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Comparison of the Benzoyl-DL-Arginine-Naphthylamide (BANA) Test, DNA Probes, and Immunological Reagents for Ability To Detect Anaerobic Periodontal Infections Due to *Porphyromonas gingivalis*, *Treponema denticola*, and *Bacteroides forsythus*

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Most forms of periodontal disease are associated with the presence or overgrowth of anaerobic species that could include *Treponema denticola*, *Porphyromonas gingivalis*, and *Bacteroides forsythus* among others. These three organisms are among the few cultivable plaque species that can hydrolyze the synthetic trypsin substrate benzoyl-DL-arginine-naphthylamide (BANA). In turn, BANA hydrolysis by the plaque can be associated with periodontal morbidity and with the presence of these three BANA-positive organisms in the plaque. In this investigation, the results of the BANA test, which simultaneously detects one or more of these organisms, were compared with the detection of these organisms by (i) highly specific antibodies to *P. gingivalis*, *T. denticola*, and *B. forsythus*; (ii) whole genomic DNA probes to *P. gingivalis* and *T. denticola*; and (iii) culturing or microscopic procedures. The BANA test, the DNA probes, and an enzyme-linked immunosorbent assay or an indirect immunofluorescence assay procedure exhibited high sensitivities, i.e., 90 to 96%, and high accuracies, i.e., 83 to 92%, in their ability to detect combinations of these organisms in over 200 subgingival plaque samples taken from the most periodontally diseased sites in 67 patients. This indicated that if *P. gingivalis*, *T. denticola*, and *B. forsythus* are appropriate marker organisms for an anaerobic periodontal infection, then the three detection methods are equally accurate in their ability to diagnose this infection. The same statement could not be made for the culturing approach, where accuracies of 50 to 62% were observed.

Periodontal disease (periodontitis) is the loss of tooth-supporting tissue that results from either the presence or the numerical overgrowth of certain bacterial types on the tooth surface in microbial communities known as dental plaque (7, 31, 34, 41). A rare form of periodontitis known as localized juvenile periodontitis (48) and possibly some forms refractory to treatment (12) appear to be associated with *Actinobacillus actinomycetemcomitans*, a microaerophilic, saccharolytic organism that is not commonly found in plaque samples. Most other forms of periodontitis appear to be associated with the presence or overgrowth of anaerobic species that include spirochetes (23, 31), *Treponema denticola* (23, 35, 39), *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Prevotella intermedia*, *Wolinella recta*, and several other species either alone or in combination (7, 11, 14, 18, 31, 34, 41, 42).

The treatment of periodontitis traditionally has relied exclusively on the debridement of the plaque from the tooth and root surfaces. However, if periodontitis is in reality a group of specific infections, then antimicrobial agents would also be indicated. Debridement plus systemic tetracycline in the treatment of an *A. actinomycetemcomitans*-associated infection provides benefits beyond that obtained with debridement or surgery alone (4, 20). In periodontitis associated with spirochetes, several double-blind studies in which metronidazole or placebo was superimposed upon debridement resulted in significant improvement in clinical health (17, 21, 30) and a reduction in the need for periodontal surgery (29).

These findings indicate that the use of systemic antimicrobial agents in the treatment of periodontitis can be successful. However, the use of these or other systemic agents should be predicated upon a diagnosis of infection. Cultural diagnostic procedures (40) and services (36), immunological reagents (2, 10, 18, 33, 39, 41, 47, 49), DNA probes (6, 8, 38), and enzyme tests (13, 22) are being recommended or developed to facilitate the diagnosis of this infection. These newer diagnostic procedures have to be evaluated, at some point in their development, against the culturing procedure, which is traditionally considered to be the primary standard (46).

