EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
T2Candida Panel and T2Dx® Instrument

DECISION SUMMARY

A. DEN Number:

DEN140019

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the T2Candida Panel and T2Dx® Instrument

C. Measurands:

The assay amplifies and detects nucleic acids of the following species:
Candida albicans and/or Candida tropicalis
Candida parapsilosis
Candida krusei and/or Candida glabrata

D. Type of Test:

The T2Candida Panel, performed on the T2Dx® Instrument, is a molecular diagnostic assay for the detection of the above listed *Candida* species from whole blood specimens obtained from patients with signs and symptoms of invasive *Candida* infection.

E. Applicant:

T2 Biosystems, Inc.

F. Proprietary and Established Names:

T2Candida Panel and T2Dx® Instrument

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3960

2. Classification:

   Class II

3. Product code(s):

PII

NSU

4. Panel:

83- Microbiology

H. Intended Use:

1. Intended use(s):

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:

1. *Candida albicans* and/or *Candida tropicalis*,
2. *Candida parapsilosis*
3. *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 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.

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 T2Candida Panel is performed on the T2Dx® Instrument.

I. Device Description:

The T2Candida panel and T2Dx® Instrument is comprised of the T2Candida Panel performed on the T2Dx® Instrument. The T2Candida Panel is a qualitative molecular diagnostic assay that employs a whole blood compatible PCR amplification followed by T2 magnetic resonance (T2MR) detection. The T2Candida Panel is performed on the T2Dx® Instrument which executes all steps after specimen loading. A K_2 EDTA whole blood specimen is loaded onto the T2Candida Sample Inlet, which is then placed on the T2Candida Base along with the T2Candida Reagent Pack. The Reagent Pack contains the internal control, amplification reagent, enzyme and the probe-coupled superparamagnetic particles for each *Candida* target. Three milliliters of the blood specimen is transferred to the T2Dx® Instrument where lysis of the red blood cells, concentration and lysis of the *Candida* cells and amplification of the *Candida* DNA takes place. Amplification products are detected by T2MR detection using species-specific probes which are attached to the superparamagnetic particles. At the end of each assay, the T2Dx® Instrument uses a bleach solution to neutralize all liquids on the cartridge to mitigate the risk of amplicon contamination. The assay provides an identification of *Candida albicans* and/or *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* and/or *Candida krusei*. The test does not distinguish between *C. albicans* and *C. tropicalis*. The test does not distinguish between *C. glabrata* and *C. krusei*.

J. 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
- CISPR 11:2003 Group 1 Class A. Industrial, scientific and medical equipment – Radiofrequency disturbance characteristics – Limits and methods of measurement. 2003
- CLSI MM03-A2. Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline. 2008
- 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-A2. Evaluation of Precision Performance of Quantitative Measurement Methods. 2005
- CLSI EP25-A. Evaluation of Stability of In Vitro Diagnostic Reagents. 2013.
- LIS01-A2. Standard Specification for Low Level Protocol to Transfer Messages Between Clinical Laboratory Instruments and Computer Systems; Approved Standard – Second Edition. Maintained by CLSI. 2009
- LIS02-A2. Standard Specification for Transferring Information Between Clinical Laboratory Instruments and Information Systems; Approved Standard – Second Edition. Maintained by CLSI. 2008
- ISTA 7E. Testing Standard for Thermal Transport Packaging Used in Parcel Delivery System Shipment. 2010
- ISTA 2A. Partial Simulation Performance Test (ASTM D5276 – Drop Test). 2011.
- CLSI EP12-A2. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition. 2014.

K. Test Principle:

In the T2Candida Panel and the T2Dx® Instrument a single pair of pan-*Candida* primers hybridizes to conserved sequences within the 5.8S and 26S ribosomal RNA operon of *Candida* species and amplify the intervening transcribed spacer 2 sequence (ITS2) released from the lysed *Candida* cells contained in the specimen. The primers are mixed in a ratio such that an asymmetric product produces a predominantly single stranded nucleic acid after amplification. The primers are designed to also amplify the internal control. Species-specific and internal control-specific probes provide the specificity of the assay. The instrument detects the amplified PCR product directly in the whole blood matrix by amplicon-induced agglomeration of superparamagnetic particles to which the species-specific and internal control-specific probes are attached. The detection method measures the spin-spin relaxation signals of water molecules. For these measurements, the T2Dx® Instrument utilizes a small permanent magnet and a specialized radio frequency coil to measure the T2MR signal from the water molecules. T2MR measures the disorder of the nuclear spins of the water molecules contained in the sample and this disorder is directly proportional to the superparamagnetic particle clustering state. The T2Dx® Instrument reports a positive or negative result for each detection channel [*C. albicans/C. tropicalis* (A/T), *C. parapsilosis* (P*), C. krusei/ C. glabrata* (K/G) and internal control (IC)].

L. Performance Characteristics:

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 three *Candida* species (*C. albicans, C. parapsilosis* and *C. glabrata*), each tested at two concentrations (1 – 2X

LoD, 3 – 4X LoD) using two reagent lots. Testing was performed for six non-consecutive days with two runs and two operators per day. Organisms were tested in triplicate. A total of 108 data points were determined for each analyte at each concentration.

The organisms were prepared in fresh negative human blood samples and the concentration of organisms that were spiked in the samples was confirmed using colony count testing. Spiked samples were prepared by T2 Biosystems, deidentified and shipped to the testing sites under refrigerated conditions and stored at 2 - 8° C until testing. Reproducibility results were acceptable with a range of 95.4 to 100% agreement with expected results for each *Candida* species tested.
A summary of the results is shown in Table 1 below.

Of the total 830 tests obtained, an analysis was conducted to assess the rate of invalid results, instrument failure or false positives. Those were as follows:

Invalid Results: 13/830 tests (1.6%)
Instrument Failures: 17/830 tests (2.1%)
False Positive: 3/830 (0.4%)

Table 1. Summary of Reproducibility Results Across Sites, Reagents and Operators

Organism	Concentration	Test site	No. Detected	No. Not Detected	% Agreement with Expected
C. parapsilosis	1 – 2 X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
	3 – 4 X LoD	Site 1	36	0	108/108 100%
		Site 2	36	0	
		Site 3	36	0	
		All sites	108	0	
C. glabrata	1-2 X LoD[a]	Site 1	35	1	105/108 97.2%
		Site 2	34	2	
		Site 3	36	0	
		All sites	105	3	
	3-4 X LoD	Site 1	36	0	106/108 98.1%
		Site 2	35	1	
		Site 3	35	1	
		All sites	106	2	
C. albicans	1-2 X LoD[b]	Site 1	35	1	103/108 95.4%
		Site 2	35	1	
		Site 3	33	3	
		All sites	103	5	

	3-4 X LoD	Site 1	35	1	107/108 99.1%
		Site 2	36	0	
		Site 3	36	0	
		All sites	107	1	
Negative	N/A	Site 1	0	36	108/108 100%
		Site 2	0	36	
		Site 3	0	36	
		All sites	0	108	

[a] Two false positive results in the P channel
[b] One false positive result in the P channel

b. Linearity/assay Reportable Range:

Not Applicable

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

The T2Candida Panel and T2Dx® Instrument require two types of controls, the internal control and the external controls. The Internal Control (IC) is introduced into each specimen during sample processing on the T2Dx® instrument; the pan-*Candida* primers are designed to amplify the target *Candida* species and the internal control DNA. The IC is designed to report on the presence of any inhibitors in the clinical specimen and on any instrument-related errors. The signal cut-off for the IC is a(b)(4) (b)(4) If the IC signal is below its cutoff and if the patient specimen is negative for all *Candida* targets, the specimen result cannot be determined and the IC result will be flagged as "Invalid" by the software resulting in an invalid test. If any of the *Candida* targets is determined to be positive, the specimen will be reported as positive for that target, regardless of the performance of the IC.

