Annals of Internal Medicine ORIGINAL RESEARCH

Performance of the T2Bacteria Panel for Diagnosing Bloodstream Infections

A Diagnostic Accuracy Study

M. Hong Nguyen, MD; Cornelius J. Clancy, MD; A. William Pasculle, ScD; Peter G. Pappas, MD; George Alangaden, MD; George A. Pankey, MD; Bryan H. Schmitt, DO; Altaf Rasool, MD; Melvin P. Weinstein, MD; Raymond Widen, MD†; Diana R. Hernandez, PhD; Donna M. Wolk, PhD; Thomas J. Walsh, MD; John R. Perfect, MD; Mollie N. Wilson, MS; and Eleftherios Mylonakis, MD

Background: Blood cultures, the gold standard for diagnosing bloodstream infections (BSIs), are insensitive and limited by prolonged time to results. The T2Bacteria Panel (T2 Biosystems) is a direct-from-blood, nonculture test that identifies the most common ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*).

Objective: To assess performance of the T2Bacteria Panel in diagnosing suspected BSIs in adults.

Design: Prospective patient enrollment (8 December 2015 through 4 August 2017).

Setting: Eleven U.S. hospitals.

Patients: 1427 patients for whom blood cultures were ordered as standard of care.

Intervention: Paired blood culture and T2Bacteria testing.

Measurements: Performance of T2Bacteria compared with a single set of blood cultures in diagnosing proven, probable, and possible BSIs caused by T2Bacteria-targeted organisms.

Results: Blood culture and T2Bacteria results were positive for targeted bacteria in 3% (39 of 1427) and 13% (181 of 1427) of patients, respectively. Mean times from start of blood culture incubation to positivity and species identification were 38.5 (SD, 32.8) and 71.7 (SD, 39.3) hours, respectively. Mean times to species identification with T2Bacteria were 3.61 (SD, 0.2) to 7.70 (SD, 1.38) hours, depending on the number of samples tested. Per-patient sensitivity and specificity of T2Bacteria for proven BSIs were 90% (95% CI, 76% to 96%) and 90% (CI, 88% to 91%), respectively; the negative predictive value was 99.7% (1242 of 1246). The rate of negative blood cultures with a positive T2Bacteria result was 10% (146 of 1427); 60% (88 of 146) of such results were associated with probable (n = 62) or possible (n = 26) BSIs. If probable BSIs and both probable and possible BSIs were assumed to be true positives missed by blood culture, per-patient specificity of T2Bacteria was 94% and 96%, respectively.

Limitation: Low prevalence of positive blood cultures, collection of a single set of culture specimens, and inability of T2Bacteria to detect nontargeted pathogens.

Conclusion: The T2Bacteria Panel rapidly and accurately diagnoses BSIs caused by 5 common bacteria.

Primary Funding Source: T2 Biosystems.

Ann Intern Med. doi:10.7326/M18-2772 **Annals.org**
For author affiliations, see end of text.
This article was published at Annals.org on 14 May 2019.
† Deceased.

Early institution of appropriate antimicrobial therapy is a crucial determinant of improved outcomes in patients with bloodstream infections (BSIs), particularly those causing sepsis (1–4). Blood cultures are the gold standard for diagnosing BSIs but are limited by prolonged time to results and sensitivities ranging from 10% or less to about 50% in cases of suspected bacteremia, febrile neutropenia, severe sepsis, and septic shock (5, 6). The inadequacies of blood cultures and the need for early treatment of BSIs provide a rationale for empirical use of broad-spectrum antibiotics. In a meta-analysis of sepsis treatment studies, empirical antibiotic therapy was inappropriate in 46.5% of patients, and the mortality rate in these patients was higher than among those receiving appropriate treatment (7). The development of nonculture diagnostic tests for BSIs that are rapid and accurate is therefore a top priority (8–10).

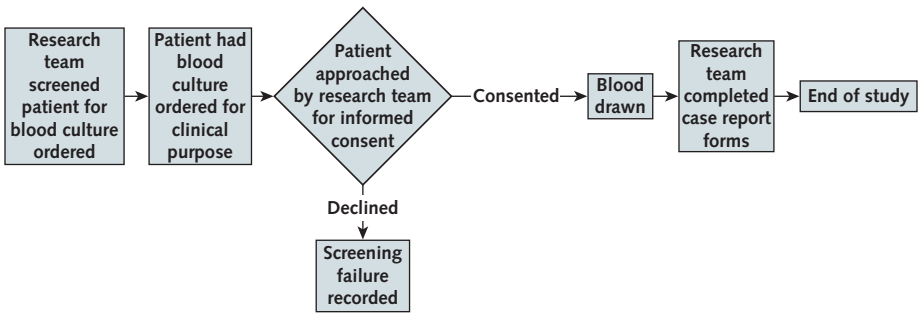
Recently, the U.S. Food and Drug Administration (FDA) cleared 2 nonculture diagnostic tests that use T2 magnetic resonance to identify pathogens in whole blood samples. The T2Candida and T2Bacteria Panels (T2 Biosystems) are the first FDA-cleared tests that directly detect multiple species of fungi and bacteria in whole blood samples without the need for cultivating organisms. In both panels, fully automated testing of whole blood collected in a standard Vacutainer (Becton Dickinson) is performed by a dedicated instrument platform (T2Dx). T2Dx amplifies microbial cell–associated DNA using a thermostable polymerase and target-specific primers and detects signals by amplicon-induced agglomeration of superparamagnetic particles and T2 magnetic resonance. The T2Bacteria Panel detects the 5 most common ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*). These species generally account for about 50% of organisms re-

See also:

Editorial comment

Web-Only
Supplement

Figure 1. Flow chart for patient recruitment and enrollment.



covered from positive blood cultures and are notable for their propensity for resistance to multiple antibiotics (11–13). In a single-center pilot study, a research prototype of T2Bacteria had sensitivity of 83% and specificity of 98% for diagnosing BSIs caused by targeted bacteria (14).

The objective of this study was to determine the performance of the commercially available, FDA-cleared version of the T2Bacteria Panel in identifying 5 target bacteria among patients for whom blood cultures were ordered as standard of care.

