510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY

I Background Information:

A 510(k) Number

K231336

B Applicant

T2 Biosystems, Inc.

C Proprietary and Established Names

T2Biothreat Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QVR	Class II	21 CFR 866.4000 - Device To Detect And Identify Biothreat Microbial Agents In Human Clinical Specimens	MI - Microbiology
QBX	Class II	21 CFR 866.3960 - Nucleic acid-based device for the amplification, detection, and identification of microbial pathogens directly from whole blood specimens	MI - Microbiology
NSU	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to obtain a substantial equivalence determination for the T2Biothreat Panel on the T2Dx Instrument.

B Measurand:

The assay amplifies and detects nucleic acids of the following species: *Bacillus anthracis, Francisella tularensis, Burkholderia* spp. (*B. mallei/B. pseudomallei*)*, Yersinia pestis,* and *Rickettsia prowazekii.*

C Type of Test:

Qualitative multiplex nucleic acid amplification test

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) 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 *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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

IV Device/System Characteristics:

A Device Description:

The T2Biothreat Panel is a qualitative nucleic acid-based test for the detection of microorganisms in K_2EDTA whole blood specimens using PCR amplification followed by T2 magnetic resonance (T2MR) detection. The assay detects six species of biothreat pathogens and categorizes the reported results using the following detection channels:

Targets
Bacillus anthracis plasmid pXO1 (Ba pXO1)*
Bacillus anthracis plasmid pXO2 (Ba pXO2)*
Francisella tularensis (Ft)
Burkholderia species (*B. mallei/B. pseudomallei*) (Bu)
Yersinia pestis (Yp)
Rickettsia prowazekii (Rp)
Internal Control

*The presumptive identification of *Bacillus anthracis* requires detection of both pXO1 and pXO2 plasmids.

Panel testing is performed on the T2Dx Instrument (K172708), a benchtop, fully automated sample-to-result system, which performs all assay steps after specimens are loaded into the instrument. To use the T2Biothreat Panel, a 4 mL K_2EDTA tube is first directly loaded onto the Sample Inlet module, which is then placed on the T2Biothreat Cartridge with the T2Biothreat Reagent Tray and loaded into the T2Dx Instrument. An Internal Control is included in each assay cartridge to monitor the integrity of the Panel results.

The T2Biothreat Panel performs sample concentration, lysis, and DNA amplification for direct detection of six target organisms in 4.5 hours. The test incorporates an Internal Control for monitoring test performance in each amplification reaction.

Device Components

The T2Biothreat Panel is comprised of a cartridge that contains all needed consumables, an inlet module for loading sample into the assay and a reagent tray that contains all reagents needed to run the assay.

The detection particles contained in the T2Biothreat Reagent Tray and used in the T2MR detection system are superparamagnetic particles that have been functionalized with oligonucleotide capture probes for each organism detected.

Principle of Operation:

Amplicons from target organisms are aliquoted into individual tubes containing target-specific probes conjugated to superparamagnetic particles. The probes hybridize to target sequences causing clustering of the particles. The hybridization occurring in individual tubes is analyzed in the MR reader and a signal for each target is generated and detected by T2 Magnetic Resonance (T2MR) indicating the presence of the target organism.

V Substantial Equivalence Information:

A Predicate Device Name(s):

FilmArray NGDS Warrior Panel

B Predicate 510(k) Number(s):

K170883

C Comparison with Predicate(s):

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.*	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

	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	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. 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,

	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.	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

VI Standards/Guidance Documents Referenced:

- CLSI Guideline EP07-A3, Interference Testing in Clinical Chemistry; Approved Guideline, Third Edition. Volume 38, Number 7
- CLSI Guideline EP37, Supplemental Tables for Interference Testing in Clinical Chemistry, First Edition.
- Protection of Laboratory Workers from Occupationally Acquired Infections. Approved Guideline-Third Edition. CLSI Document. Clinical and Laboratory Standards Institute (CLSI). M29-A3 Wayne, PA: CLSI, 2005.
- CLSI EP25-A, Vol 29 No 20. Evaluation of Stability of In Vitro Diagnostics Reagents; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2009
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- 2nd Edition
- FDA Guidance: The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)] (July 28, 2014)
- FDA Guidance: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (May 11, 2005)
- FDA Guidance: Content of Premarket Submissions for Device Software Functions Draft Guidance for Industry and Food and Drug Administration Staff
- FDA Guidance: Recommended Content and Format of Non-Clinical Bench Performance Testing Information in Premarket Submissions (December 20, 2019)
- FDA Guidance: Content of Premarket Submissions for Management of Cybersecurity in Medical Devices (October 2, 2014)
- FDA Guidance: Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions
- FDA Guidance: Off-The-Shelf Software Use in Medical Devices
- FDA Guidance: Cybersecurity for Networked Medical Devices Containing Off-The-Shelf (OTS) Software
- FDA Guidance: General Principles of Software Validation
- ISO 15223-1 Fourth edition 2021-07 Medical devices - Symbols to be used with information to be supplied
- ISO 14971 Medical Devices – Application of Risk Management to Medical Devices
- IEC 62304 Edition 1.1 2015-06 CONSOLIDATED VERSION Medical device software - Software life cycle processes

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A multi-center reproducibility study was performed to determine the run-to-run, reagent lot, day-to-day and site-to-site reproducibility. Result agreement was analyzed across multiple sites over six non-consecutive days, with a minimum of two operators per site, two Reagent Tray Kit lots, and two instruments. Several contrived sample panels were prepared in K_2EDTA-treated whole blood (a *B. anthracis, B. pseudomallei*, and *Y. pestis* triple-species panel, a *B. mallei* and *R. prowazekii* dual-species panel, and an *F. tularensis* single-species spike) at 2-3x LoD and 1-1.5x LoD. Negative K_2EDTA-treated whole blood samples were also prepared. Reproducibility results across instruments, operators, reagent lots, and sample panels were evaluated. A summary of the reproducibility results is shown in **Table 1** below. The reproducibility of the T2Biothreat Panel was found to be acceptable.

Table 1. Summary of Reproducibility Study Results

Organism	Conc.	Expected Result	Agreement with Expected Result [95% CI]	
B. anthracis	1-1.5x LoD	Detected	BaPXO1 Channel 128/128 100% [97.7-100]	BaPXO2 Channel 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]	-
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]	-

2. Linearity:

   N/A

3. Analytical Sensitivity

   To confirm detection of multiple clinically relevant strains of the organisms detected by the T2Biothreat Panel, 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* were wet tested on the T2Dx instrument. Isolates were selected to represent variations in phylogenic, temporal, and geographic diversity. Isolates were inoculated into human whole blood at a concentration of 2-3X LoD. Testing of each isolate was performed in triplicate. If a set was not positive for 3/3 replicates, a repeat sample set of 20 replicates was prepared and tested. A strain was defined as inclusive if testing resulted in 3/3 positive detections or ≥ 19/20 positive detections. 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 not be detected by the T2Biothreat Panel. Results of the inclusivity study are shown in **Table 2**. Inclusivity testing demonstrated reactivity against multiple strains of each target organism on the T2Biothreat Panel and the test results are acceptable.

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
Burkholderia mallei	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

8 strains	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[1]	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[1]	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

[1]*Y. pestis* strains Bombay and Pestoides G do not harbor the pPCP plasmid which contains the *Y. pestis* target sequence

4. Analytical Specificity:

Analytical specificity testing of the T2Biothreat Panel was conducted to assess the cross reactivity of the panel with non-panel species. 31 bacterial, fungal, and viral organisms commonly found to cause bloodstream infections or to be genetically similar to pathogens detected by the Panel were tested. Organisms were tested at 1000 CFU/mL, 1000 CAGe/mL, or international units (IU)/mL in K_2EDTA-treated human blood on the T2Biothreat Panel in 3 replicates using three production equivalent lots of T2Biothreat reagents. Organisms shown to be potentially cross-reactive at the initial concentration were then titrated to a concentration at which a negative result was achieved. Organisms that exhibited no cross-reactivity with the T2Biothreat Panel target organisms are identified in **Table 3** below.

Organisms for which cross-reactivity with the T2Biothreat Panel was observed are included in **Table 4** below. *B. cereus* strain G-9241 harboring a pXO1-like plasmid exhibited cross-reactivity with the Ba pXO1 channel at the initial concentration of 1000 CFU/mL and all titrated concentrations. *Francisella tularensis* subsp. *novicida* (also referred to as *F. novicida*) is similar genetically to *F. tularensis* yet is rarely associated with human disease. This subspecies is not considered a Select Agent and cross-reacts with the Ft channel.