The external controls are provided in a kit and include both positive and negative controls prepared in a whole blood matrix. The T2 positive external control includes two blends of *Candida* species which can interrogate each T2Candida detection channel. The T2Candida A/P/G positive external control is comprised of a blend of *C. albicans, C. parapsilosis* and *C. glabrata*. The T/P/K positive external control is comprised of a blend of *C. tropicalis, C. parapsilosis* and *C. krusei*. The *Candida* concentration in the positive external controls is at least 3-5X LoD. The T2 negative external control is a whole blood specimen free of any *Candida* cells.

As noted in sections 3a and 3b below, clinical studies were conducted using prospectively collected specimens and contrived specimens. During the clinical and analytical studies for the T2Candida assay, fresh whole blood external controls were used; a positive and negative control was run each day of the contrived and prospective arms of the study; positive controls alternated between the APG and the TPK controls. A summary of the quality control test results across all sites is listed in Table 2 below.

Table 2. Summary of Quality Control Testing, Including Testing Performed During the Clinical (Prospective and Contrived) Testing Arms.

Target Species	Site	No. Tested	No. Positive	No. Negative	No. Invalid
A/P/G control[a]	Site 1	67	67	0	0
	Site2	68	66	1[c]	1
	Site 3	56	56	0	0
	Site 4	49	46	0	3
	Site 5	99	97	0	2
	Site 6	16	15	1[c]	0
	Site 7	11	10	0	1
	All Sites	**366**	**357 (97.5%)**	**2 (0.6%)**	**7 (1.9%)**
T/P/K Control[b]	Site 1	69	67	0	2
	Site2	73	72	0	1
	Site 3	58	56	0	2
	Site 4	60	58	0	2
	Site 5	102	98	0	4
	Site 6	13	12	0	1
	Site 7	14	13	0	1
	All Sites	**389**	**376 (96.7)**	**0**	**13 (3.3%)**
Negative Control	Site 1	138	0	136	2
	Site2	140	2[d]	137	1
	Site 3	115	3[d]	109	3
	Site 4	109	2[d]	105	2
	Site 5	200	4[d]	194	2
	Site 6	29	0	25	4
	Site 7	26	0	24	2
	All Sites	**757**	**11 (1.4%)**	**730 (96.4%)**	**16 (2.1%)**

[a] A/P/G control contains: *C. albicans, C. parapsilosis* and *C. glabrata.*
[b] T/P/K control contains *C. tropicalis, C. parapsilosis* and *C. krusei.*
[c] One of three channels negative.
[d] One of three channels positive.

Fresh vs. Frozen Control Equivalence. Due to the limited shelf life of the fresh whole blood external controls, the positive and negative controls will be provided in a separate package as frozen whole blood external controls containing the same organism blends as is found in the fresh whole blood controls. These controls are intended to be stored at -20° C, thawed and equilibrated to room temperature prior to testing. In order to evaluate the equivalence of the frozen external controls with the fresh whole blood external controls, a study was conducted with two lots each of fresh and frozen controls using 65 samples (each, fresh and frozen) of the A/P/G positive control, 65 samples (each, fresh and frozen) of the T/P/K positive control and 65 samples (each, fresh and frozen) of the negative control. Results confirmed the equivalence of the fresh and frozen controls. Results for each of the three channels (A/T, P, K/G) are shown in Table 3 below.

Table 3. Control Lots 1 and 2, Fresh vs. Frozen Equivalence

Control	Lot	No tested	A/T Channel Pos (%)	P Channel Pos (%)	K/G Channel Pos (%)	IC Pos (%)	Invalid
A/P/G Fresh	1	65	65 (100)	65 (100)	65 (100)	65 (100)	0
	2	65	65 (100)	65 (100)	65 (100)	65 (100)	0
A/P/G Frozen	1	65	65 (100)	65 (100)	64 (98.5)	65 (100)	0
	2	65	65 (100)	65 (100)	64 (98.5)	65 (100)	0
T/P/K Fresh	1	65	64 (98.5)	65 (100)	64(98.5)	65 (100)	0
	2	65	64 (98.5)	65 (100)	64(98.5)	65 (100)	0
T/P/K Frozen	1	65	65 (100)	65 (100)	65 (100)	65 (100)	0
	2	65	65 (100)	64 (98.5)	65 (100)	65 (100)	0
Negative fresh	1	65	0	0	0	65 (100)	9*
	2	65	0	0	0	65 (100)	0
Negative frozen	1	65	0	0	0	65 (100)	3*
	2	65	0	0	0	65 (100)	3*

*Invalid tests were repeated and provided negative results.

External Control Reproducibility. The lot-to-lot reproducibility of the frozen external controls was further assessed by evaluating the T2 signal obtained from four production lots of the frozen controls. Ten replicates of each of the three frozen controls (A/P/G positive, T/P/K positive and negative) from each of the four lots were evaluated. The average T2 signal, %CV and % positive was evaluated for each lot. Results indicated that the four lots provided equivalent results.

Frozen Control Stability. The stability of the frozen positive and negative controls was also assessed. Three lots of each of the three controls (A/P/G positive, T/P/K positive and negative) were tested using the T2Candida Panel after 0 days, 30 days and 60 days of storage at -20° C. For each time point, the mean of the measured T2 signals from the A/T, P and K/G channels for each of the controls was expected to be statistically equivalent to the mean T2 signals measured in those channels on Day 0. At the time of clearance, all controls showed acceptable performance for at least 60 days.

d. Detection Limit:

LoD testing was performed using two strains of each species targeted by the T2Candida Panel and performed on the T2Dx® Instrument. LoD testing consisted of an initial screening phase and a confirmatory phase. In the initial screening phase triple or double spiked samples (A/G/P or T/K) were prepared at 5 concentrations (8, 4, 2, <1 CFU/mL and negative) and stored at 2 – 8° C until testing. (The applicability of using multiple-spiked samples was evaluated in a separate study, see below). Seven replicates per spike level/*Candida* strain were tested to determine the putative LoD, which was the lowest concentration of *Candida* in which the positive detection rate met or exceeded 95%. These putative concentrations were used in the confirmatory phase to determine the exact LoD for each species. In the confirmatory phase, a total of at least 20 replicates of each of two strains per species were tested using single-organism-spiked samples. The LoD was confirmed at the putative LoD concentration if no fewer than 19 of the 20 replicates gave a positive signal for the appropriate organism (95.0% detection rate). For one strain each of two species, *C. albicans* and *C. parapsilosis*, the confirmatory testing did not validate the putative LoD determined in the initial studies. Those strains were retested at the next higher dilution, resulting in a 100% positive detection rate; this concentration was determined to be the LoD for the species. Results of the confirmatory LoD testing are shown in Table 4 below.