METHODS

Study Design

This was a prospective multicenter study to evaluate the performance of the T2Bacteria Panel in diagnosing BSI. Participants were enrolled at 11 U.S. acute care hospitals (**Appendix Table 1**, available at Annals.org). The institutional review board at each site approved the study protocol, which was finalized on 20 August 2015. Patients were enrolled from 8 December 2015 through 4 August 2017.

Study Population

Hospitalized patients aged 18 years or older who had a diagnostic blood culture ordered as standard of care for suspected BSI or sepsis were eligible for the study (**Figure 1**). No specific criteria were used to define suspected BSI or sepsis; blood culture was ordered at the discretion of the treating physician. Potential participants were excluded if they had previous specimens tested by the T2Bacteria Panel; if they had a comorbid condition that, in the opinion of the investigator, could limit participation; or if they had received any investigational, novel drug compound within 30 days before enrollment.

Measurements

Aerobic and anaerobic blood cultures (1 bottle each, referred to as "companion blood cultures") and whole blood samples (n = 3 for T2Bacteria testing) were collected in that order and from the same anatomical site. Companion blood cultures (5 to 10 mL of whole blood per bottle) were performed in accordance with hospital practices and manufacturer recommendations (BacT/ALERT [BioMérieux], BACTEC FX [Becton Dickinson], or VersaTREK [Thermo Fisher Scientific]). Bacteria in positive cultures were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltronics or BioMérieux) or VITEK 2 (BioMérieux). Blood cultures that did not yield an organism were incubated for at least 5 days. For T2Bacteria, at least 3 mL was collected in 4-mL dipotassium EDTA Vacutainer tubes supplied by T2 Biosystems. The first tube was tested on a T2Dx instrument at each site. T2Bacteria was tested on a 50/50 mix of samples that were freshly collected and those that were thawed after storage at −70 °C. The 95% CIs for positive and negative percentage agreement with blood cultures overlapped between fresh and frozen samples. The second and third T2Bacteria tubes were stored at −70 °C for backup test runs. A synthetic DNA target was included with each run as an internal control. T2Bacteria results were reported as positive or negative for each targeted species. In analytic verification studies, the limit of detection was determined by spiking whole blood specimens with multiple concentrations of the species detected by the T2Bacteria Panel. The limit of detection was defined as the lowest bacterial concentration (in colony-forming units per milliliter) detected in at least 95% of contrived samples, as established by testing of at least 20 replicates of 2 strains of each species. The limits of detection ranged from 2 to 11 CFU/mL (**Appendix Table 2**, available at Annals.org).

T2Bacteria results were not available to health care teams caring for study patients and did not affect clinical decision making. Health care teams were permitted to order additional blood cultures and other types of cultures at their discretion or at any time without input from the research team; these are referred to as "clinical cultures" to distinguish them from companion blood culture specimens collected concurrently with T2Bacteria samples. Demographic, clinical, and microbiological data were extracted by using a case report form.

Definitions

For purposes of data analysis, a companion blood culture was positive if an organism targeted by the

T2Bacteria panel was recovered from at least 1 of the 2 bottles in the set. The T2Bacteria result was considered positive if 1 or more target bacteria were detected and negative if none were detected. "Proven BSI" was defined as a positive blood culture using a concurrently drawn specimen. "Probable BSI" was defined as a negative blood culture but a positive T2Bacteria result if the T2Bacteria-detected organism was isolated within 21 days from a clinical blood culture specimen collected at a different time or from another site (such as the abdomen, urine, or lungs), indicating a plausible cause of infection. "Possible BSI" was defined as a negative blood culture but a positive T2Bacteria result in the absence of supporting culture data if the T2Bacteria-detected organism was a plausible cause of disease (for example, *E coli* in a patient with cholangitis). Definitions of active antibiotics against indicated bacteria were taken from the *Sanford Guide to Antimicrobial Therapy 2017* (47th edition). Patients were considered to be receiving an active antibiotic at the time of testing if they received at least 1 dose in the 2 days before sample collection. For each patient, definitions were applied by the senior author (M.H.N.) and adjudicated by a committee of investigators (M.H.N., C.J.C., M.P.W., and E.M.).

Statistical Analysis

The primary outcomes were sensitivity and specificity of the T2Bacteria Panel, which were calculated using positive blood culture results for a T2Bacteria-targeted organism (that is, proven BSI) as the reference. For per-patient calculations, each patient's sample was considered positive or negative on the basis of results for the 5 organisms in the T2Bacteria Panel. For per-assay calculations, results for individual organisms in each sample were considered separately. Test results were classified as concordant (positive result on blood culture and T2Bacteria Panel for the same species or negative result on both) or discordant (positive blood culture and negative T2Bacteria result or vice versa) for organisms detected by T2Bacteria. Negative blood cultures with a positive T2Bacteria result were defined as putative false positives if criteria for proven, probable, or possible BSI were not met. The 95% CIs for sensitivity and specificity were calculated using the exact Clopper–Pearson method. Analyses were conducted using Stata, version 15 (StataCorp).

Role of the Funding Source

The study was designed by the investigators in conjunction with the sponsor (T2 Biosystems), with input from the FDA. The sponsor assisted the investigators with protocol development and creation of the case report form. Data were collected by the investigators and curated in the sponsor's FDA-compliant database. The investigators analyzed and interpreted data and generated conclusions without input from the sponsor. The sponsor was not involved in the decision to publish the results. A draft of the manuscript was submitted to the sponsor. The investigators are solely responsible for the content of the article.

RESULTS

Patient Enrollment and Blood Culture Results

Informed consent was obtained from 1502 patients. Five percent (75 of 1502) were excluded because of deficiencies in sample collection (56% [42 of 75]) or tube storage (41% [31 of 75]) or because of previous enrollment (3% [2 of 75]). Samples from 1427 patients were tested in the study.

