ADVANCED DIAGNOSTIC AIDS

Dr. Parag Hadge

- Clinical diagnosis of periodontitis - measuring the loss of connective tissue attachment to the root surface (*clinical attachment loss*) and loss of alveolar bone (*radiographic bone loss*)
- Provides evidence of past periodontal destruction, its extent and severity

- Does not provide any information on the cause of the condition, on the patient's susceptibility to disease, whether the disease is progressing, whether it is in remission; whether the response to therapy will be positive or negative
- Consideration should be given to including microbiologic, immunologic, systemic, genetic, and behavioral factors, in addition to the traditional clinical and radiographic parameters, when assessing patient status

- Advances in Clinical diagnosis
- Advances in Radiographic Assessment
- Advances in Microbiologic Analysis
- Advances in Characterizing the Host Response

Advances in Clinical diagnosis

Gingival bleeding

- Gingival bleeding is a sensitive clinical indicator of early gingival inflammation
- Clinical **advantage** of being more **objective**
- Severity of bleeding increases with the severity of the inflammation
- Requirement: Periodontal Probe

- Gingival bleeding universally considered indicator of gingival inflammation
- *Lang et al* - retrospective study, reported that sites that bled on probing at several visits had a higher probability of losing attachment than those that bled at one visit or did not bleed.

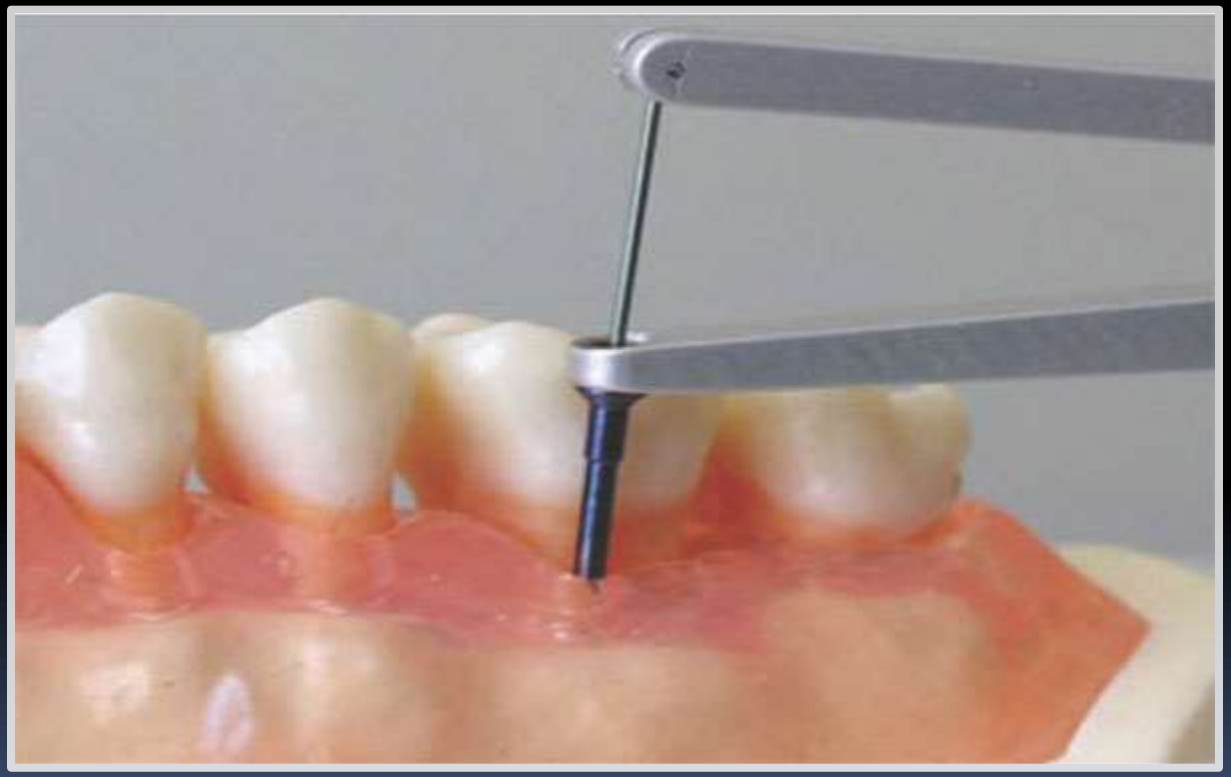
- Limitation - healthy sites may bleed on probing when force is not caliberated
- Relationship to disease progression- unclear