Table 4. LoD Results

Species	Strain 1		Strain 2		Final LoD CFU/mL
	# Positive/Total (%)	CFU/mL	# Positive/Total (%)	CFU/mL	
C. albicans	19/20 (95)	1	21/21 (100)	2	2
C. tropicalis	20/20 (100)	1	21/21 (100)	1	1
C. parapsilosis	20/20 (100)	2	20/20 (100)	3	3
C. glabrata	20/20 (100)	2	20/20 (100)	2	2
C. krusei	19/20 (95)	1	19/20 (95)	1	1

Invalid results: 23/845 (2.7%)
False positives: 5/845 tests (0.6%)

e. Single Species Spike, Multi-species Spike Equivalence.
A comparison of the equivalence of results obtained for samples spiked with a single species of *Candida* vs. samples spiked with three species of *Candida* was performed. This study was performed to determine the applicability of testing multiple species in a single blood specimen during the analytical studies.

A single strain of each of the five *Candida* species was tested at concentrations of 2-3X LoD. Twenty replicates of each single spike and multi-spike sample was tested; multi-spiked samples contained the combinations of either C. *albicans, C. parapsilosis*, and *C. glabrata* (each at 2–3 X their specific LoD) or *C. tropicalis, C. parapsilosis*, and *C. krusei* (each at 2–3 X their specific LoD). The combinations

were chosen to provide a specific result for each detection channel of the instrument.

Results from samples containing three *Candida* species were positive in each channel and were determined to be equivalent to the results for the same species when tested using a blood specimen spiked with only a single species.

Invalid Results: 5/281 (1.8%)
Instrument failure: 4/281 (1.4%)
False positive: 2/281 (0.7%)

f. Analytical Sensitivity:

Fifteen human strains of each target species were tested the T2Candida Panel and T2Dx® Instrument. The identification of all isolates was confirmed by sequence analysis of the ITS2 region of the ribosomal operon. Isolates were tested in triplicate at 2-3X LoD; testing was repeated for strains which were not detected. Results of the analytical sensitivity study are shown in Table 5 below.

Table 5. Analytical Sensitivity Results

Species	No. strains tested/no. positive (%)
C. albicans	15/15 (100%)
C. tropicalis	14/15 (93.3%)*
C. krusei	15/15 (100%)
C. glabrata	15/15 (100%)
C. parapsilosis	15/15 (100%)

*Repeat testing of 20 replicates of the *C. tropicalis* isolate that was not detected gave positive results in all replicates.

False positives: 2/225 (0.9%)

g. Co-infection Studies:

A competitive inhibition study was performed to evaluate the sensitivity of the T2Candida Panel and T2Dx® Instrument to detect *Candida* present at a concentration of 1-2X LoD in the presence of other clinically relevant organisms that may be present in a co-infection. Three combinations of organisms were tested in whole blood samples: 1) samples containing two *Candida* target species, both in concentrations at 1-2X LoD; 2) samples containing a *Candida* species at 1-2X LoD in combination with a different *Candida* species at a concentration of 100 CFU/ML; 3) samples containing a *Candida* species at (b)(4) TS/CCI in combination with members of other genera at a concentration of 100 CFU/mL. The high concentration of 100 CFU/mL was considered to be clinically relevant as two mL of blood is used in the assay resulting in an organism concentration of 200 CFU/assay.

Thirty one pairs of different *Candida* species, both at a concentration of 1-2X LoD, were tested in replicates of four for a total of 124 tests. Of these, 118 tests were positive for both *Candida* species (95.2%).

Sixty three pairs of two *Candida* species were tested in replicates of four with one species spiked at 1–2X LoD and the other species spiked at 100 CFU/mL for a total of 252 tests. The *Candida* species spiked at 100 CFU/mL were detected in all samples. Two hundred forty four tests were positive for the *Candida* species spiked at 1-2X LoD (96.8%).

Fifty samples including *Candida* species (spiked at 1–2X LoD) in combination with representatives of other genera (*Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus* and *Streptococcus pneumoniae*, spiked at 100 CFU/mL) were tested in replicates of four for a total of 200 tests. Of these, the *Candida* species was detected in 189 (94.5%) tests. Results of the co-infection study are shown in Table 6 below.

Invalid Results: 24/1597 (1.5%)
False Positives: 5/1597 (0.3%)

Table 6. Results of Competitive Inhibition Studies

Organism combinations	**Concentration**	**No. Pairs Tested**	**Total No. of Tests (4 Replicates Per Organism Combination)**	**No. of Positive Tests/Total No. of Tests (%)**	**95% CI**
***Candida* sp./*Candida* sp.**	Both at 1 - 2X LoD	31	124	118/124 (95.2%)	89.8 – 97.8
	1-2X LoD/ 100 CFU/mL	63	252	244/252 (96.8%)	93.8 – 98.4
***Candida* sp./ other genus**	1-2X LoD/ 100 CFU/mL	50	200	189/200 (94.5%)	90.4 – 96.9

f. Analytical Specificity:

A cross reactivity study was performed using 80 non-target, clinically relevant or environmental organisms including 21 yeast species, nine viruses, 25 fungi and 25 species of bacteria. Isolates were initially tested in triplicate at a concentration of 10^6 CFU/mL for yeast, molds and bacteria, and viruses were tested at a concentration of 10^5 PFU/mL. Any strain which showed cross reactivity or gave an invalid result was further evaluated at lower, more clinically relevant concentrations of organisms in blood (100, 33 and 10 CFU/mL).

Cross reactivity was defined as an increase in the T2 signal above the established cutoff for the *Candida* detection channels when tested at clinically relevant

concentrations. Cross reactivity required both amplification of the organism with the pan-*Candida* PCR primers and subsequent detection with any of the capture probes.

Forty one species tested at a concentration of 10^6 CFU/mL (10^5 PFU/mL for viruses) showed no cross reactivity and had valid IC values. These species are listed below in Table 7.

Table 7. Species Providing Valid IC Values and No Cross Reactivity

Bacteria	
Acinetobacter baumannii	*Staphylococcus aureus* MRSA
Bacteroides fragilis	*Staphylococcus auricularis*
Clostridium perfringens	*Staphylococcus epidermidis*
Enterobacter cloacae	*Staphylococcus haemolyticus*
Klebsiella oxytoca	*Staphylococcus hominis*
Klebsiella pneumoniae	*Staphylococcus intermedius*
Morganella morganii	*Staphylococcus saprophyticus*
Pseudomonas aeruginosa	*Staphylococcus warneri*
Serratia marcescens	*Streptococcus mutans*
Enterococcus faecalis	*Streptococcus pneumoniae*
Staphylococcus aureus	*Streptococcus pyogenes*
Fungi	**Viruses**
Acremonium kiliense	*Adenovirus*
Malassezia furfur	*Cytomegalovirus*
Malassezia pachydermatis	*Enterovirus*
Mucor oblongiellipticus	*Epstein-Barr Virus*
Phialophora richardsiae	*Hepatitis A*
Rhizomucor microsporous	*Hepatitis B*
Rhizopus pusillus	*Herpes simplex Virus 1*
Rhizopus oryzae	*Herpes simplex Virus 2*
Scedosporium prolificans	*Varicella zoster Virus*
Candida haemulonii	

Thirty species tested at a concentration of 10^6 CFU/mL gave an invalid result in at least one replicate due to a low IC value but gave no positive signals in any of the *Candida* channels. Further testing with these strains at clinically relevant concentrations (100, 33 and 10 CFU/mL) provided negative T2Candida results with acceptable IC values and were determined to be non-cross reacting (See Table 8 below).