The median patient age was 56 years, 63% (893 of 1427) were white, and 57% (809 of 1427) were men. Companion blood cultures were positive for 85 organisms in 6% (82 of 1427) of patients (**Appendix Table 3**, available at Annals.org). Organisms included in the T2Bacteria Panel were identified in 48% (39 of 82) of positive blood cultures, including *S aureus* (n = 16), *E coli* (n = 11), *K pneumoniae* (n = 6), *P aeruginosa* (n = 5), and *E faecium* (n = 1). Coagulase-negative staphylococci, diphtheroids, and *Corynebacterium* species were recovered from 28% (23 of 82) of positive blood cultures. The mean times from the start of blood culture incubation to positivity and species identification for T2Bacteria-targeted organisms were 38.5 (SD, 32.8) and 71.7 (SD, 39.3) hours, respectively (**Table 1**).

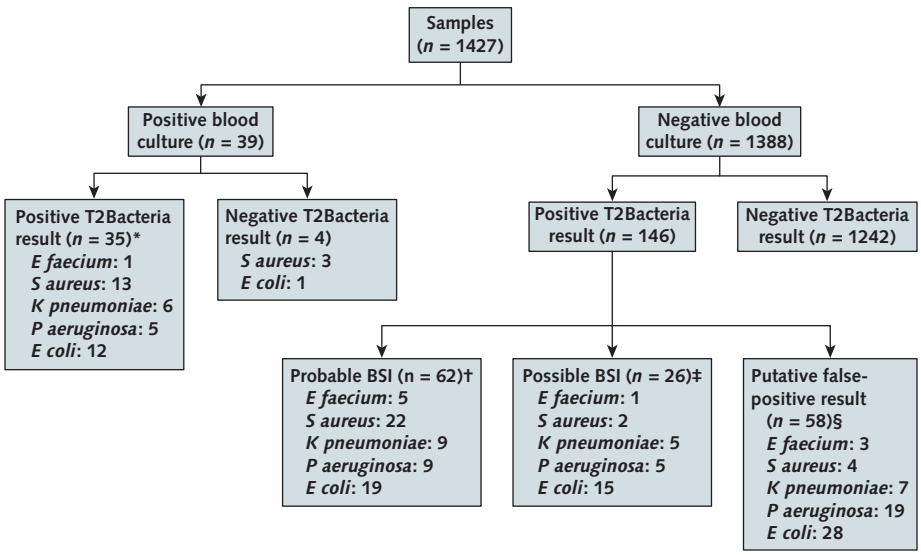
Performance of the T2Bacteria Panel

T2Bacteria results were invalid in samples from 0.3% (4 of 1427) of patients. Testing of thawed backup samples from these patients yielded valid negative results for all samples, which were included in our data analysis. T2Bacteria results were positive in 13% (181 of 1427) of samples, with identification of 190 bacteria (**Figure 2**). The performance of T2Bacteria in diagnosing BSIs caused by targeted bacteria is summarized in **Table 2**, and combined blood culture and T2Bacteria results are shown in **Figure 2**. Blood culture and T2Bacteria results were concordant in 90% (1277 of 1427) of samples and discordant in 10% (150 of 1427).

Table 1. Time to Positive Blood Culture and Species Identification of T2Bacteria-Targeted Organisms

Organism	Positive Blood Cultures, *n*	Median (Mean) Time to Detection, *h*	Median (Mean) Time to Species Identification, *h*
Enterococcus faecium	1	17.2	116.4
Staphylococcus aureus	16	37.7 (51.6)	60 (77.4)
Klebsiella pneumoniae	6	17.9 (24.7)	74.2 (75.9)
Pseudomonas aeruginosa	5	67.1 (53.9)	48.3 (56.7)
Escherichia coli	11	15.4 (21.9)	53.2 (63.8)
Total	39	24.2 (38.5)	54.0 (71.7)

Figure 2. Combined performance of blood culture and the T2Bacteria Panel for diagnosis of BSI caused by T2Bacteria-targeted organisms.



Data are from 1427 patients, from whom companion blood culture and T2Bacteria samples were collected concurrently. Negative blood culture and positive T2Bacteria results were obtained for 146 samples. BSI = bloodstream infection; *E coli* = *Escherichia coli*; *E faecium* = *Enterococcus faecium*; *K pneumoniae* = *Klebsiella pneumoniae*; *P aeruginosa* = *Pseudomonas aeruginosa*; *S aureus* = *Staphylococcus aureus*.
* In 2 samples, a second T2Bacteria-targeted organism was identified that was not identified in the companion blood culture. Therefore, 37 organisms were identified in 35 positive samples.
† In 2 samples, 2 T2Bacteria-targeted organisms were identified. Therefore, 64 organisms were identified in 62 positive samples.
‡ In 2 samples, 2 T2Bacteria-targeted organisms were identified. Therefore, 28 organisms were identified in 26 positive samples.
§ In 3 samples, 2 T2Bacteria-targeted organisms were identified. Therefore, 61 organisms were identified in 58 positive samples.

Time to T2Bacteria results depended on the number of samples loaded onto the T2Dx instrument for testing. Mean time was 3.61 hours (SD, 0.2) for 1 sample and 7.70 hours (SD, 1.38) for a full load (7 samples).

Sensitivity of T2Bacteria for proven BSI was 90% (95% CI, 76% to 96%) (Table 2). In 2 samples with positive blood culture and T2Bacteria results, the T2Bacteria result was also positive for a second species that was not identified in the companion blood culture. For 20% (7 of 35) of samples with positive blood culture and T2Bacteria results, patients were receiving an antibiotic that was active in vitro against the bloodstream pathogen at the time of sample collection. Within 24 hours after collection of companion blood culture and T2Bacteria samples, 80% (25 of 31) of patients were receiving an in vitro–effective antibiotic (antibiotic data were not available for 4 patients).

In 10% (4 of 39) of patients with a positive companion blood culture for a T2Bacteria-targeted organism, the T2Bacteria result was negative. The companion blood culture was positive for *S aureus* in 3 of these patients and for *E coli* in the fourth patient. In 2 patients, T2Bacteria retesting of thawed backup samples revealed the bacteria identified in the companion blood culture (*S aureus* and *E coli* [*n* = 1 each]); in the other 2 patients, backup samples also tested negative.

