JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1991, p. 2193–2196
0095-1137/91/102193-04$02.00/0

Vol. 29, No. 10

Direct Identification of Bacterial Isolates in Blood Cultures by Using a DNA Probe

THOMAS E. DAVIS* AND DEANNA D. FULLER

Wishard Memorial Hospital, Indiana University Medical Center, Indianapolis, Indiana 46202

Received 11 March 1991/Accepted 2 July 1991

This study involved the rapid, direct identification of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Haemophilus influenzae*, *Enterococcus* sp., and *Streptococcus agalactiae* from positive blood culture bottles (BACTEC, Johnston Laboratories, Inc.) by using the AccuProbe (Gen-Probe, San Diego, Calif.) culture confirmation test. This method uses a chemiluminescent DNA probe that detects the rRNA of the target organisms. The manufacturer's instructions were modified to use a pellet of bacteria made directly from positive blood culture broth rather than a colony from an agar plate. Two separate procedures of selective centrifugation were employed in order to obtain the pellet. The first utilized a routine clinical centrifuge and a large volume of broth (10 to 12 ml) from the blood culture bottle. The second method used a microcentrifuge and less volume (1 to 1.5 ml). A total of 196 clinical specimens taken directly from positive blood culture broths were correctly identified by AccuProbe from pellets made by using the clinical centrifuge technique, while 166 clinical specimens used as negative controls failed to show hybridization. The microcentrifuge technique for obtaining pellets was performed on 105 patient specimens, and all were correctly identified. When combined with the microcentrifuge technique for pellet preparation, the AccuProbe test has several advantages: (i) direct identification of bacteria from blood culture broths, (ii) rapid turn-around time (30 min), (iii) simplicity of the procedure, and (iv) relative low cost.

The detection and identification of microorganisms from blood culture is important because of their clinical significance. Rapid identification of blood culture isolates has the potential to optimize antimicrobial treatment in septic patients and to prevent overtreatment when positive cultures contain contaminants (6). The most common bacterial isolates from blood cultures in our laboratory, in order of descending frequency, are coagulase-negative staphylococci, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp., and *Streptococcus pneumoniae*; these isolates are similar to those reported by others (3, 28). Isolated less frequently are *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and alpha-hemolytic streptococci. Methods used for the rapid identification of bacterial isolates from blood culture broths have included modified conventional tests (1), commercial immunologic kits (25), and the direct inoculation of commercial biochemical identification kits (23). The direct identification of *S. aureus* and *S. pneumoniae* has been achieved (21), but the rapid identification of other gram-positive cocci and gram-negative rods has been less successful (25).

Numerous studies have shown the accuracy of DNA probes for the direct identification of a variety of bacteria in clinical specimens (8, 9, 13, 15, 18, 20) and as culture confirmation tests (5, 12, 14, 17, 22, 26, 27). This report assesses the potential usefulness of commercially available DNA probe kits for the direct, rapid identification of bacteria from positive blood culture broths.

MATERIALS AND METHODS

Identification of isolates. We studied 362 positive blood cultures collected from patients at Wishard Memorial Hospital, Indiana University Hospital, and Richard L. Roudebush Medical Center, Veterans Administration (all located in Indianapolis, Ind.), evaluating the ability of DNA probes to identify microorganisms directly from blood culture bottles. Gram stains were performed on blood culture broths that were positive as determined by the BACTEC system. For the presumptive identification of bacteria directly from blood culture bottles, the following AccuProbe culture confirmation tests (Gen-Probe, Inc., San Diego, Calif.) were employed: those for *Enterococcus* sp., *E. coli*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *S. aureus*, *S. agalactiae*, and *S. pneumoniae*. All blood culture isolates included in this study were identified by our standard laboratory methods (7, 10, 11, 16) at the hospitals noted above.

