever and plague in response to suspected or confirmed bioterrorism events or outbreaks. Diagnosis of infection must be made in conjunction with clinical, epidemiologic and other laboratory data. Results are for the presumptive identification of Bacillus anthracis, Francisella tularensis, Burkholderia spp. (B. mallei/B. pseudomallei), Yersinia pestis and Rickettsia prowazekii. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The definitive identification of Bacillus anthracis, Francisella tularensis, Burkholderia mallei, Burkholderia pseudomallei, Yersinia pestis or Rickettsia prowazekii requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required. Positive results do not rule out co-infections with pathogens not included on the T2Biothreat Panel. 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.

The T2Biothreat Panel is indicated for use in laboratories that have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities, and personnel trained in the safe handling of clinical specimens potentially containing biothreat organisms.

The T2Biothreat Panel is indicated for use in laboratories that follow public health guidelines 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.

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.

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510(k) Summary

Date of Summary	May 8, 2023
Product Name	T2Biothreat Panel
Sponsor	T2Biosystems, Inc. 101 Hartwell Avenue Lexington, MA 02421
Correspondent	Rachel Gilbert Manager, Regulatory Affairs 781-226-2767, 1970 rgilbert@t2biosystems.com
Device Trade or Proprietary Name	T2Biothreat Panel
Regulation	21 CFR 866.4000
Common Name	Multiplex Nucleic Acid Detection System For Biothreat Agents
Product Code	QVR
Classification	Class II

Intended Use

The T2Biothreat Panel is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with the T2Dx Instrument. The T2Biothreat Panel detects nucleic acids from the following organisms directly from K2EDTA whole blood samples:

1. *Bacillus anthracis* (plasmids pXO1 and pXO2)
2. *Francisella tularensis*
3. *Burkholderia* spp. (*B. mallei*/*B. pseudomallei*)
4. *Yersinia pestis*
5. *Rickettsia prowazekii*

The T2Biothreat Panel will not distinguish between detection of *Burkholderia mallei* and *Burkholderia pseudomallei* but will present valid detections as a positive detection of *Burkholderia* species.

The T2Biothreat 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 T2Biothreat Panel is indicated as an aid in the diagnosis of anthrax, tularemia, melioidosis, glanders, typhus fever and plague in response to suspected or confirmed bioterrorism events or outbreaks. Diagnosis of infection must be made in conjunction with clinical, epidemiologic and other laboratory data. Results are for the presumptive identification of *Bacillus anthracis, Francisella tularensis, Burkholderia* spp. (*B. mallei*/*B. pseudomallei*), *Yersinia pestis* and *Rickettsia prowazekii*. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The definitive identification of *Bacillus anthracis*, *Francisella tularensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Yersinia pestis* or *Rickettsia prowazekii* requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required.

Positive results do not rule out co-infections with pathogens not included on the T2Biothreat Panel. 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.

The T2Biothreat Panel is indicated for use in laboratories that have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities, and personnel trained in the safe handling of clinical specimens potentially containing biothreat organisms.

The T2Biothreat Panel is indicated for use in laboratories that follow public health guidelines 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.

Limitations

For prescription use only. Refer to the Biothreat Panel labeling for a more complete list of warnings, precautions, and contraindications.

Device Technology Overview

The T2Biothreat Panel is run on the T2Dx, a fully automated, benchtop instrument. During processing on the T2Dx, intact pathogen cells are concentrated directly in whole blood, then lysed to release the target DNA. After amplification, target amplicon is hybridized with superparamagnetic particles and then detected by T2MR. The Internal Control on the T2Biothreat Panel monitors performance for each sample.

The T2Biothreat Panel is a qualitative molecular diagnostic assay that employs whole blood compatible PCR amplification followed by T2 Magnetic Resonance (T2MR) detection. The T2Biothreat Panel is performed on the T2Dx Instrument, which executes all steps after specimen loading, with the capability of loading up to seven blood specimens at the same time. Individually, a K_2EDTA whole blood specimen containing a minimum of 3 mL is loaded directly onto the T2Biothreat Sample Inlet, which is then placed on the T2Biothreat Cartridge along with the T2Biothreat Reagent Tray. The Cartridge and Reagent Tray contain the lysis reagent, internal control, primers, enzyme, buffer and probe-coupled superparamagnetic particles for each detected target.

