Rapid Rule Out of Culture-Negative Bloodstream Infections by Use of a Novel Approach to Universal Detection of Bacteria and Fungi

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Background: Currently it can take up to 5 days to rule out bloodstream infection. With the low yield of blood cultures (approximately 10%), a significant number of patients are potentially exposed to inappropriate therapy that can lead to adverse events. More rapid rule out can accelerate deescalation or cessation of antimicrobial therapy, improving patient outcomes.

Methods: A method is described, termed enzymatic template generation and amplification (ETGA), that universally and sensitively detects DNA polymerase activity liberated from viable bacteria and fungi isolated from blood culture samples as a measure of bloodstream infection. ETGA was applied in a diagnostic test format to identify negative blood cultures after an overnight incubation. Performance data for a prototype (Cognitor) and automated (Magnitor) version of the test are presented.

Results: The Cognitor manual assay displayed analytical reactivity for a panel of the 20 most prevalent causes of bloodstream infection, with a detection range of 28–9050 CFU/mL. Validation with 1457 clinical blood cultures showed a negative predictive value of 99.0% compared to blood culture incubation for 5 days. Magnitor showed an improved detection range of 1–67 CFU/mL, allowing for detection of bacteria-supplemented blood cultures after 2–8 h incubation, and *Candida albicans*-supplemented blood cultures at 16–22 h, 5–15 h faster than blood culture. Removing an aliquot from a blood culture bottle and replacing the bottle into the incubator was shown not to result in contaminating organisms being introduced.

Conclusions: The described method displays excellent breadth and detection for microbial cells and demonstrates the capability of confirming negative blood cultures after an overnight incubation in a blood culture instrument.

IMPACT STATEMENT

Antibiotics are widely prescribed for patient populations suspected of having bacterial infections. Earlier deescalation or cessation of therapy in culture-negative patients should limit adverse effects including development of antibiotic-related *Clostridium difficile* or multidrug resistant pathogen infections generally, and specifically neonates suspected of sepsis may show lowered rates of mortality and necrotizing enterocolitis. Herein, a method is described that can determine blood culture negativity with high negative predictive value in <24 h, which is 1–4 days faster than current standards of care. This approach will be potentially useful to guide stewardship efforts to direct antimicrobial therapy appropriately.

Overuse of antibiotics has contributed to a rapid rise of antibiotic-resistant pathogens (*1*, *2*). In addition, considering antibiotic toxicity (*3*, *4*) and poorer outcomes for inappropriate therapy (*5*), these problems indicate a need for tests to support antimicrobial decision-making. Rapid diagnostic techniques such as PCR-based pathogen identification have been linked to lowered treatment costs and shortened hospitalizations (*6*, *7*). In this study we investigate a test to address the neglected opportunity for earlier confirmation of culture-negative samples as an aid in deciding on deescalation or cessation of therapy (*8*, *9*).

For patients at risk of bloodstream infection, blood drawn into a blood culture bottle is incubated for 5 days to determine if any microbes are present. Of these samples, 90% are negative, and patients remain on antibiotics unnecessarily. Moreover, supportive diagnostic tests are lacking that could add confidence to a clinical decision that antibiotics are no longer required.

A diagnostic test to rule out all potential bacterial and fungal causes of a bloodstream infection must perform with a very high negative predictive value. Currently, there is no rapid approach that can do this; for example, PCR-based diagnostic panels are limited by the capabilities of multiplex primer combinations, with no detection coverage beyond the most prevalent pathogens (*10*, *11*, *12*, *13*). There has been some success in detection and rule out using biomarkers that are upregulated in host response to severe bacterial infection, particularly C-reactive protein and procalcitonin (*14*, *15*). C-reactive protein, however, is upregulated in response to inflammatory events generally, so specificity is poor (*16*). Procalcitonin is more specific to bacterial infection, but it can respond to trauma and treatment with immune stimulating cytokines and is not reliable in neonates (*17*).