Processing positive blood cultures. Blood cultures were analyzed from January 1990 to November 1990. Three to five milliliters of blood were collected in BACTEC NR6A and NR7A nonradiometric and/or NR16A and NR17A nonradiometric blood culture bottles (Johnston Laboratories, Inc., Towson, Md.). Some cultures from Indiana University Hospital and Roudebush Medical Center were collected in BACTEC Plus 26 and 27 nonradiometric and BACTEC 6B and 7D radiometric bottles. A blood culture bottle with a growth index of 30 or greater by the BACTEC method was considered positive, and an aliquot was then Gram stained. Blood from all positive bottles was subcultured to blood agar and chocolate agar plates and incubated in a CO_2 incubator at 35°C. The morphology after Gram staining was used to determine which probe(s) would be performed. By using the flow chart shown in Fig. 1, identification was attempted on 217 positive blood cultures. An additional 145 blood culture isolates whose identities had been determined by conventional methods were also tested. Only bottles containing gram-positive cocci, gram-negative diplococci, gram-negative pleomorphic coccobacilli, or gram-negative bacilli in the blood culture broth were included in the probe evaluation. Bacterial identification by using AccuProbe was performed

* Corresponding author.

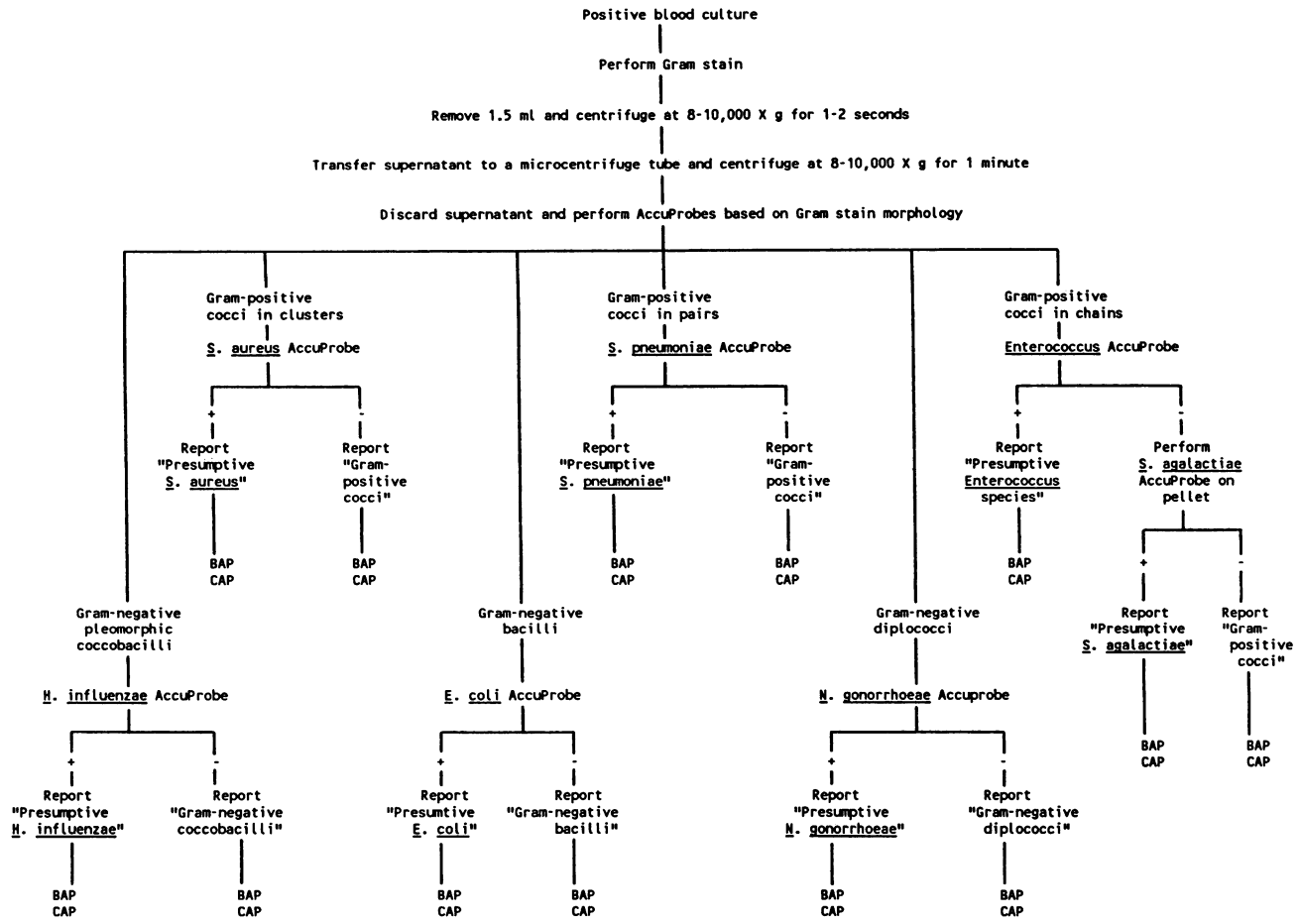

FIG. 1. Flow chart demonstrating identification of bacterial isolates by using a DNA probe. BAP, blood agar plates; CAP, chocolate agar plates.

