ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at:www.ijdsir.com

Volume – 6, Issue – 1, January - 2023, Page No. : 251 - 261

AIDE to Chairside Diagnosis in Periodontology

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Citation of this Article: Dr. Shashikanth Hegde, Dr. Shreya Shetty, Dr. Rajesh K.S., Dr. Vinita Boloor, "AIDE to Chairside Diagnosis in Periodontology", IJDSIR- January - 2023, Volume –6, Issue - 1, P. No.251– 261.

Type of Publication: Review Article

Conflicts of Interest: Nil

Abstract

Periodontal diagnosis is made using data from patient's records, radiographic, histological, and clinical examination, largely focused on conventional methods. Because of the limitations of these methods, a number of novel techniques have been suggested. The rationale for the development of diagnostic tests is that earlier the active disease is recognized, less traumatic, time-consuming, and less expensive the required therapy may be achieved. The present article reviews the recent advances in chairside diagnostic tests in periodontal diagnosis.

Keywords: Chairside tests, biomarkers, diagnosis, periodontal disease

Introduction

The majority of the time in modern practice, a Periodontal diagnosis is made using data from the patient's medical and dental records, radiographic, histological, and clinical examination records.[1]The diagnosis and classification of the condition are still largely focused on conventional clinical assessments, despite our enhanced understanding of the etiology and pathogenesis. The dentist must consider elements including the presence or absence of clinical evidence of inflammation, probing depth, and other considerations to make a periodontal diagnosis.[2] As the shortcomings of these conventional methods became clear, a number of novel techniques were suggested as periodontal disease diagnostic tests, focusing mostly on the following categories: bacteria in plaque, evaluation of metabolic alterations brought on by the discovery of periodontopathic bacteria, evaluation of the host's susceptibility and the detection of necrosis, tissue injury, or anatomical alterations in the Periodontium.[3]

These conventional tools have the benefits of being non-invasive, simple to use, and economical. Only the history of disease activity can be evaluated, which is the main flaw in these diagnostic methods. Therefore, it is necessary to develop newer diagnostic tests that can identify the existence of current disease, forecast how the disease will proceed in the future, and assess the effectiveness of periodontal therapy.

The rationale for the development of various diagnostic tests is that the earlier the active disease is recognised, the less traumatic, time-consuming, and hence less expensive the required therapy may be achieved.[4]

Objectives of chair side tests

1. They are less invasive than traditional diagnostic tools, giving them an advantage.
2. Since the appointment time is shortened, the patient finds it to be relatively less tedious.
3. They are easier to use since they are less complicated or technique-sensitive.
4. Assistance with early diagnosis and therapy preparation.
5. It is a tool for encouraging and motivating patients.

Advantages of chairside diagnostic kits:

- Simple to use.
- Can be read after relatively short periods.
- Can be used to educate the patient.

Microbiologic Test Kits

Evalusite

A sandwich enzyme-linked immunosorbent test for the detection of A. actinomycetemcomitans and P. gingival isis available commercially under the name Evalusite Test™ (Eastman Kodak Company, Rochester, NY) (Figure 1). The target bacterial species' particular antigens are coated with antibodies in the sample wells following which antibody-antigen reactions are monitored.

In a study by Synder et al.,[5] the assay demonstrated a detection limit of 10^5 A. actinomycetemcomitans cells and 10^6 P. gingival is cells. However, the assay only recognised 16 of 31 culture-positive samples of P. gingival is, with a sensitivity of 52%, and only 4 of 20 samples of A. actinomycetemcomitans, yielding a sensitivity of 20%. Over 80% more sensitivity was detected in samples containing $\geq 10^5$ A. actinomycetemcomitans cells or $\geq 10^6$ P. gingival is cells.

Advantages

- Since there is little sample dilution when many paper points are put in a single tube, the Evalusite test can be used for multiple samples at once.
- It has been demonstrated that deeper pockets have a higher likelihood of detecting bacterial colonisation than shallower pockets.[6]

Disadvantages

- Multistage test.
- Calorimetric end point is arbitrary.
- Outcome obtained is not permanently recorded.
- Makes the underlying supposition that all three organisms are responsible for the disease development.