Five species (*Candida rugosa, Candida sojae, Acinetobacter lwoffii, E. coli and E. faecalis*) gave positive *Candida* results (and either valid or invalid IC results) in one or more replicates when tested at a concentration of 10^6 CFU/mL. Repeat testing at clinically relevant organism concentrations (100, 33 or 10 CFU/mL) showed no cross reactivity (Table 8 below).

Table 8. Species Providing an Invalid IC or Positive *Candida* Results When Tested at 10^6 CFU/mL but Not When Tested at Clinically Relevant Concentrations; Not Considered to be Cross Reactive

Organisms Giving Invalid IC Results at 10^6 CFU/mL	
Candida albidus	*Aspergillus flavus*
Candida dubliniensis	*Aspergillus fumigatus*
Candida gigantensis	*Aspergillus niger*
Candida guilliermondii	*Aspergillus terreus*
Candida kefyr	*Exophiala xenobiotica*
Candida lunata	*Fusarium proliferatum*
Candida lusitaniae	*Fusarium oxysporum*
Candida nivariensis	*Fusarium solani*
Candida norvegensis	*Kluyveromyces delphensis*
Candida pelliculosa	*Pichia anomala*
Candida utilis	*Paecilomyces variotii*
Candida viswanathii	*Scopulariopsis brevicaulis*
Cryptococcus neoformans	*Trichosporon asahii*
Rhodotorula glutinis	*Trichosporon inkin*
	Trichosporon mucoides
	Trichoderma reesei
Organisms Giving Positive *Candida* Results at 10^6 CFU/mL	
Candida rugosa	*Acinetobacter lwoffii*
Candida sojae	*Escherichia coli*
	E. faecalis

The following species were shown to cross react when tested at any concentration: *C. bracarensis, S. cerevisiae, C. metapsilosis* and *C. orthopsilosis* (Table 9 below).

In order to determine the effect of the presence of bacteria in the specimen on the IC value in actual clinical specimens, a review of the T2 signal data for 63 prospectively analyzed specimens that were positive for bacteria (as determined by the corresponding blood culture) was performed. The T2 signals for the internal control for all specimens was greater than the cutoff value, indicating that for samples containing clinically relevant organism concentrations, the internal control would be within acceptable levels and would not result in an invalid test result.

Table 9. Species determined to be Cross Reactive

Organism	T2Candida Results at Various Concentrations															
	10^6 CFU/mL				100 CFU/mL				33 CFU/mL				10 CFU/mL			
	A/T	P	K/G	IC	A/T	P	K/G	IC	A/T	P	K/G	IC	A/T	P	K/G	IC
Candida bracarensis	N	N	P	Invalid	N	N	P	Valid	N	N	P	Valid	N	N	P	Valid
*Saccharomyces cerevisiae**	N	N	P	Invalid	N	N	P	Valid	N	N	P	Valid	N	N	P	Valid
Candida orthopsilosis	N	P	N	Invalid	N	P	N	Valid	N	P	N	Valid	N	P	N	Valid
Candida metapsilosis	N	P	N	Invalid	N	P	N	Valid	N	P	N	Valid	N	P	N	Valid

*Five strains evaluated

g. Interfering Substances

An interfering substances study was performed to determine and characterize the effects of potential endogenous and exogenous interfering substances on the performance of the T2Candida Panel and the T2Dx® Instrument. Interference testing was performed using a paired-difference format; the potentially interfering substance was added to a *Candida*-spiked sample at high concentration (to simulate worse case) and the bias relative to a *Candida* spiked control containing no interfering substances was determined. Substances were considered to be interfering if the T2 signal (in msec) was decreased by 30% or greater as compared to the sample containing no interferent. After testing average T2MR signal, standard deviation, %CV and % positive were evaluated for each sample.

A total of seven substances representing possible patient underlying conditions were tested at concentrations from 1X to 38X above clinical levels, with most at 3X clinical levels (Table 10).

Table 10. Endogenous Substances Evaluated to Represent Human Underlying Conditions

Underlying Source or Condition	Endogenous Interferent	Reference Level	Test Concentration
Leukocytosis	Human DNA (Buffy Coat)	WBC 3.5-11E7 cells/mL	5E7 cells/mL
Icterus	Bilirubin (conjugated)	0-3.4 µmol/L	50 mg/L
	Bilirubin (unconjugated)	5-21 µmol/L	150 mg/L
	ALT	17-50 U/L (plasma)	≥ 3 times the upper normal limit of 50 U/L
	AST	9-48 U/L	≥ 3 times the upper normal limit of 48 U/L
Hemolysis	Hemoglobin	1-2 g/L	22.4 g/L
Lipemia	Intralipid	0.34-3.7 mmol/L or 0.065 mg/dL (triglycerides)	1.3 mg/dL
Hyperproteinemia	Protein (albumin)	39-51 g/L	40 g/L
	Immunoglobulin G	6-13 g/L	15 g/L
Renal Failure	Creatinine	6-17 mg/L	50 mg/L
	Urea	1.1-14.3 mmol/L	42.9 mmol/L (2.58 mg/mL)
Multiple	Circulating human DNA	N/A	1 µg/mL

Twenty four exogenous substances were tested at concentrations from 1X to 38X above clinical levels (Table 11). Potential exogenous interferents were tested in sets of three for a total of eight interferent pools. All interferents were added to healthy donor blood; *Candida* species were spiked into the samples at 3X LoD using inocula containing either the multi-spike combinations of *C. albicans/C. parapsilosis/C. glabrata* or *C. tropicalis/C. parapsilosis/C. krusei* and tested in triplicate. If assay interference, as measured by a decrease in T2 signal by 30% or greater, was detected with any of the interferent pools, each interferent present in the pool was prepared as an individual test to determine which substance was acting as an interferent with the assay's performance.

Results indicated interference with the signal for *Candida* species or with the signal for the internal control for the following substances: Feraheme, Magnevist, EDTA, Ablavar, and samples with an interferent simulating lipemia (Intralipid).

