

## The Use of a Rapid Enzymatic Assay in the Field for the Detection of Infections Associated with Adult Periodontitis

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### Abstract

*There are few objective assays for studies of the epidemiology of periodontal diseases. The PerioScan™ is an assay capable of detecting three periodontal pathogens, namely *T. denticola*, *P. gingivalis*, and *B. forsythus*, which have been associated with adult periodontitis. The PerioScan™ was tested in a sample of 301 Brazilians. Clinical indices—bleeding, probing depth, gingival index, and periodontal index—were recorded from four sites in each subject. Subgingival plaque samples were collected from those sites and placed on the PerioScan™ card. Color results were scored in the field after 15 minutes. The plaque samples were screened with polyclonal antibodies for the three species by an ELISA system. The PerioScan™, when compared with the ELISA system, yields a sensitivity of 91 percent, specificity of 89 percent, and an accuracy of 90 percent. When the PerioScan™ was compared to clinical indices, there was a high sensitivity (at least 93%) and a low specificity (no less than 47%), with an accuracy of at least 61 percent.*

**Key Words:** periodontal diseases, diagnosis, BANA test, *T. denticola*, *P. gingivalis*, *B. forsythus*.

Studies of the epidemiology of periodontal diseases have traditionally assessed the magnitude of periodontal inflammation and/or morbidity and often have related these parameters to oral hygiene status and age of individuals in different populations (1,2). A large body of evidence has accumulated suggesting that periodontal diseases are specific microbial infections (3). However, very few studies of the epidemiology of periodontal dis-

eases have taken this fact into consideration.

Risk factors are often causally related to the occurrence of disease. The identification of risk factors is only possible if markers or indicators of disease are developed to be tested as potential risk factors in prospective studies. Risk indicators may delineate associations between factors and disease, but do not determine a cause-and-effect relationship (4). Thus, the epidemiologic approach for the study of periodontal diseases may include the development and testing of new epidemiologic markers (risk indicators) for periodontal diseases (5), as well as the study of risk indicators prospectively to ascertain that the risk indicators are indeed risk factors that may explain the development of disease (4).

The knowledge that certain bacterial species such as *Treponema denticola*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Prevotella intermedia*, *Wolinella recta*, *Actinobacillus actinomycetemcomitans* are risk indicators for destructive periodontitis (5-10) allows for the development of assays that can detect the presence or overgrowth of one or more species in subgingival plaque samples. A synthetic substrate, n-benzoyl-DL-arginine- $\beta$ -naphthylamide (BANA), can detect the presence of proteolytic enzymes produced by *T. denticola*, *P. gingivalis*, and *B. forsythus*, which are not detected in over 60 other species present in subgingival plaque samples (11). The BANA test (PerioScan™, Oral-B Laboratories, Redwood City, CA) is a simple, quick (5-15 minutes), and inexpensive chairside assay that can give information about the presence of three of the putative periodontal pathogens in subgingival plaque samples (12).

The purpose of this study was to measure the accuracy of the PerioScan™ in epidemiologic field investigations by: (1) determining how well the PerioScan™ correlates with the detection of colonization of subgingival plaque samples by *T. denticola*, *P. gingivalis*, and *B. forsythus* in Brazilian subjects, as judged by an ELISA system (valid-

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ity of the test); and (2) determining how well the Perio-Scan™ and the ELISA system correlate with clinical indices often used in field studies of periodontal diseases (sensitivity, specificity, and accuracy of the test).

## Methods

**Study Population.** This investigation was conducted at the OdontoClinica Central da Marinha (OCM) Naval Dental Clinic in Rio de Janeiro, Brazil. Three hundred and one subjects were selected for the study. Patients seeking dental treatment at the OCM were screened for periodontal diseases at the Department of Oral Diagnosis. After an initial routine oral examination performed by Naval dental officers, patients with signs of periodontal disease either clinically or radiographically were seen by the study clinicians. They were given a brief interview, a more thorough periodontal examination by two calibrated examiners, and subgingival plaque samples were collected. For study purposes, this was considered a convenience sample.

**Demographic Data.** Of the 301 subjects, 162 were males and 139 were females. The subjects ranged in age from 18 to 69 years (mean age  $38.7 \pm 13$ ). They were predominantly of low socioeconomic class. Whites accounted for 48 percent of the sample, with blacks (26%) and others (26%) being less represented. The vast majority of the subjects (98%) were classified as untreated. These subjects had not received periodontal treatment for at least one year before the examination. The reported use of antibiotics revealed that about 93 percent of the subjects had no history of antibiotic therapy in the previous six months.

