Detection of *Streptococcus pneumoniae* in Whole Blood by PCR

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***Streptococcus pneumoniae* is a major cause of bacteremia in both children and adults. Currently, the diagnosis of pneumococcal bacteremia relies on the isolation and identification of the bacteria from blood cultures. We have developed a sensitive assay for the detection of *S. pneumoniae* in whole blood by the PCR. A specific primer-probe set (JM201 and JM202 primers with JM204 probe) designed from the penicillin-binding protein 2B gene was demonstrated to reproducibly detect between 10 and 100 fg of input purified *S. pneumoniae* DNA. This assay system was shown to be inclusive for all strains of *S. pneumoniae* evaluated, including 15 different serotypes and a battery of penicillin-resistant and -sensitive strains. The specificity of this PCR-based assay was demonstrated by its inability to support amplification from a series of human, bacterial, and yeast genomic DNAs. A general specimen preparation method which should be suitable for the purification of DNA from any pathogens in whole blood was developed. With this protocol it was possible to detect *S. pneumoniae*-specific DNA from whole blood specimens inoculated with as little as 4 CFU/ml. Copurified human blood DNA, ranging from 0 to 4.5 μg per PCR, did not affect the sensitivity of *S. pneumoniae* detection by PCR. A blinded clinical trial was used to compare the PCR-based assay with standard microbiological blood culture for the detection of *S. pneumoniae* bacteremia in 36 specimens obtained from pediatric patients seen in the emergency room of Children's Hospital of Pittsburgh. With culture as the "gold standard," the PCR-based assay had a sensitivity of 80% (4 of 5 culture-positive specimens were PCR positive) and a specificity of 84% (26 of 31 culture-negative specimens were PCR negative). However, three patients whose specimens were PCR positive and culture negative had histories suggestive of bacteremia, including recent positive blood cultures, treatment with antibiotics, cellulitis, and multiple emergency room visits for fever within a 24-h period. These data suggest that PCR-based assays for *S. pneumoniae* may prove useful to augment current methods of detection for *S. pneumoniae* bacteremia.**

Streptococcus pneumoniae is a major etiologic agent for bacteremia in both children and adults (11, 15, 19, 26). Currently, the diagnosis of pneumococcal bacteremia relies on the successful growth and identification of bacteria from blood cultures (4). The usual time for detection of *S. pneumoniae* in a blood culture is 24 to 48 h (8). Identification of the cultured bacteria is conducted with modified conventional tests, commercial immunological kits, and commercial DNA probe kits (2–4, 24). Multiple-step procedures are involved in all of these methods, usually adding an additional 24 h for final identification of the organism.

A substantial number of cases of bacteremia in children may be missed by the performance of a single blood culture (12). Factors contributing to this low sensitivity include the low density of bacteremia caused by this pathogen in children and the fastidious nature of the organism. In addition, culture techniques are inhibited by pretreatment with antibiotics. A more sensitive and rapid method for detection of *S. pneumoniae* directly from whole blood would therefore facilitate the diagnosis of pneumococcal bacteremia in both children and adults.

Assays based upon the PCR offer promise as sensitive and rapid detection methods for many human pathogens (7, 21, 27). Under ideal conditions, the PCR can detect a single copy of a target DNA sequence in a given sample. Therefore, propagation of the pathogen is not required, as it is with simple DNA probe tests. Significant advances in the development of PCR-based diagnostic tests for infectious agents have been made. Although initial efforts focused on the detection of viral agents, such as human immunodeficiency virus, human papillomavirus, human cytomegalovirus, and human T-cell leukemia virus, PCR-based detection systems for a wide variety of bacteria and parasites have recently been reported (6, 7, 21, 27).

PCR-based detection systems for blood-borne pathogens face two challenges: (i) the low density of the pathogens and (ii) the inhibitory effects of certain blood components on the PCR process. However, bacteria such as *Mycobacterium avium*, *Plasmodium falciparum*, and *Borrelia burgdorferi* have been successfully detected by PCR from blood samples (1, 10, 18), and detection of *S. pneumoniae* from whole blood by nested PCR has been reported by Rudolph et al. (25). Nested PCR, however, is not ideal for clinical diagnostic applications because of the high propensity for contamination from carryover products between the first and second rounds of amplification and the extensive technical time required for this process (7). We report the development of a PCR-based assay, using DNA directly purified from whole blood, which is sensitive and specific for *S. pneumoniae*.