T. denticola, *P. gingivalis*, and *B. forsythus* are among the few cultivable plaque species capable of hydrolyzing the synthetic trypsin substrate benzoyl-DL-arginine naphthylamide (BANA) (19, 24). BANA hydrolytic activity could be demonstrated in the subgingival plaque and correlated with the depth of the periodontal pocket and the numbers and proportions of spirochetes (32). The BANA substrate was the most useful of several synthetic peptide-naphthylamide substrates in reflecting clinical disease (13, 44), and its hydrolysis could be associated with the presence of *P. gingivalis* and *T. denticola* in the plaque (3, 25). Refinements in the original liquid BANA assay led to the commercial development of a solid-state format (PerioScan; Oral-B Laboratories, Redwood City, Calif.) which required only a 15-min incubation period (24).

Subgingival plaques from over 200 periodontally diseased sites were tested with the solid-state BANA test, and these

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findings were compared to the presence or absence of *T. denticola*, *P. gingivalis*, *B. forsythus*, and the BANA-negative *A. actinomycetemcomitans* as determined by culturing procedures, DNA probes, and immunological reagents to these organisms. In the accompanying report (28) the individual detection methods were evaluated for their ability to detect each of these organisms in the plaque. In this report, the results of the BANA test, which detects simultaneously *T. denticola*, *P. gingivalis*, and *B. forsythus*, are compared with results of the other methods for their abilities to detect combinations of these same organisms in the plaque. Emphasis has been placed on the accuracy of the various measurements relative to a reference standard, as accuracy provides the percentage of times that the test is correct {[(true positive + true negative)/total number in sample] × 100} (5, 27).

MATERIALS AND METHODS

Selection of patients and tooth sites and processing of the plaque sample. Patient and site selection and sample processing are described in detail elsewhere (28) and will be summarized here. Patients at the University of Detroit School of Dentistry (n = 42) and the University of Michigan School of Dentistry (n = 25) were examined by a periodontist for the presence of periodontally diseased sites (pockets). All selected sites bled upon probing, had an average pocket depth of 6.4 ± 1.8 mm, and were judged to be the most diseased site(s) in the patient's dentition. A periodontal curette was placed at the base of the pocket, and the subgingival plaque was removed and placed into 700 μl of a reduced transport fluid and frozen in liquid nitrogen. The samples were subsequently thawed and dispersed for 10 s with a vortex mixer, and a 10-μl portion was removed and examined by dark-field microscopy for the total numbers of bacteria and spirochetes. If there were fewer than 20 bacteria in five high-power microscopic fields, the sample was deemed too small and was not processed further. Otherwise, the sample was divided as follows: (i) 100 μl was sent to BioTechnica Diagnostics (Cambridge, Mass.) for the enumeration of *P. gingivalis*, *T. denticola*, and *A. actinomycetemcomitans* by using whole genomic DNA probes (8); (ii) 200 μl was placed in 0.5% formaldehyde contained in phosphate-buffered saline (PBS) and analyzed for *T. denticola*, *P. gingivalis*, *B. forsythus*, and *A. actinomycetemcomitans* by using both an enzyme-linked immunosorbent assay (ELISA) and a fluorescent-antibody procedure (28, 47); (iii) 80 μl was diluted in 8 ml of reduced transport fluid, serially diluted, and cultured on an enriched trypticase soy agar (ETSA) medium (28, 45) for *P. gingivalis*, *B. forsythus*, and viable count and on a selective vancomycin-polymyxin medium for *A. actinomycetemcomitans* (28, 40); (iv) 225 μl was centrifuged in a microcentrifuge tube (Microfuge), and the pellet was resuspended in 10 μl of PBS and placed on a BANA-impregnated cellulose paper (PerioScan) (24).

DNA probes. BioTechnica reported its results for *P. gingivalis*, *A. actinomycetemcomitans*, and *T. denticola* as negative ($<6 \times 10^3$ cells), low ($\geq 6 \times 10^3$ but $<6 \times 10^4$ cells), moderate ($\geq 6 \times 10^4$ but $<6 \times 10^5$ cells), and high ($\geq 6 \times 10^5$ cells). The probes for *A. actinomycetemcomitans* (8), *P. gingivalis* (43), and *T. denticola* (33a) exhibited no serious cross-reactions with more than 40 tested plaque species. (See reference 28 for a listing of the several species which demonstrated a 1% hybridization with these probes).

