510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K172708

B. Purpose for Submission:

Substantial equivalence determination for the T2Bacteria Panel on the T2Dx Instrument

C. Measurand:

The assay amplifies and detects nucleic acids of the following species:
Enterococcus faecium
Escherichia coli
Klebsiella pneumoniae
Pseudomonas aeruginosa
Staphylococcus aureus

D. Type of Test:

The T2Bacteria Panel, performed on the T2Dx Instrument, is a molecular diagnostic assay for the detection of the above listed bacterial species from whole blood specimens obtained from patients with signs and symptoms of bacterial bloodstream infections.

E. Applicant:

T2 Biosystems, Inc.

F. Proprietary and Established Names:

T2Bacteria Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3960

2. Classification:

   Class II

3. Product code(s):

   QBX, NSU

4. Panel:

   83 - Microbiology

H. Intended Use:

1. Intended use(s):

   The T2Bacteria Panel run on the T2Dx Instrument is a qualitative T2 magnetic resonance (T2MR) test for the direct detection of bacterial species in K_2EDTA human whole blood specimens from patients with suspected bacteremia. The T2Bacteria Panel identifies five species of bacteria: *Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

   The T2Bacteria Panel is indicated as an aid in the diagnosis of bacteremia and results should be used in conjunction with other clinical and laboratory data. Concomitant blood cultures are necessary to recover organisms for susceptibility testing or further identification, and for organisms not detected by the T2Bacteria Panel.

   Results from the T2Bacteria Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions in patients with suspected bacteremia.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   The T2Bacteria Panel is performed on the T2Dx Instrument

I. Device Description:

The T2Bacteria panel is a qualitative molecular diagnostic assay that employs a whole blood compatible PCR amplification followed by T2 magnetic resonance (T2MR) detection. The T2Bacteria Panel is performed on the T2Dx Instrument which executes all steps after specimen loading. A K_2EDTA whole blood specimen containing a minimum of 3 mL is loaded directly onto the T2Bacteria Sample Inlet, which is then placed on the T2Bacteria Cartridge along with

the T2Bacteria Reagent Tray. The Cartridge and Reagent Tray contain the lysis reagent, the internal control, primers, enzyme, buffer and probe-coupled superparamagnetic particles for each bacterial target. The blood specimen is mixed with the red blood cell lysing reagent and the bacterial cells are concentrated by centrifugation. The internal control is added to the concentrated bacterial cells; a bead-beating process then lyses the bacterial cells. The supernatant containing the DNA from the lysed bacterial cells and the internal control are amplified using the target and internal control-specific primers. The generated amplicons are aliquoted into individual tubes containing target-specific conjugated particles for *E. faecium, E. coli, K. pneumoniae, P. aeruginosa, S. aureus* and the internal control. Bacterial DNA is amplified with target-specific primers and amplicons are hybridized to target-specific probes attached to superparamagnetic particles 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 is detected by T2 Magnetic Resonance (T2MR) indicating the presence of the target organism. Up to seven specimens can be loaded onto the T2Dx instrument at the same time. When running the first specimen in a series or a single specimen, the result is reported in 3.5 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. For *E. coli*, results are reported as Positive, Indeterminate or "Target not Detected". An Indeterminate result is a valid result, but the presence or absence of *E. coli* in the specimen cannot be definitively assessed.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   T2Candida Panel

2. Predicate 510(k) number(s):
   DEN140019

3. Comparison with predicate:

Table 1. Comparison with the Predicate

Similarities		
Item	Device K172708 T2Bacteria Panel	Predicate DEN140019 T2Candida Panel
Intended Use	The T2Bacteria Panel run on the T2Dx Instrument is a qualitative T2 magnetic resonance (T2MR) test for the direct detection of bacterial species in K_2EDTA human whole blood specimens from patients with suspected bacteremia. The T2Bacteria Panel identifies five species of bacteria: *Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The T2Bacteria Panel is indicated as an aid in the diagnosis of bacteremia and results should be used in conjunction with other clinical and laboratory data. Concomitant blood cultures are necessary to recover organisms for susceptibility testing or further identification and for organisms not detected by the T2Bacteria Panel. Results from the T2Bacteria Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions in patients with suspected bacteremia.	The T2Candida Panel and T2Dx Instrument is a qualitative T2 Magnetic Resonance (T2MR) assay for the direct detection of Candida species in EDTA human whole blood specimens from patients with symptoms of, or medical conditions predisposing the patient to, invasive fungal infections. The T2Candida Panel identifies five species of Candida and categorizes them into the following three species groups: *Candida albicans* and/or *Candida tropicalis Candida parapsilosis Candida glabrata* and/or *Candida krusei*. The T2Candida panel does not distinguish between *C. albicans* and *C. tropicalis*. The T2Candida panel does not distinguish between C. glabrata and *C. krusei*. The T2Candida panel is indicated for the presumptive diagnosis of candidemia. The T2Candida Panel is performed independent of blood culture. Concomitant blood cultures are necessary to recover organisms for susceptibility testing or for further identification. The T2Candida positive and negative external controls are intended to be used as quality control samples with the T2Candida Panel when run on the T2Dx Instrument system. These controls are not intended for use with other assays or systems.

