Clinical Trial Data Supporting FDA Clearance of the T2Bacteria® Panel Versus Blood Culture for the Diagnosis of Bacteremia

New diagnostic test overcomes time, sensitivity, and antimicrobial interference limitations of culture-based methodologies in prospective multicenter pivotal clinical trial.

WHITE PAPER

T2Biosystems®

Background

Bloodstream infections (BSI) are associated with significant morbidity and mortality, and the timely administration of appropriate antibiotics improves outcomes.[1,2] While blood culture is considered the gold standard for diagnosing BSI, it has several well-documented limitations, including suboptimal sensitivity and long turnaround time. Given the limitations of blood culture, it may be more accurate and timely to use composite microbiologic data and clinical criteria in administering faster and targeted therapy to the patient.

The T2Bacteria® Panel (T2Bacteria) prospective multicenter pivotal clinical trial evaluated the performance of T2Bacteria for diagnosing BSIs as compared to blood culture. The prospective trial included 1427 unique adult patients who were suspected of BSIs and had a diagnostic blood culture ordered per standard of care at 11 U.S. medical centers. Paired blood culture and T2Bacteria blood samples were drawn from each patient, with the blood culture samples drawn first. T2Bacteria target organisms include those responsible for >50% of BSIs: *E. faecium*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli*.[3] The limit of detection (LoD) for each target organism is defined in Table 1.

Time to Result

In the clinical trial, the mean time to species identification for T2Bacteria was 5.4 hours, 66.3 hours faster than for species identification of T2Bacteria target organisms based on blood culture. If multiple T2Bacteria Panels were loaded at a time, the first result was reported in 3.5 hours from the time the Panel was loaded onto the T2Dx® Instrument. Time to result data from the clinical trial is depicted in Table 2. Trial data supports the use of T2Bacteria to get patients on effective therapy more than 2 days faster than the current standard of care.

The T2Bacteria Panel prospective multicenter pivotal clinical trial demonstrated distinct advantages of T2Bacteria over blood culture-based methodologies, including:

- **Diagnosis of more infections than blood culture:**
  T2Bacteria detected 127 subjects with evidence of infection as compared to 39 subjects detected by the paired blood culture as infected with T2Bacteria target organisms.
- **Faster results with the T2Bacteria Panel:**
  Bacteremia and the causative organism were detected more than 2 days earlier (3-5 hours versus 2-3 days) enabling faster targeting of therapy.
- **No antimicrobial interference:**
  T2Bacteria results are not influenced by antecedent antibiotics in the blood.
- **Potential impact on therapy:**
  Trial data reveal that about 40% of infected patients may benefit from a rapid T2Bacteria result that prompts a change to or initiation of effective therapy.

Table 1: T2Bacteria target organisms and limit of detection (LoD)

Target Organism	LoD (CFU/mL)
E. faecium	5
S. aureus	2
K. pneumoniae	2
P. aeruginosa	5
E. coli	11

Table 2: Comparative time to result. Blood culture (BC) time to result reflects only the bacteria on the T2Bacteria Panel.

Statistic	Time to result (hrs.)			
	T2B Result	BC Positive	BC Species ID	BC Negative
Mean	5.4	38.5	71.7	123.8
SD	1.6	32.8	39.3	9.84
Median	5.1	24.2	54.0	121.0
Range	3.6 –10.0	10.5 –121.8	24.3 –177.3	20.8 –200.7

Clinical Performance

During the clinical trial, the positivity rate was 2.7% by paired blood culture for target organisms versus 13.3% (190/1427) by T2Bacteria. There were 35 concordant detections between T2Bacteria (T2B) and the paired blood culture (BC). As depicted in Table 3, compared to the paired blood culture, T2Bacteria demonstrated a positive percent agreement (PPA) that ranged from 81.3% to 100% depending on target organism and a negative percent agreement (NPA) that ranged from 95.0% to 99.4%.

