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Review Article

CHAIR SIDE DIAGNOSTIC KITS IN PERIODONTICS- A REVIEW

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ABSTRACT

Traditional periodontal diagnostic methods are not precisely accurate and only allow retrospective diagnosis of attachment loss. A great deal of current periodontal research has therefore been directed towards improving this situation. This has been both aimed at accuracy of traditional clinical diagnostic methods and developing alternative methods capable of detecting and predicting periodontal disease activity. Now a days, clinical chair side tests are in use for more precise molecular diagnostics and treatments. With the advent of more efficient diagnostic techniques in recent times there is an increased predictability in the diagnosis by the clinician. Development of reliable predictive tests can foresee future periodontal status and thus enable definitive treatments to be given for each specific site before extensive damage has occurred. This review describes some diagnostic test kits in the market that can facilitate the clinical examination and the establishment of a diagnosis.

The review has been generated from articles and abstracts researched from Medline, Pubmed and Google scholar indexed journal and the US patent websites describing the inventions in the periodontal diagnostic kits.

INTRODUCTION

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth. The initiation and the progression of periodontitis are dependent on the presence of virulent micro organisms capable of causing disease. (Socransky *et al*. 1984) A number of possible pathogens have been detected on the basis of their association with disease progression and also because of their possession of virulence factors which can damage the tissues. The main bacteria associated with periodontal disease are *Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis and Tannerella forsythia,*.(Slots, Bragd, and Wikström 1986)

These bacteria tend to be present in increased numbers at active disease sites and produce products capable of damaging the tissue either directly or indirectly. However, they may also be present in healthy and inactive sites and the composition of all these sites may vary between patients or even in the same patient at different time intervals. Attempts to relate microbiological data to clinical events have proved difficult due to the variability and unreliability of clinical diagnostic methods. Another factor, which complicates the quantitative assessments of the subgingival flora, is the technical problems associated with the sampling and culturing processes. (Socransky *et al*. 1991)

The aim of periodontal diagnostic methods is to provide functional information to the practitioner regarding the present periodontal disease, type, location and severity. This essential data serves as a basis for treatment planning and provides a record during periodontal maintenance and disease monitoring phases of treatment.

During the 1990s there has been an emergence of a multitude of diagnostic tests based on physical, chemical, microbiological and immunological approaches. The philosophy behind the emergence of such tests is that the earlier the active disease is diagnosed, the less invasive treatment is required. The patient also has a reduced chair time, reduced expense and a better the long term prognosis.(KINANE 2000; A. Lee *et al*. 2012)

According to Chappel for periodontal diagnosis, the ideal diagnostic test should be:(Chapple 1997)

1. Quantitative.
2. Highly sensitive method capable of analysing a single periodontal site in health as well as disease.
3. Reproducible.
4. Highly specific.
5. Simple to perform.

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6. A rapid, one or two stage procedure.
7. Non-invasive.
8. Versatile in terms of sample handling, storage and transport.
9. Amendable to chair side use.
10. Economical.
11. Dependent upon simple and robust instrumentation.

The potential markers are usually detected by enzyme linked immunosorbent assay (ELISA) systems and biochemical and histological reactions using simple substrates are easy to scale down to a simplified kit form. Most commercially available diagnostic kits involve one of these systems. More complicated assay systems are difficult or impossible to scaled own and this may preclude some potentially good markers from being used clinically.(H.-J. Lee *et al*. 1995)

Some systems using molecular biological techniques such as DNA test kits for putative periodontal pathogens. (Zappa *et al*. 1990) Real time PCR have also been deployed as a means to detect the putative periodontal pathogens but they do not have practical implications in the daily practice owing to cost factor and use of specialised equipment.(Jervoe-Storm *et al*. 2005; Sanz *et al*. 2004)

A chair side kit involves producing a simplified test kit system for chair side use which involves no specialized equipment and is easy to read. Colored detection systems are usually preferred. Secondly it is important that any chemicals used in the reaction are clearly and simply labelled in plastic containers from which it is easy to dispense the correct amount. Among these tests, chair side periodontal kits provide immediate reports of the micro flora associated with the disease compared to cumber some and time-consuming traditional laboratory procedures.

