

PCR and Blood Culture for Detection of *Escherichia coli* Bacteremia in Rats

ALEXANDRA HEININGER,^{1*} MARLIES BINDER,² SIBYLLE SCHMIDT,² KLAUS UNERTL,¹
KONRAD BOTZENHART,² AND GERD DÖRING²

*Klinik für Anästhesiologie¹ and Department of General and Environmental Hygiene,² Hygiene Institute,
University of Tübingen, Tübingen, Germany*

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Critically ill patients often develop symptoms of sepsis and therefore require microbiological tests for bacteremia that use conventional blood culture (BC) techniques. However, since these patients frequently receive early empirical antibiotic therapy before diagnostic procedures are completed, examination by BC can return false-negative results. We therefore hypothesized that PCR could improve the rate of detection of microbial pathogens over that of BC. To test this hypothesis, male Wistar rats were challenged intravenously with 10^6 CFU of *Escherichia coli*. Blood was then taken at several time points for detection of *E. coli* by BC and by PCR with *E. coli*-specific primers derived from the *uidA* gene, encoding β -glucuronidase. In further experiments, cefotaxime (100 or 50 mg/kg of body weight) was administered intravenously to rats 10 min after *E. coli* challenge. Without this chemotherapy, the *E. coli* detection rate decreased at 15 min and at 210 min after challenge from 100% to 62% of the animals with PCR and from 100% to 54% of the animals with BC ($P, >0.05$). Chemotherapy decreased the *E. coli* detection rate at 25 min and at 55 min after challenge from 100% to 50% with PCR and from 100% to 0% with BC ($P, <0.05$). Thus, at clinically relevant serum antibiotic levels, PCR affords a significantly higher detection rate than BC in this rat model. The results suggest that PCR could be a useful adjunct tool supplementing conventional BC techniques in diagnosing bacteremia.

Episodes of sepsis are common and frequently life-threatening (12) complications, occurring in 44.8% of all critically ill patients (38). By definition (4), sepsis is caused by microbial infection. However, bacteremia, i.e., the presence of microorganisms in the bloodstream, is diagnosed in only approximately 4 to 12% of all blood cultures (BC) (26, 37). *Escherichia coli* is one of the most common microorganisms isolated during hospital-acquired bacteremia in intensive care patients (2, 21, 38). Several factors may contribute to the low bacteremia detection rate. First, some species of bacteria are difficult to culture (9), and some, such as *Mycobacterium* spp. (5, 11, 15) or *Bartonella* spp. (6), grow very slowly. Second, bacteremia is often transient (29), meaning that the number of viable microorganisms circulating in the blood decreases rapidly after the onset of bacteremia due to phagocytic and other host defense mechanisms. Finally, up to 65% of intensive care patients showing symptoms of sepsis are already under antimicrobial treatment, thus compromising the results of microbiological culture techniques (8, 25, 31, 37).

Since the emergence of bacteremia is associated with high mortality rates and financial costs as well as a longer hospital stay (7, 27, 30), independent of the severity of the underlying disease (3, 33), improved techniques for diagnosing bacteremia are needed. We hypothesized that amplification of bacterial DNA by PCR could overcome some of the problems associated with conventional BC techniques. The potential of PCR technology to recover intracellular or nongrowing microorganisms may be useful for diagnosing bacteremia. Although PCR has already been successfully applied in the detection of fastidious microorganisms (22, 43) and, in particular, for detecting bacteremia (23, 28, 44), a direct comparison of BC tech-

niques with PCR with respect to detection levels and time course during bacteremia has yet to be carried out. Therefore, we assessed these parameters for experimental bacteremia induced by *E. coli* in rats.