**Equipment.** Equipment utilized for clinical examinations and for plaque sample collection consisted of a dental chair, artificial light, dental mirrors, Michigan probes #0 (Hu-Friedy-CP11) with a probe tip of 0.3 to 0.4 mm diameter, and subgingival scalers.

**Teeth Selection.** The two most periodontally diseased sites and the two healthiest sites within each subject's dentition were chosen for this study. This was done purposely to assess the performance characteristics of the test in clinically determined healthy and diseased sites. The examiner first determined the surfaces of the teeth to be sampled on the basis of tissue tone and appearance, and gingival sulcus probing depth. Subsequently, the bleeding tendency upon probing (yes or no), the gingival index, and the periodontal index (the presence of destructive periodontal disease was confirmed by a probe) were recorded from each site. Forty-three percent of the examined teeth were in the anterior segment of the lower and upper arches, 22 percent were bicuspid, and 35 percent were molar teeth.

**Collection of Plaque Samples.** Supragingival plaque was removed and discarded prior to sampling. Subgingival plaque samples were collected by means of a curette from the most apical portion of the pocket or sulcus being

sampled. Special care was taken to ensure that there was enough plaque on the scaler to be easily identifiable by eye.

**Microbiological Procedures.** Subgingival plaque samples were placed on a dry paper strip impregnated with BANA that is attached to a nitrocellulose card (Perio-Scan™, Oral-B Laboratories, Redwood City, CA). This card contains a second upper strip impregnated with Evans black dye. This upper strip was moistened with water to activate the reagent and folded over the lower strip. The folded strips were held with a clamp and incubated in a special heating block for 15 minutes at 55°C. Any plaque samples containing the known BANA hydrolyzing species (*T. denticola*, *P. gingivalis*, and *B. forsythus*) individually or in combination at levels of about  $10^4$  colony-forming units (CFUs) will cause a permanent blue color to appear on the upper strip (13). The intensity of the color reaction may vary based on the microbial load of BANA hydrolyzing species present in a given sample. Thus, the following scale was generated: 0=negative; 1=weak-positive ( $10^4$  CFU), a faint blue-black color; 2=positive ( $>10^4$  CFU), a distinct blue-black color. Weak-positive and positive results were grouped as positive results.

**Immunological Procedures.** The plaque samples on the BANA card were transported back to the University of Michigan, Ann Arbor, MI, and screened for select periodontal pathogens by means of immunological reagents for these periodontal pathogens. The plaque samples were placed on the lower strip of the PerioScan™ card vertically so that the strip could be cut into three strands, each strand containing a fraction of the plaque samples. Each strand was stained with highly specific hyperimmune rabbit polyclonal antibodies to *T. denticola*, *P. gingivalis*, or *B. forsythus* (13). Antibodies binding to the plaque specimens were coated with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins. The plaque-anti IgG complex was identified after incubation with a BCIP/NBT phosphatase substrate system (Kirkegaard and Perry Lab, Gaithersburg, MD). The ELISA results were read as negative or positive.

**Statistical Analysis.** Relative frequencies, means, standard deviation, and standard error rates were calculated for the clinical and microbiological variables. Two examiners each examined about half of the subjects. During the study, 10 percent of the subjects were examined by both examiners independently to determine interexaminer reliability. Five percent of the subjects were reexamined by the same examiner two hours after the first exam (total of 10% of the sample, since there were two examiners) to determine intraexaminer reliability. Similar procedures were performed for scoring the PerioScan™ results. Interexaminer reliability of clinical measurements remained over 79 percent ( $\kappa \geq .75$ ) throughout the study. Intraexaminer reliability of clinical measurements was above 88 percent ( $\kappa \geq .67$ ) for examiner no. 1 and

above 87 percent ( $\kappa \geq .70$ ) for examiner no. 2. Inter-scanner reproducibility of the PerioScan™ results was 90 percent ( $\kappa = .77$ ). Intra-scanner reproducibility was above 90 percent ( $\kappa \geq .70$ ).