Active DNA polymerase is present in all viable cells (*18*). This presence allows for an approach to the universal detection of bacteria and fungi from any normally sterile, acellular fluid such as cerebrospinal fluid or synovial fluid, as a measure of infection. For diagnosing bloodstream infection, human cells are present that create a high background of DNA polymerase activity. As described previously and herein, an approach has been developed for detection of DNA polymerase activity specifically from pathogenic bacteria and fungi in bloodstream infection, termed enzymatic template generation and amplification (ETGA)[3] (*19*, *20*). While this approach provides for universal detection of bacteria and fungi, it cannot determine the identity of the infecting organism, limiting the diagnostic utility for positive blood samples. The benefit of ETGA as a sensitive and universal detection method lies in ruling out bloodstream infection sooner than the current practice of incubating blood cultures for 2–5 days. Herein, we describe application of the ETGA approach to rapidly confirm negative blood cultures, without interference from human cells, dead organisms or extraneous DNA. A prototype manual test, termed Cognitor, was developed to confirm negative blood cultures after overnight incubation in a continuously monitored blood culture instrument. Furthermore, an automated format of this test, termed Magnitor, is presented with improved performance over Cognitor, allowing for even shorter incubation time. The goal is to create an objective tool aiding stewardship efforts to deescalate or cease antibiotic

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[3] **Nonstandard abbreviations:** ETGA, enzymatic template generation and amplification; CFU, colony forming unit; IPC, internal positive control; NPV, negative predictive value; ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species; HACEK, *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* species.

therapy in patients whose blood culture samples are negative.

MATERIALS AND METHODS

Cognitor protocol

The Cognitor test was carried out according to the manufacturer's instructions for use; 0.5 mL of blood culture was removed from each bottle as appropriate by use of a 3-mL syringe and a swabable vial adaptor (Medimop) and combined in a separate microcentrifuge tube. The combined specimen was then mixed with 0.33 mL of Cognitor Reagent A (Momentum Bioscience Ltd), inverted 5 times, and then incubated for 15 min. Specimens were centrifuged (7300*g* for 3 min) to pellet any microorganisms that may be present, then resuspended in 750 µL of Reagent B (Momentum) with pipette mixing. After incubation at room temperature for 5 min, 500 µL of Reagent C (Momentum) was added and mixed by inversion 3 times. Samples were centrifuged (7300*g*, 3 min) again, resuspended in 500 µL of Reagent C and transferred to a bead-milling tube containing glass beads. Centrifugation was performed to pellet any microbial cells along with the glass beads; then the supernatant was carefully removed without disturbing the glass beads, and 50 µL of microbial lysis mixture (Momentum) was added. Lysis mixture also contains the ETGA substrate and an internal positive control (IPC) oligonucleotide. Sample tubes were moved to the Disruptor Genie (Scientific Industries) and milled for 6 min at 2800 rpm to mechanically break open microbial cells.

After milling, sample tubes were moved to a 37 °C heating block for 20 min to allow microbial DNA polymerases, if present, to act upon the ETGA substrate and then moved to a separate heating block set to 95 °C for 5 min. After incubation, samples were cooled at room temperature for 1 min.

Finally, 3 µL of sample was added to 27 µL of real-time PCR (qPCR) mastermix (MBL Reagent Pack 2) and placed in the Cepheid Smartcycler to perform the qPCR reaction. The mastermix contained all primers and hybridization probes for the ETGA substrate and IPC and uracil deglycosylase for the inactivation of the ETGA template strand. Cycling was carried out as follows: 10 min at 40 °C, 10 min 50 °C (to allow uracil deglycosylase enzyme to act on the ETGA substrate), 5 min at 95 °C, and then PCR cycling at 95 °C, 61 °C and 72 °C for 5 s, 20 s, and 20 s, respectively, for 45 cycles.

Presumptive positive samples, termed "not-negative," were identified as having a quantitation cycle value below a set positive threshold value (set based on the lowest quantitation cycle value for a series of negative blood specimens). Negative samples were identified as having a quantitation cycle value greater than the positive threshold and amplification of the IPC.