MATERIALS AND METHODS

Rat model of bacteremia. Male Wistar rats (350 to 500 g) were anesthetized by an initial intramuscular injection of ketamine at 80 mg/kg of body weight (bw) and xylazine at 4 mg/kg of bw, separately. Following this injection, both jugular veins were cannulated with a 26-gauge cannula (Abbott, Wiesbaden, Germany) under aseptic conditions. One cannula was used exclusively for the collection of blood samples. Bacteremia was induced via the other cannula by injection of 100 μ l of a suspension of *E. coli* (ATCC 11229; 10^7 CFU/ml in 0.9% saline). *E. coli* was chosen to represent nonfastidious pathogens for comparing the sensitivities of BC and PCR because it is the most frequent gram-negative pathogen causing bacteremia. We collected 2.2-ml blood samples from 19 animals (group I) 5 min before bacterial challenge and at 5, 15, 150, 180, and 210 min afterward. One blood sample from each of 16 animals surviving 210 min was analyzed at each time point, with the exception of time points 15, 150, and 210 min after bacterial challenge, when analysis for *E. coli* was limited to 10, 15, and 13 rats, respectively, due to insufficient blood sample volumes. One milliliter of whole blood was immediately inoculated into the BC medium, and 1 ml was stored in EDTA tubes (Sarstedt, Nürnbrecht, Germany) for PCR analysis; 100 μ l of each sample was directly plated in duplicate on sheep blood agar plates for the quantitation of bacteria.

From an additional 20 animals, 2.0-ml blood samples were collected before bacterial challenge and at 5, 25, 40, and 55 min afterward for detecting *E. coli* by BC and PCR. Ten animals (group II) received 100 mg of cefotaxime/kg of bw 10 min after bacterial challenge via the cannula used to inject *E. coli*. The other 10 animals (group III) received 50 mg of cefotaxime/kg of bw. At each time point, one blood sample was analyzed from each of 10 animals. From three animals in each group, an additional 0.5 ml of blood was taken and stored in an EDTA tube (Sarstedt) to check serum cefotaxime concentrations. The experiments were approved by the Animal Ethics Committee of the University of Tübingen, Tübingen, Germany.

In order to detect *E. coli* by BC, 1-ml blood samples were inoculated into BACTEC Peds Plus F medium (Becton Dickinson, Sparks, Md.), which contains resin for neutralizing antimicrobial agents (13, 31, 45) and is designed for an inoculum of 1 to 3 ml. The vials were incubated for 7 days in a shaker incubator at 37°C. After 48 h, the vials were punctured under sterile conditions, and 100 μ l was subcultured on sheep blood (5%) agar in duplicate for 24 h (5% CO₂). If bacterial growth was negative, the vials were incubated for another 5 days, followed by subculturing as described above.

* Corresponding author. Mailing address: Klinik für Anästhesiologie, University of Tübingen, Hoppe-Seyler-Str. 3, D-72076 Tübingen, Germany. Phone: 0049 7071 2986622. Fax: 0049 7071 295533. E-mail: gerd.doering@uni-tuebingen.de.

PCR. A 1-ml blood sample was divided into three aliquots of 300 μ l each, which were transferred into sterile cups. Erythrocytes were lysed, and DNA was extracted according to the manufacturer's protocol with a Puregene DNA isolation kit (Biozym Diagnostik GmbH, Hessisch Oldendorf, Germany) for whole blood. Two pairs of oligonucleotide primers (one nested in the other) were derived from the *uidA* gene of *E. coli*, encoding β -glucuronidase specific for *E. coli* and *Shigella* spp. Oligonucleotides P1 (5'-ATC ACC GTG GTG ACG CAT GTC GC-3') and P2 (5'-CAC CAC GAT GCC ATG TTC ATC TGC-3') were used in the first round of PCR to amplify a 486-bp fragment (19). Oligonucleotides P3 (5'-TAT GAA CTG TGC GTC ACA GCC-3') and P4 (5'-CAT CAG CAC GTT ATC GAA TCC-3') were used in the nested PCR for the amplified products from the primary PCR to amplify a 186-bp fragment. For DNA amplification, 20.4 μ l of reaction mixture was added to 29.6 μ l of purified DNA. The master mixture for the first round of PCR included the following: 10 mM Tris-HCl, 50 mM KCl, 4.5 mM MgCl₂, 600 μ M each deoxynucleoside triphosphate (dNTP) (Amersham Pharmacia Biotech, Freiburg, Germany), 0.6 μ M each primer, and 1 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech). Each reaction mixture was overlaid with 1 or 2 drops of mineral oil. PCR amplifications were performed with a Hybaid Omni Gene Temperature Cycler (MWG Biotech, Ebersberg, Germany).