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TABLE 1. PCR primers and probes

Primer or probe	Nucleotides[a]	Sequence (5′→3′)
JM201	1805–1825	ATGCAGTTGGCTCAGTATGTA
JM202	1891–1872	CACCCAGTCCTCCCTTATCA
JM203	1835–1874	GCAAATAATGGTGTTCGTGTGGCTCCTCGTATTGTTGAAG
JM204	1836–1865	CAAATAATGGTGTTCGTGTGGCTCCTCGTA

[a] Data are from reference 5.

MATERIALS AND METHODS

Bacteria. (i) *S. pneumoniae.* The reference strain used in this study is a clinical isolate (91-0120) obtained from Children's Hospital of Pittsburgh. Additional clinical isolates were obtained either from Children's Hospital of Pittsburgh or from Texas Children's Hospital. Bacteria were propagated either on 5% sheep blood agar (BBL) or in Todd-Hewitt broth (BBL). Incubations were at 37°C in a humidified atmosphere supplemented with 5% CO_2. Trypticase soy broth with 20% glycerol (BBL) was used for the storage of bacteria at −80°C.

(ii) Bacterial suspensions. Freshly cultured *S. pneumoniae* organisms were washed twice with 40 ml of sterile saline (0.9% NaCl) and recovered by centrifugation at 4,300 × g for 10 min in an SS-34 rotor with a Sorvall RC centrifuge (duPont, Wilmington, Del.). The density of the bacteria was adjusted with saline to an A_{420} of 0.200 as measured with a spectrophotometer (Lambda 3B; Perkin-Elmer, Norwalk, Conn.). A series of 10-fold dilutions was prepared from this bacterial suspension and subsequently used for either quick lysis of the bacteria or inoculation of whole blood samples. Direct dilution plating of this suspension demonstrated the concentration to be 1×10^7 to 2×10^7 CFU/ml.

(iii) Inoculation of whole blood with *S. pneumoniae.* Whole blood was obtained from healthy adult volunteers with no recent clinical history of pneumococcal disease. The blood was collected via peripheral venipuncture, using aseptic technique, into acid citrate dextrose Vacutainer tubes (Becton Dickinson, Mountain View, Calif.) and immediately inoculated with the diluted bacterial suspension to obtain final concentrations of *S. pneumoniae* of 0.8 to 32 CFU/ml. Sham (sterile saline)-inoculated blood served as a negative control. Less than 0.05 ml of the bacterial suspension was used for the inoculation of each 1-ml whole blood sample. The inoculated whole blood samples were subsequently used for PCR sample preparation and quantitative culture for *S. pneumoniae.*

(iv) Quantitative culture of *S. pneumoniae.* The starting concentrations of the bacterial suspensions were determined by quantitative culture of the bacteria. Portions (100 μl) of diluted bacterial suspensions (10^{-3} to 10^{-9}) were plated out on 5% sheep blood agar plates in duplicate and incubated as described above. The plates were examined and bacteria were counted at 48 h. To monitor the bacterial load in the inoculated whole blood, 100-μl aliquots of the blood were first vortexed together with 6.7 μl of Isolator tube solution (0.8% polypropylene glycol P-2000, 0.96% polyethanolesulfonate, and 10 U of purified saponin per ml) to facilitate the recovery of both intracellular and extracellular bacteria. These lysates were then plated out in duplicate on 5% sheep blood agar and handled as described above. Uninoculated blood was also included as a control.

PCR sample preparation. (i) Bacterial DNA. The bacterial DNA used in this study was purified from freshly cultured bacteria by standard phenol-chloroform extraction methods (17).