Magnitor protocol

The Cognitor manual test was automated ("Magnitor") by use of an off-the-shelf pipetting robotic system. Briefly, the protocol for Magnitor is described below. A 1-mL sample of blood culture was mixed and incubated for 30 min with microbial binding buffer and magnetic beads. During this period, the microbial binding buffer preferentially lyses the blood cells while facilitating the binding of the microbes to the magnetic beads. After microbial binding, the beads were magnetized to allow the blood culture supernatant to be removed. The sample was washed (1 mL) in a nondenaturing wash solution. After the wash step, the beads, containing the bound microorganisms, were resuspended in a lysis buffer containing a mixture of lytic enzymes to permeabilize the microbes and to liberate the DNA polymerase enzyme. The sample is then incubated to allow the microbial DNA polymerases, if present, to act upon the ETGA substrate. After incubation, 1 µL of sample was added to 9 µL of qPCR mastermix to

perform the qPCR reaction. As with Cognitor, the mastermix contained all the primers and hybridization probes for the ETGA substrate and IPC and the uracil deglycosylase for the inactivation of the ETGA template strand. The qPCR cycling was the same as described above.

Cognitor clinical study protocol

The Cognitor clinical study was carried out at the Department of Microbiology, Royal Hampshire County Hospital, Winchester, and Basingstoke and North Hampshire Hospital, Basingstoke, UK (Hampshire Hospitals NHS Foundation Trust). The study protocol was cleared by the Trust research governance. Clinical specimens for Cognitor testing were obtained from routine blood culture in bioMérieux BacT/ALERT SA and SN blood culture media that remained negative after incubation for more than 12 h in the bioMérieux BacT/ALERT® 3D Microbial detection system. The Cognitor test was carried out as described.

In total, Cognitor results from 1457 samples were compared to results from automated blood culture after 5 days. Any false-negative results (as compared to blood culture) were further arbitrated by the clinical chief investigator to obtain assessment on whether the organism found in the blood culture was likely to be clinically significant or a contaminant. False-positive results were also assessed by the chief investigator to determine the likelihood of an infection undetected by blood culture.

Magnitor vs blood culture time-to-detection comparison

Aerobic blood culture bottles (bioMérieux BacT/ALERT SA), each containing 10 mL of sterile human blood, were supplemented at approximately 0.1–1.5 CFU/mL for the ESKAPE pathogens and *Escherichia coli*. *Candida albicans* was supplemented with approximately 1–9 CFU/mL. All bottles were incubated in the bioMérieux BacT/ALERT 3D Microbial detection system. Total viable counts were taken to verify the supplement concentration for each organism. At timepoint intervals of 0 h, 1 h, 2 h, 4 h, 6 h, and 8 h, the "sampled" bottles were removed from the blood culture instrument and a 1 mL sample was removed, as per the Magnitor protocol, before being returned to the incubator until the next timepoint. Because of the slow growth characteristics of *C. albicans*, additional timepoints of 16 h, 20 h, 22 h, 24 h, and 48 h were included. Matched "unsampled" bottles were incubated on the blood culture instrument until alarm positivity.

All samples were processed according to the automated Magnitor protocol. Time-to-detection was recorded for the Magnitor-processed samples and for the matched samples on the blood culture instrument. Data were analyzed by Student's *t*-test and analysis of variance.

Contamination study

Over the course of 3 separate experiments, 718 blood culture bottles (approximately half aerobic and half anaerobic; bioMérieux BacT/ALERT SA and SN), each containing 10 mL of sterile sheep blood (TCS Biosciences), were incubated in the bioMerieux BacT/ALERT 3D Microbial detection system. After 16 h, 1 mL of blood culture was aseptically removed (in a class II microbiological safety cabinet) from half of the aerobic bottle set and half of the anaerobic bottle set. Bottles were returned to the blood culture system and continued incubation for the remainder of the 5 days.

At the end of the incubation period, blood culture results for the sampled and unsampled sets were compared by Fisher's exact test.

RESULTS

Early confirmation of negative blood cultures

To illustrate how ETGA works for detection of DNA polymerase from microbes with the more

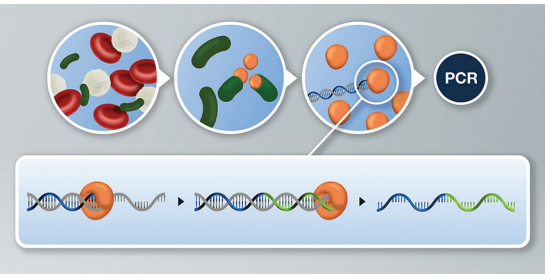

Fig. 1. Diagram showing the principle of ETGA. Red and white circles represent human cells. Green rods represent microbial cells. Brown circles represent DNA polymerase molecules. Human blood cells are lysed, and residual polymerase activity inactivated. Microbial cells are captured and concentrated by magnetic beads and then lysed to release contents. A synthetic DNA substrate is added with the lysis reagent, which is extended by microbial DNA polymerase to create a template for real-time PCR (qPCR). Amplification of the target substrate indicates the presence of living microorganisms.