Figure 1: Evalusite

Boyer et al[6], performed a study to evaluate the detection of disease-associated bacterial colonization in adult periodontitis patients by the antibody-based Evalusite Test™. An overall agreement of 94% (288 of 306) was observed when comparing test results for duplicate sets of pooled and individual samples collected from 51 patients. These studies demonstrated that the Evalusite

Test is an effective method for detecting clinically relevant colonization by the test bacteria in patients at risk for periodontal disease.

Peri Oscan

A number of periodontal pathogens, including Bacteroides Forsyth us, Porphyromonas gingivalis, and Treponema denticola, as well as those organisms that produce an enzyme similar to trypsin, including Capnocytophaga, are detected with this chairside assay (Figure 2A). The synthetic trypsin substrate N-benzoyl-DL-arginine-2-naphthylamide (BANA) can be hydrolyzed to test whether this enzyme is present in a plaque sample. The products of this reaction will appear as a blue-black substance on the reagent strip, indicating that all three species are likely to be responsible for causing the disease.[7]

Principle of BANA test

The peptide analogue BANA can be hydrolyzed by the peptididases bacterial species. B-naphthylamide, one of the hydrolytic byproducts of these three reactions, combines with an embedded reagent on the upper strip of the test to produce a permanent blue colour.[8]

Two distinct reagent matrices are connected to a plastic strip for the BANA test (Figure 2B). BANA is impregnated into the lower white reagent matrix. Samples of the subgingival plaque are placed in the lower matrix. A chromogenic diazo reagent is included in the top buff reagent matrix, and it combines with one of the hydrolytic by-products of the enzyme activity to produce a blue colour. The blue hue is constant and may be seen in the upper buff matrix. Whether it is a positive or weak reaction depends on the intensity of the hue.

Bretz et al[8] conducted a study determining whether the association between BANA hydrolysis and spirochetes could be obtained in pooled subgingival plaque samples. EDTA and $CaCl_2$ gave a slight inhibition and dithiothreitol (DTT) a slight enhancement of the BANA reaction by the pooled plaque suspensions. The majority of the reactions (85%) developed their full color after overnight incubation. Analysis of the data indicated that BANA hydrolysis by pooled subgingival plaque samples is a suitable test for the detection of spirochetes when two or three spirochetes per high power microscopic field are present in the sample.

Advantages

- Used to detect volatile sulphur compounds in patients with halitosis.

Disadvantages

- Other periodontopathogens that do not produce a trypsin-like enzyme cannot be detected by this method.
- The operator's judgement at the calorimetric end point determines the subjective results.
- It is impossible to identify the exact microorganisms that produce enzymes.

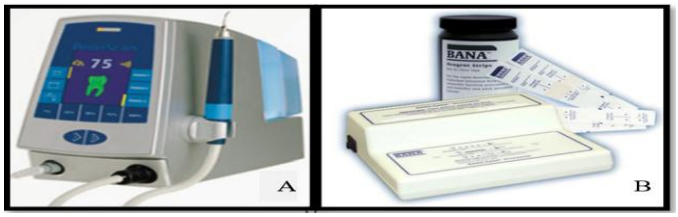

Figure 2A : Perioscan; Figure 2B: BANA test

Omni gene (DMDx)

It is a genomic probe using nucleic acid technology (Figure 3A). Purified DNA fragments can be used to identify Prevotella intermedia and Porphyromonas gingival is, however A. actinomycetemcomitans cannot be identified. The foundation for the commercial DMDx detection technology consists of the Aa cloned probe and the Pg full genomic probe. The DNA probe assay for Pg can produce false negative results.

According to Van Steen bergen et al.,[9] the DMDx detection method has an accuracy of 96% for laboratory specimens of Aa that have been spiked and an accuracy

of 86% for laboratory specimens of Pg. Sensitivity and specificity for Pg were 71% and 53%, respectively.