Similarities		
Item	Device K172708 T2Bacteria Panel	Predicate DEN140019 T2Candida Panel
Sample Type	4 mL whole blood collected in a blood collection tube with EDTA anticoagulant	Same
Test Platform	T2Dx	Same
Test Principle	Nucleic acid amplification followed by T2 magnetic resonance detection	Same
Throughput	Single cartridge test with random access with seven drawers on T2Dx	Same

Differences		
Item	Device	Predicate
Test Cartridge Format	T2Bacteria Test Cartridge and disposables	T2Candida Test Cartridge and disposables
Reagent Trays	T2Bacteria Test reagents specific for detection of bacteria	T2Candida Test reagents specific for detection of *Candida*
Targets	T2Bacteria Panel tests for six different species of bacteria implicated in bacteremia: *Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*	T2Candida Panel tests for five different species of Candida commonly associated with candidemia: *Candida albicans* and/or *Candida tropicalis, Candida parapsilosis*, *Candida glabrata* and/or *Candida krusei*

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

- IEC 61010-1:2001, (Second Edition). Safety requirements for electrical equipment for measurement control and laboratory use – General requirements. 2001
- IEC61010-2-010:2003 (Second Edition). Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 2-010. Particular requirements for laboratory equipment for the heating of materials. 2003
- IEC 61010-2-081:2001 (First Edition) + A1:2003. Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes. 2001
- IEC 61010-2-101:2002 (Second Edition). Safety requirements for electrical equipment for measurement, control, and laboratory use. Particular requirements for in vitro diagnostic (IVD) medical equipment. 2002.
- IEC 61326-1:2005. Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements. 2005
- IEC 61326-2-6:2005. Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 2-6: Particular requirements – In vitro diagnostic (IVD)

medical equipment. 2005

- CLSI EP17-A2. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. 2013.
- CLSI EP07-A2. Interference Testing in Clinical Chemistry. 2007
- CLSI EP05-A3. Evaluation of Precision Performance of Quantitative Measurement Methods. 2014
- CLSI EP25-A. Evaluation of Stability of In Vitro Diagnostic Reagents. 2013.
- CLSI EP12-A2. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition. 2014.
- ISTA 7E. Testing Standard for Thermal Transport Packaging Used in Parcel Delivery System Shipment. 2010

L. Test Principle:

During processing on the T2Dx instrument, pathogens are concentrated directly in whole blood, then lysed to release the target DNA. Bacterial DNA is amplified with target-specific primers and amplicons are hybridized to target-specific probes attached to superparamagnetic particles causing clustering of the particles. A signal is detected by T2 Magnetic Resonance (T2MR) indicating the presence of the target organism. The Internal Control on the Panel monitors performance for each patient sample or control.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   A multicenter reproducibility study was performed to determine the run to run, reagent lot, day to day and site to site reproducibility. Testing was performed at three sites (two external and one internal) with a panel of five target species, each tested in triplicate at two concentrations (1-2X LoD and 3-4X LoD) using two reagent lots. Testing was performed for six non-consecutive days with at least two operators per site for a total of 36 replicates per sample per site.

   The reproducibility panel was comprised of each target species spiked in fresh human whole blood specimens in double-spiked samples (*K. pneumoniae* and *S. aureus*) or triple-spiked samples (*E. coli, P. aeruginosa* and *E. faecium*). (The applicability of using multi-spiked samples was evaluated in a separate study, see below.) Bacterial levels were confirmed by colony count testing of the original suspension used for spiking. A total of 108 negative blood samples were included in reproducibility panel. Reproducibility results were acceptable with a range of 97.2% to 100% agreement in detection with expected results for each of the target species. A summary of the reproducibility results across sites, reagents and operators is shown in Table 2 below.

Table 2. Summary of Reproducibility Results Across Sites, Reagents and Operators

Organism	Conc.	Test Site	No. Detected	No. Not Detected	Agreement with Expected
E. faecium	1-2X LoD	Site 1	36	0	107/108 99.1%
		Site 2	35	1	
		Site 3	36	0	
		All sites	107	1	
	3-4X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
E. coli	1-2X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
	3-4X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
K. pneumoniae	1-2X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
	3-4X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
P. aeruginosa	1-2X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
	3-4X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
S. aureus	1-2X LoD	Site 1	36	0	105/108 97.2%
		Site 2	35	1	
		Site 3	34	2	
		All sites	105	3	
	3-4X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	

Organism	Conc.	Test Site	No. Detected	No. Not Detected	Agreement with Expected
Negative	Negative	Site 1	1	35	105/108 97.2%
		Site 2	2	34	
		Site 3	0	36	
		All sites	3	105	

b. Linearity/assay reportable range:

Not Applicable

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

The T2Bacteria Panel utilizes two types of controls, the internal control and the 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 contains two sample blends of bacterial species, the SaKp Positive Control (which contains cells of *S. aureus* and *K. pneumoniae*) and the EcPaEfm Positive Control (which contains cells of *E. coli, P. aeruginosa* and *E. faecium*) suspended in a buffer-based solution. These positive controls individually interrogate the respective, complementary probe-coated particles in the detection reactions. Results for the positive controls are reported for only the detection channels for species included in the control. The QCheck Negative Kit contains vials of a buffer-based solution which are used to detect contamination of the T2Bacteria regents or the instrument. Results of the negative control are reported for all five detection channels.

External controls were run each day of the analytical and clinical studies. Summaries of the quality control results obtained at all sites during the clinical study are provided in Tables 3 to 5 below.

There were a total of 4394 negative control results obtained in the clinical study at the testing sites; 4375 of these were valid and 19 were invalid. Using the original cut-off for *E. coli,* 99.2% (4340/4375) gave negative results. There were a total of 35 false positive results (0.8%) including 25 false positive results for *E. coli*. To address the occurrence of false positive results obtained for *E. coli,* the original *E. coli* cut-off for the QCheck Negative Control was modified (increased); cut-off values for other target analytes were unchanged. Using the modified cut-off for *E. coli,* 99.4% (4348/4375) gave acceptable negative results. With the revised cut-off, there was a total of 27 false positive results with the negative controls, including 17 false positive

results for *E. coli*. Results for the negative controls with both the original and revised cut-offs are shown in Table 3 below.