Cultural and microscopic procedures. The agar plates were incubated anaerobically for 7 days, and the total viable count, the count of black-pigmented colonies, and the count of speckled colonies were determined on ETSA plates containing between 50 and 250 to 300 colonies. A portion of the black-pigmented colonies was placed on the PerioScan cards and incubated for 15 min at 55°C to measure BANA hydrolysis. BANA-positive colonies were identified as *P. gingivalis* (19, 24). Portions of the speckled colonies were also placed on the PerioScan cards, and any BANA-positive colonies were identified as *B. forsythus*. This identification was confirmed by a positive ELISA reaction with the polyclonal antisera to *B. forsythus*. *A. actinomycetemcomitans* was identified by its colony appearance on the vancomycin-polymyxin medium. *T. denticola* cannot be reliably isolated (37) and thus was estimated indirectly by counting spirochetes in the 10 μl of dispersed plaque sample that was used initially to screen out small samples.

Immunological procedures. Reference strains of *A. actinomycetemcomitans* (ATCC 43718), *P. gingivalis* (ATCC 33277), and *T. denticola* (ATCC 35405) were grown as previously described (28). *B. forsythus* (ATCC 43037) was grown by A. Tanner of the Forsyth Dental Center, Boston, Mass. High-titer polyclonal rabbit antibodies were prepared by subcutaneous immunization of female New Zealand White rabbits with 2 mg of the lyophilized antigen in complete Freund's adjuvant at 0 and 2 weeks, followed by booster immunization in incomplete Freund's adjuvant at 7 weeks following the first immunization. The specificity of the antibodies was determined by indirect immunofluorescence assay (IFA) to confirm that the antibody was staining cell types with the morphologic characteristics of *T. denticola*, *P. gingivalis*, *B. forsythus*, and *A. actinomycetemcomitans*. The working titer of the antisera was 1/1,000 when tested by both the IFA and ELISA. The IFA and ELISA procedures have been described (28, 47).

PerioScan test. The pellet resulting from the centrifugation of the plaque suspension was suspended in 10 μl of water and applied to the cellulose strip on the lower half of the PerioScan card. The upper strip containing the fast black dye was activated by moistening with distilled water. The lower portion of the card was bent forward so that the two strips made contact. The card was placed into a small heating unit which contained a clip that held the two strips in contact. The card was incubated for 15 min at 55°C, and then the upper strip was examined for the presence of a blue spot. A faint or weak blue color suggests that the plaque contains about 10^4 to 10^5 BANA-positive organisms, whereas a distinct blue color is indicative of more than 10^5 BANA-positive organisms (24).

Statistical analysis. The results from the PerioScan test were compared with the results obtained with the DNA probes, immunological reagents, and culture and/or microscopic procedures. The BANA test is considered positive if *T. denticola*, *P. gingivalis*, and *B. forsythus* are either individually or collectively present at levels greater than 10^5 CFU in the sample (24). Accordingly, it was necessary to summarize the values obtained for *T. denticola*, *P. gingivalis*, and *B. forsythus* by each reference procedure in order to have a composite value which could be directly compared with the BANA value. The jack-knifing methodology (9) was used to estimate the sensitivities of the PerioScan results and compare them with the different reference tests. The PerioScan test was defined as a negative for a negative result and as a positive for a weakly positive or positive test result. The cultural data were defined as negative if the sought-after organisms could not be found on any dilution of the plaque sample, with the detection limit being set at $\geq 2 \times 10^3$ CFU.

The DNA, IFA, and ELISA data were defined as negative if the DNA probe, IFA, or ELISA results were equal to zero for all investigated organisms. The estimate of the sensitivities was a weighted average of the individual sensitivities of the individual patients. The associated variance estimate was asymptotically correct (15).

RESULTS

In the population of plaques taken from clinically diseased sites, 9.5% were PerioScan negative, 6.5% were PerioScan weakly positive and 84% were PerioScan positive. In the subsequent analysis, PerioScan weakly positive and positive results were combined to give a single PerioScan-positive value of 90%. Some portions of the plaque sample were inadequate for analysis or lost during the performance of the cultural, immunological, and DNA procedures, so that the final sample number for the various procedures was less than the 206 samples on which the microscopic count was performed, i.e., from 196 to 202 samples (Table 1).