Chair side periodontal test kits can be categorized as

- Microbiological test kits
- Biochemical test kits
- Genetic kits
- Kits under development

Microbiological test kits

Simplified immunoassay and enzymatic testing methods have been investigated as chair side supplements to reference laboratory methods. (W. J. Loesche *et al*. 1990; Boyer *et al*. 1996) The utilization of microbiological detection techniques has the potential to lead to an improved management of the periodontitis patients (Goene, Winkel, and Abbas 1990; Genco and Zambon 1989). The bacteriological tests (Microscopy, Culture, Omnigene, Affirm DP and Evalusite) are mainly aimed at spirochetes, Aa, Pg and Pi. While *P.gingivalis, A.actinomycetemcomitans* .and *P. intermedia* are often found in increased levels in diseased periodontal lesions, low levels of each species have been observed in healthy sites (Socransky *et al*. 1991). Detection methods for these bacteria generally provide information on the levels of bacteria present to facilitate the interpretation of test results. Detection cut offs have been reported on the basis of the percent of total bacterial counts, or as the absolute number of *A.actinomycetemcomitans, P. gingivalis.* and *P. inter-media* present in the sample(Bragd, Dahlén, and Wikström 1987; Peros, Savitt, and Vassos 1988). Microbial tests can also be used to monitor periodontal therapy directed towards the suppression or eradication of periodontopathogenic organisms.

Omnigene

Omnigene [figure 1] and BTD (Biotechnica Diagnostics, Inc): These are DNA probe systems developed to detect subgingival bacteria. Sample of subgingival plaque is obtained on a paper sample and is placed in the container provided and assayed. Probes are available for P.gingivalis, P.intermedia, A.acinomycetemcomitans, F.nucleatum, E.corrodens, T.denticola, T.pectinovorum and C.recta. Reports are available within few hours.

Figure 1

Evalusite (Kodak)

Evalusite is a diagnostic kit [Figure 2] that is based on a novel membrane-based enzyme immunoassay for the detection of three putative periodontopathogens: Aa, Pg and Pi. A paper point subgingival plaque sample is collected and added to a sample tube.[Figure 3] The sample is placed within the kit, which employs a sandwich-type ELISA (enzyme-linked immunosorbent)and a pink spot is displayed if the test organism is present.

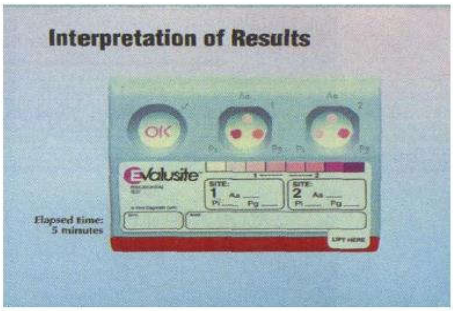
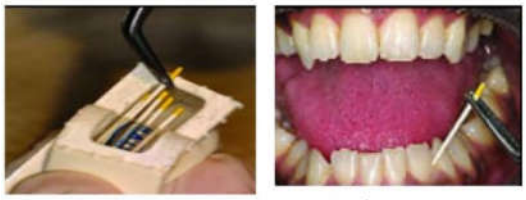


Fig 16 : Enzyme linked immunosorbent assay demonstrating positive results for Aa

Figure 2

It was designed to detect levels of colonization of bacteria present in periodontally diseased sites in number expected. Detection and differentiation of the bacteria requires approximately 5 min and the results are interpreted visually. Comparisons to bacterial culture have shown that positive test results generally indicate the Presence of greater than 10^4 recoverable Counts of *Agregatibacter Actinomyceterncomitans. Porphyromonas Gingivalis.* or *PrevotellaIntermedia* in subgingival plaque. (Snyder. B. and Son 1994) the test Format is also conducive to sample Pooling since minimal sample dilution occurs when multiple samples are placed in a single

sample tube. This feature facilitates patient-based determinations of bacterial colonization.



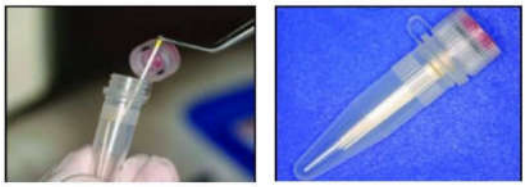


Fig 17 :

1 – A package of sterile paper points for gathering subgingival bacterial samples
2 – A sterilepoint is inserted into periodontal pocket for 10 seconds
3 – Paper points are placed ina sterile vial for transport
4 – Vial sealed for shipment

Figure 3

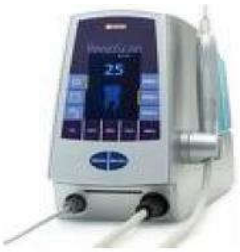
Disadvantages

1. Detects only 3 organism which can cause the disease and not others
2. It is a multistage test
3. It has a subjective calorimetric end point
4. No permanent record of the result is obtained.(Mikx and Renggli 1994)

Perioscan (oral-b laboratories)

PERIOSCAN [Figure 4] is a chair side test kit system which uses the BANA test for bacterial trypsin-like proteases. These are mainly produced by P.gingivalis, but lesser amounts are also produced by T.forsythia and T.denticola.