Table 3. Results Obtained with the QCheck Negative Control[a,b]

Site	No. valid Tests	*E. coli* original cut-off			*E. coli* revised cut-off		
		No. Correct	% Correct	No. FP[c] (%)	No. Correct	% Correct	No. FP[d] (%)
1	625	620	99.2	5 (0.8)	621	99.4	4 (0.6)
2	150	148	98.7	2 (1.3)	149	99.3	1 (0.7)
3	340	338	99.4	2 (0.6)	338	99.4	2 (0.6)
4	430	427	99.3	3 (0.7)	428	99.5	2 (0.5)
5	735	726	98.8	9 (1.2)	729	99.2	6 (0.8)
6	190	188	99.1	2 (1.1)	189	99.5	1 (0.5)
7	350	347	99.1	3 (0.9)	347	99.1	3 (0.9)
8	245	242	98.8	3 (1.2)	242	98.8	3 (1.2)
9	260	257	98.9	3(1.2)	257	98.9	3 (1.2)
10	330	329	99.7	1 (0.3)	330	100	0 (0.0)
11	405	404	99.8	1 (0.3)	404	99.8	1 (0.3)
12	315	314	99.7	1(0.3)	314	99.7	1 (0.3)
Total	**4375**	**4340**	**99.2%**	**35 (0.8%)**	**4348**	**99.4%**	**27 (0.6%)**

[a] Abbreviations: FP, False positive

[b] Includes results for all detection channels from prospective specimen and reproducibility testing during the clinical study

[c] False positives included: 25 *E. coli* results, 8 *P. aeruginosa* results and 2 *K. pneumoniae* results.

[d] False positives included: 17 *E. coli* results, 8 *P. aeruginosa* results and 2 *K. pneumoniae* results.

There were a total of 846 results obtained with the SaKp Positive control during the course of the clinical study at the testing sites; 836 of these were valid and 10 were invalid. A total of 834 (99.3%) of the results provided the expected detection. Two false negative results (one each for *K. pneumoniae* and *S. aureus*) were observed. Results obtained with the SaKp control are shown in Table 4 below.

Table 4. Results obtained with the QCheck SaKp Positive Control [a,b]

Site	No. valid Tests	No. Correct	% Correct	No. FN (%)[c]
1	124	124	100	0
2	28	28	100	0
3	60	60	100	0
4	76	76	100	0
5	142	140	98.6	2 (1.4)
6	34	34	100	0
7	68	68	100	0
8	42	42	100	0
9	58	58	100	0
10	64	64	100	0
11	78	78	100	0
12	62	62	100	0
Total	**836**	**834**	**99.8**	**2 (0.2)**

[a] Abbreviations: FN, False negative
[b] Includes results for all detection channels from prospective specimen and reproducibility testing during the clinical study
[c] False negatives included 1 *K. pneumoniae* result, 1 *S. aureus* result

There were a total of 1357 results obtained with the EcPaEfm Positive control run during the course of the clinical study at the testing sites; 1347 of these were valid and 10 were invalid. A total of 1338 (99.3%) of the results provided the expected detections. Nine false negative results (five for *E. coli* and four for *P. aeruginosa*) were observed. Results obtained with the EcPaEfm control are shown in Table 5 below.

Table 5. Results obtained with the QCheck EcPaEfm Positive Control [a,b]

Site	No. valid Tests	No. Correct	% Correct	No. FN (%)[c]
1	180	180	100	0
2	48	48	100	0
3	129	126	97.7	3 (2.3)
4	132	132	100	0
5	228	228	100	0
6	63	62	98.4	1 (1.6)
7	105	105	100	0
8	75	74	98.7	1 (1.3)
9	69	69	100	0
10	99	97	98.0	2 (2.0)
11	120	119	99.2	1 (0.8)
12	99	98	99.0	1 (1.0)
Total	**1347**	**1338**	**99.3**	**9 (0.7%)**

[a] Abbreviations: FN, False negative
[b] Includes results for all detection channels from prospective specimen and reproducibility testing during the clinical study
[c] False negatives include 5 *E. coli* results and 4 *P. aeruginosa* results

d. Detection limit:

LoD testing was performed using two strains of each target bacterial species inoculated into human whole blood. The determination of the LoD included an initial screening phase and a confirmatory phase. In the initial phase, double- or triple-spiked samples were prepared at four concentrations (1, 3, 9 and 10 CFU/mL) and a preliminary LoD was identified. (The applicability of using multi-spiked samples was evaluated in a separate study; see below.) To confirm the preliminary LoD, a minimum of 20 replicates of both strains of each species was tested at increasing concentrations until a positivity rate of 95% was achieved. The preliminary LoD for each species was confirmed using two reagent lots. For two species, *K. pneumoniae* and *P. aeruginosa*, the preliminary LoD was not confirmed with both strains of each species tested; the higher concentration that provided ≥ 95% positivity for both strains was confirmed as the LoD. For *E. coli,* the preliminary LoD of 10 CFU/mL did not provide ≥ 95% positivity during the confirmatory testing. Confirmatory testing was repeated and the LoD was confirmed at 11 CFU/mL. Results of the confirmatory LoD testing are shown in Table 6 below.

Table 6. Confirmatory LoD Testing for Target Species

Species	Strain 1		Strain 2		LoD
	Reagent Lot 1 No. Tested/ No Correct (%)	Reagent Lot 2 No. Tested/ No Correct (%)	Reagent Lot 1 No. Tested/ No Correct (%)	Reagent Lot 2 No. Tested/ No Correct (%)	
E. faecium	20/20 (100)	20/20 (100)	19/20 (95)	20/20 (100)	5
E. coli	19/20 (95)	20/20 (100)	20/20 (100)	20/20 (100)	11
K. pneumoniae	19/20 (95)[a]	20/20 (100)[a]	20/20 (100)[b]	20/20 (100)[b]	2
P. aeruginosa	20/20 (100)[c]	20/20 (100)[c]	20/20 (100)[d]	20/20 (100)[d]	5
S. aureus	20/20 (100)	20/20 (100)	20/20 (100)	20/20 (100)	2

[a] Tested at 2 CFU/mL
[b] Tested at 1 CFU/mL
[c] Tested at 3 CFU/mL
[d] Tested at 5 CFU/mL

e. Single species spike, multi-species spike equivalence

A comparison of the equivalence of results obtained for human blood samples spiked with a single bacterial species vs. samples spiked with three bacterial species was performed to determine the applicability of testing multiple species in a single blood specimen for the analytical studies.