Table 3: T2Bacteria performance characteristics by target organism

Target Organism	Sensitivity[1]		Specificity[2]	
	PPA	95% CI	NPA	95% CI
E. faecium	100% (1/1)	20.7 - 100%	99.4% (1417/1426)	98.8 - 99.7%
S. aureus	81.3% (13/16)[3]	57.0 - 93.4%	98.0% (1383/1411)	97.1 - 98.6%
K. pneumoniae	100% (6/6)	61.0 - 100%	98.5% (1399/1421)	97.7 - 99.0%
P. aeruginosa	100% (5/5)	56.6 - 100%	97.7% (1389/1422)	96.8 - 98.3%
E. coli	90.9% (10/11)[4]	62.3 - 98.4%	95.0% (1345/1416)[5]	93.7 – 96.0%

1. Sensitivity (PPA) was calculated against blood culture from a paired blood draw for T2Bacteria and blood culture.
2. Specificity (NPA) was calculated against blood culture from a paired blood draw for T2Bacteria and blood culture.
3. One subject was blood culture positive for *S. aureus* but negative in the first T2Bacteria blood sample and *S. aureus* positive in the second concurrently collected blood sample tested by T2Bacteria.
4. One subject was blood culture positive for *E. coli* but negative in the first T2Bacteria blood sample and *E. coli* positive in the second concurrently collected blood sample tested by T2Bacteria.
5. Of the total 1,416 BC negative subjects, 8 (0.6%) yielded indeterminate results for the *E. coli* channel. Excluding indeterminate *E. coli* results, the adjusted NPA for *E. coli* would be 95.5%.

Discordant Results Analysis

Overall in the trial, there were 190 T2Bacteria positive (T2B+) results consisting of:

- 35 concordant results (T2B+/BC+)
- 155 discordant T2B+/BC- results

As outlined in table 4, of the 155 discordants:

- 39 represented patients with an additional BC+ at a different blood draw within ±14 days of the T2B+ draw
- 30 represented results for which an additional blood specimen (drawn at the same time as the original T2B+ specimen) was positive by an amplification and gene sequencing method
- In total, 69 patients had "strong evidence of infection" and were identified by T2Bacteria

An additional 23 results were obtained from patients who had "other evidence of infection" from non-blood specimens positive for the same target organism as the T2B+ result (collected ± 14 days from the T2B+ specimen). These 23 patients were also identified by T2Bacteria and missed by the paired blood culture.

In total, 59% (92/155) of the discordant T2B+/BC- results proved to be associated with evidence of true infection.

- **T2B+ results were indicative of infection and offer an earlier opportunity for informed intervention that may prevent the progression of sepsis.**

Table 4: Summary of discordant results analysis (T2B+/BC-)

T2B Target Organism	TOTAL T2B+ / BC-	STRONG Evidence of Infection		TOTAL STRONG Evidence of Infection[4]	OTHER Evidence of Infection	TOTAL STRONG[4] + OTHER[3] Evidence of Infection
		Other BC Positive[1]	Sequencing Positive[2]		Non-Blood Matrices Culture Positive	
E. faecium	9	2	2	44.4% (4/9)	3	77.8% (7/9)
S. aureus	28	16	3	67.9% (19/28)	5	85.7% (24/28)
K. pneumoniae	22	6	8	63.6% (14/22)	3	77.3% (17/22)
P. aeruginosa	33	3	8	33.3% (11/33)	4	45.5% (15/33)
E. coli	63	12	9	33.3% (21/63)	8	46.0% (29/63)
Total	**155**	**39**	**30**	**44.5% (69/155)**	**23**	**59.4% (92/155)**

1. BCs positive for the T2B species identified other than the paired blood culture and processed within ± 14 days of collection of the T2B sample.
2. Sequencing from blood samples drawn at the same time as collection of the T2B sample and positive for the T2B species identified, where this sequencing assay was only run on subjects without positive evidence from other sample sources (footnote 1 and 3).
3. Other cultures from non-blood sample matrices positive for the T2B species identified within ±14 days of collection of the T2B sample.
4. Strong evidence defined as a T2B positive result associated with a BC positive from a different draw than T2B draw or a sequencing positive result from a blood sample drawn concurrently with the T2B draw.