Specificity, sensitivity, and accuracy estimates were calculated by means of the correlated binomial model (14), which takes into account site-dependence within a subject. Variables were dichotomized, as follows: (1) PerioScan™ results (+,-); (2) bleeding (yes, no); (3) probing depth ( $\leq 3$  mm,  $> 3$  mm); (4) GI ( $< 2$ ,  $\geq 2$ ); (5) *T. denticola* (+,-); (6) *P. gingivalis* (+,-); and (7) *B. forsythus* (+,-). The reference parameters against which the BANA test was compared were (1) the ELISA system results for the detection of *T. denticola*, *P. gingivalis*, and *B. forsythus*; and (2) the clinical parameters. The clinical parameters results were also treated as reference parameters when compared to the ELISA system results.

The PI was not treated as a dichotomous variable, as this index attempts to discriminate among gingival health, gingivitis, and destructive periodontitis. An analysis of variance was calculated between subjects for PerioScan™-positive and PerioScan™-negative results and their respective mean (SD) PI, for sites no. 1 and no. 2 (diseased) and sites no. 3 and no. 4 (healthy). Thus, data were analyzed by site (301 subjects per site) and not by averaging all diseased or healthy sites within a subject.

## Results

Clinical and microbiological parameters were obtained from four sites per subject, for a total of 1,204 sites in 301 subjects. The overall proportion of bleeding sites was about 33 percent. Thirty-one percent of the sites had a GI score  $\geq 2$  and 30 percent of sites had a probing depth  $> 3$  mm. The overall proportion of PerioScan™-positive sites was 66 percent and *T. denticola*, *P. gingivalis*, and *B. forsythus* were individually detected in 52 percent, 60 percent, and 50 percent of the sites, as determined by the ELISA system (Table 1). The sensitivities for the PerioScan™ against the presence of one or more of the three species ranged from 91 percent to 97 percent. The specificities ranged from 67 percent to 89 percent, and accuracies from 80 percent to 90 percent (Table 2).

The PerioScan™ was next evaluated using clinical measurements as the reference parameter (Table 3). The PerioScan™ reflected the presence of clinically determined disease in 93 percent to 95 percent (sensitivity) of the instances. Lower specificities (47%) were found for clinical measurements, indicating that the PerioScan™ detected colonization of the root surfaces by the putative periodontal pathogens where clinical signs of disease were not apparent. Accuracies ranged from 61 percent to 62 percent.

The relationship between colonization of plaque samples by one or more of the three species, as measured by the ELISA system, and clinical parameters and indices of periodontal diseases is shown in Table 4. The sensitivity

**TABLE 1**  
Clinical and Microbiological Parameters of Periodontal Sites of Brazilian Subjects

Parameter	% of Sites (Relative Frequency)
Bleeding on probing	33 (391/1204)
Gingival index $\geq 2$	31 (337/1204)
Probing depth $> 3$	30 (357/1204)
PerioScan™(+)	66 (790/1204)
<i>T. denticola</i> (+)	52 (643/1204)
<i>P. gingivalis</i> (+)	60 (713/1204)
<i>B. forsythus</i> (+)	50 (602/1204)

**TABLE 2**  
Sensitivity, Specificity, and Accuracy Estimates of the PerioScan™ when Using ELISA for *T. denticola* (TD), *P. gingivalis* (PG), and *B. forsythus* (BF), as the Reference Parameter\*

Perio-Scan™	Sensitivity		Specificity		Accuracy	
	%	SE	%	SE	%	SE
TD	97	0.7	67	2.1	82	1.1
PG	93	1.0	74	2.1	85	1.0
PF	96	0.9	64	2.0	80	1.2
TD/PG	92	0.1	82	2.0	89	0.1
TD/BF	94	0.1	80	2.0	89	0.1
PG/BF	92	1.0	85	1.8	89	0.1
TD/PG/BF	91	1.0	89	1.6	90	0.1

\*Sensitivity, specificity, and accuracy estimates were calculated using the correlated binomial model. These values differ from values calculated using ordinary binomial models.

**TABLE 3**  
Sensitivity, Specificity, and Accuracy Estimates of the PerioScan™ when Using Clinical Measurements as the Reference Parameter\*

Perio-Scan™ vs	Sensitivity		Specificity		Accuracy	
	%	SE	%	SE	%	SE
Bleeding	93	1.4	47	1.8	62	1.4
Probing depth	95	1.2	47	1.8	61	1.4
Gingival index	94	1.4	47	1.8	62	1.4

\*Sensitivity, specificity, and accuracy estimates were calculated using the correlated binomial model. These values differ from values calculated using ordinary binomial models.

