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Immunomagnetic PCR and DNA Probe for Detection and Identification of *Porphyromonas gingivalis*

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The aim of the study that we describe was to combine an immunomagnetic separation and a PCR followed by dot blot hybridization with a DNA probe for the detection and identification of *Porphyromonas gingivalis*. Immunomagnetic particles were coated with monoclonal antibody specific for *P. gingivalis* and were incubated with a suspension containing seven oral bacterial species spiked with various dilutions of *P. gingivalis*. Beads with their load of bound bacteria were boiled in water, and the target DNA in the supernatant was amplified with a primer pair to generate a 593-bp PCR fragment specific for *P. gingivalis*. Finally, the product of amplification was detected by dot blot hybridization with a digoxigenin-labeled 593-bp probe. The detection limit was determined to be 100 bacterial cells per ml. The immunomagnetic-PCR/DNA probe procedure described here should be useful for the rapid, specific, and sensitive detection and identification of *P. gingivalis* in clinical samples.

The concept that bacteria, in particular, gram-negative anaerobes, initiate the major mechanisms of destruction of the periodontium has received convincing scientific support in recent years, and the diseases are considered polymicrobial, mixed infections (24). Studies of the predominant cultivable oral microbiota have revealed that among the 150 to 200 different species that any individuals may typically harbor, perhaps as few as 10 to as many as 30 species, including *Porphyromonas gingivalis*, are frequently associated with active destruction (10). These bacteria are packed in a dense mass of cells with close physical contact, referred to as subgingival plaque. The anaerobic cultural procedure has been seen as inadequate on a routine basis, because certain of the putative periodontal pathogens are either uncultivable or extremely difficult to cultivate (13). In addition, cultural techniques are time-consuming and expensive, they require highly trained technicians and specialized equipment, and they have many sources of method error. The inability to reliably culture the putative periodontal pathogens has led to the development of either immunological reagents (30) or DNA probes (7).

Several studies have described the application of PCR to the detection of different microorganisms directly in clinical specimens (2, 9). However, PCR-based diagnostic procedures directly from clinical samples are faced with the possibility of inhibition by factors present in the crude clinical sample and usually require that impurities be sufficiently diluted so that polymerization is not inhibited (18, 26).

Immunomagnetic particle-based separation (IMS) has been shown to be an efficient tool for the separation and isolation of specific cells from heterogeneous suspensions (3, 14, 15, 23). Bacteria are specifically separated from the specimen, resulting in a useful sample for PCR with little or no nonspecific DNA or interfering factors. The so-called immunomagnetic-PCR (IM-PCR) assay allows for the selective extraction of bacteria by specific antibody; this is followed by primer-specific PCR amplification. This report describes a rapid method for the isolation and identification of *P. gingivalis* from a mixture of different bacteria by using an IM-PCR/DNA probe procedure.

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MATERIALS AND METHODS

Bacteria and culture conditions. The strains used in the study are listed in Table 1. The *P. gingivalis* strains were grown to the mid-log phase in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) enriched with hemin (10 μg/ml) and vitamin K_1 (1 μg/ml). Stock cultures were maintained by plating the organisms on the same medium containing 1.5% agar and 2% laked human blood. All cultures were grown in an anaerobic chamber (80% N_2, 10% H_2, and 10% CO_2; Coy Manufacturing Co., Ann Arbor, Mich.) at 37°C. All other bacteria used for examination of the specificities of PCR and IMS were grown by using appropriate media and conditions. The bacteria were washed, centrifuged, and resuspended in phosphate-buffered saline (PBS; pH 7.4) to approximately 10^8 bacteria per ml (optical density at 660 nm = 0.2), and the working concentrations were subsequently achieved by serially diluting the mixture 10-fold in sterile PBS.