The PerioScan results were compared initially with the presence or absence of each of the monitored BANA-positive species in order to determine whether one of the three BANA-positive species could account for the majority of the positive PerioScan results. This analysis was complicated by the variability encountered with the various detection methods. For example, *P. gingivalis* was detected in 82 to 88% of the plaques by DNA probe and ELISA analysis but in only 42% of the plaques by the cultural approach (Table 1). The PerioScan test was 85% accurate when compared with the ELISA results for *P. gingivalis* but only 54% accurate when compared with the culture results for *P. gingivalis*. The PerioScan was 89% accurate when compared with the results from microscopic detection of spirochetes but was only 52% accurate when compared with the presence of *T. denticola* as detected by IFA (Table 1). The PerioScan was 84% accurate for *B. forsythus* when compared with the IFA results for this organism but only 34% accurate when compared with the culture results for *B. forsythus*.

These findings indicated that none of the BANA-positive species uniquely accounted for the positive PerioScan reaction of the plaques, but they suggested that all three species were contributory to the PerioScan result. A more appropriate analysis would be to compare the combined results for *P. gingivalis*, *T. denticola*, and *B. forsythus* obtained by each of the four detection methods with the PerioScan results. It was of interest to determine whether the levels of these organisms played a role in the PerioScan result. Accordingly, the PerioScan results were compared to the levels of the BANA-positive species as determined by the DNA, ELISA, IFA, and culture and/or microscopic methods. For example, in comparing the DNA results for *T. denticola* plus *P. gingivalis* with the PerioScan results, three cutoff levels were used. Cutoff level 1 was defined as absent ($<6 \times 10^3$ CFU) versus present ($\geq 6 \times 10^3$ CFU); cutoff level 2 was defined as absent or present at low levels ($<6 \times 10^4$ CFU) versus present at moderate or high levels ($\geq 6 \times 10^4$ CFU); cutoff level 3 was defined as absent or present at low or moderate levels ($<6 \times 10^5$ CFU) versus present at high levels ($\geq 6 \times 10^5$ CFU). Similar cutoffs were established for the ELISA, IFA, and culture and/or microscopic results (Table 2).

The PerioScan test was most accurate when the comparison was based on a present-absent configuration (cutoff level 1; Table 2). When *P. gingivalis* and/or *T. denticola* could be detected by the DNA probes, the accuracy of the PerioScan test was 83%. When *P. gingivalis*, *T. denticola*, and/or *B. forsythus* could be detected by the ELISA or IFA procedure, the accuracy was 85 or 86%, respectively. When *P. gingivalis* and *B. forsythus* and/or spirochetes were greater than 0.2% of the respective culture and microscopic counts, the accuracy of the PerioScan was 89% (Table 2). The accuracy values dropped appreciably as the cutoff thresholds increased (Table 2). If for example, cutoff level 3 was used in the ELISA analysis, then the accuracy of the PerioScan was only 35% because of the presence of many false-positive PerioScan readings. The sensitivity values increased minimally as the cutoff thresholds increased, i.e., from 0.8% with IFA to 6% with ELISA. This analysis indicated that the PerioScan test was most accurate relative to the other detection methods when compared in a present-absent configuration for the three BANA-positive species. This suggested that the PerioScan test had approximately the same sensitivity (in the microbiological sense) as the other detection methods, which would be about 10^4 BANA-positive organisms.

In the next comparison, the PerioScan test was considered the test procedure, and each of the other detection methods was considered to be the standard reference. In this comparison, when the reference tests had to be positive for one or more of *P. gingivalis*, *T. denticola* or spirochetes, and *B. forsythus*, the accuracy of the PerioScan ranged from 83 to 89% and the sensitivity was 90 to 91% (Table 3). The 89% accuracy for the culture and/or microscopic methods is high because of the presence of spirochetes in 98% of the plaques (Table 1). This would suggest that the 83 to 86% accuracies seen with the ELISA, IFA, and DNA results are more appropriate values.