Antibiotic Analysis

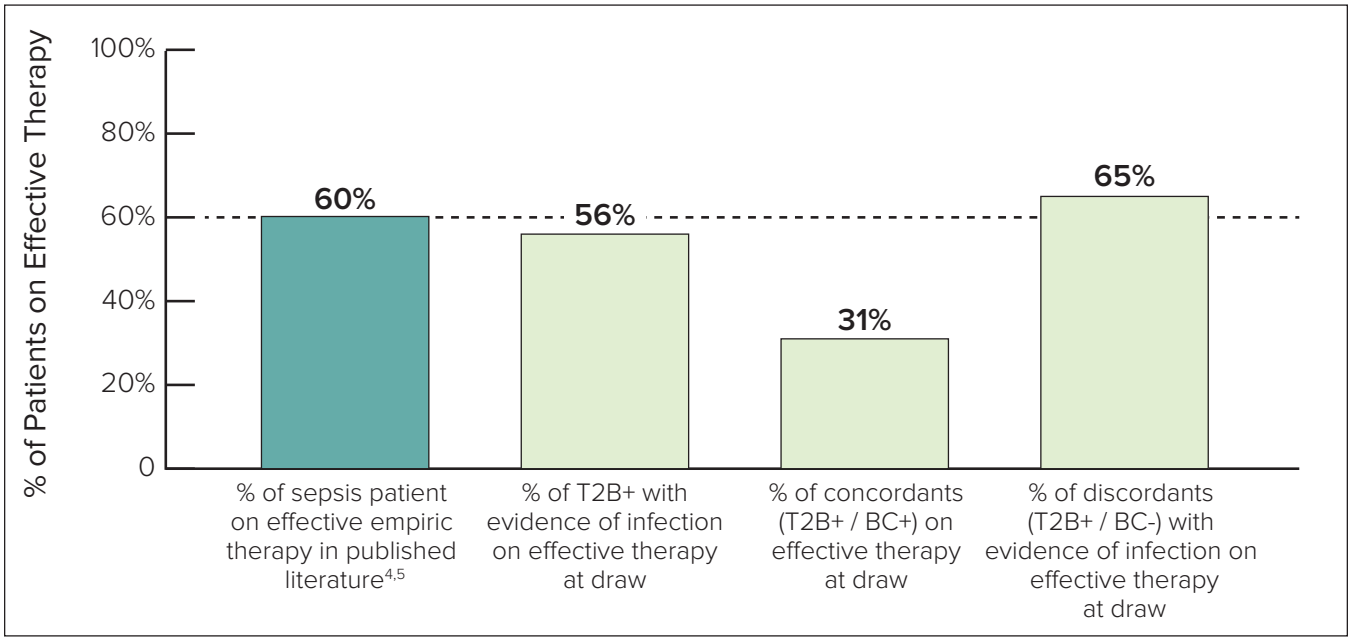
According to published literature, about 60% of sepsis patients are given effective empiric antibiotics.[4,5] Because T2Bacteria species identification results are available days earlier than blood culture-based results, T2Bacteria results offer a much earlier opportunity to target therapy and potentially improve patient outcomes and the quality of care. To evaluate this premise, an analysis of antibiotics administered to trial subjects within the 72 hour period prior to the T2Bacteria blood draw was performed. The effectiveness of therapy was evaluated across all trial subjects (n=1427) and for those trial subjects with a positive T2Bacteria result (n=190). Effective therapy was defined based on a combined antibiogram from four institutions (3 of which were trial participants), susceptibility information from published literature, and infectious diseases physician review. A therapy was deemed effective if at least 70% of isolates were susceptible based on the combined antibiogram.

Effectiveness of therapy across all trial subjects for target organisms

The antibiotic history of all trial subjects was reviewed for the 72-hour period prior to blood draw for effectiveness against T2Bacteria targets. A total of 914, or 64% of all trial subjects, were on at least one antibiotic at the time of blood draw, but only 6% of blood cultures were positive. This indicates both a potential overuse of antibiotics (or overtreatment) and a major limitation of blood culture as a means for detecting infection in the presence of antibiotics.

As depicted in Figure 1, 56% of trial subjects who were T2Bacteria positive and had evidence of infection were on effective therapy, while 44% were not on effective therapy. Interestingly, a sub-analysis of trial subjects who were positive by both T2Bacteria and the paired blood culture (T2B+/BC+), i.e. proven infected, demonstrated a far lower rate of effective therapy. 69% of these subjects were proven infected and undertreated. A rapid T2Bacteria result could help guide appropriate, targeted therapeutic intervention much earlier than the current standard of care and thereby improve the quality of patient care.

Figure 1: Effectiveness of therapy



Effectiveness of therapy in T2Bacteria positive subjects

As discussed earlier, there were 35 concordant T2Bacteria positive/blood culture positive (T2B+/BC+) subjects in the trial as shown in Table 5.

- 31% (11/35) of T2B+/BC+ subjects were on effective therapy at the time of the T2Bacteria blood draw.
- 69% (24/35) of these concordant subjects were not on an effective therapy at the time of the T2Bacteria blood draw.
  - > In fact, all but one of these patients (23/24) were not on any antibiotic at all. These subjects highlight the opportunity for early targeted therapy due to a rapid T2Bacteria result.