on blood culture pellets (BCP) of bacteria collected from positive clinical blood culture bottles. Since erythrocytes interfere with the detection method employed, a preliminary step in preparing BCP was the removal of red blood cells. A clinical centrifuge with a force of 100 × *g* was used originally, and a maximum red blood cell volume was removed in 10 min. The supernatant was then centrifuged at 1,000 × *g* for 15 min to concentrate the bacteria. The resulting supernatant was removed, and the remaining pellet was used for further testing. This method for pellet preparation was time-consuming and tedious. During the study, a more rapid modification using a microfuge was developed (Fig. 2). In this procedure, 1.5 ml of broth from the positive blood culture bottle was removed and centrifuged for 2 s in an Abbott TDx centrifuge (model LN9527-01) at approximately 9,600 × *g* to sediment the red blood cells. The supernatant was then centrifuged at 9,600 × *g* for 1 min to concentrate the bacteria. Following either concentration method, a 0.001-ml loopful of the pelleted bacteria was tested with the appropriate AccuProbe according to the manufacturer's instructions. Briefly, 50 μl of specimen diluent was combined with the loopful of pelleted bacteria in a lyophilized probe tube. If the target organism was a gram-positive coccus, a 10-min incubation period in a 35°C water bath was necessary to lyse the organisms. Fifty microliters of probe diluent was added to all tubes, and the tubes were then incubated for 15 min in a 60°C water bath. Next, 300 μl of a selection reagentwas added, and, after a 5-min incubation in a 60°C water bath, the assay results were read on a luminometer (Leader II) and recorded as relative light units (RLU). A reaction with a value of >60,000 RLU as determined by the manufacturer's instructions was catagorized as positive, while a value of <40,000 RLU was catagorized as negative. Reactions with values of >40,000 and <60,000 RLU were repeated, and only reactions with repeat values of >60,000 RLU were considered positive.

The AccuProbe for *S. aureus* was performed on BCP that showed gram-positive cocci in clumps. Gram-positive cocci in pairs and short chains were tested with the AccuProbe for *S. pneumoniae*. Bacteria that appeared in chains after direct Gram staining were first tested with the AccuProbe for *Enterococcus* sp. If the test for *Enterococcus* sp. was negative, the AccuProbe for *S. agalactiae* was performed. Tests for both staphylococci and streptococci were performed in no specific sequence when the smear showed gram-positive cocci without a predominence of clumps or chains. Polymicrobial bacteremia was rare during our study and was not tested for, since probes were not available for most bacteria in this setting (coagulase-negative staphylococci, diphtheroids, *Propionibacterium* sp., etc). Gram-negative diplococci, gram-negative pleomorphic coccobacilli, and gram-negative bacilli were also presumptively identified with the AccuProbe tests for *N. gonorrhoeae*, *H. influenzae*, and *E. coli*, respectively.

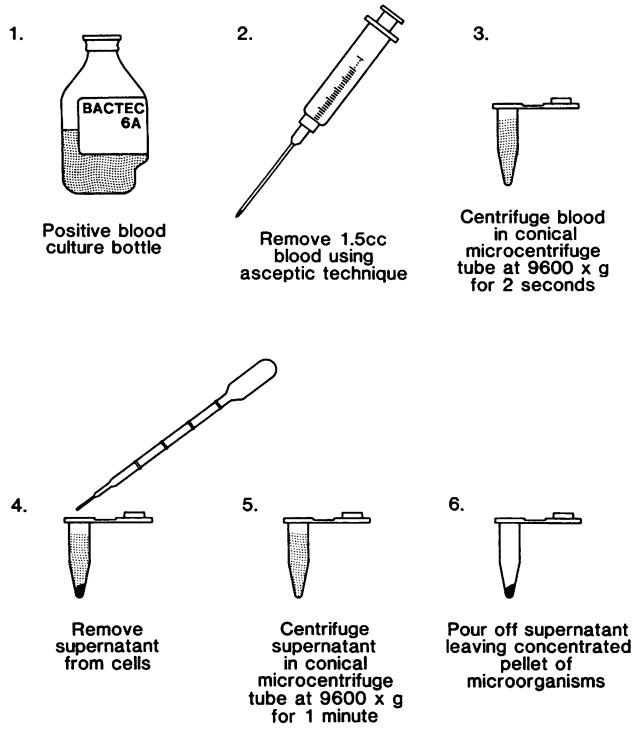

FIG. 2. Pellet preparation.