After loading into the T2Dx, the blood specimen is mixed with the red blood cell lysing reagent and the bacterial cells and human cellular debris are concentrated by centrifugation. The internal control is added to the concentrated pellet and a bead-beating process lyses the bacterial cells. The supernatant containing the DNA from the lysed bacterial cells and the internal control is amplified using the target and internal control-specific primers. The generated amplified product is aliquoted into individual tubes containing target-specific probe conjugated particles for each detected target and the internal control. The amplified DNA is hybridized to target-specific probes attached to superparamagnetic particles causing clustering of the particles. The hybridization occurring in individual tubes is analyzed in the T2MR reader and a signal for each target is generated, which indicates the presence of the target organism(s). This automated process is the same process followed by the FDA cleared T2Candida and T2Bacteria Panels performed on the T2Dx Instrument system.

When running a single specimen or multiple specimens simultaneously, the first specimen will be reported in approximately 4 hours from the time the specimen is loaded onto the instrument. The results are interpreted by the device software as valid or invalid (based on the result of the internal control or target detections), and if valid, results are reported as “Positive” or “Target not Detected” for each specific target.

Analytical Performance Characteristics

Limit of Detection (LoD)
LoD was defined as the minimum bacterial concentration at which a 95% positivity rate was achieved. The T2Biothreat Panel has a range of LoDs from 2-17 CFU/mL or 9 Cell-Associated Genomic equivalents (CAGe)/mL for the six target organisms (Table 1), as determined by measuring the percent positive detection for human whole blood spiked samples (N≥20) at a given CFU/mL level. Bacterial concentrations were assigned by quantitative CFU/mL plating of an aliquot of the final spike solution. Testing included two instruments, two reagent lots, and two different strains of each of the Panel members using K_2EDTA-treated whole blood. The final LoD for each target was determined as the highest LoD determined across all reagents and strains. The LoD for *B. anthracis* was determined based on the detection of both pXO1 and pXO2. The data is summarized below:

Table 1: LoD of T2Biothreat Panel

Species	LoD
Bacillus anthracis	6 CFU/mL
Francisella tularensis	4 CFU/mL
Burkholderia mallei	12 CFU/mL
Burkholderia pseudomallei	17 CFU/mL
Yersinia pestis	2 CFU/mL
Rickettsia prowazekii	9 CAGe/mL

Analytical Reactivity (Inclusivity)
To confirm detection of multiple clinically relevant strains of the organisms detected by the T2Biothreat Panel, an *in silico* analysis of primer and probe designs against the NCBInt data base determined that the assay was 100% inclusive of pathogens containing the target sequence. This was then confirmed with wet testing of 10 strains of *B. anthracis*, 8 strains of *B. mallei*, 10 strains of *B. pseudomallei*, 10 strains of *F. tularensis*, 5 strains of *R. prowazekii* and 12 strains of *Y. pestis* on the T2Dx instrument. The strain identities were confirmed by whole genome sequence analysis. All strains were tested at a titer consistent with approximately 2 times the LoD level for each organism. All strains were successfully detected by the T2Biothreat Panel except two *Y. pestis* strains that lacked the pPCP plasmid that contains the *Y. pestis* species target sequence. All *Y. pestis* strains that lack the pPCP plasmid will be outside the inclusivity of the T2Biothreat Panel.