Gingival Temperature

- *Kung et al* - thermal probes are sensitive diagnostic devices - early inflammatory changes in the gingival tissues.
- Studies - suspected active periodontitis lesions can create measurable elevations in sulcular temperature
- ***PerioTemp® probe*** (Abiodent, Inc., Danvers, Mass) - pocket temperature differences of 0.1^0 C.
- A naturally- temperature gradient - between maxillary and mandibular teeth and between posterior and anterior teeth.

- *Haffajee et al* used this probe to assess its predictability in identifying loss of attachment, concluding that sites with a **red** (higher) temperature indication had more than twice the risk for future attachment loss than did those with a **green** indication.
- However, the influence of pocket depth on temperature is still not clear.

Periodontal Probing



- **Gold standard** is recording changes over period of time.

Problems with probing:

- Lack of sensitivity and reproducibility (errors)
- Probing depends on: force, angulations, size of probe, precision of calibration, presence of inflammation
- Clinical pocket depth obtained - not normally coincide with the histologic pocket depth

Classification of Probes

a) first generation probes/ conventional

b) second generation probes/pressure sensitive

c) third generation probes/ computerized

d) Forth generation probes – 3 D

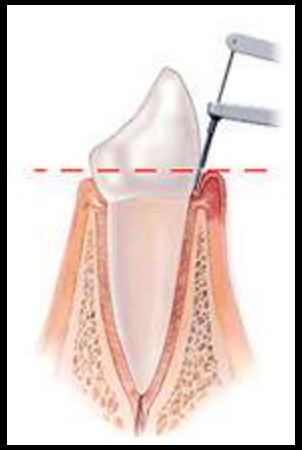
e) Fifth generation probes – ultrasonographic

e.g. Perioimager, Perioalert

Florida Probe System

Gibbs et al

- Tip is 0.4mm
- Sleeve- edge provides reference to make measurements
- Coil Spring; provides constant probing force
- Computer for data storage



FP Handpiece tip as it enters the sulcus.

FP Handpiece tip with constant force in use (tip at bottom of sulcus) and sleeve properly positioned at the top of the gingival margin allowing the computer to measure the difference

Disadvantages of Florida probe:

- Lack of tactile sensitivity
- Fixed probing force regardless of the site or inflammatory status
- Underestimation of deep periodontal pockets

Other electronic probes:

- Improvised Florida PASHA probe
- Interprobe
- Perioprobe
- Foster Miller probe by *Jeffcoat et al*
- Toronto Automated Probe (requires reproducible patient head position and difficult to record in 2nd and 3rd Molar area.)