Advantages

- Provides reports in a short amount of time.
- It assists in the detection of a variety of known periodontal pathogens

Disadvantages

- In clinical specimens, the detection of Aa revealed sensitivity as low as 21% and a specificity of 83%.[9]

IAI Pado Test 4.5

Aa, Pg, T. Forsythia, and T. denticola are the four periodontal pathogens that can be identified with the Pado RNA probe test kit (Figure 3B). To perform this test, oligonucleotide probes corresponding to conserved regions of the 16S rRNA gene, which codes for rRNA, a component of the bacterial ribosome, are mostly used. This test has a 10^3 for Aa and a 10^4 for Pg, T. forsythia, and T. denticola detection threshold.

In research, Leonhardt et al.[10] compared the prevalence of periodontopathogen identification in individuals with chronic periodontitis using the Pado Test 4.5 and the checkerboard DNA-DNA hybridization approach. 30 patients with chronic periodontitis were tested cross-sectionally. The checkerboard approach demonstrated the presence of the four periodontal pathogens in all patients using the lower score (Score 1 equal to 104 bacterial cells) and in 16 (53.3%) using a higher threshold (Score 3 corresponding to between 105 and 106 cells) using the Pado Test 4.5. According to the study's findings, there was little agreement between the two diagnostic techniques for a positive microbiological result.

Advantages

- Possible to identify and measure bacterial ribosomal 16S rRNA.
- Overall amount of bacteria can be quantified using complementary universal bacterial DNA probes as well as A. actinomycetemcomitans, T. forsythia, P. gingival is, and T. denticola specifically quantified using taxon-specific probes.
- Because RNA degrades more quickly than DNA, it can be used to determine how many bacterial cells are still alive.[11]

Disadvantages

The number of positive sites or people is frequently underestimated by the high incidence of false negatives in the Pado Test 4.5.[10]

MyPerioPath

MyPerioPath is a DNA test (Figure 3C) that uses a sample of saliva to determine the kind and quantity of the specific bacteria that cause periodontal diseases. The exact bacteria known to cause periodontal inflammation and destruction are identified from bacterial DNA. Within this category, eleven species are found. Based on known risk/virulence characteristics, the bacteria are categorised into "risk" groups: high, moderate, and low.[12]

Figure 3A; Figure 3B: IAI Pado test; Figure 3C: My Perio Path.

DNA probes

Probe hybridization is a technique that can be used to demonstrate genetic relatedness in the DNA of distinct organisms for the goal of creating phylogenetic taxonomy schemes. DNA probes are employed in the diagnostic laboratory to confirm a culture or to directly identify fastidious or difficult-to-cultivate organisms in clinical specimens. Without DNA amplification

techniques, direct probe approaches are substantially less sensitive (limit, 10^3–10^6 cells), as the quantity of target cells may be below the sensitivity of the assay. Because periodontal disease and caries are so common, oral infections are a main indication for DNA probe application.[13]

Advantages

- Extremely precise and identify phenotypic markers.
- High specificity and sensitivity.
- Unaffected by transit conditions.
- Do not insist on maintaining anaerobic conditions.
- Are possible with dead bacteria and are independent of bacterial vitality.[14]

Disadvantages

- Lowest detection thresholds for a given species are 10^3–10^5 cells.
- Diagnosis at the chairside is not feasible.
- Possible for oligonucleotide probes to exhibit cross reactivity.
- Antibiotic sensitivity is not possible.
- Hybridization involves the denaturation of double-stranded DNA.[15]

Biochemical test kits

Perio-Check (Ac Tech)

PerioCheck has received US Food and Drug Administration approval. For neutral proteases such as collagenases, elastases, and proteinases in GCF, it is the fastest chairside diagnostic test available. The GCF sample strip is incubated after being placed on a gel that contains insoluble dye-labeled collagen fibrils. The insoluble collagen-dye complex is broken down to liberate soluble dye-labeled fragments in the presence of neutral proteases which diffuse from the strip into the gel and colour the strip blue. The levels of these enzymes in GCF have been observed to rise with the onset of gingivitis and locations of existing periodontitis. It is not specific for Polymorphonuclear Leukocyte Collagenase, which is thought to be the dominant collagenase at active sites.[15] The possibility of saliva contamination prevents sampling at interproximal sites. At baseline, PerioCheck had a sensitivity of 88% and a specificity of 61%.[8]

Huang YK et al.[16]investigated the relationship between clinical status and presence of carious or periodontal pathogens among parent-child familial pairs. Supra- and sub-gingival bacteria were determined based on semi-quantitative measurements of microbial infection by using data from the Dentocult® SM test (caries-related organisms) and the PerioCheck® test (periodontal disease-related organisms). No statistically significant relationship was detected between the prevalence of periodontal pathogens and cariogenic pathogens in the oral cavity.