recent Magnitor procedure, a schematic diagram is shown in Fig. 1. The Cognitor process is similar but uses centrifugation instead of magnetizable particles for microbial isolation. Eukaryotic cells in human blood are lysed by detergent, and residual enzymatic activity in the supernatant is inactivated. Microbial cells are left intact and are then captured and concentrated by magnetic beads. The supernatant is removed, and a microbial lysis mixture is added, releasing cellular contents including DNA polymerase. A synthetic DNA substrate is added and is extended by microbial DNA polymerase to create a template for qPCR only if a living organism was present in the blood culture sample. The resulting mixture is added to a qPCR reaction containing a primer pair specific for the DNA polymerase-extended substrate. Amplification of target by DNA polymerase is indicated by fluorescent signal from an amplicon-specific hydrolysis probe. To control for PCR inhibition, a second primer set is used as an IPC. If no microorganism is present, only the IPC will be amplified and detected. No amplification of IPC or DNA polymerase-extended substrate indicates the PCR reaction failed and the result is considered invalid. The ETGA process provides for universal detection of intact microbes, allowing detection and rule out of bloodstream infection, but it does not provide species or other identity information.

Analytical validation of a prototype assay, Cognitor

A prototype manual test, Cognitor, was developed with ETGA technology to detect the presence or absence of pathogenic organism DNA polymerase as the indicator of bloodstream infection. To validate the concept of broad reactivity of the Cognitor prototype test, we determined the limit of detection of the Cognitor assay by titrating known quantities of cultured cells into negative blood cultures for representative isolates of the 20 most prevalent causes of blood stream infections, as reported for the UK in 2014 (*21*). Results show sensitivity limits with values ranging from 28 to 9050 CFU/mL detected at the time of testing (Table 1), with the majority being detectable below 200 CFU/mL.

As part of obtaining European regulatory clearance, a clinical validation study was performed on 1457 clinical blood cultures comparing Cognitor performance to the standard of care blood culture process. For blood cultures that remained negative overnight (minimum incubation time of at least 12 h; median incubation of 23.75 h), an aliquot was removed for testing by Cognitor. The bottle was replaced in time to maintain continuous monitoring according to the manufacturer's instructions and left to incubate until the alarm sounded or for 5 days total if it remained negative. Results were reported as "negative" or "not-negative" by Cognitor and compared to alarm "negative" or "positive" blood culture results. As is shown in Table 2, there was a negative predictive value (NPV) of 99.0% (1285/1298; 95% CI, 98.44%–99.36%). The 13 false-negative Cognitor results, were identified by routine biochemical identification method (Vitek 2)

Table 1. Cognitor limit of detection for 20 most prevalent pathogens detected in clinical blood cultures.[a]

Organism name	Lowest detectable density (CFU/mL)
Acinetobacter sp.	70
Bacteroides sp.	219
Candida albicans	250
Candida glabrata	257
Candida parapsilosis	201
Citrobacter sp.	50
Clostridium perfringens	900
Enterobacter cloacae	132
Enterococcus faecalis	227
Escherichia coli	75
Haemophilus influenzae	140
Klebsiella pneumoniae	52
Neisseria lactamica[b]	9050
Proteus mirabilis	156
Pseudomonas aeruginosa	28
Salmonella sp.	35
Serratia marcescens	32
Staphylococcus aureus	58
Streptococcus mitis	107
Streptococcus Group A	225

[a] Lowest detectable cell density required for a positive result in the manual Cognitor test. Cell densities were estimated by total viable counts at the time of running the Cognitor test. Organisms represent the most prevalent causes of bacteremia and fungemia in the UK, 2014 (*21*).
[b] *Neisseria lactamica* was used in place of *Neisseria meningitidis* because of health and safety restrictions on the use of *N. meningitidis* in the research laboratory.