RESULTS

Of the 217 bacteria tested by the methods outlined in the suggested flow chart (Fig. 1), 152 were positively identified by using a DNA probe. Sixty-five were correctly found to be probe negative and required identification by conventional methods. A total of 52 patient BCP were correctly identified by using the AccuProbe for *Enterococcus* sp. (Table 1), and 69 BCP were correctly identified by using the *S. aureus* AccuProbe. An additional 90 patient blood cultures containing various gram-positive bacteria were tested as controls, and no false-positive reactions were observed. The AccuProbe for *S. agalactiae* was performed on 40 pellets from clinical blood cultures, yielding 100% specificity. Twenty-four clinical blood cultures containing *S. pneumoniae* were correctly identified by AccuProbe, and 27 other streptococci gave negative results. For the direct testing of *H. influenzae* and *N. gonorrhoeae* from patient BCP, a total of 24 AccuProbe tests were performed on pellets from patient blood cultures, yielding 100% specificity.

During the course of this study, an experimental probe for *E. coli* was provided by Gen-Probe. By using this probe, 16 *E. coli* isolates were correctly identified in pellets from patient blood cultures, while 23 various gram-negative organisms gave negative results. According to the manufacturer, this probe will give positive results for both *E. coli* and *Shigella* species. We had no *Shigella* blood culture isolates during the study.

Two changes in methodology were made during this study. The initial incubation step with specimen diluent and patient specimen was increased from 5 min to 10 min at 35°C. The original shorter incubation resulted in equivocal results for 8 of 185 tests. Thus far, this change to a longer incubation has eliminated reactions with values >40,000 and <60,000 RLU. Ninety-one clinical specimens were processed by using both the clinical centrifuge and microcentrifuge methods, and the two methods yielded complete agreement in identification and there was no significant difference in the RLU observed. The microcentrifuge method alone was used for the remaining isolates because of its greater speed and simplicity.

The costs for performing AccuProbe and conventional identification for blood culture isolates of *S. aureus*, *Enterococcus* sp., and *S. pneumoniae* in our laboratory are listed in Table 2.

TABLE 1. AccuProbe tests (for *Enterococcus* sp., *S. aureus*, *S. agalactiae*, *H. influenzae*, *S. pneumoniae*, *N. gonorrhoeae*, and *E. coli*) performed directly on pellets from positive patient blood cultures[a]

Probe	No. of positive AccuProbe identifications confirmed by conventional methods	No. of bacteria tested negative by AccuProbe
Enterococcus sp.	52 (46)	35 (28)
S. aureus	69 (49)	55 (47)
S. agalactiae	20 (17)	20 (17)
H. influenzae	12 (12)	5 (4)
S. pneumoniae	24 (19)	27 (23)
N. gonorrhoeae	3 (2)	1 (1)
E. coli	16 (15)	23 (23)

[a] Isolates >60,000 RLU were categorized as positive, and isolates <40,000 RLU were categorized as negative. Isolates >40,000 and <60,000 RLU were repeated, and those with repeat values >60,00 RLU were considered positive; others were considered negative. Numbers in parentheses are total numbers of patients from whom isolates were received. Specificity for all tests was 100%.

TABLE 2. Comparison of costs of identification of blood culture isolates by DNA probe and by conventional methods

Method	Cost (in dollars)		
	Materials[a]	Labor[b]	Total[c]
AccuProbe	2.40	2.50	4.90
Conventional identification			
S. aureus	0.40	1.00	1.40
Enterococcus sp.	1.20	1.25	2.45
S. pneumoniae	2.14	1.00	3.14

[a] Prices are applicable to the clinical microbiology laboratory at Wishard Memorial Hospital.
[b] Salary plus benefit rate of $15.00 per h.
[c] Indirect costs are not included.