Bacterial sampling. The subgingival or submarginal plaque was removed with a periodontal curette and was placed in 1.0 ml of reduced transport fluid (25). Subsequently, the samples were transferred to an anaerobic chamber and were dispersed with a syringe and a 21-gauge needle (20 passes) to break up the bacterial aggregates. Tenfold serial dilutions (10^{-1} to 10^{-5}) were made in reduced transport fluid, 100 μl of the 10^{-3} to 10^{-4} dilutions were plated onto enriched Todd-Hewitt blood agar, and the plates were incubated anaerobically. The presence of black-pigmented colonies was verified over the following 14 days, and a panel of tests (5) was used for their identification.

Bacterial DNA. Extraction and purification of chromosomal DNA were performed by a standard miniprep procedure as described by Wilson (29). The concentration and quality of the DNA samples were estimated by agarose gel electrophoresis and comparison with DNA standards. This DNA was used for PCR standardization and for testing the specificity of the primer pair chosen.

Coating of IMBs. Immunomagnetic beads (IMBs; Dynabeads M-280, 2.8 μm in diameter, 5×10^8 beads per ml) are uniform superparamagnetic polystyrene beads with covalently linked sheep anti-mouse immunoglobulin G (Dynal ALS, Oslo, Norway). The beads were washed twice with PBS-bovine serum albumin (0.1%) (PBS-BSA), separated by magnetic force, and then resuspended to the original volume in PBS-BSA. Thereafter, they were coated with capture monoclonal antibody (MAb) by incubation in a 1:50 dilution of the antibody for 24 h at 4°C with rotation (50 rpm) to avoid settling of the IMBs. The MAb (immunoglobulin G1 subclass) used was a culture supernatant of hybridoma 1C4 specific for the hemagglutinating adhesin HA-Ag2 of *P. gingivalis* ATCC 33277 (4). The amount of antibody required to achieve optimal binding of the target bacteria was determined in a series of pilot experiments; the highest number of bound bacteria was obtained with 50 μg of immunoglobulin G per mg of immunomagnetic beads. The coated IMBs were stored and washed before use as described by the manufacturer.

Immunomagnetic isolation procedure. A 15-μl volume of MAb-coated Dynabeads was mixed with 1 ml of each dilution of the bacterial suspension, and the mixture was incubated at 4°C with continuous shaking for 30 min. The beads with

TABLE 1. Bacterial strains used in the study

Bacterial species	Strain no.	No.[a]
Porphyromonas gingivalis	ATCC 33277	1
	ATCC 49417	2
	W50	3
	A7A1-28	4
	W83	5
	16-1	6
	17A1	7
	E20-1	8
	23A4	9
	OMZ 409	10
Prevotella nigrescens	NCTC 9336	11
Prevotella intermedia	ATCC 25611	12
Porphyromonas salivosa	VPB 3313	13
Porphyromonas asaccharolytica	ATCC 27067	14
Bacteroides macacae	MD1-21	15
Bacteroides forsythus	ATCC 43037	16
Streptococcus mutans	ATCC 33534	17
Actinobacillus actinomycetemcomitans	ATCC 29522	18

[a] The indicated number refers to the lane of gel electrophoresis and Southern blotting in Fig. 2.

the attached bacteria were washed three times in 1 ml of PBS-BSA with the aid of magnetic separation and were finally suspended in 30 μl of deionized water. For the recovery of genomic DNA, the beads and their attached bacteria were heated at 95°C for 10 min and were briefly centrifuged (13,000 × *g*), and 5 μl of the supernatant was used in the PCR.