Table 3. Exclusivity Study Organisms with No Cross-Reactivity

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 pXO1-like plasmid that was not detected by the Panel
[2] Harbors a pXO2-like plasmid that was not detected by the Panel
[3] Tested as IU/mL

Table 4. Exclusivity Study Results - Cross-reactive Organisms

Cross-reactive Non-Panel Strain	**Panel Channel that Cross-reacts**
Bacillus cereus G-9241	BaPXO1[1]
Francisella tularensis subsp. *novicida*	Ft

[1]Panel will detect this strain, but result will report "Equivocal Detection of *B. anthracis* or detection of *B. cereus* or *B. thuringiensis*"

Five 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. If both pXO1 and pXO2 plasmids are detected, the results reported indicate a positive detection for *B. anthracis.* If only one of the plasmids is detected, the results report equivocal detection of *B. anthracis* or detection of *B. cereus* or *B. thuringiensis*.

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. Results are shown in **Table 5**. The results of the cross reactivity study are acceptable.

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*	

5. Interfering Substances

An interfering substances study was performed to determine and characterize the effects of potential endogenous and exogenous interfering substances commonly found in blood on the performance of the T2Biothreat Panel. Interferents were screened as pools of interferents in human whole blood sample panels; potential interferents were added in high concentrations to represent worst case scenarios; samples were tested in triplicate. Three contrived sample panels were prepared in K_2EDTA-treated whole blood (a *B. anthracis, B. pseudomallei*, and *Y. pestis* triple-species panel, a *B. mallei* and *R. prowazekii* dual-species panel, and an *F. tularensis* single-species spike) at 2-3x LoD and panels without interferents were include as controls. Negative blood samples were tested with each potential interferent. Interferents that caused a difference in signal from a panel without interferents were further analyzed by individual organism in blood samples. Five endogenous substances and 15 exogenous substances were screened to assess potential interference to panel performance (**Table 6**). 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 and the results of the study are acceptable.

Table 6. Interference Study Results – Substances without Interference Effect

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	

6. Competitive Inhibition

A competitive inhibition study was performed to evaluate the ability of the T2Biothreat Panel to detect target bacterial species present at 1-2X LoD in the presence of other clinically relevant organisms (on- and off- panel) that may be present in a co-infection. Three combinations of organisms were tested in human whole blood samples: 1) samples containing two target bacterial species, each in concentrations of 1-2X LoD (low concentrations); 2) samples containing one target bacterial species at a concentration of 1-2X LoD (low concentration) and a second target bacterial species at a concentration of 1000 CFU/mL (high concentration); and 3) samples containing one target bacterial species at a concentration of 1-2 X LoD (low concentration) and a non-target bacterial species, yeast, or virus species at a concentration of 1000 CFU/mL or IU/mL (high concentration). Four replicates of each combination were initially tested; any combination resulting in a false negative was repeated with 20 replicates. If ≤95% of the target species were detected in the 20 replicates, the concentration of the competing organisms was decreased to determine the level at which the detection was not inhibited. No competitive effects were observed in any combination of Panel members at ≤1,000 CFU/mL or CAGe/mL or near LoD (**Table 7**). 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**), and the data are acceptable.

Table 7. 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 8. 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
	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.

7. Assay Reportable Range:

N/A

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

Specimen Stability:
The stability of K_2EDTA blood specimens when stored at 15-30°C (room temperature) or 2-8°C (refrigerated) was evaluated with the T2Biothreat Panel on the T2Dx Instrument using contrived samples at a final concentration of 25 CFU/mL. Samples were tested at up to 31 hours when stored at room temperature or 8 days when stored at 2-8°C to determine stability. Data are supportive of the specified storage times and temperatures included in the device labeling shown in **Table 9.**

Table 9. Recommended storage times and temperatures for blood specimens

Temperature	Storage Time
2-8°C	3 days
Room temperature	8 hr

Controls:
The T2Biothreat Panel utilizes two types of controls, an internal control and two external controls. The Internal Control (IC) is automatically introduced into each specimen during sample processing on the T2Dx instrument and is carried through the lysis, amplification and detection steps of the assay. The IC monitors the amplification and detection process and detects the presence of inhibitors in the specimen.