Prognos-Stik (Dentsply)

This test kit was made available in 1993. The serine proteinase elastase is found in the GCF sample using this technique.[17] The GCF is collected on a strip of filter paper that has been coated with a known quantity of buffered elastase substrate and labelled with a fluorescent indicator. The enzyme on the test strip breaks down the substrate and releases the fluorescent-light-visible indicator during the 4–6-minute reaction period. Elastase is released from the lysosomes of polymorphonuclear leukocytes when they collect in sites of gingival inflammation. Elevated elastase levels in GCF may be a sign of active disease areas.[18] Although there exists a relationship between GCF elastase levels and Periodontal disease activity, further clinical trials are necessary to determine the use of this test kit in clinical practise.

PerioGard™

The foundation of PerioGard is the detection of aspartate aminotransferase (AST) levels in GCF samples. When a cell dies, the soluble intracellular cytoplasmic enzyme AST is released. AST levels in GCF have excellent potential as indicators of early periodontal tissue deterioration because cell death plays a significant role in periodontal aetiology. In contrast to inactive sites, elevated overall AST levels in a 30-second sample have been positively correlated with disease-active locations.[19,20] A tray with two test wells for each tooth and the necessary reagent for the test are the only components of this commercial test. The test entails gathering GCF using a filter paper strip, which is subsequently placed in a buffer containing tromethamine hydrochloride. The sample is mixed with a substrate reaction mixture including ketoglutaric acid and aspartic acid, and the mixture is given ten minutes to react. Aspartate and ketoglutaric acid are converted into oxaloacetate and glutamate in the presence of AST. The intensity of the colour produced by the addition of a dye like fast red is inversely correlated with the AST activity in the GCF sample.[21] The test is intended to be positive at AST activity levels >800 IU and negative at levels 800 IU. It is unable to distinguish between areas with high inflammation but no attachment loss and sites with attachment loss.[22]

Indications

a. To support or aid in the diagnosis of the location of active areas when a periodontal disease is present.

b. To appreciate the results of periodontal therapy.

c. To monitor high risk sites.

Persson et al[22] performed a multi-Center clinical trial to determine the relationship between measurements of the level of the enzyme AST in gingival crevicular fluid (GCF) to other measures used to detect periodontal disease and monitor outcome of treatment, including pocket depth and gingival inflammation using PerioGard™. The % of diseased sites that were PerioGard-positive decreased significantly between baseline and both post-treatment visits for patients at all 3 locations.

PocketWatch™

Aspartate transaminase is analysed at the chair side using the PocketWatch™ technique (Figure 4A). The basic idea behind this test is that when pyridoxal phosphate is present, AST catalyses the transfer of a cysteine sulfuric acid amino group from ketoglutaric acid to sulfinyl pyruvate. Inorganic sulfite is released when glutamate sulfinyl pyruvate spontaneously and quickly decomposes. Malachite green (MG), which was originally a green dye, instantly reacts with the sulfite ion to change from a green dye to a colourless one, revealing the pink rhodamine B dye underneath. The ratio of the AST concentration to the rate of MG conversion is direct. Although present in GCF of destructive pockets, extracellular matrix elements and their dissolved byproducts have the potential to release sulphide ions. With PocketWatchTM, it is possible to identify active sites apart from inactive ones. AST activity determined by PocketWatch™ provides not only an index of cell death but also the extent of the destructive pockets.[23]

Figure 4A: Pocket Watch; Figure 4B: Perio 2000 system

Perio 2000 System/Diamond Probe

By degrading the cysteine and methionine in serum proteins, several pathogenic microbes such as Pg, Pi, and T. forsythia create sulphates, which result in large

quantities of volatile sulphide compounds (VSCs). Since these VSCs can directly exacerbate periodontitis by destroying periodontal structures, their assessment can reveal the subgingival microbial burden. The properties of a periodontal probe are combined with the detection of VSC in the periodontal pocket in the Perio 2000 system.[12] Its handpiece resembles standard periodontal probes in terms of design, depth markings, and feel, but it features a special microsensor in the tip that can monitor the degree of bacterial activity at specific tooth sites even before the gingiva is bleeding.