Table 2. Clinical comparison of Cognitor to blood culture for blood cultures negative after overnight incubation.[a]

	Blood Culture	
Cognitor	Negative	Positive
Negative	1285	13[b]
Positive	131[c]	28

[a] Clinical comparison of Cognitor at >12 h to blood culture results at 5 days incubation.
[b] Six of 13 false negative results were identified to be contaminants after clinical review.
[c] Clinical review determined that 91 of 131 false positives may have been clinically significant.

as coagulase-negative staphylococci (n = 5), *Bacteroides fragilis*, *Peptostreptococcus micros*, Group G *Streptococcus*, *Propionibacterium* sp. (n = 2), and one mixed culture of *E. coli* and coagulase-negative staphylococci. Clinically 6/13 false-negative results were determined to be contaminants and insignificant. For these specimens the patient was not clinically septic and the blood culture time to positivity was >32 h. If the samples deemed contaminants are considered true negatives, the NPV increases to 99.5%. Positive predictive value for this study was low at 17.6% (28/159; 95% CI, 14.5%–21.2%). Of the 131 Cognitor false-positive results, 91 were determined to be likely true positives on the basis of clinical signs and symptoms (i.e., the patient was clinically bacteremic).

Considering the results from this study, true negatives could be reported after a mean time of 26.75 h (i.e., the mean incubation time and an allowance of 3 h for Cognitor testing) which is nearly 4 days in advance of negatives reported by blood culture at 5 days. Mean time to positivity for positive samples was 26.0 h (± 2.1 SE) and 38.1 (± 4.1 SE) for Cognitor and automated blood culture, respectively (P = 0.01).

Magnitor, an automated sample-to-result format

A prototype automated test was developed with an off-the-shelf pipetting robotic system to improve ease of use. Additionally, test reagents were further optimized including changes to the lysis mixture, the DNA substrate for DNA polymerase extension, and PCR mastermix. A sensitivity comparison between Magnitor and Cognitor was performed by titrating known quantities of cultured cells into negative blood cultures for several of the most prevalent and drug resistant bacterial species and *Candida albicans*. Detection limits for Magnitor were improved by up to 75-fold compared to Cognitor, with detection levels at time of testing ranging from 1 to 67 CFU/mL. In comparison to blood culture levels present at alarm

Table 3. Comparison of Magnitor and Cognitor to blood culture detection levels.[a]

	Cell density at the time of test positivity (CFU/mL)		
Organism	**Magnitor**	**Cognitor**	**Blood culture**
Enterococcus sp.	67	227	4.2×10^7
S. aureus	18	58	6.0×10^5
K. pneumoniae	18	52	1.9×10^8
A. baumannii	58	70	2.0×10^8
P. aeruginosa	2	28	1.4×10^8
Enterobacter sp.	3 (*E.aerogenes*)	132 (*E.cloacae*)	3.2×10^8
E. coli	1	75	1.2×10^8
C. albicans	29	250	3.1×10^4

[a] Lowest detectable cell density of organisms detected by Magnitor and Cognitor. All cell density values are quoted in CFU/mL, based on total viable counts. For comparison, approximate microbial cell density required to obtain a positive result on BACTEC automated blood culture system (*22*).

positivity (*22*), Magnitor is 5–8 orders of magnitude more sensitive (Table 3).

To determine how rapidly Magnitor could rule out bacteremia and confirm negative blood cultures, a study was performed to determine how long is required to detect low levels of cultured cells added into negative blood cultures. Bottles were supplemented and then aliquots were removed at various timepoints (1–8 h) to test by Magnitor. A matching bottle set was left on the blood culture instrument to determine the time to blood culture alarm positivity. Growth of blood cultures supplemented at 0.1–1.5 CFU/mL for the ESKAPE pathogen family in addition to *E. coli* was observed at 12–17 h in the BacT/ALERT blood culture instrument. Magnitor detected the same supplemented blood cultures after 2–8 h incubation, or 4–11 h earlier than blood culture (Table 4). Detection of *Candida albicans* by Magnitor occurred at 16 h with 9 CFU/mL and 22 h

Table 4. Magnitor time to detection vs blood culture.[a]