The primary PCR amplification consisted of an initial denaturation step at 95°C for 5 min; 35 cycles at 95°C for 30 s, 50°C for 1 min, and 72°C for 1 min; and a final elongation phase at 72°C for 5 min. After the first reaction, 1 μ l of the amplified product was added to 49 μ l of the second master mixture. The master mixture for the nested PCR contained the following: 10 mM Tris-HCl, 50 mM KCl, 4 mM MgCl₂, 400 μ M each dNTP (Amersham Pharmacia Biotech), 0.4 μ M each primer, 10 μ l of Q-solution (Qiagen, Hilden, Germany), and 1 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech). The nested PCR amplification was performed with an initial denaturation step at 95°C for 5 min; 35 cycles at 95°C for 30 s, 50°C for 1 min, and 72°C for 1 min; and a final elongation phase at 72°C for 5 min.

Each time a PCR was performed, we also ran a positive control (bacterial DNA), a negative control (rat DNA), and a reagent control (all PCR reagents without DNA) to evaluate the success of amplification, the specificity of the reaction, and the purity of the reagents, respectively. The product of the nested PCR was electrophoresed with 2 μ l of gel loading buffer (0.25 g of bromophenol blue and 40 g of saccharose dissolved in 100 ml of distilled water) through a 2% agarose gel at 110 V for 40 min in Tris-acetate-EDTA buffer. Molecular size markers (Promega, Madison, Wis.) were run concurrently. The gel, stained with ethidium bromide (0.5 μ g/ml), was examined under UV light for the presence of a 186-bp band and photographed for documentation.

In order to assess the sensitivity of a specific PCR, 1 ml of rat blood was spiked with *E. coli* (10^6 to 10^0 CFU) and immediately processed as described above.

Verification of correct fragment production by the PCR was performed by Southern hybridization. PCR fragments were Southern blotted onto positively charged nylon membranes (Boehringer GmbH, Mannheim, Germany) by use of a Turbo Blotter (Schleicher & Schuell, Inc., Keene, N.H.) as prescribed by the manufacturer for alkaline transfer. The DNA was cross-linked to the damp membranes by use of a GS Gene Linker UV chamber (BioRad Laboratories, Hercules, Calif.) according to the instructions of the provider. The gene probe used for hybridization was a digoxigenin-UTP-labeled (Boehringer) 186-bp fragment produced by PCR, resolved on a 2% agarose gel, and purified with a PCR product purification kit (Qiagen). The standard nylon membrane prehybridization and hybridization protocol was performed under stringent conditions at 65°C. Probed membranes were exposed to Hyperfilm ECL (Amersham Life Science, Little Chalfont, England).

Analysis of cefotaxime levels in serum. Cefotaxime concentrations were measured by high-performance liquid chromatography with a UV detection system by the method reported by Dell et al. (10).

Statistical analysis. In order to detect any relevant difference between the PCR and the BC methods with respect to bacteremia detection as a function of time, we chose confidence intervals of 95% ($P < 0.05$). The minimum number of experiments needed to establish a difference of at least 30% between the two techniques for group I was calculated to be 20. A preliminary data analysis was scheduled after the completion of 12 experiments. For groups II and III, the confidence intervals were also 95%. The minimum number of experiments needed to reveal a difference of at least 80% between the two methods for groups II and III was 10 (1).

