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Quantitative Point-of-Care Pathogen Detection in Septicemia

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Abstract

Pathogen nucleic acid detection exhibits faster turnaround times and higher sensitivity compared with blood culture. When antibiotics are present, blood cultures experience higher false-negative rates. In contrast, nucleic acid tests may detect pathogens irrespective of antimicrobial therapy. Thus, future point-of-care nucleic acid–based detection devices could play a role in revolutionizing pathogen detection in critically ill patients. To properly optimize clinical use, pathogen nucleic acid tests should have the capacity to quantitate pathogen DNA, be miniaturized with simplified preanalytical and postanalytical processing components, and reduce net operating costs or provide clear-cut clinical outcome benefits. These 3 elements will foster a new generation of nucleic acid tests for use at the point of need and facilitate a paradigm shift to point-of-care pathogen detection with evidence-based treatment and real-time quantitative monitoring of patients with sepsis.

Keywords

analytical sensitivity; blood culture; cost-effectiveness; loop-mediated isothermal amplification; low resource; point of care; polymerase chain reaction; quantitative nucleic acid testing

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Each year, approximately 750,000 people develop sepsis.¹ Mortality is as high as 50%.¹ If treated inadequately, sepsis can progress suddenly in a few hours. In septicemia, blood culture (BC) represents the clinically accepted method for pathogen detection. However, slow turnaround time, low sensitivity (300–3000 colony-forming units [CFU]/mL), and high false-negative rates can result from confounding factors that include antibiotic therapy and

low pathogen concentration.² Rapid and highly sensitive methods, such as nucleic acid testing (NAT), are needed to complement BC.

Studies show multiplex NAT detecting clinically significant pathogens faster (5–6 hours). In some cases, NAT detects pathogens missed by BC.³ Polymerase chain reaction (PCR), a common nucleic acid amplification method, amplifies pathogen DNA through several thermocycling steps. In PCR-based NAT, DNA products are measured via turbidity or with sequence-specific fluorescence probes. Polymerase chain reaction technology in commercial systems provides multipathogen detection of bacteria and fungi where analytical sensitivities are reported as 3 CFU/mL.⁴ However, commercial systems lack quantitation, often require extensive preanalytical and postanalytical processing, and are less cost-effective than BC.^{3,5–7}

THREE WISHES FOR NUCLEIC ACID PATHOGEN DETECTION

Quantitative NAT

Early studies using quantitative BC showed moderate correlation with severity of septic shock in patients with bacterial counts as high as 300 CFU/mL.⁷ Weil and Spink⁸ found correlation between the magnitude of bacteremia in patients with gram-negative sepsis and fatal outcome. Nonetheless, quantitative BC lacks sensitivity in cases with low-level bacteremia. For example, some symptomatic patients with endocarditis exhibited bacteremia concentrations ranging from 1 to 30 CFU/mL—well below the reported detection limits of BC.^{2,9} Antibiotic use also negatively contributes to BC sensitivity. In contrast, quantitative NAT (qNAT) may prove more sensitive and robust. A qNAT study among pediatric patients with meningococcal disease revealed severe disease as having significantly higher (8.4×10^6 DNA copies/mL) pathogen DNA concentrations than those with milder disease (1.1×10^6 DNA copies/mL; $P = 0.018$).¹⁰ Decreasing pathogen concentration may also correlate with successful antimicrobial therapy.¹¹ Therefore, qNAT could provide clinicians with a highly sensitive assay to indirectly monitor for antimicrobial efficacy and facilitate a clearer understanding of the pathophysiology of pathogen invasion and dissemination in the bloodstream.

Miniaturization and Simplification of Preanalytical and Postanalytical Processing

Whether in the hospital or in the field, NAT preanalytical and postanalytical processing steps expose samples to potential contaminants and increase both operator burden and turnaround time. In the case of the SeptiFast System (Roche Diagnostics, Indianapolis, Ind), this benchtop, multiplex, PCR-based assay simultaneously detects 25 different bacterial and fungal pathogens from whole blood. This system requires 2.5 hours of extensive preanalytical processing in a biosafety hood (Fig. 1). The PCR and postanalytical processing steps need an additional 2.5 hours in a separate room.

Automated, self-contained, and miniaturized components for preanalytical and postanalytical processing could simplify detection methods and potentially reduce sample contamination. This is illustrated in the GeneXpert Rapid Group B *Streptococcus* test (Cepheid, Sunnyvale, Calif), where semiautomated DNA purification is performed in self-contained cartridges and at the point of care.¹² Total turnaround time is 15 minutes, with

clinical sensitivity and specificity reported as 91% and 96%, respectively. Therefore, miniaturization and simplification of preanalytical and postanalytical processing reduces operator training, enhances total turnaround time, brings qNAT to the point of care, and improves the critical care workflow.

Reduction of NAT Costs

Operator training, reagents, and supplies drive up pathogen NAT costs. Studies show BC costing \$50 per bottle and an additional \$50 for antimicrobial drug-sensitivity testing.⁷ Ideally, 2 pairs of BC bottles (drawn from separate sites) are drawn, where each pair consists of an anaerobic and aerobic bottle. In certain patient groups, such as adults with cellulitis, the low sensitivity and yield of BC exhibited marginal impact on clinical management and cost-effectiveness.⁷

In comparison, the SeptiFast PCR assay costs approximately \$180 per patient and requires instrumentation costing up to \$54,000.⁶ For the SmartCycler-based methicillin-resistant *Staphylococcus aureus* test (Cepheid), instrument costs are reported at approximately \$34,000. However, a newer method called loop-mediated isothermal amplification (LAMP) shows promise. In a study by Misawa et al,⁵ a methicillin-resistant *Staphylococcus aureus* LAMP assay was compared with PCR. Although LAMP had less sensitivity (87% vs 95%) to PCR, the LAMP assay was faster (<1 hour), was conducted at a single temperature (6300B0C), and costs \$5 per reaction (vs \$80 per PCR reaction). This LAMP approach is presently being evaluated by 2 National Institute of Biomedical Imaging and Bioengineering-funded Point-of-Care Technology Centers for applications as a low-cost isothermal alternative to PCR (Fig. 2).^{13,14} Therefore, future qNAT systems should use novel and cost-effective methods for use in critical, emergency, disaster, and low-resource environments.¹⁵

CONCLUSIONS

Rapid detection of clinically significant pathogens will facilitate early targeted antimicrobial therapy in patients with septicemia. Quantitative NAT may revolutionize infectious disease testing in critical care by allowing us to understand the pathophysiology of pathogen invasion and dissemination in the bloodstream more clearly. Granting our 3 “wishes” for NAT can foster a paradigm shift quantitate pathogen detection as a foundation for evidence-based treatment and monitoring of patients with bacteremia and fungemia. Ultimately, the value of this new approach will depend on the net impact of critical care triage, workflow, efficiency, as well as outcomes, especially when managing difficult cases where patients already have been treated with antimicrobial or antifungal agents.

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Figure 1.

SeptiFast assay workflow. A 1.5-mL whole-blood sample is manually processed over a 2.5-hour time frame to extract the pathogen DNA. The extracted DNA is then tested in 3 capillary tubes containing primers and probes for gram-positive, gram-negative, and fungal pathogens. The PCR reaction takes 2.5 hours for a total turnaround time of 5 hours from blood draw to pathogen identification.