Primers and PCR. A previous study by arbitrarily primed PCR identified a 1,146-bp amplicon (RP1) common to all strains of *P. gingivalis* from humans (16). The amplified RP1 fragment was excised from preparative agarose gels, and the DNA was purified (GeneClean; Bio 101, Inc.) and blunt-end ligated into the Bluescript IIsk linearized vector according to the manufacturer's recommendations (Stratagene). The identity of the cloned rapid amplified polymorphic DNA (RAPD) product was verified by restriction digestion with *Eco*RI and separation of the resulting fragments on agarose gels by a standard procedure (21). Double-strand sequencing (Sequenase kit, U.S. Biochemicals) was done by the dideoxy-chain termination method with universal primers. The 1,155-bp sequence obtained was used for the selection of a primer pair to be used in a PCR amplification. Optimal upstream and downstream sequences for PCR amplification were predicted by using the primer analysis software Oligo (National Biosciences Inc.). Thus, we selected oligonucleotides Pg 593-1 (5′-AATCGTA ACGGGCGACACAC-3′) and Pg 593-2 (5′-GGGTTGCTCCTTCATCACAC-3′) to generate a 593-bp segment flanked by the primers. PCRs were performed in a reaction mixture containing 1× PCR buffer (10 mM Tris-HCl [pH 9], 50 mM KCl, 1.5 mM $MgCl_2$, 0.4% Triton X-100, 200 μM [each] the four deoxynucleoside triphosphates, 12.5 pmol of each primer Pg 593-1 and Pg 593-2, 2.5 U of *Taq* DNA polymerase [Cetus, Emeryville, Calif.]), and 50 ng of genomic DNA in a final volume of 25 μl. The reaction mixture was overlaid with a drop of mineral oil and was incubated in a programmable DNA thermal cycler (Perkin-Elmer Cetus). The samples were initially denatured at 94°C for 5 min and were then subjected to 30 cycles, with 1 cycle consisting of 60 s at 94°C, 60 s at 70°C, and 30 s at 72°C. One negative control (absence of template DNA) was performed for each set of amplifications. The amplified DNA was resolved in a 1.5% agarose gel (SeaKem GTG; FMC, Rockland, Maine) in 0.04 M Tris-acetate–0.002 M EDTA (pH 8.5), stained with ethidium bromide, and photographed on a UV transilluminator.

IM-PCR detection of *P. gingivalis* in spiked mixed bacterial suspensions. A series of three experiments was used to assess the ability of the IM-PCR procedure followed by detection of the amplification product by dot blot hybridization to specifically detect *P. gingivalis*. In the first experiment a serial dilution of *P. gingivalis* ATCC 33277 in PBS was used to obtain bacterial concentrations ranging from 10^7 bacteria to 1 bacterium per ml. In the second experiment aliquots of a mixed suspension of seven oral species were prepared so that the final total concentration of each species in each aliquot was 10^6 bacteria per ml. The selected species were the five gram-negative bacteria *Prevotella nigrescens* NCTC 9336, *Prevotella intermedia* ATCC 25611, *Porphyromonas salivosa* 3313, *Fusobacterium nucleatum* ATCC 10953, and *Wolinella recta* ATCC 33238 and the two gram-positive bacteria *Streptococcus mutans* ATCC 25175 and *Actinomyces naeslundii* WVU 627. The aliquots were spiked with *P. gingivalis* ATCC 33277 serially diluted from 10^7 bacteria to 1 bacterium per ml. With this dilution scheme, the concentration of the spiked bacteria decreased while the concentration of the other bacteria remained the same. Finally, a pool of submarginal plaque samples obtained from three periodontally healthy subjects was constituted by suspending approximately 5 mg of plaque in 10 ml of reduced transport fluid. Anaerobically culturing of an aliquot of the pooled plaque sample revealed that it was negative for *P. gingivalis*. Aliquots of the pooled plaque sample were spiked with *P. gingivalis* ATCC 33277 serially diluted from 10^7 bacteria to 1 bacterium per ml and subjected to the IM-PCR and dot blot hybridization procedure. All experiments were conducted in triplicate.

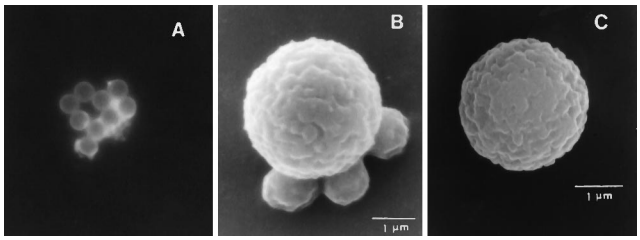

FIG. 1. (A) Fluorescence microscopy of acridine orange-stained *P. gingivalis* ATCC 33277 attached to MAb-coated IMBs. Magnification, ×1250. (B) Scanning electron micrograph of *P. gingivalis* attached to MAb-coated IMB. Bar, 1 μm. (C) Scanning electron micrograph of MAb-coated IMB. Bar, 1 μm.