The external controls include the QCheck Positive Kit and the QCheck Negative Kit. The QCheck Positive Kit is composed of eight positive samples formulated in proteinaceous buffer-based matrix. The samples contain non-pathogenic bacterial strains containing the T2Biothreat Panel target sequences that can interrogate the respective, complementary probe-coated particles in the corresponding T2Biothreat Panel detection reactions. The QCheck Negative Kit contains eight negative samples containing the proteinaceous buffer-based matrix alone, which can be used to detect contamination of the T2Biothreat Panel reagents and/or the T2Dx Instrument by one or more of the target species detected by the T2Biothreat Panel.

9. Detection Limit:

Limit of detection (LoD) determinations were performed using two T2Dx instruments, two reagent lots, and two different strains of each of the T2Biothreat Panel members in K_2EDTA-treated whole blood. Cell concentrations for all species except *Rickettsia prowazekii* were measured in CFU/mL. *R. prowazekii* does not grow on solid media because it is an obligate intracellular organism and therefore cannot be measured in CFU/mL. *R. prowazekii* cultures were enumerated as Cell Associated Genomic equivalents (CAGe). A preliminary LoD determined during test development was used as the starting concentration for confirmation studies. A minimum of 20 replicates per reagent lot and isolate (minimum of 80 samples total

per species) were tested at increasing concentrations until a positivity rate of 95% was achieved. The LoD value for each target detected by the T2Biothreat Panel was defined as the lowest CFU/mL or CAGe/mL as appropriate, for which a positive detection rate of ≥95% with n ≥20 was obtained. The final LoD for each species will be based on the highest CFU/mL or CAGe/mL for which a positive detection rate of ≥95% was obtained from the different isolates and reagent lots for that species. The LoD for *B. anthracis* was determined based on the detection of both pXO1 and pXO2. The LoD for each target species is listed in **Table 10**. The limit of detection study results are acceptable.

Table 10. LoD for Targets Detected by the T2Biothreat Panel

Organism	Channel	Tray Lot 1				Tray Lot 2				Tray Lot 3[2]				Final LoD
		Strain 1		Strain 2		Strain 1		Strain 2		Strain 1		Strain 2		
		LoD[1]	Hit Rate	LoD[1]	Hit Rate	LoD[1]	Hit Rate	LoD[1]	Hit Rate	LoD[1]	Hit Rate	LoD[1]	Hit Rate	
B. anthracis	BaPOX1	6	95%	6	100%	6	100%	6	100%	N/A	N/A	N/A	N/A	6 CFU/mL
	BaPOX2	5	100%	6	100%	6	100%	6	100%	N/A	N/A	N/A	N/A	
B. pseudomallei	Bu	10	95%	12	95%	15	100%	17	95%	N/A	N/A	N/A	N/A	17 CFU/mL
B. mallei	Bu	10	100%	10	95%	12	95%	10	100%	N/A	N/A	N/A	N/A	12 CFU/mL
F. tularensis	Ft	1	100%	2	100%	4	100%	4	100%	N/A	N/A	N/A	N/A	4 CFU/mL
R. prowazekii	Rp	N/A	N/A	N/A	N/A	9	100%	9	100%	9	100 %	9	100%	9 CAGe/mL
Y. pestis	Yp	1	100%	2	100%	2	100%	2	100%	N/A	N/A	N/A	N/A	2 CFU/mL

[1.] Units are CFU/mL for all except Rp, which is CAGe/mL
[2.] Insufficient quantities of Lot 2 remained to complete testing of Rp. Testing with Lot 3 was used only for Rp LoD determination.

10. Accuracy (Instrument):
N/A

11. Carry-Over:

The T2Biothreat Panel is run on the previously cleared T2Dx Instrument (K172708), which demonstrated a lack of cross-contamination between positive and negative samples when co-loaded on the same instrument for the T2Bacteria Panel (K172708) and the T2Candida Panel (K173536). A co-load is defined as an instrument load that had 2 or more of any combination of a positive sample (contrived whole blood or control) and negative sample (whole blood or control) loaded at the same time. The T2Dx can process up to 7 samples simultaneously. A retrospective analysis was conducted to assess potential carry-over/cross-contamination events throughout T2Biothreat verification and validation studies. Results from instrument loads that contained any combination of positive samples and negative were compiled from T2Biothreat verification and validation studies listed in **Tables 11** and **12**. The purpose of this analysis was to demonstrate a lack of cross-contamination between positive and negative samples when co-loaded on the same instrument.