If a negative blood culture was considered the gold standard for absence of BSI caused by a T2Bacteria-targeted organism, the overall per-patient and per-assay specificities were 90% (1242 of 1388) (CI, 88% to 91%) and 98% (6941 of 7096) (CI, 97% to 98%), respectively (Table 2). Specificities for T2Bacteria-targeted organisms ranged from 96% for *E coli* to 98% or higher for other species. The per-patient negative predictive

Table 2. Performance of T2Bacteria for Diagnosis of Proven BSI Caused by Targeted Organisms*

Organism	Positive Blood Culture and T2Bacteria Result, *n*	Positive Blood Culture and Negative T2Bacteria Result, *n*	Negative Blood Culture and Positive T2Bacteria Result, *n*	Negative Blood Culture and T2Bacteria Result, *n*
All	35	4	146	1242
Enterococcus faecium	1	0	9	–
Staphylococcus aureus	13	3	28	–
Klebsiella pneumoniae	6	0	21	–
Pseudomonas aeruginosa	5	0	33	–
Escherichia coli	10	1	63	–

BSI = bloodstream infection.
* Proven BSI was defined as positive companion blood culture for T2Bacteria-targeted bacterium. Per-patient sensitivity was 90% (35 of 39) (95% CI, 76% to 96%), per-patient specificity was 90% (1242 of 1388) (CI, 88% to 91%), per-assay sensitivity was 90% (35 of 39) (CI, 76% to 96%), and per-assay specificity was 98% (6941 of 7096) (CI, 97% to 98%).

value of the T2Bacteria Panel was 99.7% (1242 of 1246).

T2Bacteria results were positive in 10% (146 of 1427) of patients with negative blood cultures for a T2Bacteria-targeted organism. In 5% (7 of 146) of these patients, a second organism was also identified by T2Bacteria. Including the 2 samples mentioned earlier in which T2Bacteria identified a second organism that was not identified in the positive companion blood culture, the distribution of negative blood cultures with a positive T2Bacteria result was 41% (64 of 155) for *E coli*, 21% (33 of 155) for *P aeruginosa*, 18% (28 of 155) for *S aureus*, 14% (21 of 155) for *K pneumoniae*, and 6% (9 of 155) for *E faecium*.

Arbitration of Negative Blood Cultures With a Positive T2Bacteria Result

In 60% (88 of 146) of patients with negative blood culture and positive T2Bacteria results, composite microbiological and clinical criteria for probable (n = 62) or possible (n = 26) BSI were met (Table 3). Seventy-eight percent (69 of 88) of these patients were receiving an active antibiotic against the T2Bacteria-targeted organism at the time of collection of the companion blood culture and T2Bacteria samples, compared with the 20% (7 of 35) of patients, discussed earlier, who had positive blood culture and T2Bacteria results (P < 0.001) (Table 4). The remaining 40% (58 of 146) of patients with negative blood culture and positive T2Bacteria results did not meet criteria for probable or possible BSI, and samples were defined as putative false positives.

In 27% (40 of 146) of patients with probable BSI, negative blood cultures, and positive T2Bacteria results, the same bacterium was recovered from an earlier or subsequent clinical blood culture. In 42% (17 of 40) of these patients, the same organism was also recovered from a nonblood site (Table 3). The median time between a positive clinical blood culture and a sample with a negative companion blood culture and a positive T2Bacteria result was 2 days (range, 7 days before to 19 days after the companion sample) (Appendix Figure 1, available at Annals.org).

Another 15% (22 of 146) of patients with probable BSI, negative companion blood cultures, and positive T2Bacteria results did not have a positive clinical blood culture at a different time but did have the same bacterium recovered from cultures at a nonblood site (Table 3). The median time between a positive nonblood culture and a sample with a negative companion blood culture and a positive T2Bacteria result was 1 day (range, 10 days before to 6 days after the companion sample) (Appendix Figure 2, available at Annals.org).

If probable BSIs were assumed to be true positives that were missed by blood culture, per-patient specificity of the T2Bacteria Panel was 94% (1242 of 1326). This

Table 3. Detailed Descriptions of Negative Blood Cultures With Positive T2Bacteria Results, by BSI Classification

Variable	Samples in Which T2Bacteria-Targeted Organisms Were Identified, *n*					
	Enterococcus faecium	***Staphylococcus aureus***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Escherichia coli***	**Total**
Probable BSI						
Site of positive cultures for T2Bacteria-targeted organism						
Clinical blood culture	2	17	6	4	11	40
Nonblood culture	3	5	3	5	8	24
Lung	0	1	1	3	0	5
Wound/abscess	3	2	0	0	3	8
Urine	0	0	2	2	5	9
Tendon/bone	0	1	0	0	0	1
Multiple	0	1	0	0	0	1
Total	5	22	9	9	19	64*
Possible BSI						
Clinical syndrome						
Intra-abdominal processes	1	0	2	1	4	8
Pneumonia	0	1	1	2	3	7
Pancreatobiliary process	0	0	0	0	3	3
Pyelonephritis/urinary tract infection	0	0	1	0	1	2
Soft tissue and bone infection	0	0	0	1	2	3
Neutropenic fever	0	0	0	0	2	2
Sepsis	0	0	1	1	0	2
Central line–associated bacteremia	0	1	0	0	0	1
Total	1	2	5	5	15	28†
Putative false-positive results on T2Bacteria samples	3	4	7	19	28	61‡

BSI = bloodstream infection.
* Identified in 62 positive T2Bacteria samples. In 40 patients, the T2Bacteria-targeted organism was identified in a clinical blood culture. In 22 patients, the T2Bacteria-targeted organism was identified in a nonblood culture. Twenty-four organisms were identified in the 22 positive samples from these patients.
† Identified in 26 positive T2Bacteria samples.
‡ Identified in 58 positive T2Bacteria samples.

Table 4. Antibiotic Treatment at the Time of Collection of Companion Blood Culture and T2Bacteria Samples Among Patients With Positive T2Bacteria Results

Variable	Proven BSI	Probable BSI		Possible BSI	Putative False-Positive T2Bacteria Result
		Positive Clinical Blood Culture	Positive Nonblood Culture		
Positive companion blood culture and T2Bacteria result, *n*	35	40	22	26	58
Receiving any antibiotic, *n/N (%)*	11/35 (31)	37/40 (92.5)	16/22 (73)	22/26 (85)	33/58 (59)
Receiving active antibiotic, *n/N (%)*	7/35 (20)	29/40 (72.5)	13/22 (59)	19/26 (73)	24/58 (41)

BSI = bloodstream infection.

increased to 96% (1242 of 1300) if both probable and possible BSIs were assumed to be true-positives.