Table 2: Inclusivity Study Results

Organism	Strains	Positivity	Repeat Testing	Result
Bacillus anthracis 10 strains	2002013094	3/3	N/A	Inclusive
	A0489	3/3	N/A	Inclusive
	A0707	3/3	N/A	Inclusive
	A0809	3/3	N/A	Inclusive
	Ames	3/3	N/A	Inclusive
	Buffalo	3/3	N/A	Inclusive
	Canadian Bison	3/3	N/A	Inclusive
	G-28	3/3	N/A	Inclusive
	SK-31	3/3	N/A	Inclusive
	Vollum	3/3	N/A	Inclusive

Organism	Strains	Positivity	Repeat Testing	Result
Burkholderia mallei 8 strains	AMC Strain China 5	3/3	N/A	Inclusive
	GB8 Horse 4	3/3	N/A	Inclusive
	NCTC 10230	3/3	N/A	Inclusive
	NCTC10245	3/3	N/A	Inclusive
	NCTC 10260	3/3	N/A	Inclusive
	NCTC 120	3/3	N/A	Inclusive
	NCTC 3708	3/3	N/A	Inclusive
	NCTC 3709	3/3	N/A	Inclusive
Burkholderia pseudomallei 10 strains	Human/Blood/OH/US/1994	3/3	N/A	Inclusive
	7894	3/3	N/A	Inclusive
	China 3	3/3	N/A	Inclusive
	ATCC 23343	3/3	N/A	Inclusive
	Environment/Thailand/1990	3/3	N/A	Inclusive
	HBPUB10134A	2/3	19/20	Inclusive
	JCU-NCTC 13178	2/3	20/20	Inclusive
	MSHR840	3/3	N/A	Inclusive
	NAU20B16	3/3	N/A	Inclusive
	NCTC 13392	3/3	N/A	Inclusive
Francisella tularensis 10 strains	425	3/3	N/A	Inclusive
	100892A	2/3	20/20	Inclusive
	ATCC Vaccine Strain	3/3	N/A	Inclusive
	GA99-3549	3/3	N/A	Inclusive
	HN63	3/3	N/A	Inclusive
	JAP (Cincinnati)	3/3	N/A	Inclusive
	KY99	3/3	N/A	Inclusive
	OR96	3/3	N/A	Inclusive
	MO#1	3/3	N/A	Inclusive
	MR#2	3/3	N/A	Inclusive
Rickettsia prowazekii 5 strains	103-2P	3/3	N/A	Inclusive
	Addis Abbas	3/3	N/A	Inclusive
	Cairo	3/3	N/A	Inclusive
	GvF	3/3	N/A	Inclusive
	ZRS	3/3	N/A	Inclusive
Yersinia pestis 12 strains	195/P (India)	3/3	N/A	Inclusive
	Angola	3/3	N/A	Inclusive
	AZ94-0666	3/3	N/A	Inclusive
	Bombay	0/3	N/A	Not Detected
	El Dorado 2572-1	3/3	N/A	Inclusive
	Harbin	3/3	N/A	Inclusive
	MAD115	3/3	N/A	Inclusive
	Pestoides G	0/3	0/20	Not Detected
	Shasta	3/3	N/A	Inclusive
	ZE94-2122	3/3	N/A	Inclusive
	PBM19	3/3	N/A	Inclusive
	Nicholisk	3/3	N/A	Inclusive

Analytical Specificity (Exclusivity)
To establish the analytical specificity of the T2Biothreat Panel, 31 bacterial, fungal, and viral strains commonly found to cause blood stream infections or to be genetically similar to pathogens detected by the Panel were tested. All organisms were prepared at 1,000 CFU/mL, CAGe/mL, or IU/mL K2EDTA-treated whole blood followed by titration to determine if any repeatable reactivity was detected. The results are outlined below:

Table 3: Pathogens for Which no Reactivity was Detected

Non-Reactive Species/Strains		
Bacillus cereus NR-608	*Burkholderia thailandensis*	Human Immunodeficiency Virus[3]
Bacillus cereus BAG1X1-1[1]	*Candida albicans*	*Klebsiella pneumoniae*
Bacillus cereus BAG4X2-1[2]	*Citrobacter koseri*	*Pseudomonas aeruginosa*
Bacillus cereus VD115[2]	*Clostridium perfringens*	*Rickettsia rickettsia*
Bacillus tropicus	*Enterobacter aerogenes*	*Rickettsia typhi*
Bacillus thuringiensis	*Enterococcus faecalis*	*Staphylococcus epidermidis*
Bacillus anthracis A0006 (pXO1 plasmid only)	*Enterococcus faecium*	*Staphylococcus lugdunensis*
Bacillus anthracis 4229 (pXO2 plasmid only)	*Escherichia coli*	*Staphylococcus aureus*
Bacteroides fragilis	*Francisella hispaniensis*	*Yersinia pseudotuberculosis*
Burkholderia cepacia	*Francisella philomiragia*	*Yersinia enterocolitica*