(ii) Quick lysis of *S. pneumoniae.* Portions (100 μl) of the bacterial suspensions were harvested by centrifugation at 2,800 × g for 10 min in a microcentrifuge (Microspin 24S; Sorvall). After the supernatant was carefully removed, the pellet was resuspended in 40 μl of quick lysis buffer A (100 mM KCl, 10 mM Tris [pH 8.3], 2.5 mM $MgCl_2$)–10 μl of lysozyme (20 mg/ml; Gibco BRL, Grand Island, N.Y.). After incubation for 30 min at room temperature, 40 μl of quick lysis buffer B (10 mM Tris [pH 8.3], 2.5 mM $MgCl_2$, 1% Nonidet P-40) and 10 μl of proteinase K (5 mg/ml; Gibco BRL) were added. The proteinase K digestion was performed at 60°C for 1 h. To inactivate the proteinase K and denature the DNA, the samples were incubated in a boiling water bath for 10 min. Samples were used immediately for PCR or stored at −20°C until used.

(iii) Purification of total DNA from whole blood inoculated with *S. pneumoniae.* Whole blood inoculated with *S. pneumoniae* underwent DNA preparation for PCR with the QIAamp blood kit (QIAgen, Chatsworth, Calif.). In order to obtain sufficient lysis of the bacteria, whole blood was first mixed with an equal volume of glass beads (0.1 mm in diameter; Biospec Products, Bartlesville, Okla.) and vortexed continuously for 5 min with a microtube adaptor. A 500-μl aliquot of this treated blood sample was further processed with the QIAamp blood kit according to the manufacturer's instructions.

PCR primers and probes. The primers JM201 and JM202 were derived from the penicillin-binding protein 2B gene on the basis of the sequence published by Dowson et al. (5) (Table 1). Both primers and probes (JM203 and JM204) were synthesized with a PCR-MATE 391 DNA synthesizer (ABI, Foster City, Calif.) and subsequently cleaved and purified according to the manufacturer's instructions by using oligonucleotide purification cartridges (ABI). All primers and probes were diluted to 10 pmol/μl (100 nM) with fresh TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and stored at −20°C until used.

One microliter of the probe (10 pmol/μl) was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs, Beverly, Mass.) according to the manufacturer's instructions. The labeled oligonucleotides were purified by gel exclusion chromatography with a Sephadex G-50 (Pharmacia, Piscataway, N.Y.) spin column format. All oligonucleotides were stored at −20°C.

PCR amplification. All PCR amplifications were performed in a 100-μl volume with the JM201-JM202 primer set. A 2× PCR cocktail of reaction buffer (1× is 50 mM KCl; 10 mM Tris [pH 8.3]; 2.5 mM $MgCl_2$; 0.2 mM [each] dATP, dCTP, dGTP, and UTP; 2 U of *Taq* polymerase [Perkin-Elmer]; and the primers at 10 pmol) was adjusted to a volume of 50 μl with sterile water. Fifty microliters of specimen was added to the 2× cocktail, and thermal cycling was performed in a 9600 thermal cycler (Perkin-Elmer). The PCR process included an initial 10-min incubation at 94°C to denature the target DNA. This was followed by 30 cycles of PCR, which included primer annealing at 55°C for 30 s, primer extension at 72°C for 60 s, and denaturation of the PCR products at 94°C for 30 s. A 7-min extension at 72°C was included at the end of the final cycle. The PCR products were stored at 4°C prior to analysis.

Liquid hybridization-gel retardation analysis. The PCR products were detected by liquid hybridization-gel retardation analysis as described previously (6). A positive PCR result with the experimental specimen was determined by the presence of a retarded probe band comigrating with amplified purified *S. pneumoniae* control DNA.

Clinical trial. The PCR-based assay for *S. pneumoniae* was applied in a prospective fashion to 36 clinical whole blood specimens collected from children with suspected bacteremia at Children's Hospital of Pittsburgh. Venous peripheral blood was collected via syringe and aliquoted into sodium citrate Vacutainer (blue top) tubes (Becton Dickinson) for PCR and into separate Bactec bottles (Peds Plus 660; Becton Dickinson) for aerobic and anaerobic cultures. The specimens for PCR were placed at 4°C for 6 to 24 h before being placed in cryostorage at −80°C until processing for DNA extraction. The entire process was conducted in a blinded fashion such that the individuals performing the PCRs were not aware of the culture results and vice versa.