A



B

Fig 18 :
A – the incubator
B – the BANA test strip

Figure 4

The plaque sample is exposed to a substrate that can only be hydrolyzedby a specific enzyme. (Dhalla *et al*. 2015) Since some of these species grow poorly in cultures and account for a significant proportion of the protease activity of the subgingival flora, these enzyme as says provide a rapid and in expensive method of screening samples of these bacteria. (Schmidt *et al*. 1985; W. Loesche, Lopatin, and Giordano 1992)

The PERIOSCAN works by detecting the activity of this enzyme and it can be measured with the hydrolysis of the colourless substrate N-benzoyl-dL arginine-2-naphthylamide (BANA).When the hydrolysis takes place, it releases the chromophore β -naphthylamide, which turns orange red when a drop of Fast Garnet added to the solution. The system is particularly simple to use. This method has recently been made more sensitive.(Ishihara *et al*. 1992)

Disadvantage

1. There is presence of plaque sampling and on the assumption that the test organisms identified represent the active disease. This is valid for all patients and sites.
2. Results are qualitative and rely upon the operator's assessment of the calorimetric end point.
3. It may give a false positive result at clinically healthy sites and might remain so even after treatment.

Meridol periodiagnostics

It is a real – time PCR [Figure 5] for the quantitative determination of the six most important marker organisms of periodontitis and peri- implantitis and the total bacterial load. (Jervoe-Storm *et al*. 2005) The marker organisms are Agregatibacteractinomycetemcomitans,

Figure 5

Porphyromonasgingivalis, Tanerellaforsythus, Treponema denticola, Fusobacterium nucleatum and Prevotella intermedia. It combines high specificity with high sensitivity and a precise quantification. The detection limit, at100 bacterial cells per type of pathogen, is far below the limits of methods available. Making it highly sensitive.

Advantages of periodontal diagnostic test systems based on subgingival bacteria and their by-products:

1. Predict disease activity in long term studies.
2. Convenient to use.
3. Results of chair side test kits are available in a short time.
4. Chair side test kits produce visual results which can be shown to the patient.

Disadvantages of periodontal diagnostic test systems based on subgin gival bacteria and their by-products:

1. Poly microbial nature of the disease.
2. Most are not predictive of the disease activity.
3. The site to be sampled needs to be known.

Biochemical test kits

Commericial kits based on host cell death and tissue

Degradation products

The only commercial kit based on factors released from tissue degradation is that based on GCF Aspartate aminotransferase transferase (AST) which is released from dead cells. (Persson, Rouen, and Page 1990) It is briefly described below.

Periogard

Test kit uses paper point GCF samples and calorimetric detection. This is based on Aspartate Transaminase (AST). Gingival crevicular fluid AST levels have been shown to increase during the development of ligature induced experimental periodontitis. (Chambers *et al*. 2012). In a cross sectional study GCF AST was also shown to correlate with clinical indices of disease severity. (Persson, Rouen, and Page 1990) In longitudinal studies, GCF AST levels have been related to confirmed attachment loss. (Chambers *et al*. 1991)

The test kit consists of a tray with two test wells for each tooth and appropriate reagents for conducting the test. The GCF sample is obtained on a strip and is placed in to a suitable test well with two drops of one reagent. At the same time positive and negative control wells are prepared using strips provided. Two drops of a solution provided are added to the wells and are allowed to incubate at room temperature. The test results can be visually appreciated by comparing the test well colour to the colour of the positive control. A colour of greater intensity to that of the negative control is scored as positive and one of lesser or equal intensity as a negative result. The test is designed to be positive at 800mlU of AST activity and negative at values<800mlU.(GR *et al*. 1995)

Disadvantages

1. The colour differentiation in PERIOGARD is difficult.
2. Complex procedure with multiple steps.

Pocket Watch

It is one of the simplest tests for analysing Aspartate amino transferase (AST) at the chair side and it is suggested that AST levels may help to attune the clinical measurements in subjects with chronic periodontitis [Figure 6]. The GCF sample paper strip is placed in a well on the reagent coated test tray that is a part of pocket watch kit and 1 drop of AST positive control solution is added to another non-sample well. The tray is incubated for 10 minutes at room temperature for colour development. If the GCF sample after incubation shows the same colour or lighter colour than AST positive control, it is given a score of 2.A sample is given a score of 1 if its colour is the same as or lighter than the AST standard sample. A score 0 is given if it is darker than AST standard sample. Pocket watch provides an index of cell death.