DISCUSSION

The T2Bacteria Panel is the first direct-from-blood, nonculture test cleared by the FDA for diagnosis of BSI caused by multiple bacteria. In this multicenter study of patients for whom blood cultures were ordered as standard of care, T2Bacteria showed sensitivity and specificity of 90% in identifying BSIs caused by 5 common bacterial pathogens. Specificity increased to 94% if probable BSIs were considered as true positives that were not identified by companion blood cultures and to 96% if both probable and possible BSIs were considered as true positives. The negative predictive value in this large cohort approached 100%. Time from initiation of testing to detection and identification of pathogens was shorter for T2Bacteria (mean, 3.61 [SD, 0.2] to 7.70 [SD, 1.38] hours, depending on how many samples were loaded for testing) than for blood cultures (means, 38.5 [SD, 32.8] and 71.7 [SD, 39.3] hours, respectively). Taken together, the data suggest that T2Bacteria may improve management of BSI and sepsis by providing results more rapidly than blood cultures and identifying some pathogens that are missed by blood cultures.

As in all studies of nonculture diagnostic tests for BSI (8), a central question is whether negative blood cultures with positive T2Bacteria results were false positives or true positives missed by blood cultures. Such results were observed in 10% of samples, 60% of which were associated with probable or possible BSI, based on a composite of clinical findings, blood culture results at other times, or culture results from nonblood sites. In 78% of probable or possible BSIs, patients had negative blood cultures and positive T2Bacteria results despite treatment with active antibiotics. In contrast, only 20% of patients with positive blood cultures and T2Bacteria results were receiving active antibiotics. Therefore, T2Bacteria may have particular value over blood cultures in patients who are already receiving antimicrobial therapy.

A positive T2Bacteria result with a negative blood culture could be explained by the presence of nonviable, nonproliferating, or latent bacteria; intracellular organisms within circulating phagocytic cells; inhibition of bacterial growth by antibiotics; or contamination (8, 15). Bacterial DNA may persist after bloodstream sterilization in cases of transient or intermittent bacteremia. Transient bacteremia generally stems from short-term disturbances of colonized or infected mucosal surfaces and tissue sites (as may occur during medical procedures), whereas intermittent bacteremia is often seen with closed-space infections (such as abscesses) or focal infections (such as pneumonia and osteomyelitis) (16, 17). Transient or intermittent bacteremia may account for cases in which negative blood culture and positive T2Bacteria results are obtained but clinical blood cultures at other time points or nonblood cultures are positive. In some cases of continuous BSI, microbial concentrations may be too low for reliable detection by blood culture but are sufficiently high for detection by a target amplification-based test, such as the T2Bacteria Panel (18, 19). It is noteworthy that T2Bacteria results were positive in 3.5-fold more patients with proven, probable, or possible BSIs (n = 123) compared with blood cultures (n = 35).

Results were defined as putative false positives for the 40% of samples that had negative blood culture and positive T2Bacteria results and did not meet criteria for probable or possible BSI. However, because there was enough suspicion of BSI or sepsis for clinicians to order a diagnostic blood culture in all patients, it is possible that at least some putative false-positive results were true positives. Fifty-seven percent (33 of 58) of patients with putative false-positive T2Bacteria results were receiving antibiotics at the time of sample collection, indicating significant concerns about active infection. The T2Dx platform is a closed system, and the T2Bacteria Panel targets bacterial cell–associated DNA rather than free DNA; these properties are designed to minimize contamination and false positivity compared with polymerase chain reaction assays (15). Of note, only 0.4% (4 of 1427) of samples had positive blood culture and negative T2Bacteria results. For 2 of these cases, T2Bacteria showed a positive result on a backup sample. It is likely that low circulating bacterial DNA concentrations in false-negative T2Bacteria samples were below the limit of detection of the assay. As is the practice with collection of blood culture specimens, additional samples may improve sensitivity of the T2Bacteria Panel.

This study was limited by the low prevalence (3% [39 of 1427]) of positive blood cultures for T2Bacteria-targeted organisms and that only 1 set of aerobic and anaerobic specimens for the companion blood culture

was collected. A second set of companion cultures may have identified more patients with proven BSIs caused by T2Bacteria-targeted organisms. Because T2Bacteria results were not available to health care teams, we cannot assess their effect on treatment decisions or patient outcomes. Finally, T2Bacteria is limited to detection of 5 bacterial species. T2Bacteria samples should always be collected in conjunction with blood culture specimens because cultures identify bacteria not included in the panel and yield organisms for phenotypic antibiotic susceptibility testing.

A MEDLINE search of articles published in English was conducted on 24 February 2019 using the terms *T2Bacteria*, *T2 magnetic resonance diagnostic(s)*, *T2MR diagnostic(s)*, and *T2Candida*. The search revealed only 1 study that evaluated the performance of the T2Bacteria Panel in diagnosing suspected BSI (14). The study included 140 samples from 129 adult patients in the emergency department, infectious diseases unit, or intensive care unit at a hospital in Italy. The investigators used a research prototype of the T2Bacteria Panel, which detected the 5 bacteria in our study and *Acinetobacter baumannii*. Three percent of results were invalid compared with 0.3% in our study, which may reflect use of a different version of the assay. Bacteria targeted by T2Bacteria accounted for about 50% of organisms recovered from companion blood cultures in both studies. In the previous study, T2Bacteria results were positive for a target bacterium in 16% of samples; sensitivity and specificity were 83% and 98%, respectively, for proven BSIs caused by target bacteria. The mean time to detection or species identification was 5.5 hours (SD, 1.4), which was significantly shorter than corresponding times for blood cultures. Therefore, performance of the T2Bacteria Panel was similar in both studies.