Invalid Results: 12/1647 (0.7%)
False Positives : 5/1647 (0.3%)

Table 11. Exogenous Substances Evaluated

Exogenous Interferent	Reference Level	Test Concentration
EDTA	2 mg/mL	6 mg/mL
Heparin	350-1000 U/L	3000 U/L
Calcium Hypochlorite	NA	8% (80 mg/mL)
Fluconazole	65.2-81.5 µmol/L	245 µmol/L (75 µg/mL)
Micafungin	100 mg dosage/day	60 mg/L
Ferumoxytol (Feraheme)	510 mg/dosage, 30 mg/mL	306 mg/L
MRI Contrast Agent: Magnevist (gadopentetate dimeglumine, Gd-DTPA)	0.01 mmol/kg	5.4 mmol/L (6.86 mg/mL)
MRI Contrast Agent: Ablavar (gadofosveset or Vasovist)	0.03 mmol/kg	1.6 mmol/L (1.56 mg/mL)
Amphotericin B Trihydrate	1 mg/kg/day	54.5 mg/L
Amphotericin B, liposomal (Ambisome)	3-5 mg/kg/day	270 mg/L
Piperacillin/Pipril (Piperacillin)	6g	3.6 g/L
Vancomycin	12.4-27.6 µmol/L	69.0 µmol/L (100 µg/mL)
Imipenem/Cilastatin (Primaxin)	1 g	600 mg/L
Ciprofloxacin	1.51-15.1 µmol/L	30.2 µmol/L (10µg/mL)
Tazobactam (Tazobac)	4.5 g	8.1 g/L
Gentamycin sulfate	10.5-20.9 µmol/L	21 µmol/L (10 µg.mL)
Linezolid	600 mg	360 mg/L
Azithromycin (Zithromax)	0.4-5.1 µmol/L	15.3 µmol/L (11.5 µg/mL0
Clindamycin (Cleocin)	4.0-29.7 µmol/L	89.1 µmol/L (41.4 µg/mL)
Metronidazole	35-234 µmol/L	701 µmol/L (119.9 µg/mL)

Exogenous Interferent	Reference Level	Test Concentration
Caspofungin	70 mg	42 mg/L
Lisinopril	0.25 µmol/L	0.74 µmol/L (326.7 µg/L)
Cytarabine	6 mg/kg	324 mg/L

h. Assay Cut-off:

To establish the cutoff values for the A/T, P, K/G and internal control detections, a limit of blank study was conducted using negative blood specimens from healthy (N=113) and unhealthy (N=39) patients, with two different reagent lots. The T2MR signal, in milliseconds (msec), was evaluated to establish the upper limit of T2 signal distribution on negative measurements and lower limit of T2 signal of positive internal control measurements. These limits encompassed 99% of the no target signals and 99% of the positive internal control signals, respectively. The mid-point of the interval between these upper and lower limits was 65 ms and was set as the clinical cutoff for A/T, P and K/G detection. The upper limit was 85 ms and was set as the cutoff for the internal control detection. Validation of the cutoff was completed by analysis of results obtained during the prospective clinical studies. Cutoff values are indicated in Table 12 below.

In the LoD studies, T2MR signals were typically (b)(4) TS/CCI msec for negative detections and ranged from (b)(4) TS/CCI msec to (b)(4) TS/CCI msec for positive detections.

Table 12. Assay Cutoff Values

Detection Channel	Lower limit of valid signal (ms)	Clinical cutoff (ms)
A/T	20	65
P	20	65
K/G	20	65
IC	85	85

Application of assay cutoff to patient specimens. In the event that one or more of the A/T, P, and K/G particle detection signals is below the lower limit of valid signal (20ms) for a patient specimen, that entire specimen is deemed invalid because the specific detection reactions that were below the lower limit of valid signal had an associated failure.

i. Carryover/Cross Contamination Studies

A cross contamination study was performed to determine the rate of false positivity caused by organism carryover within the T2Dx® Instrument. A checkerboard study (alternating high positives and negatives) was performed using two runs on five instruments using multi-spiked samples prepared to contain 100 CFU/mL (200 CFU/assay or 'test") interspaced with negative samples. An additional study was

performed using samples spiked with 1000 CFU/mL (2000 CFU/assay).

A possible total of 175 negative samples were run. Three of these samples were false positive (all with samples spiked at 100 CFU/mL) for a false positive rate of 1.7%.

j. Specimen Stability at 2-8° C

In order to support a labeling recommendation for storage of blood samples at 2 - 8°C prior to testing, a specimen stability study was performed. Ten replicates of whole blood specimens were spiked with each of the target *Candida* species at a concentration of 100 CFU/mL. The organism concentration was determined by colony count at Day 0, Day 1 and Day 3 of storage. Results indicated that the organism concentration in samples held for 1 day at 2 – 8 °C showed a change in organism concentration of -9% to +12.4%. Colony count results obtained after three days of storage showed a decrease in organism concentration of -4% to -17%. Device labeling indicates that blood specimens should be tested as soon as possible after collection and that specimens held for longer than one day at 2 - 8° C may result in a decrease of viable organisms in the specimen.

k. Lysis Tube and Reagent Stability

A stability study was performed to determine the shelf-life of the reagents and the lysis tubes at the recommended temperatures. Reagents were removed from the kits and each component was evaluated using quality control methods applied to the bulk reagents prior to kit fill. Results were compared to results obtained with recently manufactured reagents; all acceptance criteria were met indicating all reagents included in the kit were stable for at least seven months from date of manufacture. The following storage instructions will be recommended for the packaging and labeling: T2Candida Reagent Packs should be stored at 2-8°C; the T2Candida Cartridge should be stored at 15-30°C. The expiration date of each component will be indicated on the component label.

l. Shipping Stability

The T2Candida shipping containers containing Reagent Packs, the Cartridge Kit and the External Controls were tested for component, labeling and packaging damage according to the ISTA 2A drop test. Containers were dropped in several package orientations from a drop height of 38 inches. All shipping configurations passed the pre-specified acceptance criteria.

To evaluate the effects of thermal exposure, packaged components were exposed to temperatures between 24.5 and 30.4° C for 36 hours and between 4.3 and 14.2°C for 36 hours. After exposure the packaged T2Candida Panel components were functionally tested on the T2Dx® Instrument. Results were compared to results obtained by the same lot of T2Candida panel components stored at the recommended storage temperatures. All components passed the pre-specified acceptance criteria, that thermal cycle exposed T2Candida components were not statistically different

(p>0.5) than the control components.

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 low prevalence of *Candida*-positive blood cultures from clinical specimens (prevalence <0.5%), the sensitivity of the T2Candida Panel and T2Dx® Instrument was determined using contrived samples. Contrived samples were prepared with representatives of each of the target *Candida* species and were tested with the T2Candida panel. Fifty unique *Candida* isolates of each of the five T2Candida targets were spiked into whole blood at specific concentrations for a total of 250 positive samples. In addition 50 unspiked blood specimens were included as negative samples and were tested randomly and in a blinded fashion throughout the study.

   Isolates. Isolates were obtained from (b)(4) TS/CCI (b)(4) TS/CCI), a reference laboratory and a clinical laboratory. The ITS region of each isolate was sequenced to confirm its identification; for all isolates the BLAST results of the determined sequence showed > 99.2% similarity to *C. albicans, C. tropicalis, C. krusei, C. glabrata* or *C. parapsilosis,* as appropriate.

   Blood specimens. Three K_2EDTA tubes containing 4 mL of blood in each tube were drawn from patients for whom blood cultures were ordered at three clinical sites. Thirty-four percent of specimens were obtained from patients determined to have some level of immunocompromise. Tubes were transported to T2Biosystems within 24 hours of collection under refrigeration conditions. In the event that a blood culture (from the same patient draw as the K_2EDTA tubes) was determined to be *Candida* positive for the patient blood specimen, the contrived specimen's T2 analytical result was declared invalid and not analyzed as part of the T2 study.