Organism	Bottle inoculum (CFU/mL)	Magnitor TTD (h:min)	Blood culture TTD (h:min)	ΔTime
E. faecium	1.5	8:00	12:44	4:44
S. aureus	1.5	2:00	12:10	10:10
S. aureus	0.2	6:00	13:10	7:10
K. pneumoniae	0.4	6:00	13:08	7:08
A. baumannii	0.4	8:00	14:17	6:17
P. aeruginosa	0.5	4:00	17:05	13:05
E. cloacae	2	2:00	12:43	10:43
E. cloacae	0.2	4:00	14:03	10:03
E. aerogenes	0.5	2:00	13:04	11:04
E. coli	0.1	6:00	12:45	6:45
C. albicans	9	16:00	29:43	13:43
C. albicans	0.9	22:00	37:23	15:23

[a] Comparison of detection time for Magnitor vs blood culture on supplemented blood samples for the ESKAPE pathogen group, *E. coli*, and *C. albicans*. All organisms were grown from stationary (overnight) cultures and periodically tested with Magnitor. Simultaneously, matched unsampled bottles were incubated in blood culture. The difference in detection time (ΔTime) is shown. All cell density values, added into the blood culture bottles, are quoted in CFU/mL, based on total viable counts.

Table 5. Effect of sampling running blood culture on contamination rate.[a]

Blood culture	Sampled	Unsampled
Positive @ 5 days	5	10
Negative @ 5 days	354	349

[a] The effect of taking samples from simulated running blood cultures. Final blood culture results (after 5-day incubation) from negative blood cultures after a sample was removed at 16 h ("sampled"), compared to replicate sets that were left "unsampled" after initial placement in the blood culture system. Differences were not found to be significant (Fisher's exact test, $P = 0.297$, $n = 359$).

for 0.9 CFU/mL or 13–15 h faster than blood culture.

Because the proposed approach requires removing an aliquot from a running blood culture bottle that may be replaced for additional incubation, there is the possibility of introducing a contaminating organism that might create a false-positive blood culture. To test the impact of sampling at 16 h on contamination, 2 blood culture sets (n = 359 bottles each set) were supplemented with negative blood. At 16 h, one set was sampled as would occur in the Magnitor protocol and replaced to continue incubating for 5 days, whereas the other set was untouched for 5 days. No evidence of contamination due to sampling was measured (Table 5); the contamination rate for the bottle set that was sampled (1.4%) was similar to the unsampled set (2.8%); the difference was not statistically meaningful ($P = 0.2966$).

DISCUSSION

Herein a high-performance rapid negative blood culture confirmation method has been described including initial clinical validation of a prototype format, termed Cognitor. An incubation overnight before testing fits well with a clinical team schedule allowing early decision-making in the postadmission ward where antimicrobial dosing decisions might be made. Clinical performance for Cognitor was encouraging with an NPV of 99.5% when adjusted to remove presumed contaminants compared with 5-day incubation on a blood culture instrument. These results are very similar to a recently published clinical study on Cognitor confirming the clinical capabilities of the technology to rule out bloodstream infection (*23*). Under many circumstances in which deescalation is under consideration, a negative blood culture after 48 h will add support to the clinical decision to deescalate or cease treatment. At 48 h blood culture incubation, the sensitivity is reported to be 95% (*24*). Assuming a rate of positivity of 10%, this suggests an NPV of roughly 99.5%, suggesting Cognitor can perform equally to blood culture with results 32 h sooner. Additionally, we have shown analytically that sampling a running bottle does not introduce contamination to the blood cultures.

The described assay has demonstrated an NPV that is much better than tests in clinical practice today, which will provide clinicians with a tool to manage clinical decision-making for antimicrobial deescalation. Further analysis of individual clinical workflows will highlight areas in which the Magnitor rule-out test could be used to greatest effect and deliver the best result. Furthermore, combination with existing tests or newer, rapid state-of-the-art tests may prove to have the greatest value.

Cognitor found an additional 9% of samples that were not negative after testing but negative by blood culture, of which two-thirds (6%) were considered to be from clinically septic patients. Being able to detect a substantially higher number of clinically significant bloodstream infections than current blood culture provides a further opportunity for ETGA-based tests. Unfortunately, the identity of these pathogens could not be determined on the basis of the study design, so it is not known if they were from the class of slower growing pathogens, such as the HACEK group or anaerobes. It was not within the scope of the clinical study described herein to identify Cognitor "positives" for samples that were blood culture negative, and future studies will provide more complete

clinical adjudication of discordant results. Significantly more positives are detected with use of sensitive PCR-based techniques than with blood culture (*25*, *26*). Furthermore, it has been reported that only 40%–60% of septic patients have positive blood culture results (*27*), suggesting that blood culture sensitivity may be poor. There are several explanations for this including the initiation of antibiotic therapy before drawing blood for culturing and slow-growing organisms. Future studies on clinical specimens are planned for Magnitor to perform more extensive microbiological adjudication along with potential analysis by broth and plate culture and molecular identification panels developed for positive blood cultures (*11*, *12*).