DISCUSSION

Our preliminary study of 362 positive blood cultures shows that direct identification of bacteria by using the AccuProbe methodology is possible. Although the number of clinical isolates is relatively small for some of the species, the accuracy of these probes from blood culture broth is excellent. The probe test is rapid and simple (approximately 30 min), but the preparation of the bacterial pellet by using a standard clinical centrifuge is time-consuming (two centrifugation steps total approximately 30 min). The faster microfuge method takes less than 5 min and requires less volume.

Since probes or pools of probes for all clinically significant causes of bacteremia are not available, evaluation of the blood culture Gram stain by an experienced microbiologist is essential. Using our flow chart, we identified 70% of the blood culture isolates with selected DNA probes, and no

misidentifications were recorded. Direct identification of bacteria from blood cultures by using commercial DNA probe kits has not been previously reported, but identification of mycobacteria from BACTEC broths by using a DNA probe has been successful (2, 19, 24, 27), and often the liquid media had been inoculated with blood. Therefore, the successful probe identification of bacteria from blood cultures was not unexpected.

In his review of DNA probes used for microbial identification, Kohne (12) stated "DNA probes will allow the timely diagnosis of diseases caused by infectious agents" and "will contribute greatly to both the patient's health and cost containment." The cost of direct identification is slightly greater by DNA probe than by conventional methods. The increased cost may be justified by what is saved in antibiotic therapy and shortened hospital stay, although we have no data to substantiate this at present. For example, direct identification of *Enterococcus* sp. in blood culture by using a DNA probe may be more expensive ($4.90 versus $2.45 by conventional methods), but results are available 24 to 48 h sooner. The impact of rapid blood culture identification on patient management and cost of care merits future study.

It has been our experience and that of others (4) that polymicrobial bacteremias often involve skin and other environmental contaminants. We have not performed rapid identifications on polymicrobic bacteremias, because most cases include organisms for which we do not have diagnostic probes. We currently report positive DNA probe tests on blood culture pellets as "presumptive" when they are called to the clinician. It is hoped our early success will continue and that these methods can be accepted as definitive identifications. In that event, subculture to solid media will be performed only to rule out mixed bacteremias and to provide isolates for conventional susceptibility testing.

ACKNOWLEDGMENTS

We gratefully acknowledge Patricia Lineback, Marsha Milish, and Patricia Nolan for their technical assistance, Ed Harris and Julie Lindsley for providing clinical specimens, Beverly Jarrett for her secretarial assistance, and Joseph Brickler and James W. Smith for their review of this manuscript. In addition, we would like to thank Gen-Probe for supplying the AccuProbe test kits.