RESULTS

Sensitive detection of *S. pneumoniae* DNA. The sensitivity of the PCR-based assay with the JM201-JM202 primer pair and the JM203 and JM204 probes for *S. pneumoniae* DNA was determined. Ten-fold dilutions of purified *S. pneumoniae* genomic DNA were subjected to amplification and liquid hybridization followed by gel retardation analysis and autoradiography. In all instances we were able to detect 100 fg of input DNA (approximately 30 genomic equivalents), and in most cases we could detect 10 fg of genomic DNA (3 genomic equivalents) (Fig. 1). Liquid hybridization-gel retardation assays of JM201-JM202-amplified DNA with the JM203 and JM204 probes yielded equivalent sensitivities. However, because of the greater resolution between the hybridized bands
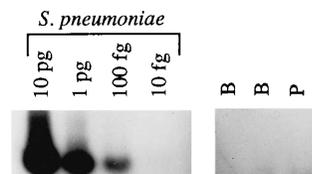

FIG. 1. Sensitivity of *S. pneumoniae* PCR-based assay. An autoradiograph of gel retardation products following liquid hybridization of amplified *S. pneumoniae* DNA with a ^{32}P-end-labeled probe (JM203), demonstrating the ability to detect 10 fg of input purified genomic DNA (approximately three genomic equivalents), is shown. Reagent blanks (B) and unbound probe (P) served as negative controls.

Detection of *S. pneumoniae* in saline dilutions (CFU/PCR reaction)

Detection of *S. pneumoniae* in the presence of human DNA (*S. pneumoniae* DNA [pg] / human DNA [μg])
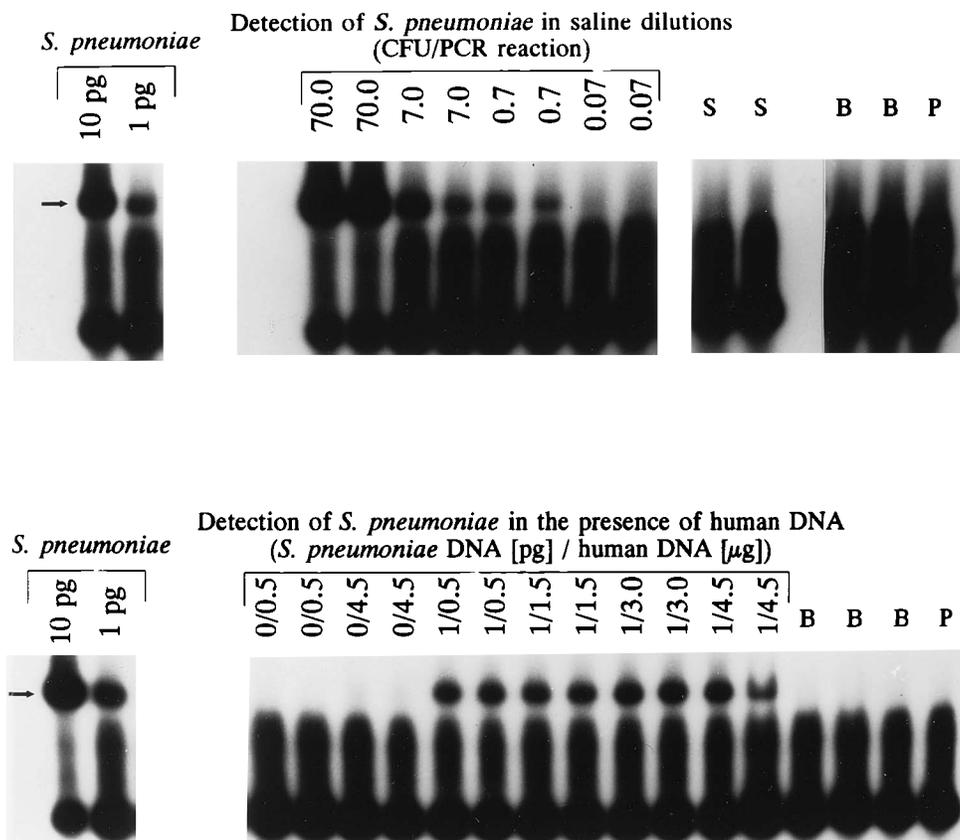