The Diamond Probe/Perio 2000 System (Figure 4B) was used to evaluate the relationship between volatile sulfur compounds (VSC) and gingival health status by Pavolotskaya et al.[24] A split-mouth design with randomly selected quadrants of the mandibular arch enabled 39 participants to serve as their own controls. At baseline and at three subsequent appointments (days 7, 14, and 21) gingival inflammation (GI), bleeding on probing (BOP), and sulfide levels (SUL) were measured using the Gingival Index and the Diamond Probe/Perio 2000 System. For three weeks, participants refrained from brushing and flossing one randomly selected quadrant of the mandibular arch. Data suggest that SUL correlate positively to GI and BOP on both sides; however, the strength of the correlation was stronger for the non-hygiene side. Based on study outcomes, the Diamond Probe/ Perio 2000 System demonstrated the ability to detect sites with elevated SUL.

Dip Stick Test

The immunochromatography principle is the foundation of the matrix metalloproteinase-8 (MMP-8) test stick, which employs two monoclonal antibodies that are targeted to various MMP-8 epitopes. Results from the test stick can be found in five minutes. Both neutrophils and non-PMN type MMP-8 isoforms are detected by the antibody.[25] The GCF sample will be inserted into a test tube with 0.5 ml of pH 7.4 buffer. The dipstick absorbs liquid when it is inserted in the extracted sample, and liquid then begins to run up the dipstick. MMP-8 from the sample binds to the antibody on the latex particles when it is present. If MMP-8 is coupled to the particles, they are carried by the liquid flow and bind to the capturing antibody. A positive line will show up in the result area if the sample's MMP-8 concentration is higher than the test's cut-off value.[26]

Toxicity Prescreening Assay (TOPAS)

Two indicators of gingival infection—bacterial toxins and bacterial proteins—are used in the toxicity pre-screening assay to indirectly detect the presence of bacteria (Figure 5A). This test serves as an indirect indicator for metabolic activity in the actively replicating micro-organisms.

This test has a relationship to both the degree of inflammation and the development of the destructive process. Based on the fact that metabolic activity increases as the concentration of these toxins increase, it distinguishes between an active and an inert periodontal disease as evidenced by the shift in the colour intensity scale of the test.

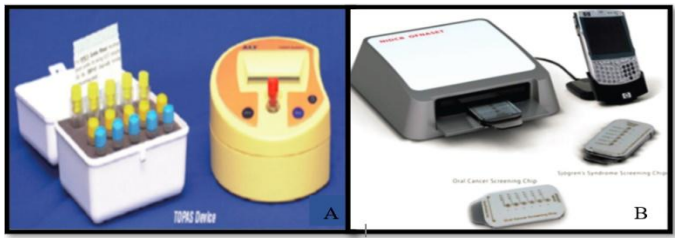

Figure 5A: Toxicity pre-screening assay; Figure 5B: Oral fluid nano sensor test

Integrated Microfluidic Platform for Oral Diagnostics

A clinical point-of-care diagnostic test that allows for the quick detection of a biomarker for an oral disease in human saliva using a disposable monolithic cartridge

that is intended to work with a small, portable analytical device. It evaluates MMP-8 in saliva from healthy and periodontally diseased patients quickly (within 10 minutes). It calls for little sample volume (10 L).[28]

Oral Fluid Nano Sensor Test (OFNASET)

It is a portable, automated, and user-friendly integrated system (Figure 5B). It allows for the quick and simultaneous detection of a variety of salivary proteins and nucleic acid targets. It is built on an electrochemical sensing platform that can detect salivary protein and RNA biomarkers in real-time with extreme sensitivity and specificity. Salivary indicators for oral cancer are detected multiplex at the point of service using OFNASET. With high specificity and sensitivity, it examines saliva for the presence of two salivary proteomic biomarkers (thioredoxin and IL8) as well as four salivary mRNA biomarkers (SAT, ODZ, interleukin [IL]-8, and IL-1). Multiple salivary proteins and nucleic acids may be detected at the same time.[29]

Gau V et al.,aimed to develop and validate a solution for requirement and also to develop a portable system. They demonstrated that the combination of two salivary proteomic biomarkers and four salivary mRNA biomarkers can detect oral cancer with high specificity and sensitivity.