DNA probe for detection of amplified products. The presence of the expected 593-bp product obtained by PCR was confirmed by Southern and dot blot hybridizations. For construction of a nonisotopic probe, the 593-bp DNA fragment resulting from amplification with the primer pair Pg 593-1 and Pg 593-2 was labeled by random priming with digoxigenin-dUTP (Boehringer, Mannheim, Germany) according to the manufacturer's recommendations. For dot blot hybridization, 10 μl of the amplified samples was heat denatured and spotted onto Hybond N nylon membranes (Amersham Inc., Oakville, Ontario, Canada). The amplified DNA immobilized on the nylon membranes was then processed for hybridization under stringent conditions at 65°C. Colorimetric detection was conducted with anti-digoxigenin–alkaline phosphatase, with Nitro Blue Tetrazolium–5-bromo-4-chloro-3-indolylphosphate used as the substrate according to the Boehringer manual.

Microscopy. A drop of each IMB-*P. gingivalis* preparation was put on a slide together with a drop of a 0.3-mg/ml solution of acridine orange (Sigma), and a coverslip was applied. The slide was examined after a couple of minutes by fluorescence microscopy at ×1,250 magnification.

A drop of a suspension of IMBs to which *P. gingivalis* cells were attached was processed for scanning electron microscopy as described previously (17). Preparations were examined in an electron microscope (JEOL JSM-840 A) at 10 kV.

RESULTS

Control of bacterial attachment. The attachment of the target bacteria to the beads was verified microscopically both by staining with acridine orange (Fig. 1A) and by scanning electron microscopy (Fig. 1B). The bacteria that attached to the surfaces of the beads were visible. The bacteria tended to bind several particles simultaneously, thus forming aggregates. A control bacterium-free bead is shown in Fig. 1C. The attachment of the target bacteria to the beads was also verified by culture. A sample of beads that went through the immunomagnetic procedure was plated: we observed specific growth of *P. gingivalis.*

Specificity and sensitivity of PCR. To evaluate the specificity of the PCR amplification, DNAs from a panel of strains (Table 1) were analyzed by PCR. The primer pair Pg 593-1 and Pg 593-2 amplified a specific 593-bp DNA fragment present only in *P. gingivalis* strains. No amplification product from non-*P. gingivalis* strains was detected by gel staining and Southern blot hybridization (Fig. 2).

To evaluate the sensitivity of the PCR, the lower detection limit of the template DNA was determined by comparing the capacities of agarose gel electrophoresis and dot blot hybridization to detect the amplified products. Genomic DNA extracted from *P. gingivalis* ATCC 33277, serially diluted from 0.5 μg to 0.5 pg, was amplified, and 10-μl aliquots were analyzed by gel electrophoresis or were dot blotted for hybridization with the labeled probe. On ethidium bromide-stained agarose gels, the limit of detection was 0.5 ng of template DNA, which

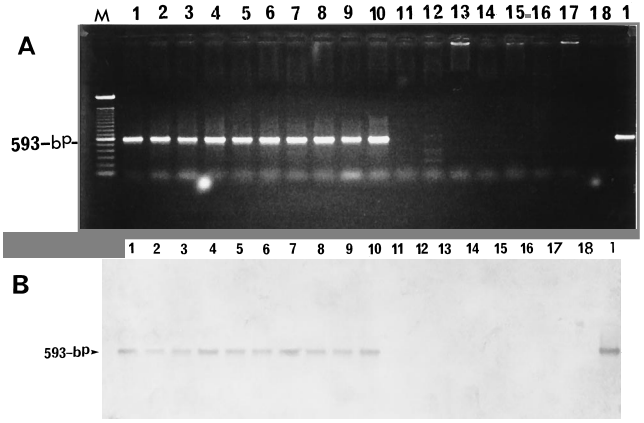

FIG. 2. Specificity of the PCR assay for the detection of *P. gingivalis*. (A) Agarose gel showing the PCR amplification products of DNA obtained from 10 strains of *P. gingivalis* (lanes 1 to 10, respectively) and from 8 other oral bacteria (lanes 11 to 18, respectively) by using primers Pg 593-1 and Pg 593-2. (B) Southern blot of the gel in panel A hybridized with the digoxigenin-labeled 593-bp probe. The molecular size standard is 100 bp (Gibco).