**TABLE 4**  
Sensitivity, Specificity, and Accuracy Estimates of ELISA for *T. denticola*, *P. gingivalis*, and *B. forsythus* when Using Clinical Measurements as the Reference Parameter\*

Measurements	Sensitivity		Specificity		Accuracy	
	%	SE	%	SE	%	SE
Bleeding	95	1.2	45	1.7	60	1.4
Probing depth	95	1.1	41	1.7	58	1.4
Gingival index	96	1.0	43	1.7	60	1.4

\*Sensitivity, specificity, and accuracy estimates were calculated using the correlated binomial model. These values differ from values calculated using ordinary binomial models.

for the clinical measurements against the ELISA system was about 95 percent, indicating that the ELISA system reflected clinically defined disease in 95 percent of the instances. The specificities ranged from 43 percent to 45 percent, also indicating that the ELISA system detected colonization of the species where clinical disease was not apparent. Accuracies ranged from 58 percent to 60 percent.

The relationship between the variation of the PerioScan™ results and the PI responses were submitted to an analysis of variance (Table 5). Ninety percent of the plaque samples from the diseased sites were PerioScan™-positive and had a mean PI that ranged from 3.9 to 4.1. Conversely, the few PerioScan™-negative plaque samples from the diseased sites had a mean PI of 2.0. These differences were statistically significant ( $P < .0001$ ). No association could be found between PerioScan™-positive and PerioScan™-negative plaque samples in the healthy sites, and their respective average PI. PerioScan™-positive plaque samples in the healthy sites and PerioScan™-negative plaque samples in the healthy sites showed a comparable mean PI score of about 0.9. This shows that the PI indicated health of these sites when over 50 percent of the plaque samples coming from those sites tested positive for the PerioScan™.

### Discussion

In one study involving American subjects seen at four university dental clinics (Detroit, Missouri, Florida, and Harvard), the PerioScan™ agreed with the ELISA system in the detection of *T. denticola* and *P. gingivalis* in 84 percent of the instances (12). The relative contribution of *B. forsythus* to the PerioScan™'s performance (accuracy) could not be obtained because a polyclonal antibody for this organism was not available at the time the studies were performed. The accuracy of the BANA test in detecting *T. denticola*, *P. gingivalis*, or *B. forsythus* in the Brazilian subjects was 90 percent (Table 2), indicating a moderate contribution of *B. forsythus* to the BANA test's accuracy. The sensitivity and specificity for the American subjects were 92 percent and 70 percent, respectively. The

**TABLE 5**  
Analysis of Variance of PerioScan™ Results and Periodontal Index in Diseased and Healthy Sites

Total # of	Diseased Sites		Healthy Sites	
	Tooth #1 (n=301)	Tooth #2 (n=301)	Tooth #3 (n=301)	Tooth #4 (n=301)
Plaques PerioScan™ Results				
Positive no. of plaques	4.1 (2.3)* n=276	3.9 (2.2) n=261	0.9 (0.4) n=168	0.9 (0.5) n=181
Negative no. of plaques	2.0 (1.6) n=25	2.0 (1.6) n=40	0.9 (0.6) n=133	1.0 (0.5) n=120

\*Mean periodontal index (SD).

† $P < .00001$ .

‡NS=not significant.

low specificity of the BANA test in this case may be due to the absence of an antibody for *B. forsythus*. Sensitivity and specificity for the Brazilian subjects were about 91 percent and 89 percent, respectively. These measures of the diagnostic value of a test indicated that the BANA test under field conditions was a valid objective instrument for the detection of colonization of root surfaces by at least one of the putative periodontal pathogens in Brazilian subjects, as shown by its high agreement with the ELISA system. The PerioScan™ agreed with the ELISA in 90 percent of the instances (accuracy), indicating that both the PerioScan™ and the ELISA are detecting comparable levels of the BANA-positive organisms, as was noted previously in US subjects (13).