   Spiking. Contrived samples were spiked with a specific concentration of *Candida* based on Table 13 below to represent clinically relevant organism concentrations; a majority of samples contained *Candida* levels near the limit of detection.

Table 13. Number of Contrived Specimens/*Candida* Species/Titer Level

	Number of Isolates Tested at Each Concentration			
Organism (LoD*)	**< 1 CFU/mL**	**1 – 10 CFU/mL**	**11 – 30 CFU/mL**	**31 – 100 CFU/mL**
***C. albicans* (2)**	10	18	17	5
***C. tropicalis* (1)**	10	18	17	5
***C. krusei* (1)**	10	18	17	5
***C. glabrata* (2)**	10	18	17	5
***C. parapsilosis* (3)**	10	18	17	5
Number (%) of Total	50 (20)	90 (36)	85 (34)	25 (10)

*LoD in CFU/mL

Cell bullets were prepared for each isolate and stored frozen. The concentration of organism in the cell bullets was determined by (b)(4) CCI The (b)(4) CCI s was used to assign an organism concentration value (b)(4) CCI was used to guide contrived sample preparation. Cell viability and cell death was evaluated by comparing cell counts obtained before and after a single freeze/thaw cycle. Results were consistent with minimal cell death after a single freeze/thaw cycle.

Within 48 hours of specimen draw, contrived samples were prepared using a randomized organism list to determine the species and strain to be used each day of preparation. Cell bullets were diluted to appropriate levels and the concentration was verified by colony count testing; three replicates, each in 4 mL of K_2EDTA blood, were prepared for each organism/inoculum concentration. For each three-tube set, one tube was retained by T2 Biosystems for quality control purposes and the remaining two tubes were distributed to a testing site. Duplicate samples were shipped to assure the availability of a sample in the event one of the sample tubes was damaged during transit or otherwise malfunctioned. Only one sample was tested by the site, the untested sample was discarded.

In addition to positive spiked samples, an additional 50 un-spiked blood specimens were sent to testing sites as blinded negative samples.

Contrived samples and negative samples were distributed across four clinical sites within 24 hours of preparation and analyzed with the T2Candida Panel and T2Dx® Instrument within 48 hours of receipt. Instructions for performance of contrived sample testing specified that as part of the loading procedure, refrigerated (i.e. controls and contrived) samples should be allowed to equilibrate to room temperature for ~20 minutes prior to loading. In all cases, samples were tested within five hours of equilibration to room temperature.

Assay. Contrived samples were tested with the T2Candida Panel on a T2Dx® instrument at four clinical sites per the testing protocol. For each specimen, T2Candida results for all four detection reactions *(C. albicans/C. tropicalis, C. krusei/C. glabrata, C. parapsilosis* and internal control) were collected yielding T2Candida sets for 300 different samples (250 spiked and 50 negative) for a total of 900 organism channel results. Positive percent agreement (PPA) across all organism concentrations was 94.0% for the A/T and P channels and 88.0% for the K/G channel. Results for the contrived samples by detection channel for all concentrations are shown in Table 14 below.

Results were also analyzed relative to *Candida* spike concentration (Table 15) and relative to species LoD (Table 16). PPA for samples spiked at concentrations ≥ LoD showed 97.4 to 100.0% PPA across all detection channels.

All 50 negative samples gave negative results for all channels. A single false positive result (1/250, 0.4%) was seen in the parapsilosis channel with *C. krusei*.

Table 14. Contrived Specimen Performance by Detection Channel

Detection Channel	PPA	95 %CI	NPA	95%CI
A/T	94/100 (94.0%)	87.5-97.2	200/200 (100.0%)	98.1-100.0
P	47/50 (94.0%)	83.8-97.9	249/250 (99.6%)	97.8-99.9
K/G	88/100 (88.0%)	80.2-93.0	200/200 (100.0%)	98.1-100.0

Abbreviations: PPA, Positive percent agreement; NPA, Negative percent agreement; A/T, *C. albicans/C. tropicalis* channel; P, *C. parapsilosis* channel; K/G, *C. krusei, C. glabrata* channel

Table 15. Contrived Specimen PPA by Detection Channel and Relative to *Candida* Spike Concentration[a]

Detection Channel	< 1 CFU/mL		1 – 10 CFU/mL		11 - 30 CFU/mL		31 – 100 CFU/mL	
	PPA	95% CI	PPA	95% CI	PPA	95% CI	PPA	95% CI
A/T	16/20 (80.0%)	58.4 - 91.9	34/36 (94.4%)	81.9 - 98.5	34/34 (100%)	89.8 - 100	10/10 (100.0%)	72.2 - 100
P	8/10 (80.0%)	49.0 – 94.3	17/18 (94.4%)	74.2 – 99.0	17/17 (100%)	81.6 - 100	5/5 (100%)	56.6 - 100
K/G	11/20 (55.0%)	34.2 – 74.2	34/36 (94.4%)	81.9 – 98.5	33/34 (97.1%)	85.1 – 99.5	10/10 (100%)	72.2 - 100

[a]Abbreviations: A/T, *C. albicans/C. tropicalis* channel; P, *C. parapsilosis* channel; K/G, *C. glabrata, C. krusei* channel

Table 16. Contrived Specimen PPA by Detection Channel and Relative to each Species LoD[a]

Detection Channel	Species	LoD (CFU/mL)	PPA at ≥ LoD[b]	95% CI	PPA at < LoD[c]	95% CI
A/T	*C. albicans*	2	77/79 (97.5%)	91.2 – 99.3	17/21 (81.0%)	60.0 – 92.3
	C. tropicalis	1				
P	*C. parapsilosis*	3	32/32 (100%)	89.3 -100.0	15/18 (83.3%)	60.8 – 94.2
K/G	*C. krusei*	1	75/77 (97.4%)	91.0 – 99.3	13/23 (56.5%)	36.8 – 74.4
	C. glabrata	2				

[a]Abbreviations: A/T, *C. albicans/C. tropicalis* channel; P, *C. parapsilosis* channel; K/G, *C. glabrata, C. krusei* channel
[b] The calculations of PPA at ≥ LoD included a portion of the samples spiked at 1-10 CFU/mL (depending on the LoD of the species) and all samples spiked at 11-30 CFU/mL and 31-100 CFU/mL).
[c]The calculations of PPA at < LoD included all samples spiked at < 1 CFU/mL and a portion of the samples spiked at 1-10 CFU/mL (depending on the LoD of the species)

b. Clinical specificity:

The specificity of the T2Candida Panel and T2Dx® Instrument was determined by a prospective comparison of the results of the T2Candida Panel with results from blood culture collected from the same draw at the same anatomical site.

Patients. A total of 1501 blood specimens were drawn from adult patients who had been referred for a diagnostic blood culture per routine standard of care. Informed consent was obtained. Forty-eight percent of specimens were obtained from patients determined to have some level of immunocompromise.

Study Sites. Specimens were collected at nine geographically diverse sites; testing was performed at seven sites.