gave a faintly stained band (Fig. 3A). In contrast, amplification of as little as 5 pg of DNA gave a visible spot by dot blot hybridization (Fig. 3B).

Detection of *P. gingivalis* in spiked mixed bacterial suspensions. The sensitivity of the IM-PCR/DNA probe procedure for detecting specific bacteria was first investigated with serial dilutions of *P. gingivalis* in PBS. The immunomagnetically isolated cells were subjected to PCR, and the amplified products were detected by dot blot hybridization with the 593-bp probe. The lowest concentration of *P. gingivalis* that gave a positive signal was 10^2 cells per ml, i.e., approximately 16 bacteria in the assay (Fig. 4A). In aliquots containing a mixture of 7×10^6 heterologous bacteria spiked with a serial dilution of *P. gingivalis*, the same sensitivity was obtained (Fig. 4B). The same threshold of sensitivity was also obtained by using IMS-isolated cells from a pooled *P. gingivalis*-negative plaque sample spiked with a serial dilution of *P. gingivalis* (Fig. 4C).

Detection of *P. gingivalis* in clinical samples. To assess the potential usefulness of the IM-PCR/DNA probe procedure for laboratory diagnosis, we examined its performance with 19 clinical samples. The dispersed subgingival plaque samples were subjected to immunomagnetic separation, and cell lysates

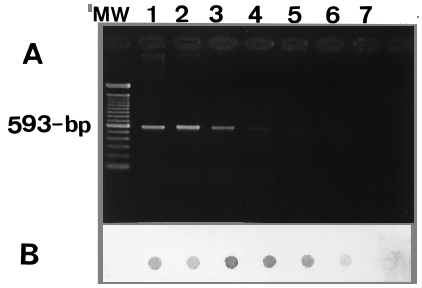

FIG. 3. Sensitivity of the PCR assay for the detection of *P. gingivalis*. Comparison of two methods of assessing the limit of detection of PCR-amplified products from *P. gingivalis* DNA: ethidium bromide staining after agarose gel electrophoresis (A) and dot blot hybridization (B). The quantities of DNA loaded in the gel are as follows: 0.5 μg (lane 1), 50 ng (lane 2), 5 ng (lane 3), 0.5 ng (lane 4), 50 pg (lane 5), 5 pg (lane 6), 0.5 pg (lane 7); lane MW, 100-bp molecular size standard (Gibco). The limit of detection was 0.5 ng of DNA by gel staining and 5 pg by DNA hybridization.

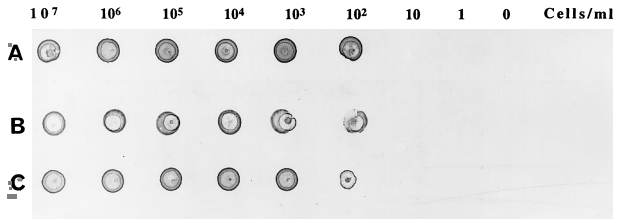

FIG. 4. DNA probe detection of *P. gingivalis* in serial dilutions (10^7 bacteria per ml down to eight dilutions) after the IM-PCR procedure and dot blot hybridization of the amplification product with the 593-bp probe. Rows: A, lysate of cells isolated by IMS from a homologous suspension of *P. gingivalis*; B, lysate of cells isolated by IMS from a mixed suspension of seven heterologous oral bacteria spiked with a serial dilution of *P. gingivalis*; C, lysate of cells isolated by IMS from a pooled plaque sample spiked with a serial dilution of *P. gingivalis*. The results are typical of those from three independent experiments.

amplified by PCR were detected with the 593-bp probe (Fig. 5). The seven samples which were culture positive for *P. gingivalis* were identified by the IM-PCR/DNA probe procedure. In addition, four samples (samples B5, C3, C4, and D3) that were *P. gingivalis* negative by culture were identified as positive by IM-PCR and dot hybridization. The results derived from the 19 clinical samples assayed either by the IM-PCR/DNA probe procedure or by culture are summarized in Table 2.