Table 11. Descriptive information of the co-load analysis for the T2Biothreat Panel

Study Analyzed	Positive Sample Titer	# Samples in Analysis	Number of Co-Loads

Limit of Detection	1X LoD	59	11[1]
Single versus Multi	2.5X LoD	13	2
Reproducibility	QCheck and 2.5X LoD	500	78[1]
Exclusivity	1000 CFU/mL	6	1
Inclusivity	2-3X LoD	64	10
Mix and Match	QCheck	178	27
Tray Stability	QCheck	118	26
Sample Stability	25 CFU/mL	77	16
Fresh versus Frozen	1X LoD	34	4[1]
Clinical: Contrived Febrile Arm[2]	QCheck and 1-5X LoD	262	53[1]
Reproducibility and Clinical: Contrived Febrile Arm	N/A	N/A	3
Limit of Detection and Fresh vs. Frozen	N/A	N/A	2
TOTAL		1311	233

[1.] Value does not include combined study loads
[2.] Data collected as of 10/21/2022

Table 12. Number of samples in the co-load analysis

Sample Type	# of Samples Analyzed	Contained *B. anthracis*	Contained *B. mallei*	Contained *B. pseudomallei*	Contained *F. tularensis*	Contained *R. prowazekii*	Contained *Y. pestis*
Positive Blood	568	167	131	133	155	124	178
Negative Blood	430						
Positive Control	182						
Negative Control	132						
Total	1312						

The false positive sample rates from each T2Biothreat verification and validation study were compiled and demonstrated that blood samples had a false positive rate of 0% and Negative QChecks had a false positive rate of < 0.44% for any one study, supporting a low-risk for carryover contamination. **Table 13** lists a summary of the results. The results of the carry-over contamination evaluation are acceptable.

Table 13. Summary of co-load analysis results on the T2Biothreat Panel

Channel		Ba pXO1	Ba pXO2	Bu	Rp	Ft	Yp	Overall
False Positives[1] (FP)	# FP/total samples	0/936	0/936	0/866	0/1006	0/975	0/952	0/5671
	FP Rate	0%	0%	0%	0%	0%	0%	0%
	95% Confidence Interval	0-0.32%	0-0.32%	0-0.35%	0-0.30%	0-0.31%	0-0.30%	0-0.05%
False Negatives[2] (FN)	# FN/total samples	0/583	1/583	4/486	0/626	3/595	2/572	10/3612
	FN Rate	0%	0.16%	0.65%	0%	0.50%	0.35%	0.28%
	95% Confidence Interval	0-0.51%	0.004%-0.95%	0.22%-2.09%	0-0.48%	0.10%-1.47%	0.04%-1.26%	0.13%-0.51%

[1.] Negative blood and negative control samples
[2.] Positive blood and positive control samples

B Comparison Studies:

1. Method Comparison with Predicate Device:
   N/A

2. Matrix Comparison:
   N/A

C Clinical Studies:

1. Clinical Sensitivity:
   Clinical performance of the T2Biothreat Panel was evaluated in a prospective study designed as a dual arm investigation that included a "negative arm" and a "positive contrived arm". The study tested whole blood samples from K_2EDTA tubes from two different subject populations (heathy and febrile). Due to the low prevalence of positive blood cultures for the target bacterial species, evaluation of the positive performance of the T2Biothreat Panel used contrived sample testing. K_2EDTA whole blood from febrile subjects (patients with temperature ≥100.4°F at time of collection) was collected concurrently at 7 geographically diverse sites with samples to be used to evaluate negative performance. Blood samples were collected from 294 febrile subjects, 29 samples were excluded from the study resulting in a total of 265 samples tested and included in the clinical sensitivity analysis. Samples collected from each febrile subject were either contrived for use in the contrived study arm to evaluate positive performance or used in the arm to evaluate negative performance. Contrived samples were prepared in BSL-3 laboratories using 6 strains per target species resulting in a total of 300 tested positive samples (50 samples for each panel species). Contrived sample concentrations were designed to evaluate the T2Biothreat Panel Positive Percent Agreement (PPA) at various levels (10 samples prepared at 0.1-1x LoD, 35 samples prepared at 1-3x LoD, 5 samples prepared at 3-5x LoD). All contrived samples and negative febrile samples were blinded and processed on a T2Dx Instrument. PPA was calculated by comparing T2Biothreat Panel results to the positive contrived clinical samples. Positive and negative controls (QChecks) were tested daily for each instrument used in the study. Controls must pass on each instrument for the clinical samples tested to be considered valid.