RESULTS

For the detection of *E. coli* DNA in rat blood samples, PCR was performed with primers for the *uidA* gene of *E. coli* (19). PCR products from rat blood samples were verified to be specific for the target gene by Southern hybridization with a dUTP-labeled probe for *uidA*. No differences in the sizes of the PCR fragments from rat blood samples on agarose gels and the hybridization product on the Southern blot were observed (data not shown).

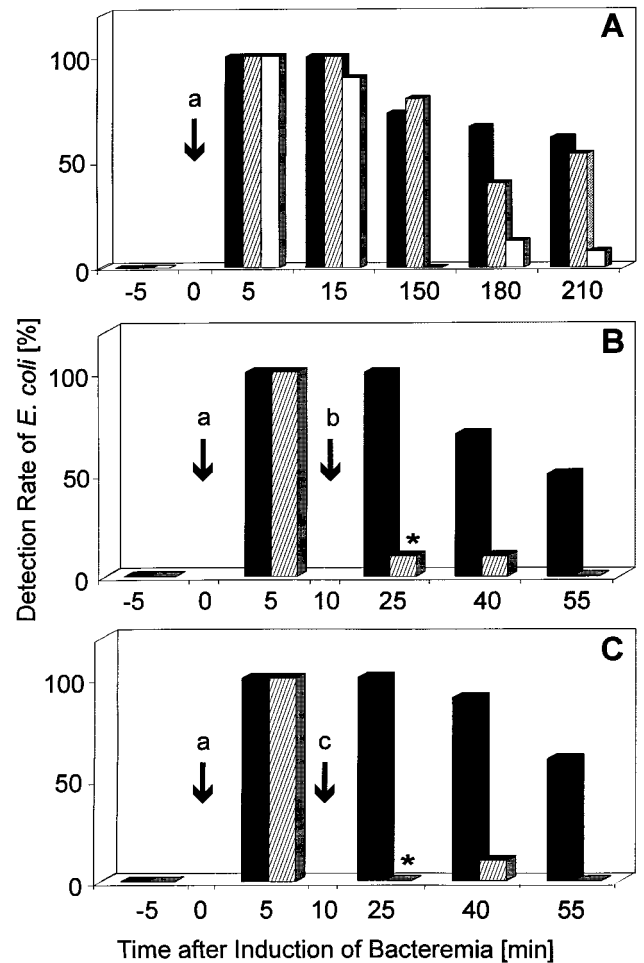


FIG. 1. Time course of *E. coli* detection in experimental bacteremia in rats by PCR, BC, and the direct plating method. (A) Sixteen rats were challenged intravenously with 10^6 CFU of *E. coli* at time zero (arrow a) without further intervention. (B and C) Another 10 rats each received 100 mg (B) (arrow b) or 50 mg (C) (arrow c) of cefotaxime/kg of bw 10 min after bacterial challenge. For *E. coli* detection, blood was collected 5 min before challenge and at the indicated times after challenge. *E. coli* was detected by PCR (A, B, and C) (filled bars), BC (A, B, and C) (hatched bars), and the direct plating method (A) (open bars). Bars represent the percentages of *E. coli*-positive blood samples. *, $P < 0.05$.

The sensitivity of PCR and BC for detecting *E. coli* in whole rat blood was 1 CFU/ml (data not shown). Before bacterial challenge, BC, the direct plating method, and PCR all returned negative results, indicating the absence of bacterial contamination during the animal experiment and during the PCR procedure (Fig. 1A). After bacterial challenge, the rates of detection of *E. coli* by the three methods decreased in a time-dependent manner (Fig. 1A). Even after 210 min, the *E. coli* PCR was positive for 61% of the 13 blood samples, whereas BC with the BACTEC system and the direct plating method were positive for 53 and 8% of the samples, respectively. No significant difference was observed between PCR and BC for the detection of *E. coli* at any of the examination times, but the rate of detection by the direct plating method was noticeably lower than that by either PCR or BC. Quantitatively, the direct plating method showed mean numbers of bacteria per milliliter of 27, 15, 0.0, 0.6, and 0.4 at 5, 15, 150, 180, and 210 min after *E. coli* challenge.