DISCUSSION

The cultural method for the identification and enumeration of periodontal pathogens in human plaque samples is fraught with numerous technical difficulties, mostly because of the fastidious nature of the anaerobic pathogens (13, 22, 31). The inability to reliably culture these organisms has led to the development of either immunological reagents (30) or DNA probes (7) for diagnosing their presence. Because the future importance of a technology which does not require the preservation of viable microorganisms in anaerobic bacteriological culture depends on advances in the development of probe assays (11), we explored the possibility of combining immunological, PCR, and DNA probe technologies to develop a rapid, specific, and sensitive method of detecting the periodontal pathogen *P. gingivalis.*

Amplification of specific sequences of DNA by the PCR technique has been reported to provide a rapid diagnosis of

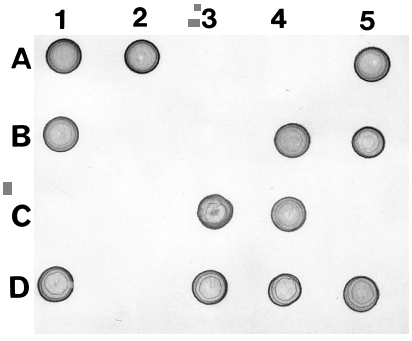

FIG. 5. DNA probe detection of *P. gingivalis* in clinical samples after the IM-PCR procedure and dot blot hybridization of the amplification product with the 593-bp probe. Dots A1, A2, A5, B1, B4, D1, and D4 were produced from plaque samples that were *P. gingivalis* positive by culture. Dots B5, C3, C4, and D3 were produced from culture-negative plaque samples. Dots A3, A4, B2, B3, C1, C2, C5, and D2 were negative both by culture and by use of the probe. Dot D5 was produced from a pure culture of *P. gingivalis*, which was used as a positive control.

TABLE 2. Frequency distribution of results by the IM-PCR/DNA probe procedure or culture for the detection of *P. gingivalis* in 19 clinical samples

Clinical origin of sample	No. of samples			
	Culture positive	Culture negative	IM-PCR/ DNA probe positive	IM-PCR/ DNA probe negative
Healthy gingiva, 7 sites in 7 patients	0	7	1	6
Gingivitis, 3 sites in 3 patients	1	2	1	2
Periodontitis, 9 sites in 3 patients	6	3	9	0

many diseases and is especially useful for the rapid detection of fastidious or slowly growing pathogens (20). However, direct PCR of clinical samples is known to be less sensitive than PCR of purified DNA because of the presence of inhibitory compounds in the clinical material (28). To avoid these inhibitory factors, several investigators working directly with samples (1, 6, 8, 12) perform PCR after DNA extraction, a method that is rather laborious. This can be circumvented by immunomagnetic separation of the target microorganisms from the samples (3, 14, 15, 23, 26, 27) to eliminate all nonbacterial contaminants potentially acting as inhibitors in the subsequent PCR amplification step.

The IM-PCR/DNA probe procedure described here is a rapid, specific, and sensitive method for the detection of *P. gingivalis*. This can be performed within 2 working days, from sampling to dot blot hybridization. The procedure involves the following three steps: (i) IMS for the isolation of the target bacteria from a complex sample by using specific MAb-coated IMBs, (ii) PCR amplification of the recovered target DNA released by boiling the captured bacteria, and (iii) detection by dot blot hybridization of the amplified products with a nonisotopic DNA probe.