[1] Harbors a pXO-1 like plasmid that was not detected by the Panel;
[2] Harbors a pXO-2-like plasmid that was not detected by the Panel;
[3] Tested as IU/mL

Table 4: Cross-Reactive Species/Strains at Concentrations from 1,000 to 10 CFU/mL

Cross-reactive Non-Panel Strain	**Panel Channel that Cross-reacts**
Bacillus cereus G-9241	BaPXO1

Five (5) strains of *Bacillus cereus* were tested, two of which contained a pXO1-like plasmid and two that contained a pXO2-like plasmid. None of these strains were detected except strain G-9241, known to harbor a plasmid similar to pXO1 in *Bacillus anthracis*. The T2Biothreat Panel distinguishes between the detection of a single virulence plasmid in a *Bacillus spp*. and detection of fully virulent *B. anthracis* harboring both the pXO1 and pXO2 plasmids and the software identifies results accordingly.

To evaluate higher concentrations of analyte which may be encountered in whole blood samples, $1x10^6$ copies/mL of DNA from the exclusivity strains were spiked individually into whole blood samples and tested in triplicate with the T2Biothreat Panel. No cross-reactivity was observed at this higher analyte concentration.

Table 5: Exclusivity Study Organisms Evaluated at 1x 10^6 copies/mL

Non-Reactive Species/Strains		
Bacillus cereus NR-608	*Candida albicans*	*Pseudomonas aeruginosa*
Bacillus cereus BAG1X1-1	*Citrobacter koseri*	*Rickettsia rickettsia*
Bacillus cereus BAG4X2-1	*Clostridium perfringens*	*Rickettsia typhi*
Bacillus cereus VD115	*Enterobacter aerogenes*	*Staphylococcus epidermidis*
Bacillus circulans	*Enterococcus faecalis*	*Staphylococcus lugdunensis*
Bacteroides fragilis	*Enterococcus faecium*	*Staphylococcus aureus*
Bacillus tropicus	*Escherichia coli*	*Yersinia enterocolitica*
Bacillus thuringiensis	*Francisella hispaniensis*	*Yersinia pseudotuberculosis*
Burkholderia cepacia	*Francisella philomiragia*	
Burkholderia thailandensis	*Klebsiella pneumoniae*	

Reproducibility

Results agreement was analyzed at multiple sites over six non-consecutive days for each sample type, minimum of two operators per site, two Reagent Tray Kit lots, and two instruments. Sample types consisted of a negative K_2EDTA-treated whole blood sample, and three types of positive K_2EDTA-treated whole blood samples (*B. anthracis, B. pseudomallei*, and *Y. pestis* triple species spike, *B. mallei* and *R. prowazekii* dual species spike, and *F. tularensis* single species spike) at 2-3x LoD or 1-1.5 LoD. Reproducibility results across instruments, operators, reagent lots, and sample type showed an overall agreement of 98.4% with expected positive results and an overall agreement of 100% for expected negative results.

Table 6: Summary of Reproducibility Results

Organism	**Conc.**	**Expected Result**	**Agreement with Expected Result [95% CI]**	
B. anthracis	1-1.5x LoD	Detected	BaPXO1 Channel	BaPXO2 Channel
			128/128 100% [97.7-100]	127/128 99% [95.7-100]
	2-3x LoD	Detected	104/104 100% [97.2-100]	103/104 99% [94.8-100]
B. pseudomallei	1-1.5x LoD	Detected	Bu Channel	-
			126/128 98% [94.5-99.8]	
	2-3x LoD	Detected	103/104 99% [94.8-100]	-
B. mallei	1-1.5x LoD	Detected	96/96 100% [96.9-100]	-
	2-3x LoD	Detected	95/96 99% [94.3-100]	-
Y. pestis	1-1.5x LoD	Detected	Yp Channel	-
			126/128 98% [94.5-99.8]	
	2-3x LoD	Detected	104/104 100% [97.2-100]	-