FIG. 2. Detection of *S. pneumoniae* by PCR. An autoradiograph from liquid hybridization-gel retardation analysis of PCR-amplified *S. pneumoniae* DNA with the JM201-JM202 primer pair is shown. The probe used in this experiment was JM203, which is longer than JM204. Less resolution between the unbound probes and the hybridized products was seen with JM203 than with JM204. Reagent blanks (B) and unbound probe (P) served as negative controls. (Top) PCR amplification of *S. pneumoniae* DNA with quick lysates of an *S. pneumoniae* suspension diluted in saline. Saline (S) was used as a negative control. (Bottom) PCR amplification of *S. pneumoniae* DNA in the presence of DNA purified from whole blood via the glass bead-QIAamp process. Arrows, *S. pneumoniae* DNA.

and unhybridized probe in the JM204 system than in the JM203 system, we adopted the JM204 probe for most of the later work.

To determine the sensitivity of the assay under mock clinical conditions, saline suspensions with 10-fold dilutions of *S. pneumoniae* bacteria, prepared from a fresh overnight culture, were prepared and tested for their abilities to support amplification. These bacterial dilutions were also plated on 5% sheep blood agar to compare colony-forming ability with PCR sensitivity. We could reproducibly detect *S. pneumoniae* DNA by PCR at less than 1 CFU equivalent (Fig. 2, top panel), since we could obtain a signal from a 200-μl aliquot of whole blood that had been inoculated with 4 CFU/ml.

JM201 and JM202 do not support amplification of human DNA, and human DNA does not decrease the efficiency of the *S. pneumoniae* amplification process. A unique procedure using mechanical disruption, based upon glass bead lysis, combined with the QIAAmp kit for the processing of whole blood was developed to obtain ultra-high-purity DNA. This sample preparation procedure obviated any need to fractionate the blood prior to DNA purification, thus ensuring that all *S. pneumoniae* sequences, whether in the cellular or humoral fraction, would be recovered for analysis (unpublished observations). Typically, 2 to 4 μg of DNA was obtained from 0.5 ml of whole blood.

S. pneumoniae PCR was conducted in the presence of human DNA isolated from whole blood (0 to 4.5 μg/100 μl of PCR mixture) to test whether the JM201-JM202 primer pair

would be inhibited by high concentrations of DNA. The results indicated that the *S. pneumoniae* amplification signal achieved at all levels of input human DNA, prepared as described above, was equivalent to the signal obtained when no human DNA was added to the reaction mixture. Further, it was demonstrated that human DNA sequences did not at any concentration support amplification and detection by the *S. pneumoniae* primer-probe set (Fig. 2, bottom panel).

JM201-JM202 is specific for *S. pneumoniae* DNA. The specificity of the JM201-JM202–JM204 primer-probe set was analyzed with a series of gram-positive bacteria, gram-negative bacteria, and yeast isolates obtained from pediatric patients at Children's Hospital of Pittsburgh. No amplification was achieved with 10 pg of DNA (>3,000 genomic equivalents) from any of the species in the specificity panel (Fig. 3). The specific *S. pneumoniae* DNA was amplified strongly at this input level.