The accuracy of the PerioScan™ and the ELISA system against clinical parameters and indices (GI, bleeding, probing depth) in the Brazilian subjects was comparable and never exceeded 62 percent (Tables 3 and 4). This is in agreement with the study performed in American subjects where the accuracy of the BANA test and of the ELISA system against selected clinical parameters of periodontal diseases ranged from 53 percent to 72 percent (12). The PerioScan™ and the ELISA system results agreed with the presence of clinical disease in Brazilian and American subjects, indicating that both assays were highly sensitive (Tables 3 and 4) (12). However, low specificities were observed in Brazilian and American subjects. Possible explanations for the low specificity observed in American and Brazilian subjects may relate to colonization by at least one of the three species where clinical disease was not apparent. The fact that teeth can be colonized by these organisms without evidence of clinical disease was suggested by a study in children (15). Approximately 86 percent of the children aged 3–11 years were colonized by either *T. denticola* or *P. gingivalis*, using the same antibodies used in this study. These data collectively indicate that the detection level of these assays at about  $10^4$  CFU is too low. The PerioScan™ can be made

more specific by incubating at 35°C for 5 min. When this was done, the specificity improved to about 96 percent (16).

The periodontal index (PI) has been the most widely used index in studies of the epidemiology of periodontal diseases. Much of the information on the distribution of periodontal diseases across populations is based on the PI. It has been argued that this index underestimates the true prevalence of periodontal destruction. In this study, a periodontal probe was used to confirm the presence of pockets in the PerioScan™ sites, and as such, was a modification of the PI. The mean PI for PerioScan™-positive plaque samples and PerioScan™-negative plaque samples differed significantly in the diseased sites (Table 5). However, no mean PI differences could be found between PerioScan™-positive and PerioScan™-negative plaque samples in the healthy sites. Thus, the PerioScan™ detected colonization by the putative periodontal pathogens where clinical disease was not apparent, as determined by the PI.

What would a simple test such as PerioScan™ add to the public health planner while targeting individuals at higher or lower risk for periodontal diseases? Limited resources and the limited availability of diagnostic tests for dental diseases in developing countries has led to the use of inadequate treatment modalities, i.e., an annual dental prophylaxis, given to all available subjects, instead of focusing treatment on those who are at risk for developing disease. The ability to use diagnostic tests in the field is an important consideration, because in developing countries data are usually obtained in the field. Because developing countries have limited resources, and in order to render its use cost effective, a diagnostic test must be simple, quick to manipulate, accurate, acceptable to the patient, and of low cost. Based on available information (13), the PerioScan™ has an accuracy comparable to that found for immunological reagents and DNA probes, when compared to reference parameters (ELISA system, cultural methods). The test can be performed in the field in 5–15 minutes, and does not require specialized personnel to manipulate it. A projected cost of \$10.00 per card (each PerioScan™ card can give information on up to 28 teeth) renders the use of the PerioScan™ in the field more likely to be accepted in developing countries than the more expensive and laboratory-based immunologic reagents, DNA probes, and cultural and microscopic procedures. It is possible that the cost of the PerioScan™ can be further lowered if the test is produced locally with local resources. In addition, by sampling four teeth per patient, as was the case in this study, several subjects can be assessed, per card, further lowering the per-subject cost.

The cost effectiveness of any treatment in dentistry increases when it is specifically targeted at individuals who are at risk for disease. The targeting of high-risk for periodontal diseases is an important economic consider-

ation that may considerably affect estimates of periodontal treatment needs. Thus, the economics of preventive programs in periodontology may require health care providers to consider new and improved methods to detect high-risk groups and individuals. Some of the periodontal diseases do not need refinements in diagnosis, since clinical symptoms can readily identify the clinical entity (e.g., ANUG, LJP, periodontal abscesses). Similarly, there is not a need for an improved diagnostic test for gingivitis because visual inspection of the gingival tissue provides immediate information on the gingival status, and this condition can be reversed by common oral hygiene methods. There is, however, a real need to gather information on the dynamics of host-microbial interactions in the destructive periodontal diseases to arrive at a more objective diagnosis of these infections. Information obtained from these findings can then be transferred to, and readily applied in, community-based preventive programs.

Future research directions regarding the use of the PerioScan™ test in field surveys should include (1) cost-effectiveness and cost-benefit analyses of the PerioScan™; (2) prospective cohort studies, where risk indicators such as a positive PerioScan™ and the subsequent development of clinical disease could be followed throughout time; (3) school-based screening programs to identify risk groups for periodontal diseases at an early age; and (4) studies to monitor the efficacy of selected clinical or antimicrobial procedures.

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