In Table 4, the combined cultural or microscopic results for *P. gingivalis*, *B. forsythus*, and spirochetes are treated as the test result and then compared with the combined results for *P. gingivalis*, *B. forsythus*, and/or *T. denticola* obtained with the DNA probes as the reference standard. The comparison is then made with the ELISA results as the reference standard, followed by the IFA and the PerioScan results as the reference standard. Under these conditions the sensitivity ranged from 90 to 96% and the accuracy ranged from 89 to 95%. However, these values would be inflated by including the microscopic presence of spirochetes in the analysis. When the analysis was repeated and the spirochetal data from the culture and microscopic count and the *T. denticola* data from the DNA, ELISA, and IFA studies were excluded, the sensitivities remained high, i.e., 86 to 98%, but the accuracies dropped to 50 to 62% because of the large number of false negatives seen with the culture methodology; i.e., the organisms can be detected by the various reference methods, but are not detected by the cultural procedures.

In Table 5 the DNA probe results, the ELISA results, and the IFA results are each treated as the test procedure. There were minimal differences in the sensitivity or accuracy values, indicating that all procedures were comparable.

We next determined what the PerioScan score would be when all three of the BANA-positive organisms were present in the same plaque samples. Of the 119 plaques that were positive for the three organisms, 111 were also positive by the PerioScan test (sensitivity = 93.3%; standard error = 0.0307). *A. actinomycetemcomitans* did not appear to be an important organism in these patients, as it was present in only 14% of the plaque samples. There were no plaque samples colonized with *A. actinomycetemcomitans* that

TABLE 1. Relationship between plaque BANA reactions (PerioScan) and the presence or absence of certain periodontopathic species in plaque samples as determined by various detection methods

Detection method (no. of plaques) and presence or absence	BANA reaction with organism															
	P. gingivalis				*T. denticola*				*B. forsythus*				*A. actinomycetemcomitans*			
	No. positive[a]	No. negative	% Accuracy[b]	% Present[c]	No. positive	No. negative	% Accuracy	% Present	No. positive	No. negative	% Accuracy	% Present	No. positive	No. negative	% Accuracy	% Present
Culture and/or microscopic (206)			54	42			89	98			34	24			24	13
Present	87	0			181[d]	21			49	2			27	1		
Absent	95	24			1	3			133	22			155	23		
DNA probe (201)			79	82			77	81							22	15
Present	153	13			155	19							23	7		
Absent	29	6			27	0							159	12		
ELISA (198–202)			85	88			75	82			65	64			51	48
Present	165	13			149	17			120	9			90	7		
Absent	17	7			33	3			62	10			90	11		
IFA (196–201)			62	63			52	58			84	93			52	55
Present	116	12			100	15			168	19			95	13		
Absent	65	8			79	4			12	0			82	6		

[a] Weakly positive and positive results combined.
[b] % Accuracy = [(no. of true positives + no. of true negatives)/no. of plaques in sample] × 100.
[c] % Present is the percentage of plaques positive for the indicated organism with each detection methodology.
[d] Microscopic count measured spirochetes, not *T. denticola*.

TABLE 2. Comparison of PerioScan with various cutoff points used with the reference measurements for *T. denticola*, *P. gingivalis*, and *B. forsythus*

Comparison test vs. PerioScan[a] (organisms studied) and cutoff level no.	Negative cutoff for reference standard (CFU)[b]	% Sensitivity	% Accuracy
DNA (*T. denticola*, *P. gingivalis*)			
1	$<6 \times 10^3$	89.8	83.1
2	$<6 \times 10^4$	89.7	78.4
3	$<6 \times 10^5$	93.1	53.9
ELISA (*T. denticola*, *P. gingivalis*, *B. forsythus*)			
1	$<10^4$	90.9	85.2
2	$<10^5$	91.9	61.2
3	$<10^6$	96.9	34.8
IFA (*T. denticola*, *P. gingivalis*, *B. forsythus*)			
1	$<10^4$	89.7	86.1
2	$<10^5$	90.0	84.8
3	$<10^6$	90.5	56.9
Culture and/or microscopic (*P. gingivalis*, *B. forsythus*, spirochetes)			
1	<0.2%	89.6	89.2
2	<10%	89.7	86.8
3	<30%	91.1	60.3

[a] PerioScan positive includes weakly positive and positive results.
[b] Except for culture and/or microscopic method (percentage of the viable count for *P. gingivalis* and *B. forsythus* and of microscopic count for spirochetes).

were not also colonized by *T. denticola*, *P. gingivalis*, or *B. forsythus*.