A single strain each of the five target bacterial species was tested at concentrations of 1-2 X LoD and 3-4 X LoD as controls. Four replicates of each species at each concentration level were evaluated. Multi-spiked samples included the combinations of *K. pneumoniae*/*S. aureus* and *E. faecium*/*E. coli*/

P. aeruginosa; each species in each combination was tested at two concentrations, 1-2 X LoD and 3-4 X LoD.

Samples containing two or three bacterial species gave positive results for each species; signals obtained from the single-spiked samples were equivalent to the signals obtained from the multi-spiked samples.

f. Analytical specificity:

Analytical exclusivity testing of the T2Bacteria Panel was conducted to assess the cross reactivity of the panel with non-panel species tested at 10^3 CFU/mL for fungi and bacteria, and 10^3 $TCID_{50}$ for viral pathogens. Bacterial species that were shown to be potentially cross-reactive at the initial concentration were then tested in triplicate at more physiologically relevant concentrations of 100, 33 and 10 CFU/mL. Isolates for testing were selected based on ≥96 primer homology and ≥84% probe homology with species identified in a BLAST search. Species that showed no cross-reactivity with T2Bacteria Panel target organisms when tested at a concentration of 10^3 CFU/mL or 10^3 $TCID_{50}$ are shown in Table 7 below. Species that showed cross reactivity with T2Bacteria Panel members when tested at concentrations of ≥ 10 CFU/mL are shown in Table 8.

By *in silico* analysis, *Yersinia pestis* was determined to be non-cross reactive (Table 7); *Klebsiella quasipneumoniae* and *S. argenteus* were determined to be cross reactive with *K. pneumoniae* and *S. aureus*, respectively (Table 8).

Table 7. Organisms with No Cross-Reactivity with T2Bacteria Panel Targets

Fungi					
Aspergillus fumigatus	*Aspergillus niger*	*Candida albicans*	*Candida glabrata*	*Candida krusei*	*Candida parapsilosis*
Candida tropicalis	*Cryptococcus albidus*	*Cryptococcus neoformans*	*Fusarium moniliforme*	*Fusarium oxysporum*	*Rhizomucor meihei*
Rhizopus microsporus		*Rhizopus oryzae*		*Rhodotorula glutinis*	
Viruses					
Adenovirus type 1	Cytomegalovirus	Epstein-Barr Virus	Hepatitis A Virus	Hepatitis B Virus	
Herpes Simplex Virus 1		Herpes Simplex Virus 2		Varicella Zoster Virus	
Gram Positive Bacteria					
Actinomyces israelii	*Clostridium sphenoides*	*Enterococcus avium*	*Enterococcus caccae*	*Enterococcus casseliflavus*	*Enterococcus cecorum*
Enterococcus dispar	*Enterococcus faecalis*	*Enterococcus gallinarum*	*Enterococcus gilvus*	*Enterococcus hirae*	*Enterococcus italicus*
Enterococcus malodoratu	*Enterococcus mundtii*	*Enterococcus pallens*	*Enterococcus pseudoavium*	*Enterococcus raffinosus*	*Finegoldia magna*
Lactobacillus acidophilus	*Lactococcus lactis*	*Leuconostoc mesenteroides*	*Listeria monocytogenes*	*Parvimonas micra*	*Pediococcus pentosaceus*
Peptoniphilus harei	*Peptostreptococcus anaerobius*	*Propionibacterium acnes*	*Staphylococcus auricularis*	*Staphylococcus capitis*	*Staphylococcus epidermidis*
Staphylococcus haemolyticus	*Staphylococcus hominis*	*Staphylococcus lugdunensis*	*Staphylococcus saprophyticus*	*Staphylococcus warneri*	*Staphylococcus xylosus*
Streptococcus agalactiae	*Streptococcus anginosus*	*Streptococcus bovis*	*Streptococcus constellatus*	*Streptococcus dysgalactiae*	*Streptococcus mutans*
Streptococcus pneumoniae	*Streptococcus pyogenes*	*Streptococcus salivarius*		*Streptococcus oralis*	
Gram Negative Bacteria					
Acinetobacter calcoaceticus	*Acinetobacter lwoffi*	*Acinetobacter nosocomialis*	*Acinetobacter pittii*	*Acinetobacter radioresistans*	*Aeromonas hydrophila*
Bacteroides fragilis	*Burkholderia cepacia*	*Chryseobacterium indologenes*	*Citrobacter koseri*	*Corynebacterium jeikeium*	*Cupriavidus pauculus*
Enterobacter aerogenes	*Enterobacter cloacae*	*Enterobacter hormaechei*	*Fusobacterium necrophorum*	*Fusobacterium nucleatum*	*Klebsiella oxytoca*
Leptotrichia trevisanii	*Moraxella catarrhalis*	*Morganella morganii*	*Myroides odoratus*	*Ochrobactrum anthropi*	*Oligella urethralis*
Pantoea agglomerans	*Plesiomonas shigelloides*	*Proteus mirabilis*	*Proteus vulgaris*	*Providencia stuartii*	*Pseudomonas alcaligenes*
Pseudomonas fluorescens	*Pseudomonas luteola*	*Pseudomonas oryzihabitans*	*Pseudomonas pseudoalcaligenes (oleovorans)*		*Pseudomonas putida*
Pseudomonas stutzeri	*Ralstonia pickettii*	*Raoultella ornithinolytica*	*Raoultella planticola*	*Salmonella enterica Enteritidis*	
Salmonella enterica Typhimurium	*Serratia marcescens*	*Shewanella putrefaciens*	*Stenotrophomonas maltophilia*	*Weeksella virosa*	
Yersinia pseudotuberculosis		*Yersinia pestis*[a]			

[a] Determined to be non-cross reactive by *in silico* analysis

Table 8. Organisms with Cross Reactivity with T2Bacteria Targets

T2Bacteria Panel Target	Cross Reactive Species Predicted by Wet Testing	Cross Reactive Species Predicted by In Silico Analysis
E faecium	*Enterococcus durans*	NA
E. coli	*Escherichia albertii*	
	Escherichia fergusonii	
	Shigella boydii	
	Shigella dysenteriae	
	Shigella flexneri	
	Shigella sonnei	
K. pneumoniae	*Klebsiella variicola*	*Klebsiella quasipneumoniae*[a]
S. aureus	NA	*S. argenteus*[a]