   When samples were grouped based on concentration all targets had 100% detection at 1-3x LoD except *F. tularensis*, which was detected at 94.3% at these concentrations (**Table 14**). In samples above the LoD for each target, the resulting PPA was ≥ 95% (ranged 95-100%) for each channel (n = 40 per channel except n=80 for Bu channel) (**Table 15**). These data met the acceptance criteria of ≥ 90% PPA for each target channel.

Table 14. PPA by organism relative to concentration (positive contrived)

Organism	LoD	Titer Bucket	Titer Range (CFU/mL)[1]	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 15. PPA by organism relative to LoD (positive contrived)

Organism	LoD	Detection Channel	< LoD		≥ LoD		All Concentrations	
			PPA (TP/TP+FN)	95% CI	PPA (TP/TP+FN)	95% CI	PPA (TP/TP+FN)	95% CI
B. anthracis	6 CFU/mL	Ba pXO1	100% (10/10)	74.11% - 100%	100% (40/40)	92.78% - 100%	100% (50/50)	94.18% - 100%
		Ba pXO2	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%

2. Clinical Specificity:

Negative performance of the T2Biothreat Panel was evaluated by determining the negative concordance between T2Biothreat Panel results and blood samples that were presumed to be negative for target biothreat pathogens. These studies included K_2EDTA whole blood specimens from both febrile (temperature ≥100.4ºF) and non-febrile donors and patients. Whole blood samples from 362 unique healthy subjects were collected, with 58 samples excluded from the study that did not meet screening criteria, resulting in 304 samples from 304 unique subjects included in the analysis. A total of 194 whole blood samples from febrile patients which were not used to make contrived samples for the sensitivity study were included in the clinical specificity study, with 179 samples producing valid results. The negative percent agreement (NPA) for healthy subjects, febrile subjects, and combined healthy and febrile subjects is shown in **Table 16**. The NPA of negative healthy subject samples was calculated from 304 valid, unique donor sample results. Each target channel had

0/304 positive detections giving an NPA of 100% with a 95% CI of 99.02% - 100% for each channel. For febrile negative samples the NPA was calculated from 179 valid, unique subject sample results. Each target channel had 0/179 positive detection giving an NPA of 100% with a 95% CI of 98.34% - 100%. The results of the clinical testing are acceptable.

Table 16. Results from Presumed Negative Specimens (among healthy and febrile subjects)

Organism	Channel	Healthy Negatives		Febrile Negatives	
		NPA (TN/(FP+TN))	95% CI	NPA (TN/(FP+TN))	95% CI
B. anthracis	Ba pXO1	100% (304/304)	99.02% - 100%	100% (179/179)	98.34% - 100%
	Ba pXO2	100% (304/304)	99.02% - 100%	100% (179/179)	98.34% - 100%
Burkholderia spp.	Bu	100% (304/304)	99.02% - 100%	100% (179/179)	98.34% - 100%
F. tularensis	Ft	100% (304/304)	99.02% - 100%	100% (179/179)	98.34% - 100%
R. prowazekii	Rp	100% (304/304)	99.02% - 100%	100% (179/179)	98.34% - 100%
Y. pestis	Yp	100% (304/304)	99.02% - 100%	100% (179/179)	98.34% - 100%

D Clinical Cut-Off:

N/A

E Expected Values/Reference Range:

None of the rare T2Biothreat Panel organisms were detected during the prospective clinical evaluation of the T2Biothreat Panel.

F Other Supportive Instrument Performance Characteristics Data:

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.