In conclusion, the T2Bacteria Panel accurately identified or excluded BSIs caused by 5 common ESKAPE pathogens in about 4 to 8 hours versus about 24 to 72 hours and 5 days, respectively, for blood cultures. Overall, T2Bacteria results correlated well with those from blood cultures. T2Bacteria will most likely be useful if it is used in conjunction with blood and nonblood cultures and results are interpreted with careful consideration of patients' clinical status and antibiotic use. To date, T2Bacteria and T2Candida are the only culture-independent tests cleared by the FDA for direct detection of multiple bacteria and fungi in whole blood. We hope the promising performance and FDA clearance of these tests will encourage continued investment, research, and development in this area of pressing medical need. Now that performance characteristics of both panels have been established in multicenter clinical trials (20), a top priority is to define their precise roles in clinical practice and their effect on patient outcomes.

From University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania (M.H.N., A.W.P., M.N.W.); University of Pittsburgh, Pittsburgh, Pennsylvania (C.J.C.); University of Alabama at Birmingham, Birmingham, Alabama (P.G.P.); Henry Ford Health System, Detroit, Michigan (G.A.); Ochsner Health System, New Orleans, Louisiana (G.A.P.); Indiana University School of Medicine, Indianapolis, Indiana (B.H.S.); Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey (A.R., M.P.W.); Tampa General Hospital, Tampa, Florida (R.W.); Geisinger Health, Danville, Pennsylvania (D.R.H., D.M.W.); Weill Cornell Medicine of Cornell University, NewYork–Presbyterian Hospital, New York, New York (T.J.W.); Duke University, Durham, North Carolina (J.R.P.); University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and Alpert Medical School of Brown University, Providence, Rhode Island (E.M.).

Acknowledgment: The authors thank the patients who participated in this study; the research staff (Debbie Ashcraft, Kailey Hughes, Theresa Lingg, Ines Hamilton, Fei Chen, Amanda Styer, Michail Alevizakos, Styliani Karanika, and Myrto E. Flokas), who assisted with recruitment and review of medical records; and Drs. Tom Lowery and Joseph E. Marturano for helpful discussions.

Financial Support: By T2 Biosystems.

Disclosures: Dr. Clancy reports other support from T2 Biosystems during the conduct of the study. Dr. Schmitt reports nonfinancial support from T2 Biosystems during the conduct of the study and personal fees from T2 Biosystems outside the submitted work. Dr. Weinstein reports other support from T2 Biosystems during the conduct of the study. Dr. Wolk reports grants from Cepheid, OpGen, Streck, GenMark, La Jolla Pharmaceutical Company, Accelerate Diagnostics, DiaSorin, and Roche and other support from OpGen, Streck, DiaSorin, and Safeguard outside the submitted work. Dr. Perfect reports grants from Pfizer, Merck, Minnetronix, Amplyx, and Mayne; nonfinancial support from Astellas Pharma; and other support from Astellas Pharma, Merck, Viamet, Minnetronix, F2G, Amplyx, Vical, Matinas, and Cidara during the conduct of the study. Authors not named here have disclosed no conflicts of interest. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M18-2772.

Reproducible Research Statement: *Study protocol:* See the **Supplement** (available at Annals.org). *Statistical code:* Available from Dr. Nguyen (e-mail, mhn5@pitt.edu). *Data set:* Relevant data are provided in the tables, figures, and appendix materials. More details are available from Dr. Nguyen (e-mail, mhn5@pitt.edu).

Corresponding Author: M. Hong Nguyen, MD, University of Pittsburgh School of Medicine, 3550 Terrace Street, Suite 871, Pittsburgh, PA 15261; e-mail, mhn5@pitt.edu.

Current author addresses and author contributions are available at Annals.org.

References

1. **Cohen J, Vincent JL, Adhikari NK, Machado FR, Angus DC, Calandra T, et al.** Sepsis: a roadmap for future research. Lancet Infect Dis. 2015;15:581-614. [PMID: 25932591] doi:10.1016/S1473-3099(15)70112-X

2. **Lee CC, Lee CH, Hong MY, Tang HJ, Ko WC.** Timing of appropriate empirical antimicrobial administration and outcome of adults

with community-onset bacteremia. Crit Care. 2017;21:119. [PMID: 28545484] doi:10.1186/s13054-017-1696-z
3. **Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al.** Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34:1589-96. [PMID: 16625125]
4. **Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al.** Assessment of clinical criteria for sepsis: for the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315:762-74. [PMID: 26903335] doi:10.1001/jama.2016.0288
5. **Murray J, Brent A.** Responding to NICE - developing a regional sepsis pathway [Letter]. Clin Med (Lond). 2018;18:263-4. [PMID: 29858442] doi:10.7861/clinmedicine.18-3-263
6. **Angus DC.** Early, goal-directed therapy for septic shock - a patient-level meta-analysis [Letter]. N Engl J Med. 2017;377:995. [PMID: 28877020] doi:10.1056/NEJMc1708979
7. **Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L.** Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. Antimicrob Agents Chemother. 2010;54:4851-63. [PMID: 20733044] doi:10.1128/AAC.00627-10
8. **Clancy CJ, Nguyen MH.** T2 magnetic resonance for the diagnosis of bloodstream infections: charting a path forward. J Antimicrob Chemother. 2018;73:iv2-iv5. [PMID: 29608754] doi:10.1093/jac/dky050
9. **Opota O, Jaton K, Greub G.** Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Clin Microbiol Infect. 2015;21:323-31. [PMID: 25686695] doi:10.1016/j.cmi.2015.02.005
10. **Bissonnette L, Bergeron MG.** Diagnosing infections–current and anticipated technologies for point-of-care diagnostics and home-based testing. Clin Microbiol Infect. 2010;16:1044-53. [PMID: 20670286] doi:10.1111/j.1469-0691.2010.03282.x
11. **Karchmer AW.** Nosocomial bloodstream infections: organisms, risk factors, and implications. Clin Infect Dis. 2000;31 Suppl 4:S139-43. [PMID: 11017863]
12. **Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB.** Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309-17. [PMID: 15306996]
13. **Rice LB.** Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE [Editorial]. J Infect Dis. 2008;197:1079-81. [PMID: 18419525] doi:10.1086/533452
14. **De Angelis G, Posteraro B, De Carolis E, Menchinelli G, Franceschi F, Tumbarello M, et al.** T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood. J Antimicrob Chemother. 2018;73:iv20-iv26. [PMID: 29608753] doi:10.1093/jac/dky049
15. **Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D, et al.** T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med. 2013;5:182ra54. [PMID: 23616121] doi:10.1126/scitranslmed.3005377
16. **Reimer LG, Wilson ML, Weinstein MP.** Update on detection of bacteremia and fungemia. Clin Microbiol Rev. 1997;10:444-65. [PMID: 9227861]
17. **Seifert H.** The clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. Clin Infect Dis. 2009;48 Suppl 4:S238-45. [PMID: 19374579] doi:10.1086/598188
18. Classics in infectious diseases. Observations on the sites of removal of bacteria from the blood of patients with bacterial endocarditis. By Paul B. Beeson, Emmett S. Brannon, and James V. Warren. Rev Infect Dis. 1985;7:565-73. [PMID: 3898311]
19. **Beeson PB, Brannon ES, Warren JV.** Observations on the sites of removal of bacteria from the blood in patients with bacterial endocarditis. J Exp Med. 1945;81:9-23. [PMID: 19871447]
20. **Clancy CJ, Pappas PG, Vazquez J, Judson MA, Kontoyiannis DP, Thompson GR 3rd, et al.** Detecting Infections Rapidly and Easily for Candidemia Trial, Part 2 (DIRECT2): a prospective, multicenter study of the T2Candida panel. Clin Infect Dis. 2018;66:1678-86. [PMID: 29438475] doi:10.1093/cid/cix1095