Organism	Conc.	Expected Result	Agreement with Expected Result [95% CI]	
R. prowazekii	1-1.5x LoD	Detected	Rp Channel 95/96 99% [94.3-100]	-
	2-3x LoD	Detected	96/96 100% [96.9-100]	-
F. tularensis	1-1.5x LoD	Detected	Ft Channel 96/96 100% [96.9-100]	-
	2-3x LoD	Detected	95/96 99% [94.3-100]	-
Negative	Negative	Not Detected	379/379 100% [99.2-100]	-

Interfering Substances

To determine and characterize the effects of potential endogenous and exogenous interfering substances on the performance of the T2Biothreat Panel, 7 endogenous and 15 exogenous substances were tested with contrived positive and negative samples in K_2EDTA-treated whole blood by the Panel on the T2Dx. Potentially interfering substances were tested at or above clinically relevant concentrations based on the CLSI guidelines. None of the tested substances demonstrated interference with the detection of any T2Biothreat Panel targets nor in the validity of samples tested. Based on previous studies, Feraheme is considered an interferent at ≥21µg/mL, Magnevist is considered an interferent at ≥1.7 mg/mL, and Ablavar is considered an interferent at ≥0.39 mg/mL. No interference was detectable at the specified test concentration for all other substances.

Table 7: Substances Tested for Interference with the T2Biothreat Panel – No Interference Observed

Endogenous Substances & Concentrations		Exogenous Substances & Concentrations			
Bilirubin (conjugated)	475 µmol/L	Ampicillin	215 µmol/L	Levofloxacin	99.6 µmol/L
Bilirubin (unconjugated)	684 µmol/L	Chloramphenicol	241 µmol/L	Meropenem trihydrate	884 µmol/mL
Creatinine	150 mg/L	Ciprofloxacin	36.2 µmol/L	Novobiocin	450 µmol/mL
Hemoglobin	>20 g/dL	Ceftazidime pentahydrate	606 µg/mL	Penicillin G sodium salt	0.777 µg/mL
Intralipid (to mimic triglycerides)	1703 mg/dL	Doxycycline hyclate	40.5 µmol/L	Streptomycin sulfate	444 µmol/L
Urea	42.9 mmol/L	Gentamicin sulfate	62.8 µg/mL	Tetracycline hydrochloride	54 µmol/L
White Blood Cells	≥16.5x10^6 cells/mL	K_2EDTA	9 mg/mL	Trimethoprim	145 µmol/L
		Kanamycin B sulfate	186 µmol/L		

Competitive Inhibition

Sensitivity of the T2Biothreat Panel was tested in whole blood samples spiked with two organisms to mimic co-infection in a patient sample. The conditions tested included: co-infection with two Panel target species both at, or near, the LoD; co-infection with two Panel target species where one species is at high titer (1,000 CFU/mL or CAGe/mL) and the other is at or near LoD; and co-infection with one Panel species at or near LoD and a non-Panel species at high titer (1,000 CFU/mL or IU/mL). Non-Panel species tested were *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *C. albicans,* and HIV. This study assessed the impact on the positive detection of Panel members by competitive inhibition from other potential co-infecting species. No competitive effects were observed in any combination of Panel members at ≤1,000 CFU/mL or CAGe/mL or near LoD. No competitive effects were observed in any combination of non-Panel members and Panel members at ≤1,000 CFU/mL or IU/mL.