Further, by including those subjects that also had "Strong Evidence" or "Other Evidence" of infection, an additional 32 subjects with evidence of infection, missed by the paired blood culture, and not on effective therapy in the 72 hours prior to the T2Bacteria blood draw could have been identified and placed on effective targeted therapy. These results are outlined in Table 5.

Table 5: Analysis of effectiveness of therapy in T2Bacteria positive subjects with evidence of infection.

Level of Evidence	Result Type	Coverage in T2B+ Subjects	Eci	Efm	Kp	Pa	Sa	Total	Overall NOT Covered by Effective Therapy
Concordant	T2B+/BC+ matched draw	**Total Subjects (#)**	**10**	**1**	**6**	**5**	**13**	**35**	**68.6% (24 / 35)**
		Covered by effective therapy (#)	3	0	1	1	6	11	
		NOT covered by effective therapy (#)	7	1	5	4	7	24	
Strong Evidence	T2B+/BC- & BC+ for other blood draw	**Total Subjects (#)**	**12**	**2**	**6**	**3**	**16**	**39**	**33.3% (23 / 69)**
		Covered by effective therapy (#)	11	0	4	2	15	32	
		NOT covered by effective therapy (#)	1	2	2	1	1	7	
	T2B+/BC- & Sequencing+ from other tube	**Total Subjects (#)**	**9**	**2**	**8**	**8**	**3**	**30**	
		Covered by effective therapy (#)	4	0	4	6	0	14	
		NOT covered by effective therapy (#)	5	2	4	2	3	16	
Other Evidence	T2B+/BC- & non-blood matrix culture positive	**Total Subjects (#)**	**8**	**3**	**3**	**4**	**5**	**23**	**39.1% (9 / 23)**
		Covered by effective therapy (#)	6	1	2	3	2	14	
		NOT covered by effective therapy (#)	2	2	1	1	3	9	
							TOTAL	**127**	**44% (56 / 127)**

Antibiotic analysis summary findings

T2Bacteria results may enable changes in therapy to occur more than 2 days prior to the current standard of care.

- Across all subjects in the trial, 3.9% (56/1427) could have potentially been placed on an effective therapy faster after receiving a positive T2Bacteria result.
- Further, 5.0% (71/1427) could have benefited from confirmation that the therapy they were receiving was effective for their infectious species.
- In total, 8.9% (127/1427) of all trial subjects could have benefited from a positive T2Bacteria result, allowing changes in therapy or confirmation of correct therapy.

Conclusion

The T2Bacteria Panel prospective multicenter pivotal clinical trial demonstrated the distinct advantages of T2Bacteria over culture-based methodologies, including: faster time to result, improved sensitivity, and freedom from antimicrobial interference.

- **Faster results:** The T2Bacteria Panel detects bacteremia and the causative organism more than 2 days before blood culture-based methodologies (3-5 hours versus 2-3 days) enabling faster targeting of therapy.
- **Diagnosis of infections missed by BC:** The T2Bacteria Panel detects the five most common and deadly sepsis-causing bacteria species accounting for more than 50% of BSIs with an overall PPA of 90% and NPA of 98% as compared to blood culture. Further, T2Bacteria detects infections that may be missed by blood culture - **T2Bacteria detected 127 subjects with evidence of infection, compared to 39 subjects by blood culture for T2Bacteria target organisms**.
- **No antimicrobial interference**: Unlike culture-based methodologies, T2Bacteria Panel results are not influenced by antecedent antibiotics in the blood.
- **Potential impact on therapy:** T2Bacteria pivotal clinical study data demonstrates that a significant proportion of patients stand to benefit from a rapid T2Bacteria positive result.

Based on the findings from the clinical trial, T2Bacteria shows promise for broad application in testing patients suspected of bloodstream infection to prevent the progression of sepsis. The ability to access clinically relevant results within hours offers an opportunity to improve patient outcomes and the quality of care.

1. Seymour CW et al. N Engl J Med 2017;376:2235-44.
2. Kumar A et al. Crit Care Med. 2006 Jun;34(6):1589-96.
3. Pien BC et al. Am J Med. 2010 Sep;123(9):819-28.
4. Paul M et al. Antimicrob Agents Chemother. 2010 Nov;54(11):4851-63
5. Marquet K et al. Crit Car. 2015 Feb 16:19:63.

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