As expected, the sensitivity of BC decreased significantly during experimental *E. coli* bacteremia in rats receiving clinically relevant doses of cefotaxime (Fig. 1B and C). In antibiotic treatment groups II and III, peak serum cefotaxime levels ranged between 121 and 137 mg/liter and between 94 and 86 mg/liter 15 min after injection, respectively (data not shown). When blood was taken 15 min after cefotaxime was administered intravenously, i.e., 25 min after bacterial challenge, only 10% of the samples were found positive by BC, whereas 100% of the samples were found positive by PCR (Fig. 1B and C). Data analysis established an 80% higher detection rate for the PCR method 25 min after bacterial challenge with both cefotaxime doses. At subsequent sampling times, the advantage of the PCR technique was also evident but did not reach the 80% range.

DISCUSSION

BC is considered the "gold standard" for detecting microorganisms in the blood (29, 37). Nevertheless, many clinicians and microbiologists are concerned that BC for critically ill patients receiving antimicrobial chemotherapy could indicate lower rates of bacteremia than are actually present (8, 34). Antibiotic-binding BC devices, such as the BACTEC Plus F system, were developed in response to these concerns (13, 31, 41). Still, the rate of detection of bacteria in blood specimens has not changed substantially. The results of the present study strongly suggest that the clinicians' suspicions have a scientific basis. In an experimental rat model for *E. coli* bacteremia, a significantly higher rate of detection of *E. coli* was achieved with PCR technology than with BC when the animals were treated with clinically relevant levels of cefotaxime.

Interestingly, this difference in the detection rate between PCR and BC was not due to a higher sensitivity of our PCR than of BC. Regardless of whether PCR or BC is used, the ability to detect bacteremia depends on the presence of at least one microorganism in the blood being sampled. When tested in vitro, both methods detected as little as 1 CFU of *E. coli* per ml of blood, a volume which is normally used for diagnosing bacteremia, particularly in neonatal intensive care units (26). Furthermore, no significant difference in *E. coli* detection rates was observed between PCR and BC at any time point during experimental bacteremia in animals without antibiotic treatment. The sensitivity of our PCR was comparable to that previously reported for PCR used to detect bacteremia (17, 23, 28, 32, 35, 44). To our knowledge, however, minimal detection levels of BC systems in vitro have yet to be reported. Since the number of pathogens is less than 1 microorganism per ml of blood in 62% of all adult patients with *E. coli* bacteremia (37) and can be less than 0.04 organism per ml of blood (18), large blood volumes (20 to 30 ml) have to be used in order to avoid false-negative results. Thus, even though the PCR method was designed for smaller volumes from the very outset, it has to be adapted accordingly. This problem might be solved in the near future, because the rapidly increasing clinical use of PCR technology to detect pathogens which could not be isolated by conventional methods (5, 15, 24) has already prompted the development of a variety of different DNA isolation kits.

Our finding that BC is much less efficient than PCR in detecting bacteremia during antimicrobial treatment is most probably due to the killing of *E. coli* by cefotaxime; killed bacteria are not detectable by BC, while PCR detects bacterial DNA independently of viability.

Similarly, in a rabbit model of endocarditis, recovery rates for *E. coli* and other bacteria were reduced during antimicrobial treatment (25). In addition, the higher sensitivity of PCR

technology than of conventional BC techniques is due to the fact that intracellular or phagocytosed microorganisms are also detectable by PCR.

In order to quantify bacterial numbers in *E. coli*-challenged rat blood, the direct plating method was used. Direct plating corroborated the results obtained by PCR and BC with respect to the rapid clearance of *E. coli* from the bloodstream. The lower sensitivity of the direct plating method compared with PCR or BC may be due to its smaller sample volume and a lack of dilution of bacterial growth-inhibiting factors in the blood, which are known to influence bacterial recovery rates in BC (29, 37). Thus, since direct plating is clearly less sensitive than BC in detecting *E. coli* in blood samples, BC is a better standard than direct plating for the evaluation of PCR technology (20).