Current Author Addresses: Drs. Nguyen and Clancy: University of Pittsburgh School of Medicine, 3550 Terrace Street, Suite 871, Pittsburgh, PA 15261.
Dr. Pasculle and Ms. Wilson: Microbiology Laboratory, UPMC Presbyterian, 3477 Euler Way, Pittsburgh, PA 15213.
Dr. Pappas: University of Alabama at Birmingham, 1900 University Boulevard, 229 THT, Birmingham, AL 35294-0006.
Dr. Alangaden: Henry Ford Hospital, 2799 West Grand Boulevard, CFP 316, Detroit, MI 48202.
Dr. Pankey: Ochsner Health System, 1514 Jefferson Highway, New Orleans, LA 70121.
Dr. Schmitt: Indiana University School of Medicine, 350 West 11th Street, Room 6027D, Indianapolis, IN 46202.
Drs. Rasool and Weinstein: Rutgers Robert Wood Johnson Medical School, 125 Patterson Street, New Brunswick, NJ 08903.
Drs. Hernandez and Wolk: Geisinger Health, 100 North Academy Avenue, Danville, PA 17822.
Dr. Walsh: Weill Cornell Medicine of Cornell University, NewYork–Presbyterian Hospital, 1300 York Avenue, Room A-421, New York, NY 10065.
Dr. Perfect: Duke University Medical Center, Hanes House, Trent Drive, Durham, NC 27710.
Dr. Mylonakis: Division of Infectious Diseases, Rhode Island Hospital, 593 Eddy Street, Physicians Office Building, 3rd Floor, Suite 328/330, Providence, RI 02903.

Author Contributions: Conception and design: M.H. Nguyen, C.J. Clancy, P.G. Pappas, R. Widen, D. Wolk.
Analysis and interpretation of the data: M.H. Nguyen, C.J. Clancy, A.W. Pasculle, G. Alangaden, G.A. Pankey, B.H. Schmitt, A. Rasool, M.P. Weinstein, D.R. Hernandez, J.R. Perfect, E. Mylonakis.
Drafting of the article: M.H. Nguyen, C.J. Clancy, G. Alangaden, G.A. Pankey, B.H. Schmitt, E. Mylonakis.
Critical revision of the article for important intellectual content: M.H. Nguyen, C.J. Clancy, P.G. Pappas, B.H. Schmitt, M.P. Weinstein, D.R. Hernandez, T.J. Walsh, E. Mylonakis.
Final approval of the article: M.H. Nguyen, C.J. Clancy, A.W. Pasculle, P.G. Pappas, G. Alangaden, G.A. Pankey, B.H. Schmitt, A. Rasool, M.P. Weinstein, D.R. Hernandez, D.M. Wolk, T.J. Walsh, J.R. Perfect, M.N. Wilson, E. Mylonakis.
Provision of study materials or patients: M.H. Nguyen, A.W. Pasculle, P.G. Pappas, G. Alangaden, G.A. Pankey, B.H. Schmitt, M.P. Weinstein, R. Widen, D.R. Hernandez, E. Mylonakis.
Statistical expertise: M.H. Nguyen, C.J. Clancy.
Administrative, technical, or logistic support: A.W. Pasculle, R. Widen, D.R. Hernandez, E. Mylonakis.
Collection and assembly of data: M.H. Nguyen, A.W. Pasculle, G.A. Pankey, B.H. Schmitt, A. Rasool, M.P. Weinstein, R. Widen, D.R. Hernandez, M.N. Wilson, E. Mylonakis.

Appendix Table 1. Description of the 11 Medical Centers Involved in the Study

Name	City	Description
Duke University Hospital	Durham, North Carolina	Tertiary, 988-bed acute care teaching hospital at Duke University School of Medicine
Geisinger Health System	Danville, Pennsylvania	Tertiary, 574-bed acute care teaching hospital at Geisinger Commonwealth School of Medicine
Henry Ford Hospital	Detroit, Michigan	Tertiary, 877-bed acute care hospital
Miriam Hospital	Providence, Rhode Island	Community/tertiary, 250-bed acute care teaching hospital affiliated with the Warren Alpert Medical School of Brown University
NewYork–Presbyterian Hospital	New York, New York	Tertiary, 2478-bed acute care teaching hospital at Weill Cornell Medicine of Cornell University
Ochsner Health System	New Orleans, Louisiana	Tertiary, 767-bed acute care teaching hospital
Rhode Island Hospital	Providence, Rhode Island	Tertiary, 719-bed acute care teaching hospital at the Warren Alpert Medical School of Brown University
Robert Wood Johnson University Hospital	New Brunswick, New Jersey	Tertiary, 620-bed acute care teaching hospital at Rutgers Robert Wood Johnson Medical School
Sidney and Lois Eskenazi Hospital	Indianapolis, Indiana	Community, 315-bed acute care teaching hospital
University of Alabama Hospital	Birmingham, Alabama	Tertiary, 1157-bed acute care teaching hospital at the University of Alabama at Birmingham
University of Pittsburgh Medical Center	Pittsburgh, Pennsylvania	Tertiary, 954-bed acute care teaching hospital at the University of Pittsburgh