They sequentially delivered a serial dilution of IL-8 antigen, probe solution' wash, enzyme solution, wash, and mediator solution to sensor reaction chambers housed in a prototype cartridge and demonstrated strong signal separation at 50 pg./mL above a negative control.[30]

Electronic Taste Chips

They are lab-on-a-chip bead microreactors that have been chemically sensitive, and they were used to assess salivary C reactive protein and other inflammatory indicators. The usual laboratory technology (ELISA) for assessing C reactive protein in saliva was compared to the electronic taste chips methodology, which showed a 20-fold lower limit of detection than the ELISA. With this method, it is quantitatively possible to distinguish C-reactive protein levels between healthy people and patients with periodontal disorders, and it is also possible to monitor multiple indicators at once.[31]

Genetic test kits

Periodontitis susceptibility trait test

The first genetic susceptibility test for severe periodontitis is the periodontitis susceptibility trait test. It evaluates the simultaneous occurrence of allele 2 at the IL-1α +4845 and 1β +3954 loci.[32] IL1 genetic susceptibility may not start or cause the disease, but it may make symptoms appear sooner or worse. Before the age of 60, individuals at risk for severe periodontal disease can be identified using the IL1 genetic test to differentiate between specific IL1 genotypes linked to various inflammatory responses.

My Perio ID

Saliva is used in the MyPerioID test to identify a patient's genetic predisposition to periodontal disorders. It evaluates patients who are more likely to have more severe periodontal infections.[12] It recognises genetic polymorphism or variation within the IL1 gene. One of the main inflammatory mediators is IL-1. People who are IL1 positive typically experience more severe infections that are aggressive. It identifies patients who are most at risk for developing severe illness, particularly if the individuals smoke. When present, this genetic variant can 2–7 times increase the risk of developing serious disease or tooth loss.

PerioPredict

PerioPredictTM, the next generation genetic risk test for periodontal disease, was released by Interleukin Genetics, Inc. in 2013 (Figure 6A). PerioPredictTM

detects polymorphisms in the genes for Interleukin-1 (IL-1). An easy-to-use cheek swab is part of the improvised sample collection method included in the kit. The new test also makes use of an expansion of earlier genetic markers, which now encompass all significant ethnic groupings, including Caucasian, African-American, Hispanic, and Asian.

Figure 6A: PerioPredict; Figure 6B: OraQuick

Other advances in chairside diagnostic kits

OraQuick

It is a test made to identify HIV infection quickly (Figure 6B). Results are available in 20 minutes. The developing solution is combined with the fluid to be diagnosed in a vial, and the results are shown on a testing apparatus. This test is the first oral swab at-home test for HIV-I and HIV-2 that has received FDA approval.

Pant Pai N et al[33]reported a field evaluation of the diagnostic accuracy, client preference, and feasibility for the oral fluid-based OraQuick® Rapid HIV1/2 test in a rural hospital in India. The OraQuick test on oral fluid specimens had better performance with a sensitivity of 100% and a specificity of 100%, as compared to the OraQuick test on finger stick specimens with a sensitivity of 100%, and a specificity of 99.7%. The OraQuick oral fluid-based test was preferred by 87% of the participants for first time testing and 60% of the participants for repeat testing.

Conclusion

The key to a successful treatment is a precise diagnosis of the disease. Accuracy in diagnosis has increased as a result of advances in diagnostic techniques. We can determine the microbiological and immunological activity occurring in the periodontal pocket by using several chairside diagnostic kits to identify different bacterial and host-derived products. This has further enhanced the treatment planning, enabling us to get better outcomes after periodontal therapy.

Chairside diagnostic kits provide a quick, repeatable method of testing, and the outcomes can also be used to inspire patients. They are particularly helpful in following up with patients after treatment to assess the effectiveness of the medication and the risk of disease recurrence. It is critical to incorporate new salivary diagnostic techniques into clinical practise to support dental professionals in making patient-Centered healthcare decisions.