From the clinical point of view, it is vital to develop improved techniques for diagnosing bacteremia, because the emergence of bacteremia is of substantial prognostic and therapeutic importance (2, 16, 39). The occurrence of secondary bacteremia (14) is a signal that the host's defenses have failed to contain an infection at its primary site or that the physician has failed to control the infectious process (40, 42). Thus, the detection of bacteria in the patient's blood, regardless of whether they are still viable or have been killed by antibiotics, implies that the treatment regimen might be insufficient and has to be augmented.

Extracellular bacterial DNA has been considered to be immunologically inert in mammals. However, it was shown recently that bacterial DNA has substantial immunostimulatory properties comparable to those of endotoxin (36) and that its presence can cause sepsis-like symptoms in mice (36). Thus, methods for detecting DNA might improve our understanding of the frequently life-threatening systemic inflammatory response syndrome (4) in intensive care patients. In summary, our results suggest that PCR could prove to be a useful adjunct tool supplementing conventional BC techniques in diagnosing bacteremia.

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REFERENCES

- Altman, D. G. 1991. Comparing groups—categorical data, p. 229–276. In D. G. Altman (ed.), *Practical statistics for medical research*. Chapman & Hall, Ltd., London, England.
- Bachur, R., and G. L. Caputo. 1995. Bacteremia and meningitis among infants with urinary tract infections. *Pediatr. Emerg. Care* 11:280–284.
- Bates, D. W., K. E. Pruess, and T. H. Lee. 1995. How bad are bacteremia and sepsis? *Arch. Intern. Med.* 155:593–598.
- Bone, R. C., R. A. Balk, F. B. Cerra, R. P. Dellinger, A. M. Fein, W. A. Knaus, R. M. H. Schein, and W. J. Sibbald. 1992. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 101:1644–1655.
- Böttger, E. C., A. Teske, P. Kirschner, S. Bost, H. R. Chang, V. Beer, and B. Hirschel. 1992. Disseminated *Mycobacterium genevase* infection in patients with AIDS. *Lancet* 340:76–80.
- Brenner, S. A., J. A. Rooney, P. Manzwetsch, and R. L. Regnery. 1997. Isolation of *Bartonella (Rochalimaea) henselae*: effects of methods of blood collection and handling. *J. Clin. Microbiol.* 35:544–547.
- Crowe, M., P. Ispahani, H. Humphreys, T. Kelley, and R. Winter. 1998. Bacteraemia in the adult intensive care unit of a teaching hospital in Nottingham, UK, 1985–1996. *Eur. J. Clin. Microbiol. Infect. Dis.* 17:377–384.
- Darby, J. M., P. Linden, W. Pasculle, and M. Saul. 1997. Utilization and