To assess the ability of the JM201-JM202–JM204 primer-probe set to amplify and detect various strains of *S. pneumoniae*, PCR amplifications were conducted with DNAs extracted from 51 additional *S. pneumoniae* clinical isolates (Table 2). This group of specimens included DNAs obtained from 15 *S. pneumoniae* isolates that had been serotyped, as well as those from a number of penicillin-sensitive and -resistant isolates. At input levels of 1 pg of DNA per PCR, amplification equivalent to that for our reference strain was observed in all of the clinical isolates.
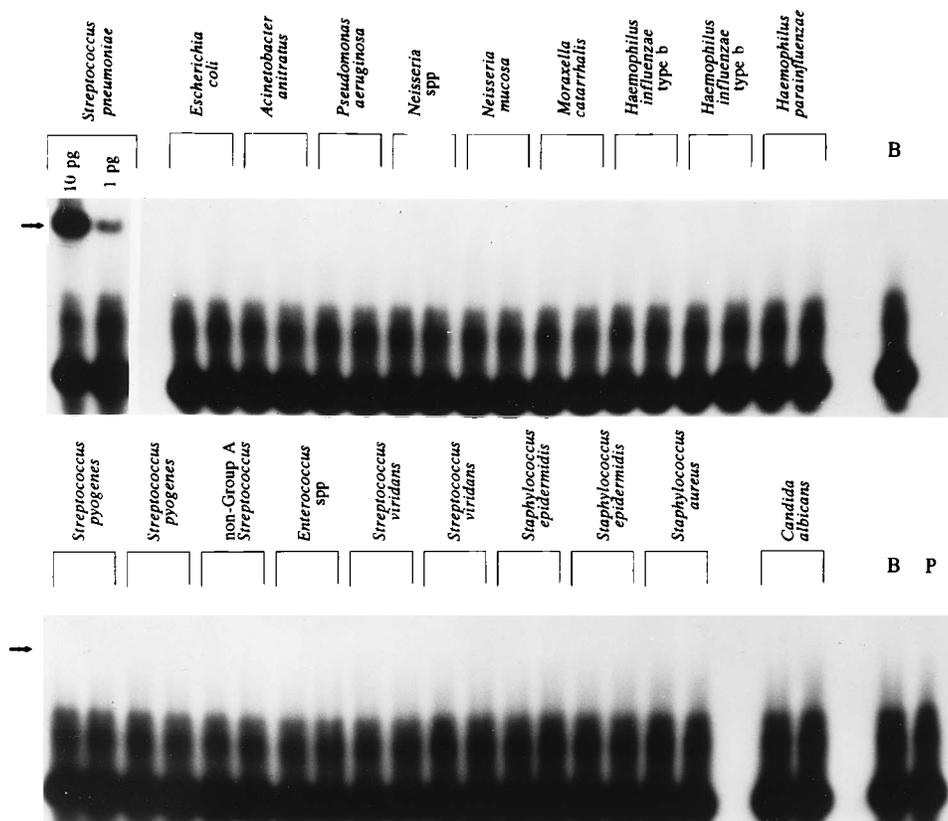
FIG. 3. Specificity of *S. pneumoniae* PCR-based assay. An autoradiograph from liquid hybridization-gel retardation analysis following JM201-JM202-based amplification of bacterial and yeast DNAs, demonstrating the inability of this primer pair to support amplification of non-*S. pneumoniae*-derived DNAs, is shown. PCRs were conducted with 10 pg of purified DNAs from the organisms indicated. Purified *S. pneumoniae* DNA was used for the positive control; the arrows indicate the *S. pneumoniae*-specific signals. Reagent blanks (B) and unbound probe (P) served as negative controls. Probe JM204 was used.

TABLE 2. PCR-based detection of *S. pneumoniae* clinical isolates

Specimen no.[a]	Serotype	Phenotype[b]	Source[c]
12533–12537 (5)	NA[d]	Resistant	TCH
12540–12547 (8)	NA	Resistant	TCH
14070–14082 (13)	NA	Sensitive	CHP
14711, 14712 (2)	1	NA	CHP
14713, 14714 (2)	3	NA	CHP
14715, 14716 (2)	4	NA	CHP
14717, (1)	5	NA	CHP
14718, 14719 (2)	6B	NA	CHP
14720, 14721 (2)	7F	NA	CHP
14722, 14723 (2)	9V	NA	CHP
14724 (1)	10A	NA	CHP
14725, 14726 (2)	11A	NA	CHP
14727, 14728 (2)	14	NA	CHP
14729 (1)	15	NA	CHP
14730 (1)	17F	NA	CHP
14731 (1)	18C	NA	CHP
14732, 14733 (2)	19F	NA	CHP
14734, 14735 (2)	23F	NA	CHP

[a] All specimens showed a positive PCR result. The number in parentheses is the number of isolates tested that fit all criteria for that row.

[b] Sensitivity or resistance to penicillin.

[c] TCH, Texas Children's Hospital and Baylor College of Medicine; CHP, Children Hospital of Pittsburgh.

[d] NA, data not available.