[a] Determined to be cross-reactive by *in silico* analysis

For one species, *Enterovirus* Type 68, 0 of 3 replicates tested at 1,000 $TCID_{50}$/mL were positive for *E. coli*; 1 of 6 replicates tested at 316 $TCID_{50}$/mL were positive on the *E. coli* channel of the Panel. This species was deemed not cross reactive.

g. *Assay cut-off:*

To establish the limit of blank (LoB) and cut-off values for the bacterial target and internal controls detections, negative blood specimens from healthy donors and blood specimens from patients suspected of bacteremia were tested using multiple instruments and reagent lots across multiple days. T2MR signals were evaluated to establish the upper limit of the signal distribution for negative samples; the T2MR signal values that encompassed ≥ 99% of all signals from negative samples was determined to be the LoB.

The assay cut-off values were determined on a per channel basis using spiked positive and negative samples; the lower limit of the signal distribution for each target channel and for the internal control measurements determined the assay cut-off. Established cut-offs were reevaluated utilizing data obtained with prospectively-collected and contrived samples evaluated during the clinical study to validate the cut-off with a larger specimen pool; analysis verified the cut-off for all targets except *E. coli*. The cut-off for detection of *E. coli* was redefined using the larger data set and all results were reanalyzed with the new cut-off. The cut-off values were established to produce ≥ 95.6% specificity and ≥ 90.0% sensitivity.

Because of the occurrence of false positive results with *E. coli* in the clinical study, the cut-off for *E. coli* was reassessed to include an indeterminate range for which the presence or absence of *E. coli* cannot be definitively assessed.

h. Analytical Sensitivity

A total of 57 clinical isolates were evaluated for inclusivity in the T2Bacteria Panel. Isolates were selected to represent variations in antimicrobial resistance, and 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. In the event of a false negative result, testing was repeated with 20 replicates. Results of inclusivity testing combined with results from contrived specimen testing in the clinical study (for which 50 unique isolates of each target species were tested) indicate that the T2Bacteria Panel can detect a variety of strains of target organisms with >95% accuracy. Results of the inclusivity study are shown in Table 9 below.

Table 9. Results of Inclusivity Testing

Species	No. Strains Tested	No. Positive/Total (%)
E. faecium	11	33/33 (100)
E. coli	12	36/36 (100)
K. pneumoniae	13	77/79 (97.5)
P. aeruginosa	13	39/39 (100)
S. aureus	8	42/44 (95.5)

i. Co-infection Studies

A competitive inhibition study was performed to evaluate the ability of the T2Bacteria 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 or yeast species at a concentration of 1000 CFU/mL (high concentration). Four replicates of each combination were initially tested; any combination resulting in a non-detection 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.

On Panel Combinations. All target organisms were detected with four replicates at all concentrations except for the following combinations: *E. coli* (low concentration)/ *P. aeruginosa* (high concentration), *P. aeruginosa* (low concentration)/ *E. coli* (low concentration) and *P. aeruginosa* (low concentration)/*S. aureus* (low concentration). Repeat testing of 20 replicates of these combinations resulted in 100% detection. For the combination of *P. aeruginosa* (low concentration) combined with *E. coli* (high concentration), *P. aeruginosa* was initially detected in 2/4 replicates; repeat testing

showed detection of 18/20 replicates. This combination was further evaluated with decreasing concentrations of *E. coli* (100 CFU, 33 CFU and 10 CFU); both *P. aeruginosa* and *E. coli* were detected in all replicates with these concentrations. A limitation was included in the labeling indicating that high concentrations of *E. coli* in a blood specimen may prevent the detection of low concentrations of *P. aeruginosa* when present in the same specimen.

Non-Panel Combinations. Combinations of non-panel and panel species were tested as noted above. Non-panel member species were selected based on clinical relevance and included *Staphylococcus epidermidis, Streptococcus pneumoniae, Enterobacter cloacae, Streptococcus salivarius* and *Candida albicans*. Non-panel species were tested at a concentration of 1000 CFU/mL. Initial testing with four replicates showed 100% detection for all targets except *P. aeruginosa* with *S. pneumoniae* for which *P. aeruginosa* was detected in three of the four replicates. Additional testing with 20 replicates of this combination resulted in 100% detection.

j. 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 T2Bacteria Panel. Interferents were screened as pools of interferents in human whole blood samples; potential interferents were added in high concentrations to represent worst case scenarios; samples were tested in triplicate. Samples were inoculated with T2Bacteria target species at a concentration of 2-3 X LoD and samples without interferents were include as controls. Interferents that caused a difference in signal from a sample without interferents were further analyzed individually in blood samples.

Evaluation of potential endogenous substances included 13 interferents that represented underlying disease conditions (Table 10). None of the endogenous substances tested showed interference with the T2Bacteria assay.

Table 10. Endogenous Substances (Representing Human Underlying Conditions) Evaluated with the T2Bacteria Panel

Underlying Source or Condition	Endogenous Interferent	Reference Level	Test Concentration	Interference Noted
Leukocytosis	Human DNA (Buffy Coat)	WBC 4.5-11 X 10^6 cells/mL	2.08 X 10^7 to 2.48 X 10^7 cells/mL	No
Icterus	Bilirubin (conjugated)	0-3.4 µmol/L	342 µmol/L	No
	Bilirubin (unconjugated)	5-21 µmol/L	342 µmol/L	No
Hepatitis	ALT	5-40 U/L	120 U/L	No
	AST	9-48 U/L	144 U/L	No
Hemolysis	Hemoglobin	11-17.4 g/dL	>20 g/dL	No