- diagnostic yield of blood cultures in a surgical intensive care unit. *Crit. Care Med.* **25**:989–994.
9. **Davies, S., and R. Eggington.** 1991. Recovery of *Mycoplasma hominis* from blood culture media. *Med. Lab. Sci.* **48**:110–113.
 10. **Dell, D., J. Chamberlain, and F. Coppin.** 1981. Determination of cefotaxime and desacetylcefotaxime in plasma and urine by high-performance liquid chromatography. *J. Chromatogr.* **226**:431–440.
 11. **Esteban, J., A. Molleja, R. Fernandez-Roblas, and F. Soriano.** 1998. Number of days required for recovery of mycobacteria from blood and other samples. *J. Clin. Microbiol.* **36**:1456–1457.
 12. **Fagon, J. Y., A. Novara, F. Stephan, et al.** 1994. Mortality attributable to nosocomial infections in the ICU. *Infect. Control Hosp. Epidemiol.* **15**:428–434.
 13. **Fuller, D. D., and T. E. Davis.** 1997. Comparison of Bactec plus Aerobic/F, Anaerobic/F, Peds Plus/F and Lytic/F media with and without fastidious organism supplement to conventional methods for culture of sterile body fluids. *Diagn. Microbiol. Infect. Dis.* **29**:219–225.
 14. **Garner, J. S., W. R. Jarvis, T. G. Emori, T. C. Horan, and J. M. Hughes.** 1988. CDC definitions for nosocomial infections. *Am. J. Infect. Control* **16**:128–140.
 15. **Haas, W. H., P. Kirschner, S. Ziesing, H. J. Bremer, and E. C. Bottger.** 1993. Cervical lymphadenitis in a child caused by a previously unknown mycobacterium. *J. Infect. Dis.* **167**:237–240.
 16. **Isaacman, D. J.** 1998. Strategies for improving the detection of bacteremia in children. *Infect. Dis. Clin. Pract.* **7**:28–31.
 17. **Isaacman, D. J., Y. Zhang, J. Rydquist-White, R. M. Wadowsky, J. C. Post, and G. D. Ehrlich.** 1995. Identification of a patient with *Streptococcus pneumoniae* bacteremia and meningitis by the polymerase chain reaction (PCR). *Mol. Cell. Probes* **9**:157–160.
 18. **Jonsson, B., A. Nyberg, and C. Henning.** 1993. Theoretical aspects of detection of bacteraemia as a function of the volume of blood cultured. *APMIS* **101**:595–601.
 19. **Juck, D., J. Ingram, M. Prevost, J. Coallier, and C. Greer.** 1996. Nested PCR protocol for the rapid detection of *Escherichia coli* in potable water. *Can. J. Microbiol.* **42**:862–866.
 20. **Kane, T. D., S. R. Johnson, J. W. Alexander, G. F. Babcock, and C. K. Ogle.** 1996. Detection of intestinal bacterial translocation using PCR. *J. Surg. Res.* **63**:59–63.
 21. **Kiehn, T. E., B. Wong, F. F. Edwards, and D. Armstrong.** 1983. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. *J. Clin. Microbiol.* **18**:300–304.
 22. **Kox, L. F. F., D. Rhienthong, A. M. Miranda, N. Udomsantisuk, K. Ellis, J. van Leeuwen, S. van Heusden, S. Kuijper, and A. H. J. Kolk.** 1994. A more reliable PCR for detection of *Mycobacterium tuberculosis* in clinical samples. *J. Clin. Microbiol.* **32**:672–678.
 23. **Ley, B. E., C. J. Linton, D. M. C. Bennett, H. Jalal, A. B. M. Foot, and M. R. Millar.** 1998. Detection of bacteraemia in patients with fever and neutropenia using 16S RNA gene amplification by polymerase chain reaction. *Eur. J. Clin. Microbiol. Infect. Dis.* **17**:247–253.
 24. **Martin, A. B., S. Webber, J. Fricker, R. Jaffe, G. Demmler, D. Kearney, Y.-H. Zhang, J. Bodurtha, B. Gelb, J. Ni, T. Bricker, and J. A. Towbin.** 1994. Acute myocarditis: rapid diagnosis by PCR in children. *Circulation* **90**:330–339.
 25. **McKenzie, R., and L. G. Reimer.** 1987. Effect of antimicrobials on blood cultures in endocarditis. *Diagn. Microbiol. Infect. Dis.* **8**:165–172.