Disadvantages

1. It does not predict disease activity
2. False positive results may be obtained if any other disease is associated with inflammation masking periodontitis.(Arturo Sánchez-Pérez, María José Moya-Villaescusa, and Raúl Guillermo Caffesse 2012)

A

Fig 19: Pocket watch test kit with (A) displaying the result

Figure 6

Commericial kits based on GCF proteolytic and hydrolytic enzyme levels

Periocheck (Actechinc. Usa)

This system detects the presence of neutral proteases such as collagenase in GCF [Figure 7]. The presence of neutral proteases is implicated in collagen break down, which is an important feature of periodontal disease. A paper strip is used to obtain a GCF sample. This strip is then placed in contact with a collagen gel to which a blue dye has been covalently bonded. If the neutral proteases are present in the sample they will attack the collagengel and release the blue dye. The released blue dye reproduces a blue colour in the strip, the intensity of which is proportional to the amount of the enzyme present in the sample. The intensity and the area of the blue colour are then scored on a scale of 0 to 2 by comparing it with three standards on the colour card which is provided with the test kit. The test shows predictive value for disease progression in a short-term evaluation. (Hemmings, Griffiths, and Bulman 1997; Yoneda *et al*. 2005)

Figure 7

Disadvantages

1. No account of the biological control mechanism
2. This test involves expensive procedures.

Prognostik (dentsply)

This system detects the presence of the serine proteinase, elastasein GCF sample [Figure 8]. A GCF sample is collected

on special filter paper strips which have been impregnated with the appropriate peptidylderivative of 7-amino-trifluoromethylcoumarin (AFC). The substrate used detects elastase and is linked to a fluorescent leaving, AFC. If elastase is present the sample reacts with the substrate in 4-8 minutes releasing the fluorescent leaving group, AFC. This produces green fluorescence in the strip which can be seen under ultraviolet (UV) light using a UV light box. The intensity of the fluorescence is proportional to the amount of GCF in the sample and this is scored by comparing it with AFC standards
The evolution of micro chips and micro fluidic platforms for salivary components may have great possibilities in the use of oral fluid for point-of-care testing. These systems use small sample and reagent volumes coupled with integrated detection methods to perform analyses. Researchers are design in glab-on-a-chip prototypes handheld, automated, easy-to-use and integrated systems will enable simultaneous and rapid detection of multiple salivary protein and nucleic acid targets. (CHRISTODOULIDES *et al*. 2007; Mäntylä *et al*. 2003)

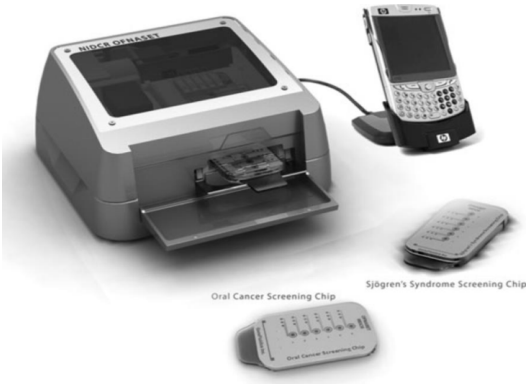


Figure 8

Biolise

Hermann *et al*. Developed a new test (SLT-Lab instruments, Craitsheim, Germany) which is used to detect the elastase kit Biolise activity in GCF.

TOPAS Tm (toxicitypre-screeningassay)

A new TOPAS TM test kit [Figure 9] has been introduced to detect elevated levels of bacterial toxins and increased levels of human and bacterial inflammatory proteins. The first generation TOPAS was a manual test and the latest second generation TOPAS TM is an automated one.

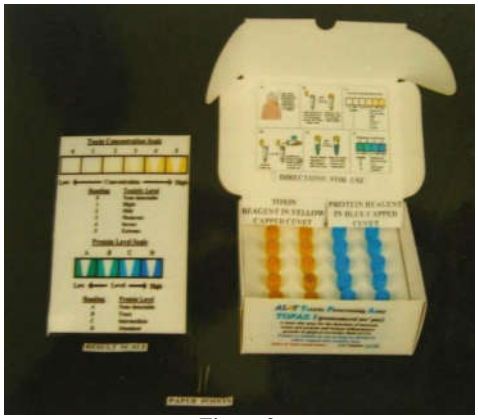


Figure 9

It is a simple, painless test which can be performed by any medical professional in only 7 minutes. The intensity of the blue colour produced by the assay is proportional to the amount of total proteins present in the GCF.(Pu°ca°u and Dumitriu 2005)