Underlying Source or Condition	Endogenous Interferent	Reference Level	Test Concentration	Interference Noted
Lipemia	Intralipid (to mimic triglycerides)	0.34-3.7 mmol/L	3270 mg/dl	No
Hyperproteinemia	Protein (albumin)	39-51 g/L	60 g/L	No
	Immunoglobulin G	6-13 g/L	60 g/L	No
Renal Failure	Creatinine	6-13 mg/L	50 mg/L	No
	Urea	1.1-14.3 mmol/L	42.9 mmol/L	No
Multiple	Circulating human DNA	3.1-730.5 ng/mL (plasma)	2.2 µg/mL	No
Systemic Inflammatory Response	Lactoferrin (87kDa)	0.005-2.5 µM	7.5 µmol/L	No

A total of 28 exogenous potentially interfering substances were tested at high concentrations (Table 11). Only Feraheme was shown to interfere with the T2Bacteria assay at the test concentration of 618 µg/mL. Further testing was performed with lower concentrations of Feraheme; concentrations of Feraheme ≥21 µg/mL were shown to interfere with the performance of the T2Bacteria Panel. The interference of Feraheme with the T2Bacteria Panel was noted as a limitation in the device labeling.

Table 11. Exogenous Substances Evaluated with the T2Bacteria Panel

Exogenous Interferent	Reference Level	Test Concentration	Interference
EDTA	1.8 mg/mL	5.4 mg/mL	No
Feraheme	206 µg/mL	618 µg/mL	Yes
Heparin	350-1000 U/L	3000 U/L	No
Iopamidol	10-225 mL cumulative injections per individual	180 µL per 4 mL Vacutainer	No
MRI Contrast Agent: gadopentetate dimeglumine	0.01 mmol/kg	1.5mM	No
Dexamethasone	0.51 µg/mL	1.53 µg/mL	No
Lisinopril	0.25 µmol/L	0.74 µmol/L	No
Cytarabine	10.8 µg/mL	32.4 µg/mL	No
Amphotericin B Trihydrate	80 µg/mL	240 µg/mL	No
Caspofungin	8.0 – 17.6 µg/mL	52.8 µg/mL	No
Fluconazole	65.2-81.5 µmol/L	245 µmol/L	No
Metronidazole	35-234 µmol/L	701 µmol/L	No
Micafungin	30 µg/mL	90 mg/L	No
Ampicillin	7.59-50.6 µmol/L	152 µmol/L	No
Azithromycin	0.4-5.1 µmol/L	15.3 µmol/L	No
Cefazolin, sodium salt	37.4-881 µmol/L	2.643 mmol/L	No
Cefepime Hydrochloride	164 µg/mL	492 µg/mL	No
Cefoxitin Sodium Salt	60 µg/mL	180 µg/mL	No

Exogenous Interferent	Reference Level	Test Concentration	Interference
Ceftazidime Pentahydrate	46.9 mg/L 162.3 mg/L	487 µg/mL	No
Ciprofloxacin	1.51-15.1 µmol/L	30.2 µmol/L	No
Clindamycin	4.0-29.7 µmol/L	89.1 µmol/L	No
Gentamycin sulfate	10.5-20.9 µmol/L	21 µmol/L	No
Imipenem/Cilastatin (Primaxin)	Imipenem 21-83 µg/mL Cilastin 31-88 µg/mL	528 µg/mL	No
Linezolid	18.6 µg/mL	55.8 µg/mL	No
Meropenem trihydrate	55-62 µg/mL	186 µg/mL	No
Piperacillin	39 µg/mL	117 µg/mL	No
Tazobactam	6.3 µg/mL	18.9 µg/mL	No
Vancomycin	12.4-27.6 µmol/L	103 µg/mL	No

k. Carryover/Cross Contamination Studies

A carryover/cross contamination study was performed to evaluate potential assay contamination caused by operator or carryover in the T2 Dx instrument. Whole blood specimens were spiked at concentrations of 100 CFU/mL (61 samples) and 1000 CFU/mL (56 samples) and were loaded on the T2 Dx instrument alternately with negative whole blood specimens (123 negative specimens).

Of the 123 negative specimens, 3 (2.4%) gave false positive results; two with *E. coli* (1.6%) and one with *K. pneumoniae* (0.8%). The occurrence of false positive results was evaluated in follow-up studies related to reagent contamination (see below).

l. Specimen Stability

The stability of K_2EDTA blood specimens when stored at room temperature (15-30° C), refrigerator temperature (2-8° C) and frozen (≤65° C) using samples spiked at a concentration of 20 CFU/mL and tested over a range of days was evaluated. In addition, studies were performed to evaluate the effect of storage at room temperature followed by refrigeration. Results indicated that storage at all evaluated temperatures and tested within the recommended times provided expected results for target and internal control detections. The acceptable storage times and temperatures included in the device labeling are shown in Table 12.

Table 12. Recommended Storage Times and Temperatures for Blood Specimens Prior to Testing with the T2Bacteria Panel

Temperature	Storage Time from collection
Room Temperature (15 - 25° C)	Up to12 hours
Refrigeration Temperature (2 - 8° C)	Up to 72 hours
Room Temperature (15 - 25° C), then Refrigeration (2 - 8° C)	Room Temperature up to 12 hours Refrigeration up to 72 hours
Freezer Temperature ≤65° C	Up to 3 months

To determine the equivalence of fresh vs. frozen specimens, human whole blood samples were spiked with 20 replicates of two strains of each target species at various concentrations around the LoD concentration and tested with two lots of reagents (a total of 980 tests). A total of 70 tests were also performed for a single target organism at 5X LoD concentrations. All samples were tested with the T2Bacteria Panel at the time of preparation (fresh) and after freezing for at least 24 hours. Results from both preparations showed 100% detection with both specimen types at the established LoD. In addition, a portion of the contrived samples (minimum of 15 for each target species) and eight prospectively collected T2 blood specimens with positive concurrently-collected blood cultures were tested fresh and frozen. Results were equivalent for all samples tested.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable.

   b. *Matrix comparison:*

      Not applicable

3. Clinical studies:

   a. *Clinical Sensitivity:*

      Because of the expected low prevalence of positive blood cultures for the target bacterial species, evaluation of the sensitivity of the T2Bacteria Panel required the use of contrived samples. Contrived sample testing was conducted at three clinical sites. A total of 300 organism-spiked contrived samples and 50 negative samples were prepared using human whole blood specimens collected in K_2EDTA tubes from healthy adults and were verified to be negative for the T2Bacteria target species. Each contrived sample was prepared using a single unique bacterial strain; a total of 50 unique strains for each target species were tested at specific concentrations for each target organism. Samples were spiked at four concentration levels, <1 CFU/mL, 1-10 CFU/mL, 11-30 CFU/mL and 31-100 CFU/mL as shown in Table 13 below. The concentrations were determined to approximate clinically relevant concentrations of organisms in a blood specimen. Because of the higher LoD for *E. coli* as compared to other target species, only 22 samples were prepared for *E. coli* at or above the LoD. All samples were tested in a blinded fashion and negative samples were tested randomly throughout the study.