References

1. D'Ercole S, Catamo G, Piccolomini R. Diagnosis in Periodontology: a further aid through microbiological tests. Critical reviews in microbiology. 2008 Jan 1;34(1):33-41.

2. Armitage GC; Research, Science and Therapy Committee of the American Academy of Periodontology. Diagnosis of periodontal diseases. J Periodontol 2003; 74:1237-47.

3. Highfield J. Diagnosis and classification of Periodontal disease. Aust Dent J 2009;54 Suppl 1: S11-26.

4. Kinane DF. Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. Periodontol 2000 2000; 24:215-25.

5. Snyder B, Ryerson CC, Corona H, Grogan EA, Reynolds HS, Contestable PR, Royer BI: Mayer J, Mangan T, Nork us N, Zambon JJ, Genco RJ. Analytical performance of an immunologic-based periodontal bacterial test for simultaneous detection and

differentiation of Actinobacillus actinornycetemcomitans, Porphyrornonas gingivalis, and Prevotella intermedia. J Periodontol 1996: 67: 497-505

6. Boyer BP, Ryerson CC, Reynolds HS, Zambon JJ, Genco RJ, Snyder B. Colonization by Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia in adult periodontitis patients as detected by the antibody-based Evalusite Test. J Clin Periodontol. 1996 May;23(5):477-84.

7. Laugh on BE, Syed SA, Loesche WJ. API ZYM system for identification of Bacteroides spp., Capnocytophaga spp., and spirochetes of oral origin. Journal of clinical microbiology. 1982 Jan;15(1):97-102.

8. Bretz WA, Loesche WJ. Characteristics of trypsin-like activity in subgingival plaque samples. Journal of dental research. 1987 Nov;66(11):1668-72.

9. Van Steen bergen TJ, Timmerman MF, Mikx FH, De Quincey G, Van der Weijden GA, Van der Velden U, De Graaff J. Discrepancy between culture and DNA probe analysis for the detection of periodontal bacteria. J Clin Periodontol. 1996 Oct;23(10):955-9.

10. Leonhardt Å, Carlen A, Bengtsson L, Dahlen G. Detection of periodontal markers in chronic periodontitis. The open dentistry journals. 2011; 5:110.

11. Belibasakis GN, Schmidlin PR, Sharman P. Molecular microbiological evaluation of subgingival biofilm sampling by paper point and curette. Apmis. 2014 Apr;122(4):347-52.

12. Mani A, Anarthe R, Mara war PP, Mustilwar RG, Bhosale A. Diagnostic kits: An aid to periodontal diagnosis. Journal of Dental Research and Review. 2016 Jul 1;3(3):107.

13. Retamal-Valdes B, Soares GM, Stewart B, Figueredo LC, Faveri M, Miller S, Zhang YP, Feres M. Effectiveness of a pre-procedural mouthwash in reducing bacteria in dental aerosols: randomized clinical trial. Brazilian oral research. 2017 Mar 30;31: e21:1-10.

14. Malathi K, Sharmila K, Sable D, Ahamed S. Microbial diagnosis in Periodontics: Merits and demerits: A review. J Dent Med Sci. 2014;13(2):104-7.

15. Chapple IL, Matthews JB, Thorpe GH, Glen wright HD, Smith JM, Saxby MS. A new ultrasensitive chemiluminescent assay for the site-specific quantification of alkaline phosphatase in gingival crevicular fluid. J Perio dont Res. 1993 Jul;28(4):266-73.

16. Huang YK, Lee WF, Wang MJ, Chang YH, Tchaou WS, Chang WJ, Lee SY, Sheu JR, Teng NC. Chair-side quantitative oral-microflora screening for assessing familial correlation of periodontal status and caries prevalence. PLoS One. 2014 Jan 30;9(1): e87100

17. Eley BM, Cox SW. Advances in periodontal diagnosis. 1. Traditional clinical methods of diagnosis. BDJ. 1998 Jan;184(1):12-6.