PERIO 2000

Various pathogenic microorganisms like P. gingivalis, P. intermedia and T.forsythia produce sulphates, leading to elevated levels of volatile sulphide compounds (VSCs) by degradation of serum proteins: cysteine and methionine. Since these VSCs can directly degrade periodontal structures so they may aggravate periodontitis and their evaluation can indicate the subgingival microbial load. The Perio2000 system is designed to display the sulphide level digitally at each site. The probe tip should be hydrated using sterile wash solution provided by the manufacturer and then inserted subgingivally at peak or hold operational mode. After a positive reading, the tip is washed and reinserted in other subgingival site.

Genetic Test Kits

A lot of research now a day has been aimed at detection of various gene polymorphisms. *Kornman et al* found an association between the polymorphism in the genes encoding for interleukin-1 and increasing severity of periodontitis. (Kornman K 1997) Now chair side kits have been made available for its detection.

PST® genetic susceptibility test

Periodontal susceptibility test (PSTR) is the first and only genetic test that analyses two interleukins (IL-1α and IL-1β) genes for variations. The IL-1 genetic testing can be used to differentiate between IL-1 genotypes associated with diverse inflammatory responses to identify subjects at risk for severe periodontal disease even before the age of 60. Clinically, PSTR is used in("Periodontal Susceptibility Test (PST®)," n.d.):

1. New periodontal subjects to aid in the development treatment strategies.
2. To determine prognosis of subjects requiring extensive periodontal and/or implant therapy and to improve their acceptance and optimize the treatment outcomes.
3. As an incentive for smoking cessation.
4. Improvement of patient compliance and recall intervals in patients on maintenance.
5. Specialist referral after detection of early signs of the disease.

Commercial Diagnostic Test kits under Development

B-Glucoronidase

A diagnostic kit is being commercially developed by Abbot Laboratories, North Chicago, USA. It probably uses a his to chemical substrate for the enzyme, coupled to a colour detection system which is released if the enzyme attacks the substrate.

Cysteine and serine proteinases

The test system suitable for chair side use has been developed by Enzyme System Products/Prototek of Dublin, California, USA. This firm synthesizes (AFC) which is more sensitive

than other fluorogenic leaving groups. GCF is collected with chromatography filter paper strips. This has been applied to the detection of bacterial proteases in gingivalcrevicular fluid.

A typical green fluorescence is produced which can be detected by ultraviolet (UV) light. The amount of enzyme present is proportional to the intensity of the fluorescence or colour. The colour system is more sensitive than fluorescence and requires no special apparatus in the clinical setting.(Stella Lorraine Martin 2010)

Advantages of diagnostic test systems based on proteolytic and hydrolytic enzymes

1. Convenient to use, especially the colour detection system.
2. Can be read within a short time.
3. Can be shown to the patient along with the involved tooth site.
4. The markers used are predictors of disease activity.

Disadvantages of diagnostic system based on proteolytic and hydrolytic enzymes

1. Difficulty in choosing an appropriate biomarker due to insufficient studies.
2. Difficulty in sampling of the sites and the time period of the sample.
3. False positive results in cases of associated of a disease with inflammation, m asking its association with the destructive disease.
4. No account of biological control mechanisms are taken in the present tests.
5. Costs of developing that kit and implying it in the private practice.

Summary

It can be interpreted that new technologies that have been developed or are in development can be used to enhance the ability to predict, diagnose& treat periodontitis. Moreover, new diagnostic technologies like nucleic acid and protein microarrays & micro fluidics are under development for risk assessment & comprehensive screening of biomarkers. These will provide practitioners with more effective means of prevention, detection, & treatment of periodontitis than are currently available. Hence, periodontists & dentists will take on the role of physicians dedicated to prevention & treatment of oral diseases &rely less on mechanical or non-biologically based treatment modalities. These recent advances are leading to the development of more powerful diagnostic tools for practitioners to optimize their treatment predictability. If a reliable predictive test is developed then it can be used to predict future periodontal activity & thus enable administration of the treatments tailored to specific sites before irreversible damage has occurred. However as periodontal disease is site specific & its progression may beep is odicitis difficult to determine which sites to test or when to test them. This will therefore always ways be a problem demanding sound clinical judgment. (Stathopoulou, Buduneli, and Kinane 2015; Barros *et al*. 2016)

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