The first step of our method relies on the use of beads coated with a specific antibody that allows for the capture and separation of the bacterial cells by application of a magnet. In addition to specifically concentrating the target bacteria, IMS also provides a purer specimen suitable for PCR. It is known that *Taq* polymerase is susceptible to inhibition by compounds that may be present in crude clinical samples. IMS provides an efficient way of avoiding PCR inhibitors. The presence of IMBs did not adversely affect PCR amplification.

One particularity of our approach was that our PCR primers were not designed on the basis of published sequences of *P. gingivalis*. Indeed, we used a primer pair derived from the sequence of a species-specific amplicon identified by arbitrarily primed PCR in a previous study (16). The species-specific random amplified polymorphic DNA marker RP1, with an estimated size of 1,146 bp, was cloned and sequenced to yield a so-called sequence-characterized amplified region (19) of 1,155 bp. This sequence was used to design the specific PCR primer pair Pg 593-1 and Pg 593-2 to amplify a 593-bp fragment internal to the region spanned by the random amplified polymorphic DNA primer. In addition, the primer pair allowed the synthesis of quantities of the 593-bp fragment which was labeled with digoxigenin to yield a nonisotopic DNA probe.

To assess the potential usefulness of the IM-PCR/DNA probe procedure for laboratory diagnosis, we determined that it allowed for the detection of low numbers of target bacteria from large volumes of highly contaminated samples. We showed that against a background of 7×10^6 diverse nontarget bacteria the MAb-coated beads efficiently captured as few as 100 *P. gingivalis* cells and that this low number of target bacteria was sufficient to yield a positive signal following PCR amplification and detection with a DNA probe. The high degrees of sensitivity and specificity of the IM-PCR/DNA probe procedure reported here suggest that this method, which is fast compared with culture, should be useful for the reliable detection and identification of *P. gingivalis* in polymicrobial infections.

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REFERENCES

1. **Boondeekhun, H. S., V. Gurtler, M. L. Odd, V. A. Wilson, and B. C. Mayall.** 1993. Detection of *Clostridium difficile* enterotoxin gene in clinical specimens by the polymerase chain reaction. J. Med. Microbiol. **38:**384–387.
2. **Brakstad, O. G., K. Aasbakk, and J. A. Maeland.** 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. **30:**1654–1660.
3. **Cudjoe, K. S., L. I. Thorsen, T. Sorensen, J. Reseland, O. Olsvik, and P. E. Granum.** 1991. Detection of *Clostridium perfringens* type A enterotoxin in faeces and food samples using immunomagnetic separation (IMS)-ELISA. Int. J. Food Microbiol. **12:**313–321.
4. **Deslauriers, M., and C. Mouton.** 1991. Targeting the production of monoclonal antibodies to the hemagglutinating adhesin of *Bacteroides* (*Porphyromonas*) *gingivalis* by injection of an immunoprecipitate from crossed immunoelectrophoresis. J. Biol. Buccale **19:**155–160.
5. **Fournier, D., and C. Mouton.** 1993. Phenotypic characterization of human and animal biotypes within the species Porphyromonas gingivalis. Res. Microbiol. **144:**435–444.
6. **Frankel, G., L. Riley, J. A. Giron, J. Valmassoi, A. Friedmann, N. Strockbine, S. Falkow, and G. K. Schoolnik.** 1990. Detection of *Shigella* in feces using DNA amplification. J. Infect. Dis **161:**1252–1256.
7. **French, C. K., E. D. Savitt, S. L. Simon, S. M. Eklund, M. C. Chen, and L. C. Klotz.** 1986. DNA probe detection of periodontal pathogens. Oral Microbiol. Immunol. **1:**58–62.
8. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z. Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
9. **Greisen, K., M. Loeffelholz, A. Purohit, and D. Leong.** 1994. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. J. Clin. Microbiol. **32:**335–351.
10. **Haffajee, A. D., and S. S. Socransky.** 1994. Microbial etiological agents of destructive periodontal diseases. Periodontology **5:**78–111.
11. **Hofstad, T.** 1994. Utility of newer techniques for classification and identification of pathogenic anaerobic bacteria. Clin. Infect. Dis. **18**(Suppl. 4)**:**S250–S252.
12. **Jensen, J. S., S. A. Uldum, J. Sondergard-Andersen, J. Vuust, and K. Lind.** 1991. Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. J. Clin. Microbiol. **29:**46–50.
13. **Loesche, W. J., D. E. Lopatin, J. Stoll, N. van Poperin, and P. P. Hujoel.** 1992. Comparison of various detection methods for periodontopathic bacteria: can culture be considered the primary reference standard? J. Clin. Microbiol. **30:**418–426.
14. **Luk, J. M. C., and A. A. Lindberg.** 1991. Rapid and sensitive detection of Salmonella (O:6,7) by immunomagnetic monoclonal antibody-based assays. J. Immunol. Methods **129:**243–250.
15. **Lund, A., A. L. Hellemann, and F. Vartdal.** 1988. Rapid isolation of $K88^+$ *Escherichia coli* by using immunomagnetic particles. J. Clin. Microbiol. **26:** 2572–2575.
16. **Ménard, C., and C. Mouton.** 1994. Polymerase chain reaction using arbitrary primers for the design and construction of a DNA probe specific for *Porphyromonas gingivalis*. Res. Microbiol. **145:**595–602.
17. **Mouton, C., H. S. Reynolds, E. A. Gasiecki, and R. J. Genco.** 1979. In vitro adhesion of tufted oral streptococci to *Bacterionema matruchotii*. Curr. Microbiol. **3:**181–186.
18. **Muir, P., F. Nicholson, M. Jhetam, S. Neogi, and J. E. Banatvala.** 1993. Rapid diagnosis of enterovirus infection by magnetic bead extraction and polymerase chain reaction detection of enterovirus RNA in clinical specimens. J. Clin. Microbiol. **31:**31–38.
19. **Paran, I., and R. W. Michelmore.** 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. **85:**985–993.