DISCUSSION

If periodontal disease is mainly a mixed infection involving multiple organisms in various, possibly interchangeable, combinations, then diagnostic tests based on the detection of a single species should have a low accuracy in relating to the clinical situation. This suggests that a useful diagnostic test for periodontal infections will have to monitor for the presence or increased levels of more than one species. The challenge, then, is to find if there are any common denominators that separate the putative periodontal pathogens from the myriad of nonperiodontopathic species found in the plaque. If such can be found, then diagnostic tests could be set up as a single test to detect one or more of the putative periodontopathogens. The overwhelming majority of the putative periodontopathogens are anaerobic species (exceptions: *A. actinomycetemcomitans* and *Eikenella corrodens*), and the majority of the anaerobes are gram-negative species (exceptions: *Eubacterium* species and *Peptostreptococcus*

TABLE 3. Relationship between a positive PerioScan result and comparable results obtained with the various detection methodologies

Comparison with PerioScan[a]	% Sensitivity	Variance	% Accuracy
DNA probe	90	0.10	83.0
ELISA	91	0.10	85.2
IFA	90	0.09	86.1
Culture and/or microscopic	90	0.01	89.2

[a] The PerioScan was positive while the reference tests were also positive for one or more of *P. gingivalis*, *T. denticola* or spirochetes, and *B. forsythus*.

TABLE 4. Relationship between a positive culture and/or microscopic result for one or more of the BANA-positive species and comparable results obtained with the various detection methodologies

Comparison with culture and/or microscopic method[a]	% Sensitivity[b]	% Accuracy[b]
DNA probe	92 (86)[b]	91.5 (56)
ELISA	92 (88)	91.1 (51)
IFA	96 (92)	95.1 (50)
PerioScan	90 (98)	89.2 (62)

[a] The culture procedures were positive for *P. gingivalis* and *B. forsythus* and/or the microscopic examination was positive for spirochetes, while the reference tests were positive for one or more of *P. gingivalis*, *T. denticola*, and *B. forsythus*.
[b] Values in parentheses were obtained by omitting the spirochetal and *T. denticola* values.

species). There is no diagnostic test for anaerobes or for gram-negative anaerobes, nor is it likely that such a test would adequately distinguish plaques from diseased and nondiseased sites.

Our choice of *P. gingivalis*, *T. denticola*, and *B. forsythus* as key organisms was based on the finding that these organisms appear to be among the few plaque species which possess an enzyme capable of hydrolyzing the synthetic peptide chromophore BANA (24). The ability of plaque samples to hydrolyze BANA has been related both to the presence of clinical disease (13, 25) and to the presence of spirochetes (32), *T. denticola*, and *P. gingivalis* (3, 25) in the plaque samples. The present investigation extended prior findings by comparing the BANA test in the commercially developed PerioScan format with combinations of DNA probes and highly specific antibodies to *P. gingivalis*, *T. denticola*, and *B. forsythus*. The PerioScan, the DNA probes, and the antibodies gave accuracies of 83 to 92%,

TABLE 5. Comparison of the various detection methods for one or more of the BANA-positive species

Comparison test vs. reference	% Sensitivity	% Accuracy
DNA probe[a] vs.:		
ELISA	93	87.6
IFA	96	88.6
Culture and/or microscopic	99	91.5
PerioScan	90	83.1
ELISA[b] vs.:		
DNA probe	94	87.6
IFA	96	88.1
Culture and/or microscopic	99	91.1
PerioScan	91	85.2
IFA[c] vs.:		
DNA probe	92	88.6
ELISA	92	88.1
Culture and/or microscopic	99	95.1
PerioScan	90	86.1