Sensitive detection of *S. pneumoniae* in in vitro-inoculated whole blood. To determine whether our method was suitable for the detection of *S. pneumoniae* in whole blood specimens, an in vitro model system was developed. In this assay, whole blood from a healthy adult donor was inoculated with a known concentration of *S. pneumoniae* and subsequently processed for PCR amplification and culture. An aliquot of purified DNA (0.8 to 2.0 μg) from the whole blood was used to support PCR. When the initial sample was inoculated with *S. pneumoniae* at a concentration of 4 CFU/ml or greater, specific amplified products could be detected by our system, although the signal at 4 CFU/ml was faint (Fig. 4). No signal was detected in the negative control blood specimens or the reagent blanks.

Detection of *S. pneumoniae* in clinical specimens. To further evaluate the usefulness of the *S. pneumoniae* PCR-based assay as a diagnostic test, a blinded prospective clinical trial in which 36 pediatric blood specimens were evaluated by culture and PCR for evidence of bacteremia was conducted. The results of this study demonstrated a sensitivity of 80% (4 of 5 culture-positive specimens were PCR positive) and a specificity of 84% (26 of 31 culture-negative specimens were PCR negative) for the PCR-based assay when it was compared with blood culture as the "gold standard". The clinical data for the discordant cases are summarized in Table 3.

DISCUSSION

The detection of human pathogens by PCR has been achieved with specimens of many body fluids, including cere-
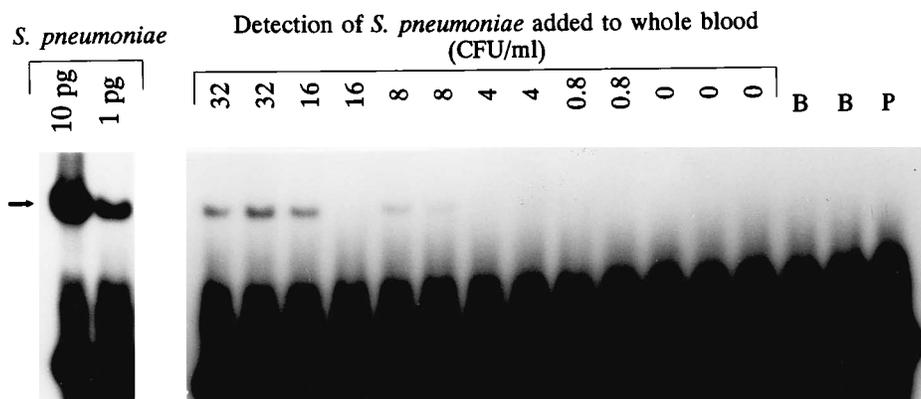
FIG. 4. Detection of *S. pneumoniae* in whole blood by PCR. PCR amplification of DNA purified from whole blood that had been inoculated with *S. pneumoniae* is shown. Uninoculated blood, reagent blanks (B), and unbound probe (P) were used as negative controls. Probe JM204 was used. Arrow, *S. pneumoniae* DNA.

brospinal fluid (CSF), blood, sputum, tears, middle-ear fluid, and urine (1, 10, 14, 16, 18, 22, 23). With few exceptions, PCR samples have been prepared from these specimens by organic extraction and ethanol precipitation of the DNA, a labor-intensive process involving multiple steps. Therefore, simplified sample preparation methods, such as the one described here, represent improved methods for the PCR-based diagnosis of infectious diseases.

A major barrier to the direct use of body fluids in PCR is the presence of inhibitory agents in the samples. Numerous approaches have been taken to overcome this problem. Ni et al. demonstrated a sensitivity and a specificity of 91% in the detection of *Neisseria meningitidis* by PCR after boiling of clinical CSF samples (20). This approach, of using heat denaturation of proteinaceous inhibitors, has been widely employed in the preparation of nonblood body fluids for PCR analyses. We were able to detect *S. pneumoniae* in boiled and sonicated CSF samples, which had been diluted with water up to 4×10^5-fold, from a patient with pneumococcal meningitis (13). In the case of tears, Epstein-Barr virus has been detected by PCR with boiled tear samples from patients with primary Sjögren's syndrome (22). Direct detection of cytomegalovirus has been performed with urine samples from which the PCR inhibitor has been removed by either dialysis or ultrafiltration (14). However, these examples do not involve whole blood specimens, and it has been repeatedly demonstrated that certain components of whole blood, particularly the porphyrin ring of the hemoglobin molecule, can inhibit the PCR amplification process by binding the *Taq* DNA polymerase (9). Unlike the case