20. **Persing, D. H.** 1993. In vitro nucleic acid amplification techniques, p. 51–87. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
21. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Savitt, E. D., M. W. Keville, and W. J. Peros.** 1990. DNA probes in the diagnosis of periodontal microorganisms. Arch. Oral Biol. **35:**153S–159S.
23. **Skjerve, E., L. M. Rorvik, and O. Olsvik.** 1990. Detection of *Listeria monocytogenes* in food by immunomagnetic separation. Appl. Environ. Microbiol. **56:**3478–3481.
24. **Socransky, S. S., and D. Haffajee.** 1994. Evidence of bacterial etiology: a historical perspective. Periodontology **5:**7–25.
25. **Syed, S., and W. Loesche.** 1972. Survival of human dental plaque flora in various transport media. Appl. Microbiol. **24:**638–644.
26. **Widjojoatmodjo, M. N., A. C. Fluit, and R. Torensma.** 1992. The magnetic immuno polymerase chain reaction assay for direct detection of salmonellae in fecal samples. J. Clin. Microbiol. **30:**3195–3199.
27. **Widjojoatmodjo, M. N., A. C. Fluit, R. Torensma, B. H. I. Keller, and J. Verhoef.** 1991. Evaluation of the magnetic immuno PCR assay for rapid detection of *Salmonella*. Eur. J. Clin. Microbiol. Infect. Dis. **10:**935–938.
28. **Wilde, J., J. Eiden, and R. Yolken.** 1990. Removal of inhibitory substances from human fecal specimens for detection of group A rotavirus by reverse transcriptase and polymerase chain reaction. J. Clin. Microbiol. **28:**1300–1307.
29. **Wilson, K.** 1991. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.2. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Wiley-Interscience, Philadelphia.
30. **Zambon, J. J., V. Bochacki, and R. J. Genco.** 1986. Immunological assays for putative periodontal pathogens. Oral Microbiol. Immunol. **1:**39–44.
31. **Zappa, U., M. Reinking-Zappa, H. Graf, R. Gmur, and E. Savitt.** 1990. Comparison of serological and DNA probe analyses for detection of suspected periodontal pathogens in subgingival plaque samples. Arch. Oral Biol. **35:**161S–164S.