Table 8. Competitive Inhibition – High and Low Concentrations of On-Panel Organisms

Species 1 Low Conc. (1-2x LoD)	Species 2 High and Low Conc. (1000 CFU/mL or CAGe/mL and 1-2x LoD)[1]	Positive Detection Rate		Competitive Inhibition
		Species 1	Species 2	
B. anthracis	*B. mallei*	100%	100%	No
	Y. pestis	100%	100%	No
	B. pseudomallei	100%	100%	No
	F. tularensis	100%	100%	No
	R. prowazekii	100%	100%	No
B. mallei	*Y. pestis*	100%	100%	No
	B. pseudomallei	100%	100%	No
	F. tularensis	100%	100%	No
	R. prowazekii	100%	100%	No
Y. pestis	*B. mallei*	100%	100%	No
	B. anthracis	100%	100%	No
	B. pseudomallei	100%	100%	No
	F. tularensis	100%	100%	No
	R. prowazekii	100%	100%	No
B. pseudomallei	*B. anthracis*	100%	100%	No
	Y. pestis	100%	100%	No
	F. tularensis	100%	100%	No
	R. prowazekii	100%	100%	No
F. tularensis	*B. anthracis*	100%	100%	No
	B. mallei	100%	100%	No
	Y. pestis	100%	100%	No
	B. pseudomallei	100%	100%	No
	R. prowazekii	100%	100%	No
R. prowazekii	*B. anthracis*	100%	100%	No
	B. mallei	100%	100%	No
	Y. pestis	100%	100%	No
	B. pseudomallei	100%	100%	No
	F. tularensis	100%	100%	No

[1]Units are CFU/mL for all except Rp, which is in CAGe/mL

Table 9. Competitive Inhibition – High Conc. Off-Panel Organisms

Species 1 Low Conc. (1-2x LoD)	Species 2 High Conc. (1000 CFU/mL, CAGe/mL, or IU/mL)[1]	Positive Detection Rate		Competitive Inhibition
		Species 1[2]	Species 2[2]	
B. anthracis	*Escherichia coli*	100%	0%	No
	Enterococcus faecium	75% (3/4 Ba pXO2, 4/4 Ba pXO1) 100% (20/20 both channels)	0%	No
	Klebsiella pneumoniae	100%	0%	No
	Pseudomonas aeruginosa	100%	0%	No
	Staphylococcus aureus	100%	0%	No
	Candida albicans	100%	0%	No
	Human Immunodeficiency Virus	100%	0%	No
B. mallei	*E. coli*	100%	0%	No
	E. faecium	100%	0%	No
	K. pneumoniae	100%	0%	No
	P. aeruginosa	100%	0%	No
	S. aureus	100%	0%	No
	C. albicans	100%	0%	No
	HIV	100%	0%	No
Y. pestis	*E. coli*	100%	0%	No
	E. faecium	100%	0%	No
	K. pneumoniae	100%	0%	No
	P. aeruginosa	100%	0%	No
	S. aureus	100%	0%	No
	C. albicans	100%	0%	No
	HIV	100%	0%	No
B. pseudomallei	*E. coli*	100%	0%	No
	E. faecium	100%	0%	No
	K. pneumoniae	100%	0%	No
	P. aeruginosa	100%	0%	No
	S. aureus	100%	0%	No
	C. albicans	100%	0%	No
	HIV	100%	0%	No
F. tularensis	*E. coli*	100%	0%	No
	E. faecium	100%	0%	No
	K. pneumoniae	100%	0%	No
	P. aeruginosa	100%	0%	No
	S. aureus	100%	0%	No
	C. albicans	100%	0%	No
	HIV	100%	0%	No
R. prowazekii	*E. coli*	100%	0%	No
	E. faecium	100%	0%	No
	K. pneumoniae	100%	0%	No

Species 1 Low Conc. (1-2x LoD)	Species 2 High Conc. (1000 CFU/mL, CAGe/mL, or IU/mL)[1]	Positive Detection Rate		Competitive Inhibition
		Species 1[2]	Species 2[2]	
	P. aeruginosa	100%	0%	No
	S. aureus	100%	0%	No
	C. albicans	100%	0%	No
	HIV	100%	0%	No

[1]Units are CFU/mL for all except Rp, which is in CAGe/mL, and HIV which is in IU/mL
[2]Unless otherwise listed, the positivity rate applies to the intended biothreat target channel.