Table 13. Organism Concentrations in Contrived Samples

Species	LoD CFU/mL	Number of Contrived Samples				
		<1 CFU/mL	1-10 CFU/mL	11-30 CFU/mL	31-100 CFU/mL	Total
E. faecium	5	10	18	17	5	50
E. coli	11	10	18	17	5	50
K. pneumoniae	2	10	18	17	5	50
P. aeruginosa	5	10	18	17	5	50
S. aureus	2	10	18	17	5	50
Negative	-	-	-	-	-	50
Total	-	60	108	102	30	350

Testing all target species with the T2Bacteria Panel at concentrations greater than LoD showed PPA >90% for all species (Range 90.0 – 100%); results for *E. coli* were determined using the final modified cut-off (Table 14).

Of the 250 results obtained for the negative samples included in the contrived study, 248 were negative for all targets. Two results were false positive for *E. coli*.

Table 14. T2 Results Obtained for Contrived Samples

Species	LoD	< LoD		≥ LoD	
		PPA	95% CI	PPA	95% CI
E. faecium	5	60.0% (6/10)	31.3 - 83.2	100.0% (40/40)	91.2 – 100.0
E. coli	11	67.8% (19/20)	49.3 – 82.1	90.9 (20/22)	72.2 – 97.5[a]
K. pneumoniae	2	50.0% (5/10)	23.7 - 76.3	100.0% (40/40)	91.2 – 100.0
P. aeruginosa	5	63.6% (7/11)	35.4 – 84.8	97.4 (38/39)	86.8 – 99.5
S. aureus	2	18.2% (2/11)	5.1 – 47.7	92.3 (36/39)	79.7 – 97.3

[a] For samples spiked with *E. coli* at concentrations > 1 CFU/mL, PPA is 90.0% (36/40) (95% CI, 77.0 – 96.0); one sample gave an indeterminate result (1/40).

b. Clinical specificity:

The specificity of the T2Bacteria Panel was determined by a prospective study comparing the results of the T2Bacteria Panel with results from a standard of care blood culture collected from the same draw at the same anatomical site. The comparison study was performed at 11 geographically diverse clinical sites in the U.S. A total of 2430 subjects were enrolled, specimens from 1003 subjects were excluded, with the majority excluded due to blood not collected, blood specimens frozen and not tested (to decrease the number of frozen specimens vs. fresh specimens), insufficient blood volume for testing and bloods for which the storage limits were exceeded. A total of 1427 prospectively-collected specimens were tested and included in the comparative study. For each participant, standard of care blood cultures were drawn followed by collection of three K_2EDTA blood tubes (Tubes A,

B and C) for testing with the T2Bacteria Panel. Tube A was tested with the T2Bacteria Panel and the remaining tubes were frozen for future analysis. Three blood culture systems were used in the study; a total of 39 blood cultures gave positive results for the T2Bacteria targeted organisms. Organism identification was performed on all positive blood cultures using standard of care methods. Of the 1427 specimens 672 were fresh specimens and 755 were frozen. Results obtained with the T2Bacteria Panel showed a specificity range of 95.0 to 99.4% depending on the target (Table 15).

Table 15. T2Bacteria Results Obtained in the Prospective Study as Compared to Blood Culture

Species	Sensitivity	95% CI	Specificity	95% CI
E. faecium	100.0% (1/1)	20.7 -100	99.4% (1417/1426)	98.8 – 99.7
E. coli	90.9% (10/11)[a]	62.3 – 98.4	95.0% (1345/1416)[b]	93.7 – 96.0
K. pneumoniae	100.0% (6/6)	61.0 – 100.0	98.5% (1399/1421)	97.7 – 99.0
P. aeruginosa	100% (5/5)	56.6 – 100.0	97.7 (1389/1422)	96.8 – 98.3
S. aureus	81.3% (13/16)[c]	57.0 – 93.4	98.0% (1383/1411)	97.1 – 98.6

[a] *E. coli* detected with retest using Tube B (residual specimen, drawn at the same time as the original blood specimen).
[b] Eight additional specimens gave indeterminate results
[c] *S. aureus* detected with retest using Tube B (residual specimen, drawn at the same time as the original blood specimen.

In the clinical study, there were no prospectively collected specimens that contained two or more target species; the ability of the T2Bacteria Panel to detect more than one species in a single blood specimen was not evaluated. A limitation was added to the device labeling.

Although the sensitivity (PPA) and specificity (NPA) of the T2Bacteria Panel was acceptable, false positive results were obtained at multiple clinical sites during the prospective arm of the clinical study. These occurred with multiple lots of cartridges and reagents and from both fresh and frozen specimens. The majority of false positive results were obtained for the *E. coli* and *P. aeruginosa* targets.

A review of other culture results from patients for whom a false positive T2Bacteria result was obtained was performed. Various data that may provide insight on the validity of the false positive results was reviewed and additional testing was conducted. Positive blood cultures for the same target species obtained within ± 14 days of the T2Bacteria test were considered as strong evidence of bacteremia; positive cultures from other sources were considered less predictive of bacteremia (Table 16)

To further evaluate the validity of the false positive results, alternative amplification and sequencing was performed on a frozen, residual blood tube (Tube B of the original specimen draw) collected from each patient for whom a false positive T2Bacteria result was obtained and for whom no other culture from any source

(collected within 14 ± days of the T2 positive specimen) was positive with the same detected organism. Samples that were positive by amplification and sequencing for the same species detected by the T2Bacteria Panel were also considered strong evidence of bacteremia (Table 16).