Specimens. A standard blood culture set (aerobic and anaerobic bottle set) was obtained for each patient in accordance with standard institutional practice and blood culture system manufacturer's recommendations. In addition, three 4 mL specimens in K_2EDTA vacutainers for the T2Candida Panel were obtained from the same anatomical collection site as the blood culture. The first tube drawn (Tube A) is used for the T2Candida assay and the remaining tubes were transferred to T2 Biosystems for -80 ° C storage and additional testing.

Results. Based on results from the 1501 prospective specimens, the specificity of the T2Candida Panel and T2Dx® Instrument was determined to be 98.8% for the A/T channel, 99.2% for the P channel and 99.2% for the K/G channel (Table 17). A total of six blood cultures were positive for *Candida* species (Table 18). An additional blood culture was positive for *C. parapsilosis*, T2Candida results were positive in the P (*C. parapsilosis*) channel but the specimen was excluded from the study due to a protocol violation. A total of 63 of the 1501 prospective specimens were positive with bacteria only.

The T2Candida Panel and T2Dx® Instrument correctly detected 2/4 specimens that had corresponding blood cultures positive for *C. albicans*, 2/2 and 1/1 specimens that had corresponding blood cultures positive for *C. parapsilosis* and *C. glabrata*, respectively (Table 18).

Table 17. Prospective Specimen Sensitivity and Specificity by Detection Channel

Detection Channel	Sensitivity	95%CI	Specificity	95%CI
A/T	2/4* (50.0%)	15.0 – 85.0	1479/1497 (98.8%)	98.1 – 99.2
P	2/2 (100%)	34.2 – 100.0	1487/1499 (99.2%)	98.6 – 99.5
K/G	1/1 (100%)	20.6 – 100.0	1499/1500 (99.9)	99.6 – 99.9

* an additional specimen collected at the same time was positive for *C. albicans*

Table 18. T2Candida Results for Blood Cultures Positive for Candida Species.

Specimen	T2Candida Results			Blood Culture Result
	A/T	P	K/G	
1	Positive	Positive	Negative	*C. albicans* and *C. parapsilosis*
2	Negative	Positive	Negative	*C. parapsilosis*
3	Negative	Negative	Positive	*C. glabrata*
4	Positive	Negative	Negative	*C. albicans*
5	Negative*	Negative	Negative	*C. albicans*
6	Negative	Negative	Negative	*C. albicans*

*Repeat T2Candida results from an additional blood specimen drawn at the same time were positive in the A/T channel.

False Positives. The T2Candida Panel indicated 31 positive detections in 29 patient specimens for which *Candida* was not detected in corresponding blood cultures. A chart and case history review was conducted for those patients. This review revealed that one sample was obtained from a patient who had proven intra-abdominal candidiasis with no positive blood cultures. In addition, six patients were receiving anti-fungal therapy and four patients were colonized with *Candida* species. Repeat testing of 26 of the 29 false positive specimens using a specimen drawn at the same time as the original specimen were negative for *Candida* by the T2Candida assay. Three specimens were unavailable for retesting.

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

4. Clinical cut-off:

Table 19. Cut-off values for Candida and IC Channels

Detection Channel	Lower limit of Valid Signal (msec)	Clinical Cutoff (msec)
A/T	20	65
P	20	65
K/G	20	65
IC	85	85

5. Expected values/Reference range:

The overall incidence of *Candida* species as determined by the T2Candida Panel and T2Dx® Instrument in direct blood specimens in patients tested during this study was 0.3% (4/1501). All clinical specimens collected during this study were collected between August, 2013 and October 2013 and April 2014.

M. Instrument Name:

T2Candida Panel and T2Dx® Instrument

N. System Descriptions:

1. Modes of Operation:
   The system operates in a fully automated mode with limited user intervention. Reagent packs, samples and cartridges are loading manually but all processing and analysis steps are controlled and monitored by the system.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X__ or No _____

   Level of Concern:
   Moderate

   Software Description:

   The T2Dx® instrument software controls the following:
   - T2Dx® instrument interactions with peripheral devices and systems (e.g. printer, barcode reader, portable memory device, service laptop computer and LIS system)
   - T2Dx® instrument system diagnostics, including alarms and error notifications
   - T2Dx® instrument graphical user interface, including touch screen prompts that guide an operator through the steps associated with loading and unloading test cartridges/patient specimens on the T2Dx® instrument and obtaining test results
   - T2Dx® internal operating temperatures
   - Operating parameters (e.g. time, temperature, etc.) of various T2Dx® instrument subsystems.
   - Initiation of workflows, scheduling of specimens for processing and monitoring of workflow implementation
   - Determination of the result
   - Administrative functions (e.g. Require Operator ID, Positive Sample Counter, Archive Results, Shutdown OS, and Restart Application)

   This T2Dx® instrument software is designed and intended to interface with the T2Candida workflow software.

   Device Hazard Analysis:

   A list of potential hazards was developed by conducting a bottom up analysis of potential failure modes. Potential failure modes were identified including those which could lead

to invalid or incorrect results. The RPN value for these potential hazards exceeded the threshold, which triggered a mitigation action. As a result, solutions were implemented to verify that risks were reduced.

Architecture Design Chart:

The T2Dx® instrument software and T2Candida workflow software architecture design chart was provided along with a summary of software architecture illustrations and references to description of each illustration.

Software Requirements Specification (SRS):

The software requirements for T2Dx® instrument and the T2Candida panel were based on discussions with potential users, site visits, and a review of current, similar diagnostic systems. The Software Requirements Specifications (SRS) were developed using these findings.

Software Design Specification (SDS):

Requirements defined in the SRS document are implemented according to the design specifications described.

Traceability Analysis:

A traceability matrix which links requirements, specifications, hazards, mitigations and verification & validation testing for the software was acceptable.

Software Development Environment Description:

Software development life cycle plan and software development configuration management plan for the T2Dx® instrument software and the T2Candida workflow software was acceptable.

Verification and Validation Testing:

Based on the Software Requirements Specification ("SRS") and Software Design Specifications ("SDS"), software verification test plans were devised to verify that the software meets the requirements. Each element of the SRS was tested and found to meet the requirements. This was accomplished via a series of system, software integration, and code inspection activities:

- Unit level code inspection performed by an independent reviewer and associated unit/integration level automated or manual functional testing of code
- Module level (integrated software and hardware) functional testing
- System level performance verification
- System level validation

Revision Level History:
A software revision history record for the T2Dx® instrument software and T2Candida

workflow software was acceptable.

Unresolved Anomalies:

The T2Dx® software release version 1.0.0.9 contains three unresolved anomalies. Impact analysis of these unresolved anomalies on device safety or effectiveness was acceptable.

EMC Testing:

The electrical safety of the T2Dx® Instrument has been verified in accordance with the following standards:
Safety requirements for electrical equipment for measurement, control, and laboratory use (Safety).

- IEC 61010-1:2001 (Second Edition): Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements
- IEC 61010-2-010: 2003 (2nd Ed): 100562844BOX-004: Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials
- IEC 61010- 2 - 081: 2001 (1st Edition) + A1:2003 : Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- IEC 61010-2-101: 2002 (ed.1): Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment

Electromagnetic Compatibility (EMC)

- IEC 61326-1:2005: Electrical equipment for measurement, control and laboratory use, control and laboratory use - EMC requirements - Part 1: General requirements
- 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

3. Specimen Identification:

The T2Dx® instrument interfaces with a handheld digital imager scanner. The barcode reader can be used to identify:

- the type of assay;
- assay component lot numbers;
- the specimen being analyzed;
- the T2Dx® instrument operator, if the operator uses barcode identification.