Appendix Table 2. Limit of Detection for Each Bacterium Included in the T2Bacteria Panel

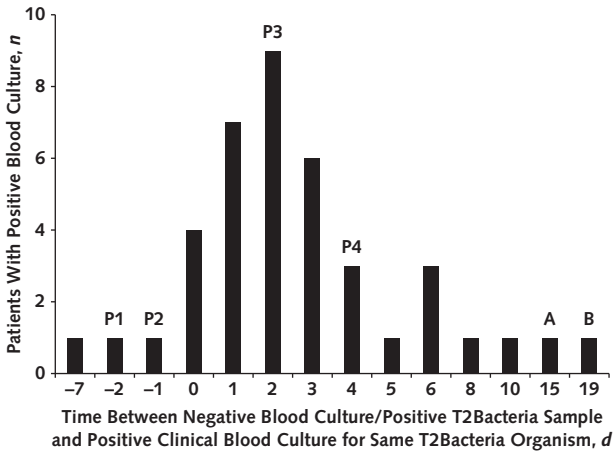
Organism	Limit of Detection, *CFU/mL*
Enterococcus faecium	5
Staphylococcus aureus	2
Klebsiella pneumoniae	2
Pseudomonas aeruginosa	5
Escherichia coli	11

Appendix Table 3. Bacterial Species Identified by Blood Cultures and T2Bacteria Panel

Type of Organism	Positive Blood Cultures, *n**	Positive T2Bacteria Samples, *n*†
T2Bacteria-targeted		
Enterococcus faecium	1	1
Staphylococcus aureus	16	12
Klebsiella pneumoniae	6	5
Pseudomonas aeruginosa	5	5
Escherichia coli	11	10
K pneumoniae and *E coli*	NA	1‡
S aureus and *E coli*	NA	1§
Other *Staphylococcus* and *Enterococcus* species and *Streptococcus* species		
Coagulase-negative *Staphylococcus*	18	NA
Streptococcus species		
Streptococcus, group A	1	NA
Streptococcus anginosus	1	NA
Streptococcus species	1	NA
Enterococcus faecalis	5	NA
Other Gram-negative bacilli		
Serratia marcescens	1	NA
Klebsiella oxytoca	2	NA
Citrobacter freundii complex	2	NA
Yeasts		
Candida dubliniensis	1	NA
Cryptococcus species	1	NA
Others		
Actinomyces odontolyticus	1	NA
Bacteroides ovatus	1	NA
Clostridium innocuum	1	NA
Fusobacterium nucleatum	1	NA
Diphtheroids	2	NA
Propionibacterium acnes	1	NA
Corynebacterium species	1	NA
Polymicrobial		
Coagulase-negative *Staphylococcus* and diphtheroids	1	NA
Coagulase-negative *Staphylococcus* (3 species)	1	NA

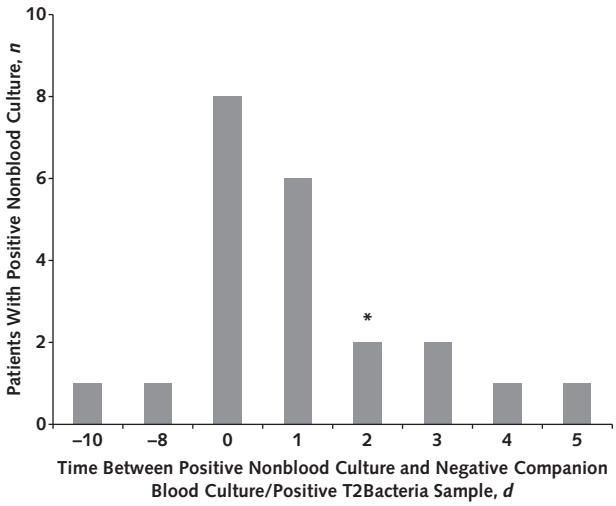
NA = not applicable.
* 39 blood cultures were positive for a T2Bacteria-targeted organism. In each of these, a single bacterial species was recovered.
† 35 T2Bacteria samples were positive for the same organism that was identified in the companion blood culture. In 2 of these, T2Bacteria detected 2 species, for a total of 37 organisms in the T2Bacteria samples.
‡ Companion blood culture was positive for *K pneumoniae*.
§ Companion blood culture was positive for *S aureus*.

Appendix Figure 1. Time between negative companion blood culture/positive T2Bacteria sample and positive clinical blood culture in 40 patients with probable bloodstream infection.



Four patients had positive clinical blood cultures with specimens that were collected both before and after the negative companion blood culture/positive T2Bacteria sample. Of note, 42.5% (17 of 40) of patients had the same T2Bacteria-targeted organism recovered from a nonblood culture and a clinical blood culture. P1, P2, P3, and P4 also had positive clinical blood cultures on days 7, 7, 2, and 11, respectively. Ninety-five percent (38 of 40) of patients had positive clinical blood cultures with specimens that were collected within 10 d of a negative companion blood culture/positive T2Bacteria sample. One patient (*A*) had a positive clinical blood culture for *Pseudomonas aeruginosa* with a specimen that was collected 15 d after a negative companion blood culture/positive T2Bacteria sample. The patient had persistent endovascular *P aeruginosa* infection and had recurrent *P aeruginosa* bacteremia therapy was discontinued after 42 d of therapy was discontinued. The second patient (*B*) had a positive clinical blood culture for *Staphylococcus aureus* with a specimen that was collected 19 d after a negative companion blood culture/positive T2Bacteria sample. She had disseminated *S aureus* infection, empyema, and pulmonary abscesses; a follow-up chest radiograph 17 d after therapy revealed persistent lung nodules and abscesses. Antistaphylococcal therapy was extended to 6 wk. P = patient.

Appendix Figure 2. Time between negative companion blood culture/positive T2Bacteria sample and positive nonblood culture for the T2Bacteria-identified organism in 22 patients.



Clinical blood cultures for these patients were negative.
* Patient had an additional positive nonblood culture on day 6.