Analysis of the additional data indicated that 44.5% of the false positives were associated with strong evidence of bacteremia, 14.8% were weakly associated with bacteremia and 40.6% were not associated with bacteremia.

Table 16. Analysis of T2 Positive/Blood Culture Negative Specimens[a]

Species	T2 Pos/ BC Neg Total	Other BC Positive[b]	Amplification and Sequence Positive[c]	No. Associated with Strong Evidence of Bacteremia[d] (%)	No. Associated with Other Evidence of Infection[e] (%)	No. Associated with No Evidence of Infection (%)
Efm	9	2	2	4/9 (44.4)	3/9 (33.3)	2/9 (22.2)
Eci	63	12	9	21/63 (33.3)	8/63 (12.7)	34/63 (54.0)
Kp	22	6	8	14/22 (63.6)	3/22 (13.6)	5/22 (22.7)
Pa	33	3	8	11/33 (33.3)	4/33 (12.1)	18/33 (54.5)
Sa	28	16	3	19/28 (67.9)	5/28 (17.9)	4/28 (14.3)
Total	**155**	**39**	**30**	**69/155 (44.5)**	**23/155 (14.8)**	**63/155 (40.7)**

[a] Abbreviations: Efm, *E. faecium*; Eci, *E. coli*; Kp, *K. pneumoniae*; Pa, *P. aeruginosa*; Sa, *S. aureus;* BC, blood culture

[b] Blood cultures positive for the T2 species detected other than the paired blood culture and collected within ± 14 days of the T2 specimen

[c] Amplification and sequencing performed on blood samples drawn at the same time as collection of the T2 specimen (Tube B) and positive for the species detected by the panel.

[d] Strong evidence, see text.

[e] Other evidence, see text.

Analysis of the results obtained during the analytical studies and with negative controls performed during the analytical and clinical studies also showed a higher than expected number of false positive results (especially for *E. coli*) that mirrored the results obtained with clinical specimens (Table 3 and Table 17).

Table 17. False Positive Results Obtained in the Analytical Studies

Analytical Study	Percent False Positives by Target[a]				
	Efm	Eci	Kp	Pa	Sa
LoD	0.0	2.4	0.1	0.2	0.0
Single vs. Multi Spike	0.0	1.6	0.0	0.0	0.0
Interfering Substances	0.2	3.5	0.9	4.3	0.4
Analytical Reactivity	0.0	3.0	0.0	0.4	0.0
Competitive Inhibition	0.4	3.9	0.0	1.7	0.2
Analytical Specificity	0.0	3.1	0.0	0.3	0.0
Reproducibility	0.0	3.7	0.0	0.6	0.0
Total	0.1	3.0	0.2	1.5	0.1

[a] Abbreviations: Efm, *E. faecium*; Eci, *E. coli*; Kp, *K. pneumoniae*; Pa, *P. aeruginosa*; Sa, *S. aureus*

An investigation into the root cause identified reagent contamination as the likely source for the false positive results from patients with no evidence of infection. T2 Biosystems implemented improved reagent testing methods and release criteria with the overall goal of reducing the rate of false positive results that can be attributed to reagents contamination. To evaluate the effect of the improved testing methods, the false positive rate obtained with testing negative controls during the clinical study (875 negative control runs) was compared to the false positive rate obtained with an additional 286 negative control runs. Results showed that after these improvements the false positive rate potentially caused by reagent contamination was ≤1.1% for all targets (Table 18).

Table l8. Positivity of T2Bacteria Panel in QCheck Negative Samples Before and After Reagent Improvements

Improvements	No. Positive/No. Tests (%)[a]				
	Efm	Eci	Kp	Pa	Sa
Before[b]	0/875 (0.0)	17/875 (1.9)	2/875 (0.2)	8/875 (0.9)	0/875 (0.0)
After[c]	0/286 (0.0)	3/286 (1.1)	0/286 (0.0)	1/286 (0.3)	0/286 (0.0)

[a] Abbreviations: Efm, *E. faecium*; Eci, *E. coli*; Kp, *K. pneumoniae*; Pa, *P. aeruginosa*; Sa, *S. aureus*
[b] Negative controls run during the clinical study
[c] Negative controls run post study

Additional testing of archived samples (Tubes B or C) for 43 samples that were originally positive for *E. coli* (30 samples) or *P. aeruginosa* (13 samples) and not associated with other evidence of infections gave negative results for all samples. Two of the archived samples were positive for *P. aeruginosa* from patients who were originally T2 positive for *P. aeruginosa*, suggesting that these 2 samples were most likely true positive results (with negative blood cultures) and the remaining samples were most likely false positive results for *P. aeruginosa*.

After reagent improvements, additional testing was completed on 286 QCheck negative controls and 120 K_2EDTA blood samples obtained from healthy donors. False positives were observed only for *E. coli* (1.7%) and *P. aeruginosa* (1.7%) in the

blood samples and only for *E. coli* (1.0%) and *P. aeruginosa* (0.3%) in the QCheck negative controls.

To address the possibility of false positive results with *E. coli*, an indeterminate zone was instituted for interpretation of results for this species only. The device labeling indicates that an indeterminate result (while valid) cannot be considered positive or negative, and no antimicrobial therapy decisions should be based on this result.

The rate of invalid results and instrument failures that occurred during the clinical study were 0.4% (8/1991 tests) and 3.1% (61/1991 tests), respectively.

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

In the prospective clinical study a total of 1427 paired blood cultures and T2Bacteria specimens were evaluated. There were a total of 36 positive blood cultures and 199 positive T2Bacteria results. Positive T2Bacteria results were evaluated as noted above.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 809.10.

O. Conclusion:

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