 26. **Paisley, J. W., and B. A. Lauer.** 1994. Pediatric blood cultures. *Clin. Lab. Med.* **14**:17–30.
 27. **Pittet, D., D. Tarara, and R. P. Wenzel.** 1994. Nosocomial bloodstream infection in critically ill patients. Extra length of stay, extra costs, and attributable mortality. *JAMA* **271**:1598–1601.
 28. **Rattanathongkom, A., R. W. Sermawan, and S. Wongratanacheewin.** 1997. Detection of *Burkholderia pseudomallei* in blood samples using polymerase chain reaction. *Mol. Cell. Probes* **11**:25–31.
 29. **Reimer, L. G., M. L. Wilson, and M. P. Weinstein.** 1997. Update on detection of bacteremia and fungemia. *Clin. Microbiol. Rev.* **10**:444–465.
 30. **Rello, J., M. Richart, B. Mirelis, et al.** 1994. Nosocomial bacteremia in a medical-surgical intensive care unit: epidemiologic characteristics and factors influencing mortality in 111 episodes. *Intensive Care Med.* **20**:94–98.
 31. **Rohner, P., B. Pepey, and R. Auckenthaler.** 1997. Advantage of combining resin with lytic Bactec blood culture media. *J. Clin. Microbiol.* **35**:2634–2638.
 32. **Rudolph, K. M., A. J. Parkinson, C. M. Black, and L. W. Mayer.** 1993. Evaluation of polymerase chain reaction for diagnosis of pneumococcal pneumonia. *J. Clin. Microbiol.* **31**:2661–2666.
 33. **Smith, R. L., S. M. Meixler, and M. S. Simberkoff.** 1991. Excess mortality in critically ill patients with nosocomial bloodstream infections. *Chest* **100**:164–167.
 34. **Smith, S. M., and R. H. K. Eng.** 1983. In vitro evaluation of the Bactec resin-containing blood culture bottle. *J. Clin. Microbiol.* **17**:1120–1126.
 35. **Song, J.-H., H. Cho, M. Y. Park, D. S. Na, H. B. Moon, and C. H. Pai.** 1993. Detection of *Salmonella typhi* in the blood of patients with typhoid fever by polymerase chain reaction. *J. Clin. Microbiol.* **31**:1439–1443.
 36. **Sparwasser, T., T. Miethke, G. Lipford, K. Borschert, H. Häcker, K. Heeg, and H. Wagner.** 1997. Bacterial DNA causes septic shock. *Nature* **386**:336–337.
 37. **Spencer, R. S.** 1988. Blood cultures: where do we stand? *J. Clin. Pathol.* **41**:668–670.
 38. **Vincent, J.-L., D. J. Bihari, P. M. Suter, H. A. Bruining, J. White, M.-H. Nicolas-Chanoin, M. Wolff, R. C. Spencer, and M. Hemmer.** 1995. The prevalence of nosocomial infections in intensive care units in Europe. *JAMA* **274**:639–644.
 39. **Washington, J. A.** 1989. Blood cultures: an overview. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**:803–806.
 40. **Weinstein, M. P.** 1996. Current blood culture methods and systems: clinical concepts and interpretation of results. *Clin. Infect. Dis.* **23**:40–46.
 41. **Wilson, M. L., and M. P. Weinstein.** 1994. General principles in the laboratory detection of bacteremia and fungemia. *Clin. Lab. Med.* **14**:69–82.
 42. **Yagupsky, P., and F. S. Nolte.** 1990. Quantitative aspects of septicemia. *Clin. Microbiol. Rev.* **3**:269–279.
 43. **Yamashita, Y., S. Kohno, H. Koga, K. Tomono, and M. Kaku.** 1994. Detection of *Bacteroides fragilis* in clinical specimens by PCR. *J. Clin. Microbiol.* **32**:679–683.
 44. **Zhang, Y., D. H. Isaacman, R. M. Wadowsky, J. Rydquist-White, J. C. Post, and G. D. Ehrlich.** 1995. Detection of *Streptococcus pneumoniae* in whole blood by PCR. *J. Clin. Microbiol.* **33**:596–601.
 45. **Ziegler, R., I. Johnscher, P. Martus, D. Lenhardt, and H.-M. Just.** 1998. Controlled clinical laboratory comparison of two supplemented aerobic and anaerobic media used in automated blood culture systems to detect bloodstream infections. *J. Clin. Microbiol.* **36**:657–661.