Even in the improved Magnitor format, variability in detection limit was observed between organism species. While copy number variations for DNA polymerase cannot be excluded, the difference is likely due to extraction variability. Studies are underway to improve extraction even further for more difficult pathogens such as *Candida* (*28*). With consistent 1–10 CFU/mL limits of detection across all pathogen types, it is conceivable to imagine using Magnitor to triage blood specimens for identifying the low percentage that are positive as candidates for testing in newer direct-from-blood molecular test formats (*25*, *26*). Even shorter incubation time on the blood culture instrument may also be possible with improved extraction, but further studies will also be required on slower growing pathogens such as *Bartonella* species and the HACEK group, as well as pathogens that are prone to autolysis in stationary phase such as *Streptococcus pneumoniae* (*29*).

The described test is applicable to support stewardship efforts to review antibiotics in febrile patient groups. Neonates with risk factors for sepsis such as prematurity, chorioamnionitis, and maternal fever are treated with antibiotics at birth (*30*). However, the rates of sepsis are very low, and the practice of antibiotic therapy for culture-negative neonates is associated with poorer outcomes when antibiotics are continued unnecessarily for extended periods (*31*, *32*). Earlier cessation of therapy for the infant can reduce treatment costs by fewer antibiotic doses and a shorter length of hospitalization. Patients undergoing treatment for cancer, particularly hematological cancers such as leukemia and lymphoma, are at increased risk for developing neutropenic fever. Because of low neutrophil levels, these patients are at increased risk for infection, and owing to immune suppression caused by treatment, often fever is the only sign of infection. As a result, these patients are widely treated with antibiotics that can lead to an increased risk of developing a multidrug resistant or *C. difficile*-associated disease infection (*33*). Rule out of bacteremia for lower risk, stable, neutropenic fever patients can inform therapeutic switch from IV to oral antibiotics and allow for discharge to complete treatment at home (*34*). For higher risk neutropenic fever patients who are expected to have a longer duration of neutropenia due to the treatment course but are hemodynamically stable, rule out of bloodstream infection may prevent escalation of therapies and possibly even hospital admission. More rapid rule out of bloodstream infection in general ward febrile patients that are stable can potentially inform discharge. For example, it was reported that for gram-positive cocci in clusters that test negative in the *S. aureus* PNA FISH test, indicating a coagulase-negative staphylococci infection, deescalation could be initiated sooner leading to lower treatment costs, fewer define daily doses, and shortened length of stay for general ward patients (*35*). For intensive care unit patients, the same benefit was not observed because coagulase-negative staphylococci were treated as significant. For clinically stable intensive care unit patients, it will be interesting to see the effect on treatment for Magnitor-negative results, which would indicate the absence of a bloodstream infection.

Blood culture has been reported to be approximately 85% sensitive by 24 h (*24*) of incubation and is likely lower at 16 h; this reduction suggests that a significant number of positive samples will not have been detected by the time Magnitor is run. To address this, an assay, termed CONFIRM, is under development. For not-negative Magnitor test samples, a second PCR reaction would be simultaneously run that contains primer sets for reporting positive results with a nominal gram status: gram-positive, gram-negative, and/or a *Candida* species result. Furthermore, because a higher proportion of later positive blood cultures are due to contamination, a call of coagulase-negative staphylococci could be added. With a robust, high-NPV test and an indicator of late positives, Magnitor can easily be formatted as a high-throughput test and could possibly be used for all blood culture bottles negative at 16 h, improving time to actionable results and freeing up valuable laboratory resources.

Magnitor can integrate into laboratory and clinical schedules, assisting stewardship efforts directed at appropriate antimicrobial therapy. With timely antimicrobial decision-making, treatment costs can be lowered by reducing length of antimicrobial treatment and length of hospitalization, as well as reducing development of antimicrobial resistance.

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