Clinical Performance Characteristics

The clinical performance of the T2Biothreat Panel was established through a two-arm study conducted using samples from healthy donors and febrile donors collected was collected from 7 geographically diverse sites. One arm comprised the Negative Arm, which tested negative whole blood samples from healthy and febrile donors and was used to assess the Panel specificity. K_2EDTA-treated whole blood samples from healthy donors were collected from patients presenting with no signs or symptoms of infection and were tested at a clinical site. Whole blood samples from febrile donors were collected from patients presenting with a fever of ≥ 100.4 °F and were tested at two (2) laboratory sites. The Positive Arm, which tested contrived positive-spiked whole blood samples from febrile donors, was designed to assess the Panel sensitivity. This arm of the study included the testing of sequence-verified clinical bacterial strains spiked at specific concentrations into whole blood collected from febrile donors. Contrived positive samples were produced to contain a single strain for each species being tested, (spiked at concentrations less than the LoD and greater than or equal to the LoD). Analysis of the Panel negative percent agreement (NPA) utilized results from healthy and febrile negative blood samples and analysis of the Panel positive percent agreement (PPA) utilized results from the positive contrived samples. The PPA was analyzed for samples above and below LoD as well as all concentrations. The PPA and NPA were assessed for each analyte at a range of concentrations. The PPA ranged from 95% to 100% for analyte concentrations at 1-3x LoD except for *F. tularensis* which had a PPA of 94.3% and the NPA was 100% for all analytes.

Table 10. PPA by organism relative to concentration (positive contrived)

Organism	LoD	Titer Bucket	Titer Range[1] (CFU/mL)	PPA[2,3,4] (TP/TP+FN)	95% CI
B. anthracis (pXO1 & pXO2)[5]	6 CFU/mL	0.1-1x LoD	0.6 - <6.0	83.3% (5/6)	35.9-99.6%
		1-3x LoD	6.0 - <18.0	100% (32/32)	91.1-100%
		3-5x LoD	18.0 - 30.0	100% (12/12)	77.9-100%
Burkholderia spp. (*B. pseudomallei* & *B. mallei*)	12 – 17 CFU/mL[6]	0.1-1x LoD	1.2 - <17.0	100% (17/17)	83.8-100%
		1-3x LoD	12.0 - < 51.0	100% (77/77)	96.2-100%
		3-5x LoD	36.0 - 85.0	100% (6/6)	60.7-100%
F. tularensis	4 CFU/mL	0.1-1x LoD	0.4 - <4.0	85.7% (6/7)	42.1-99.6%
		1-3x LoD	4.0 - <12.0	94.3% (33/35)	80.8-99.3%
		3-5x LoD	12.0 - 20.0	100% (8/8)	68.8-100%
R. prowazekii	9 CAGe/mL	0.1-1x LoD	0.9 - <9.0	N/A	N/A
		1-3x LoD	9.0 - <27.0	100% (10/10)	74.1-100%
		3-5x LoD	27.0 - 45.0	100% (40/40)	92.8-100%
Y. pestis	2 CFU/mL	0.1-1x LoD	0.2 - <2.0	96.2% (25/26)	80.4-99.9%
		1-3x LoD	2.0 - <6.0	100% (24/24)	88.3-100%
		3-5x LoD	6.0 - 10.0	N/A	N/A

[1]Rp is in CAGe/mL
[2]TP = True Positives
[3]FN = False Negatives
[4]PPA = Positive Percent Agreement
[5]The LoD for *B. anthracis* detection is the concentration needed to detect both pXO1 and pXO2 plasmids (the higher of the two LoD)
[6]*Burkholderia* species are not differentiated in the results reporting, the LoD for *B. pseudomallei* is 17 CFU/mL, the LoD for *B. mallei* is 12 CFU/mL

Table 11: PPA by organism relative to LoD (positive contrived)