18. Armitage GC, Jeffcoat MK, Chadwick DE, Taggart Jr EJ, Numabe Y, Landis JR, Weaver SL, Sharp TJ. Longitudinal evaluation of elastase as a marker for the progression of periodontitis. J Periodontol. 1994 Feb;65(2):120-8.

19. Persson GR, De Rouen TA, Page RC. Relationship between gingival crevicular fluid levels of aspartate aminotransferase and active tissue destruction in treated chronic periodontitis patients. J Perio dont Res. 1990 Mar;25(2):81-7.

20. Chambers DA, Imray PB, Cohen RL, Crawford JM, Alves ME, McSwigan TA. A longitudinal study of aspartate aminotransferase in human gingival crevicular fluid. J Perio dont Res. 1991 Mar;26(2):65-74.

21. Komiya A, Kato T, Nakagawa T, Saito A, Takahashi J, Yamada S, Okuda K. A rapid DNA probe method for detection of Porphyromonas gingivalis and

Actinobacillus actinomycetemcomitans. J Periodontol. 2000 May;71(5):760-7.

22. Persson GR, Alves ME, Chambers DA, Clark WB, Cohen R, Crawford JM, DeRouen TA, Magnusson I, Schindler T, Page RC. A multi-Center clinical trial of PerioGard in distinguishing between diseased and healthy Perio dontal sites. (I). Study design, methodology and therapeutic outcome. J Clin Periodontol. 1995 Oct 1;22(10):794-803.

23. Shimada K, Mizuno T, Ohshio K, Kamaga M, Murai S, Ito K. Analysis of aspartate aminotransferase in gingival crevicular fluid assessed by using PocketWatchTM: a longitudinal study with initial therapy. J Clin Periodontol. 2000 Nov;27(11):819-23.

24. Pavolotskaya A, McCombs G, Darby M, Marinak K, Dayanand NN. Sulcular Sulfide Monitoring: An Indicator of Early Dental Plaque-Induced Gingival Disease. Journal of Dental Hygiene. 2006 Jan 1;80(1).

25. Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luo to H, Salo T, Sorsa T. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Perio dont Res. 2003 Aug;38(4):436-9.

26. Sorsa T, Mäntylä P, Ronak H, Kallio P, KALLIS GB, Lundqvist C, Kinane DF, Salo T, Golub LM, Teronen O, Tikanoja S. Scientific basis of a matrix metalloproteinase-8 specific chair-side test for monitoring periodontal and peri-implant health and disease. Annals of the New York Academy of Sciences. 1999 Jun;878(1):130-40.

27. Pucau CG, Dumitriu A, Dumitriu HT. Biochemical and enzymatic diagnosis aids in periodontal disease. OHDMBSC. 2005; 4:19-25.

28. Herr AE, Hatch AV, Gian Nobile WV, Throckmorton DJ, Tran HM, Brennan JS, Singh AK. Integrated microfluidic platform for oral diagnostics. Annals of the New York Academy of Sciences. 2007 Mar;1098(1):362-74.

29. Priyanka N, Kalra N, Shanbhag N, Kumar K, Seema Brijet B, Avani PR. Recent approaches in saliva as a credible periodontal diagnostic and prognostic marker. AOSR. 2012;2(1):40-6.

30. Gau V, Wong D. Oral fluid nanosensor test (OFNASET) with advanced electrochemical-based molecular analysis platform. Annals of the New York Academy of Sciences. 2007 Mar;1098(1):401-10.

31. Christodoulides N, Mohanty S, Miller CS, Langub MC, Floriano PN, Dharshan P, Ali MF, Bernard B, Romanovicz D, Anslyn E, Fox PC. Application of microchip assay system for the measurement of C-reactive protein in human saliva. Lab on a Chip. 2005;5(3):261-9.

32. Greenstein G, Hart TC. A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic Perio dontitis. J Periodontol. 2002 Feb;73(2):231-47.

33. Pant Pai N, Joshi R, Dogra S, Taksande B, Kalantri SP, Pai M, Narang P, Tulsky JP, Rein gold AL. Evaluation of diagnostic accuracy, feasibility and client preference for rapid oral fluid-based diagnosis of HIV infection in rural India. PLoS one. 2007 Apr 11;2(4): e367.