for CSF, which can constitute up to 10% of the reaction mixture without significantly inhibiting the PCR process, final blood concentrations of less than 1% have been shown to be strongly inhibitory to the PCR process (5a, 9, 20). In fact, the majority of the PCR-based detection methods for blood-borne pathogens have used only selected fractions of the whole blood in order to overcome this problem. Intracellular pathogens can be detected by PCR with only leukocytes. Likewise, true extracellular pathogens can be detected by using serum or plasma. However, for complete recovery of low-copy-number pathogens which may be present in multiple blood fractions, e.g., *S. pneumoniae*, the use of a single blood component will reduce the sensitivity of the assay.

To obtain complete recovery of *S. pneumoniae* DNA, we developed a method for the preparation of DNA samples from whole blood. In this process no blood components were eliminated prior to the DNA purification. Mechanical lysis of the specimen was achieved by vortexing it with glass beads, which ensured breakage of the gram-positive bacterial cell wall. The high-efficiency purification (4 to 5 μg/0.5 ml) of DNA from this whole blood lysate via the QIAAmp column provided sufficient substrate for the routine performance of replicate assays. As judged by the total lack of inhibition at high input levels, the DNA recovered via this procedure is of very high quality. The ability to obtain amplifiable DNA from small aliquots of whole blood without having to add carrier nucleic acids was an important criterion that we had set at the outset of the study because blood draw volumes for neonatal pediatric patients are very small. The combination of the above-described fea-

TABLE 3. Summary of clinical features of cases showing discordant PCR and culture results for *S. pneumoniae*

Patient no.	Age (mo)	Temp (°C)	WBC[a]	Diagnosis	Miscellaneous	Blood culture	PCR
1	17	41.2	7.3	Fever without source	One of four bottles positive by blood culture; afebrile on repeat examination	+	−
2	6	40.7	18.2	Cellulitis	Admitted for intravenous nafcillin	−	+
3	24	40.4	10.8	Fever without source	Two emergency room visits within 24 h	−	+
4	26	38.3	19.0	Positive blood culture 24 h prior to visit	Pretreated with Rocephin; admitted for intravenous antibiotics	−	+
5	13	40.0	NA[b]	Status asthmaticus	Positive history of asthma	−	+
6	10	39.4	6.0	Gastroenteritis	NA	−	+

[a] WBC, leukocytes.
[b] NA, data not available.

tures suggests that this method has the potential for the recovery of sufficient DNA from small blood volumes to will make it generally applicable as a sample preparation method for PCR-based assays of low-copy-number infectious agents. It has been reported that average levels of *S. pneumoniae* in pediatric patients with occult bacteremia are between 5 and 20 CFU/ml, while those patients having focal infections generally have higher density of bacteremia (2).

Among the 36 clinical samples tested, only one of the specimens was blood culture positive and PCR negative, while five of the specimens were PCR positive and culture negative. Although blood culture is considered the gold standard, the clinical data on the PCR-positive, culture-negative discordant cases indicate that the PCR-based assay may be more sensitive in the detection of *S. pneumoniae* bacteremia, particularly in cases when the patient has been pretreated with antibiotics (patients 2 and 4). The sensitivity and specificity of this assay will be further evaluated as part of an ongoing clinical trial. The high sensitivity of our method suggests that it may be sufficient for the detection of the majority of pediatric bacteremia caused by this organism.

In conclusion, we have developed a PCR-based assay that utilizes a primer-probe set that targets the penicillin-binding protein 2B gene of *S. pneumoniae*. This assay is both sensitive and specific for the detection of *S. pneumoniae* DNA in purified form or in clinical specimens, including whole blood. It is broadly inclusive for all *S. pneumoniae* serotypes examined, and it detects clinical isolates regardless of their antibiotic sensitivity or resistance. The comparative evaluation of this method with standard blood cultures in a small prospective clinical trial indicates that this assay may be useful to augment current methods of detection of *S. pneumoniae* bacteremia. We are currently conducting a large-scale clinical trial in an attempt to extend these preliminary results. The blood-processing method developed for this assay should prove practical for any blood-borne pathogens.

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