4. Specimen Sampling and Handling:

Blood should be collected in K_2EDTA vacutainer tubes; the minimum volume for testing is 3 mL. Specimens should be tested as soon as possible after collection and should be

held at room temperature (15 - 25°C) for no longer than 12 hours or for up to one day at 2 - 8° C. Holding specimens longer than one day at 2 – 8° C may result in a decrease of viable organisms in the specimen. Blood specimens should be at room temperature at the time of testing and should be inverted a minimum of 8-10 times to ensure sample homogeneity. Samples that are not visually homogenous should not be tested. The vacutainer tubes are uncapped and placed on the T2Candida Sample Inlet. The specimen and sample inlet are inserted into the instrument.

5. Calibration:

   Calibration routines are performed automatically and require no user intervention

6. Quality Control:

   Users are recommended to follow all laboratory procedures, local, state, and/or federal requirements and accrediting organizations guidelines for the testing of all external positive and negative control materials regardless of source.

   The following recommendations are provided which relate to external quality control testing at least once per month:

   - A single POSITIVE (APG or TPK) control tube of either multiplex blend and a single NEGATIVE control tube from the T2Candida External Controls kit is run at least once every 30 days in order to verify the continuing performance of the T2Dx® instrument and the T2Candida panel reagents. Users should alternate the multiplex blend POSITIVE control tube with each QC check.
   - A POSITIVE APG, a POSITIVE TPK control tube and a NEGATIVE control tube from the T2Candida External Controls kit should be run when either of the following events occurs
     - A new reagent lot is received into the laboratory
     - Significant maintenance (including software upgrades) to the T2Dx® Instrument
   - A single NEGATIVE control tube from the T2Candida External Controls kit should be run upon completion of the T2Dx® instrument decontamination procedure.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the specials controls.

Q. Identified Risks and Required Mitigations:

Identified Risks	Required Mitigations
Incorrect identification of a pathogenic microorganism by the device can lead to improper patient management	Special Controls (1), (2), (3), (4), (5)
Failure to correctly interpret test results	Special Control (6)
Failure to correctly operate the instrument	Special Controls (7) and (8)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	The primary benefit provided by the T2Candida Panel and T2Dx® Instrument is the earlier diagnosis of candidemia and early initiation of appropriate antifungal treatment. Symptoms of candidemia may be nonspecific, and difficult to differentiate from other infectious causes of disease. Early diagnosis of candidemia may be associated with decreased morbidity and mortality for some patients. Additionally, negative testing may provide additional clinician confidence when delaying empiric therapy or discontinuing empiric antifungal therapy. Patients may experience fewer side effects from antifungal therapy, and there may be additional benefits to antimicrobial stewardship programs

Summary of the Risk(s)	False positive and false negative results resulting from incorrect identification of a pathogenic organism by the device, failure to correctly interpret test results, or failure to correctly operate the instrument are the primary risks associated with use of the T2Candida Panel and T2Dx® Instrument. A false positive result may result in unnecessary antifungal therapy, with associated toxicities and side effects, including potential allergic reaction. Additionally, patients may undergo unnecessary imaging studies to identify potential organ system involvement or have alternative testing delayed due to a presumptive diagnosis of candidemia. Because established treatment guidelines also recommend indwelling central line removal for certain *Candida* infections, unnecessary line removal could occur as a result of a false positive result. These side effects from antifungal therapy are generally reversible, and additional laboratory testing is often ordered simultaneously with evaluation for *Candida.* Unnecessary imaging increases potential radiation exposure, but many critically ill patients may obtain these scans as part of their evaluation. False negative results could result in delayed diagnosis of candidemia, or delayed initiation of empiric antifungal therapy. An incorrect identification could result in inappropriate anti-fungal therapy. As a result, the patient could experience a delay in effective anti-fungal therapy. However, these risks can be mitigated through appropriate labelling and do not exceed the current standard of care when used with traditional blood culture.
Summary of Other Factors	None
Conclusions Do the probable benefits outweigh the probable risks?	The anticipated probable benefits of the T2Candida Panel and T2Dx® Instrument likely outweigh the anticipated potential risks in light of the special controls and the applicable general controls. The T2Candida panel is the first of its kind, and represents a potential for patient benefit through more rapid diagnosis of candidemia. Potential risks associated with false positive or false negative results may be mitigated by use of traditional blood cultures, which would be necessary to recover the organism for further identification and susceptibility testing even without the T2Candida panel. Data obtained from additional studies with contrived specimens demonstrated high sensitivity. However, the prospective clinical study found that the sensitivity for *C. albicans*, the most prevalent species of *Candida*, is low, but given the small number of positive prospective samples (4), it is difficult to determine how the T2 will perform in real-world use. Used in conjunction with traditional cultures, much of this risk can be mitigated. End user education will also allow clinicians to make informed judgements about how they will implement the T2Candida panel in their clinical practice, and may decrease the risk of unnecessary imaging scans or central line removal. Ultimately, the majority of risks associated with the T2Candida panel may be minimized with appropriate precautions and the T2Candida panel may provide substantial benefit to patients.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3960. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PII, NSU

Device Type: Nucleic acid-based device for the amplification, detection and identification of microbial pathogens directly from whole blood specimens.
Class: II (special controls)
Regulation: 21 CFR 866.3960

(a) *Identification*. A nucleic acid-based device for the amplification, detection and identification of microbial pathogens directly from whole blood specimens is a qualitative *in vitro* device intended for the amplification, detection, and identification of microbial-associated nucleic acid sequences from patients with suspected bloodstream infections. This device is intended to aid in the diagnosis of bloodstream infection when used in conjunction with clinical signs and symptoms and other laboratory findings.

(b) Classification. Class II (special controls). A nucleic acid-based device for the amplification, detection and identification of microbial pathogens directly from whole blood specimens must comply with the following special controls:

1) Premarket notification submissions must include detailed device description documentation, including the device components, ancillary reagents required but not provided, and a detailed explanation of the methodology, including primer/probe sequence, design, and rationale for sequence selection.
2) Premarket notification submissions must include detailed documentation from the following analytical and clinical performance studies: Analytical sensitivity (Limit of Detection), reactivity, inclusivity, precision, reproducibility, interference, cross reactivity, carry-over, and cross contamination.
3) Premarket notification submissions must include detailed documentation from a clinical study. The study, performed on a study population consistent with the intended use population, must compare the device performance to results obtained from well-accepted reference methods.

4) Premarket notification submissions must include detailed documentation for device software, including, but not limited to, software applications and hardware-based devices that incorporate software.
5) The device labeling must include limitations regarding the need for culture confirmation of negative specimens, as appropriate.
6) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.
7) Premarket notification submissions must include details on an end user device training program that will be offered while marketing the device, as appropriate.
8) As part of the risk management activities performed as part of your 21 CFR 820.30 design controls, you must document an appropriate end user device training program that will be offered as part of your efforts to mitigate the risk of failure to correctly operate the instrument.