[a] The DNA probe was positive for *P. gingivalis* and/or *T. denticola*, while the reference tests were positive for one or more of *P. gingivalis*, *T. denticola*, or spirochetes, and *B. forsythus*.
[b] The ELISA antibodies were positive for one or more of *P. gingivalis*, *T. denticola*, and *B. forsythus*, while the reference tests were also positive for one or more of *P. gingivalis*, *T. denticola* or spirochetes, and *B. forsythus*.
[c] The fluorescent antibodies were positive for one or more of *P. gingivalis*, *T. denticola*, and *B. forsythus*, while the reference tests were also positive for one or more of *P. gingivalis*, *T. denticola* or spirochetes, and *B. forsythus*.

when compared with each procedure serving as either the reference standard or the test procedure (Tables 3 and 5). This indicated that if the combination of *P. gingivalis*, *T. denticola*, and *B. forsythus* is an appropriate marker for an anaerobic periodontal infection, then the three detection methods used in this investigation are equally accurate in their ability to diagnose this infection. The same statement could not be made for the culturing methods.

The PerioScan test had the same level of detection for the combination of *T. denticola*, *P. gingivalis*, and *B. forsythus* as did the DNA probes and the immunological reagents, i.e., about 10^4 CFU (Table 2). This was about 1 to 1.5 logs lower than the 10^5 detection limits seen previously with laboratory-grown strains of *T. denticola* and *P. gingivalis* (24). This suggests that in vivo *T. denticola*, *P. gingivalis*, and presumably *B. forsythus* may have more copies of the BANA hydrolytic enzyme per CFU than their in vitro-grown counterparts. As bacterial cells have a competitive phenotypic profile in vivo, this would indicate that the BANA hydrolytic enzymes are important for the cell's survival in the competitive plaque ecology.

Alternatively, the low detection level for the BANA organisms in the plaque could indicate that there are other BANA hydrolytic species in the plaque that were not accounted for, i.e., the weakly and variably BANA-positive *Capnocytophaga* species (24), *Rothia dentocariosa*, and *Bacterionema matruchotii* (16). We have not been able to associate the *Capnocytophaga* species (32) or *Rothia dentocariosa* (1) with periodontal disease. *B. matruchotii* is an aerobic species, isolated from supragingival plaque, and would not be expected to be present in the subgingival plaque samples that were used in this investigation. In other studies, a logistical regression model indicated that the presence of *T. denticola*, *P. gingivalis*, and *B. forsythus* could account for 95% of the BANA reactivity of the plaque samples (2a).

These findings indicate that the combination of DNA probes to *P. gingivalis* and *T. denticola*; the combination of highly specific antibodies to *P. gingivalis*, *T. denticola*, and *B. forsythus*; or the PerioScan test for BANA hydrolysis will equally document the presence of an anaerobic periodontal infection in about 90% of the subgingival plaques removed from periodontally diseased sites (Table 2). This finding suggests that considerations other than sensitivity and accuracy will determine the choice of any of these procedures. In this regard, the PerioScan is simple to perform, has the convenience of giving a result at chairside in 15 min (unpublished results indicate that 5 min will suffice), and is inexpensive. Other studies have shown that the presence of BANA hydrolysis by the plaque has some utility for the management of anaerobic infections in periodontal disease. For example, we have used the presence of advanced forms of periodontal disease plus a positive BANA test as the basis for diagnosing an anaerobic infection (26). These patients were successfully treated with metronidazole (29, 30). Also, a tooth site that tested BANA positive after the initial therapy lost significantly more attachment in the year following active treatment than did a tooth site that tested BANA negative (26). A positive BANA test by the plaque and the demonstration of *P. gingivalis* in the plaque by an IFA procedure were identified as risk cofactors for attachment loss observed during a longitudinal study of periodontal disease in a geriatric population (1a). Collectively, these findings suggest that the ability to diagnose an anaerobic periodontal infection will have some utility in the clinical management of periodontal disease.

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