Target Species	LoD	Detection Channel	< LoD		≥ LoD		All Concentrations	
			PPA[1]	95% CI	PPA1	95% CI	PPA1	95% CI
B. anthracis	6 CFU/mL	BaPXO1	100% (10/10)	74.11% - 100%	100% (40/40)	92.78% - 100%	100% (50/50)	94.18% - 100%
		BaPXO2	90% (9/10)	55.50% - 99.75%	100% (40/40)	92.78% 100%	98% (49/50)	89.35% - 99.95%
B. pseudomallei	17 CFU/mL	Bu	100% (20/20)	86.09% - 100%	100% (80/80)	96.32% - 100%	100% (100/100)	97.05% - 100%
B. mallei	12 CFU/mL							
F. tularensis	4 CFU/mL	Ft	90% (9/10)	55.50% - 99.75%	95% (38/40)	83.08% - 99.39%	94% 47/50	83.45% - 97.75%
R. prowazekii	9 CAGe/mL	Rp	100% (10/10)	74.11% - 100%	100% (40/40)	92.78% - 100%	100% (50/50)	94.18% - 100%
Y. pestis	2 CFU/mL	Yp	100% (10/10)	74.11% - 100%	98% (39/40)	86.84% 99.94%	98% (49/50)	89.35% - 99.95%

The analytical and clinical performance data support the T2Biothreat Panel is safe and effective for its intended use and is substantially equivalent to the predicate device.

Predicate Comparison

Table 12: Comparison between T2Biothreat Panel and Predicate Device

Device & Predicate Device(s):	Device K231336	Predicate K170883
Device Trade Name	T2Biotreat Panel	FilmArray NGDS Warrior Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	The T2Biothreat Panel is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with the T2Dx Instrument. The T2Biothreat Panel detects nucleic acids from the following organisms directly from K2EDTA whole blood samples: 1. *Bacillus anthracis* (plasmids pXO1 and pXO2) 2. *Francisella tularensis* 3. *Burkholderia* spp. (*B. mallei*/*B. pseudomallei*) 4. *Yersinia pestis* 5. *Rickettsia prowazekii* The T2Biothreat Panel will not distinguish between detection of *Burkholderia mallei* and *Burkholderia pseudomallei* but will present valid detections as a positive detection of *Burkholderia* species. The T2Biothreat 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 T2Biothreat Panel is indicated as an aid in the diagnosis of anthrax, tularemia, melioidosis, glanders, typhus fever and plague in response to suspected or confirmed bioterrorism events or outbreaks. Diagnosis of infection must be made in conjunction with clinical, epidemiologic and other laboratory data. Results are for the presumptive identification of *Bacillus anthracis, Francisella tularensis, Burkholderia* spp. (*B. mallei*/*B. pseudomallei*), *Yersinia pestis* and	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 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 even without turbidity, and is indicated to be performed with concomitant Gram stain performed on positive blood culture specimens as per normal laboratory procedure. 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 of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus.

	Rickettsia prowazekii. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The definitive identification of *Bacillus anthracis, Francisella tularensis, Burkholderia mallei, Burkholderia pseudomallei, Yersinia pestis* or *Rickettsia prowazekii* requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required. Positive results do not rule out co-infections with pathogens not included on the T2Biothreat Panel. 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. The T2Biothreat Panel is indicated for use in laboratories that have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities, and personnel trained in the safe handling of clinical specimens potentially containing biothreat organisms. The T2Biothreat Panel is indicated for use in laboratories that follow public health guidelines 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.	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. Negative results do not preclude infection with these biothreat agents and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. The FilmArray NGDS Warrior Panel is solely for use by United States Department of Defense laboratories, and laboratories designated by the Department of Defense.
Test Platform Automation	Fully automated	Same
Reagent Platform	All reagents contained within a single-use tray or pouch	Same
Sample Type	Whole blood (EDTA)	Whole blood (EDTA), positive blood culture, and sputum
General Device Characteristic Differences		
Instrument Platform	T2Dx Instrument	FilmArray 2.0 System

Test Principle	Nucleic acid amplification followed by T2 magnetic resonance detection	Nested multiplex RT-PCR followed by melting analysis
Pathogens Detected	*Bacillus anthracis, Burkholderia* species*, Francisella tularensis, Rickettsia prowazekii, and Yersinia pestis.*	*Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus.
Time to Result	About 4.5 hours	About 1 hour