REFERENCES

1. **Agger, W. A., and D. G. Maki.** 1978. Efficacy of direct Gram stain in differentiating staphylococci from streptococci in blood cultures positive for gram-positive cocci. J. Clin. Microbiol. **7**:111–113.
2. **Body, B. G., N. B. Warren, A. Spicer, D. Henderson, and M. Chery.** 1990. Use of Gen-probe and BACTEC for rapid isolation and identification of mycobacteria. Correlation of probe results with growth index. Am. J. Clin. Pathol. **93**:415–420.
3. **Bryan, C. S.** 1989. Clinical implications of positive blood cultures. Clin. Microbiol. Rev. **2**:329–353.
4. **Cooper, G. S., D. J. Havlir, D. M. Shlaes, and R. A. Salata.** 1990. Polymicrobial bacteremia in the late 1980's: predictors of outcome and review of the literature. Medicine **69**:114–123.
5. **Daly, J. A., N. L. Clifton, K. C. Seskin, and W. M. Gooch.** 1991. Use of rapid, nonradioactive DNA probes in culture confirmation tests to detect *Streptococcus agalactiae*, *Haemophilus influenzae*, and *Enterococcus* spp. from pediatric patients with significant infections. J. Clin. Microbiol. **29**:80–82.
6. **Dunagan, W. C., R. S. Woodward, G. Medoff, J. L. Gray III, E. Casabar, M. D. Smith, C. A. Lawrenz, and E. Spitznagel.** 1989. Antimicrobial misuse in patients with positive blood cultures. Am. J. Med. **87**:253–259.
7. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci, p. 154–175. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
8. **Granato, P. A., and M. R. Franz.** 1990. Use of the Gen-probe PACE system for the detection of *Neisseria gonorrhoeae* in urogenital samples. Diagn. Microbiol. Infect. Dis. **13**:217–221.
9. **Hyypia, T., P. Houvinen, M. Holmberg, and U. Pettersson.** 1989. Nucleic acid probes in the diagnosis of human microbial pathogens, p. 81–104. *In* R. H. Symons (ed.), Nucleic acid probes. CRC Press, Inc., Boca Raton, Fla.
10. **Kilian, M.** 1985. *Haemophilus*, p. 387–393. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
11. **Kloos, W. E., and J. H. Jorgensen.** 1985. Staphylococci, p. 143–153. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
12. **Kohne, D. E.** 1990. The use of DNA probes to detect and identify microorganisms. Adv. Exp. Med. Biol. **263**:11–35.
13. **Kreemola, S. R., J. E. Karjalainen, and R. Raty.** 1990. Rapid diagnosis of *Mycoplasma pneumoniae* infection: clinical evaluation of a commercial probe test. J. Infect. Dis. **162**:70–75.
14. **Lewis, J. S., P. Krang-Brow, and P. A. Trainor.** 1990. DNA probe confirmatory test for *Neisseria gonorrhoeae*. J. Clin. Microbiol. **28**:2349–2351.
15. **Malouin, E., L. E. Bryan, P. Shewciw, J. Douglas, D. Li, H. Van Den Elzen, and J. R. LaPointe.** 1988. DNA probe technology for rapid detection of *Haemophilus influenzae* in clinical specimens. J. Clin. Microbiol. **26**:2132–2138.
16. **Morella, J. A., W. M. Janda, and M. Bohnhoff.** 1985. *Neisseria* and *Branhamella*, p. 176–192. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
17. **Parsons, L. M., M. Shayegani, A. L. Waring, and L. H. Bopp.** 1989. DNA probes for the identification of *Haemophilus ducreyi*. J. Clin. Microbiol. **27**:1441–1445.
18. **Pasculle, A. W., G. E. Veto, S. Krystofiak, K. McKelvey, and K. Vrsalovic.** 1989. Laboratory and clinical evaluation of a commercial DNA probe for the detection of *Legionella* spp. J. Clin. Microbiol. **27**:2350–2358.
19. **Peterson, E. M., R. Lo, C. Floyd, A. Nakasone, G. Friedly, and L. M. de la Maza.** 1989. Direct identification of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare* from amplified primary cultures in BACTEC media using DNA probes. J. Clin. Microbiol. **27**:1543–1547.
20. **Peterson, E. M., R. Oda, R. Alexander, J. R. Greenwood, and L. M. de la Maza.** 1989. Molecular techniques for the detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **27**:2359–2363.
21. **Rappaport, T., K. P. Sawyer, and I. Nachamkin.** 1988. Evaluation of several commercial biochemical and immunologic methods for rapid identification of gram-positive cocci directly from blood cultures. J. Clin. Microbiol. **26**:1335–1338.
22. **Rossau, R., M. Duhamel, E. Van Dyck, P. Diot, and H. Van Heuverswyn.** 1990. Evaluation of an rRNA-derived oligonucleotide probe for culture confirmation of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **28**:944–948.
23. **Schifman, R. B., and K. J. Ryan.** 1982. Rapid automated identification of gram-negative bacilli from blood cultures with the AutoMicrobic System. J. Clin. Microbiol. **15**:260–265.
24. **Sherman, I., N. Harrington, A. Rothrock, and H. George.** 1989. Use of a cutoff range in identifying mycobacteria by the Gen-Probe Rapid Diagnostic System. J. Clin. Microbiol. **27**:241–244.
25. **Strand, C. L., and J. A. Shulman.** 1988. Bloodstream infections, p. 21–44. American Society for Clinical Pathologists, Chicago.
26. **Tenover, F. C.** 1988. Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin. Microbiol. Rev. **1**:82–101.
27. **Woodley, C. L., V. A. Silcox, M. M. Floyd, and G. P. Kubica.** 1990. The use of DNA probes for rapidly identifying cultures of mycobacterium. Adv. Exp. Med. Biol. **263**:51–56.
28. **Yagupsky, P., and F. S. Nolte.** 1990. Quantitative aspects of septicemia. Clin. Microbiol. Rev. **3**:269–279.