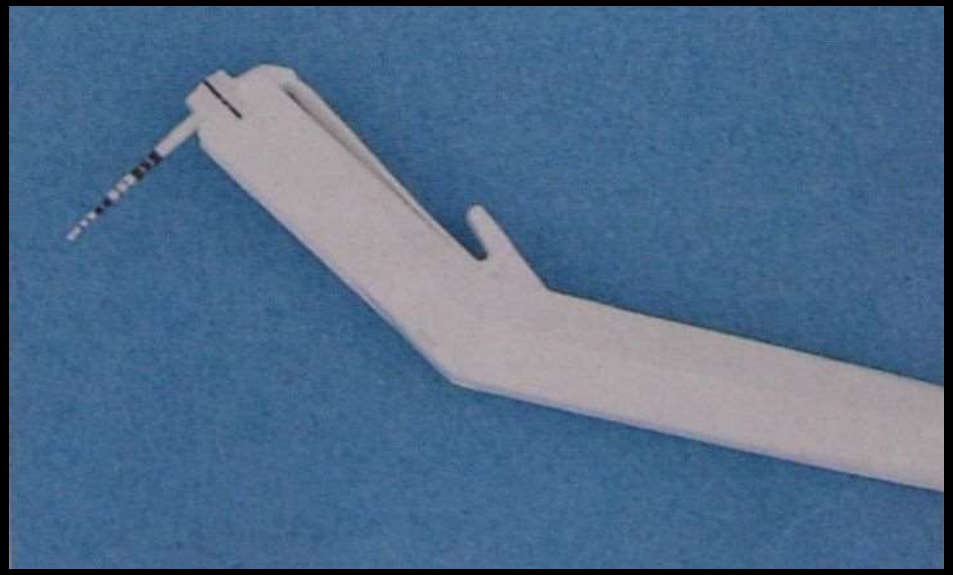
- Probing around implants is difficult

(1) the prosthetic construction may need to be removed for access

(2) standard metal instruments are unsuitable.

- Plastic or titanium probe tips should be used to avoid damage of the implant / tissue interface.

Probing around dental implants



If automatic probing is considered, the ***Florida Probe*** is available with a titanium tip that will not hurt the implant ; also, the ***Interprobe system*** comes with disposable plastic tips.

Advances in Radiographic Assessment

- Radiographs cannot accurately reflect - bone morphology buccally and lingually - provide -useful information on interproximal bone levels

- More than 30% of the bone mass at the alveolar crest must be lost for a change in bone height to be recognized on radiographs
- Therefore conventional radiographs are very specific, but lack sensitivity

Problems with conventional Radiography:

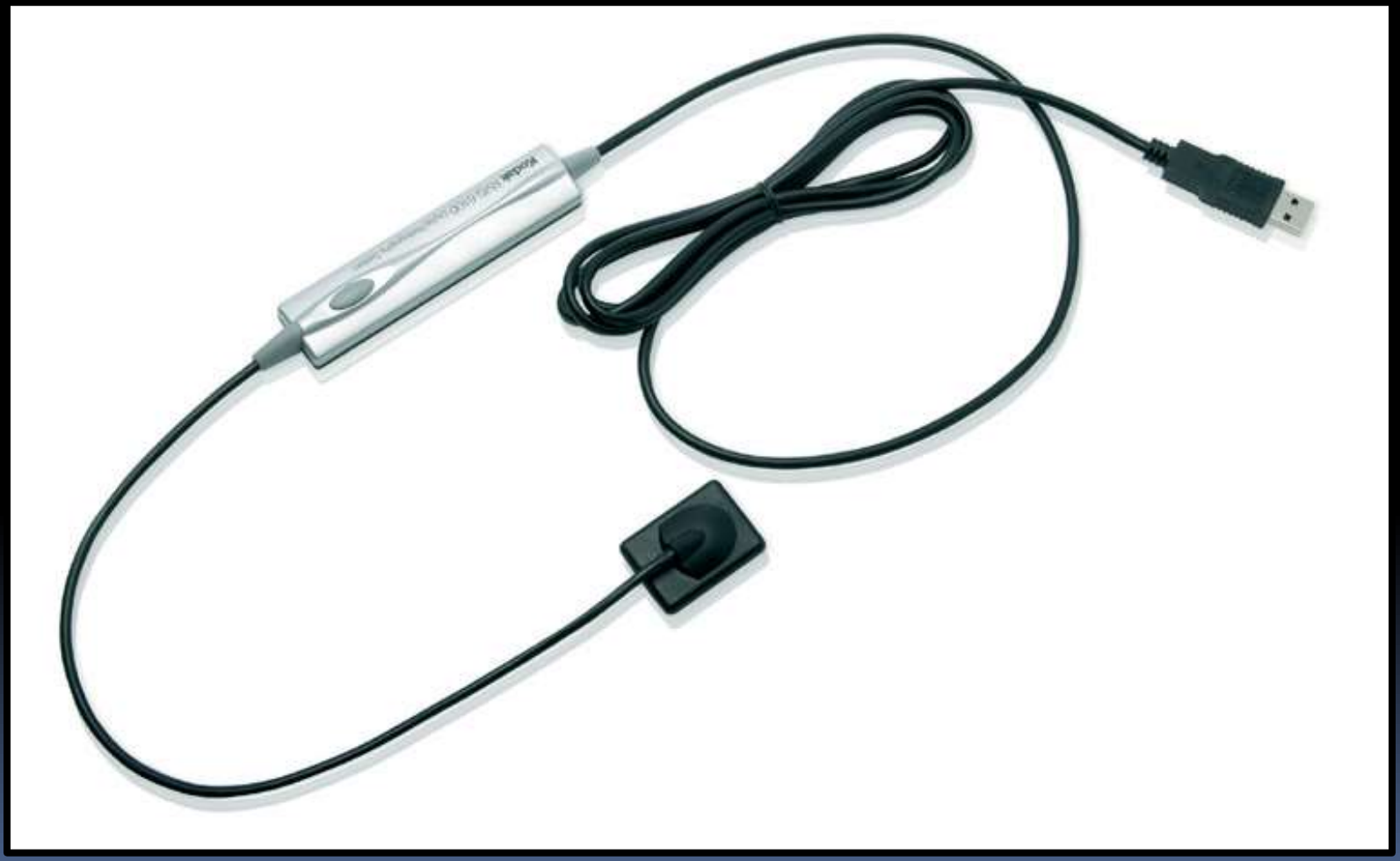
- Variation in projection geometry
- Variation in contrast and density
- Masking by other anatomic structures

Digital Radiography

Advantages:

- Digital storage
- Image enhancement
- Radiation dose reduction

- Two digital radiography systems rely on the sensor - the ***direct*** and ***indirect*** methods
- The ***direct method*** uses a charged coupled device (CCD) sensor linked with a fiber optic or other wire to the computer system – provides real – time imaging
- Disadvantage - limited sensor area

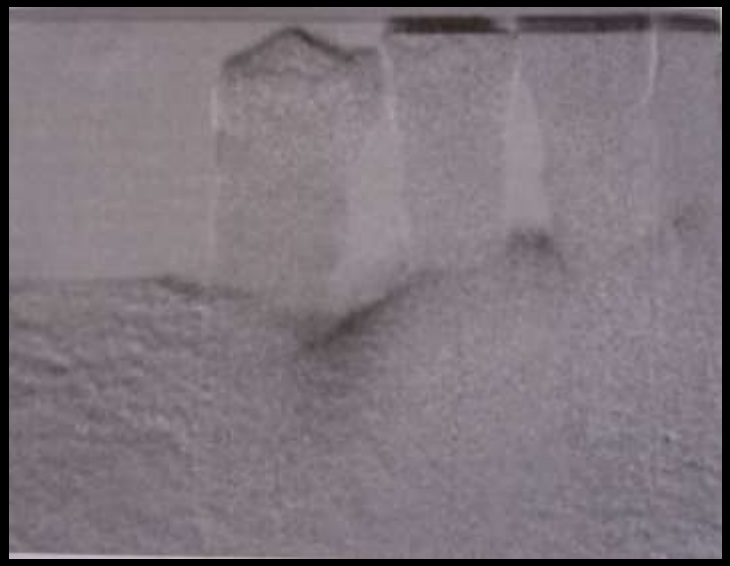


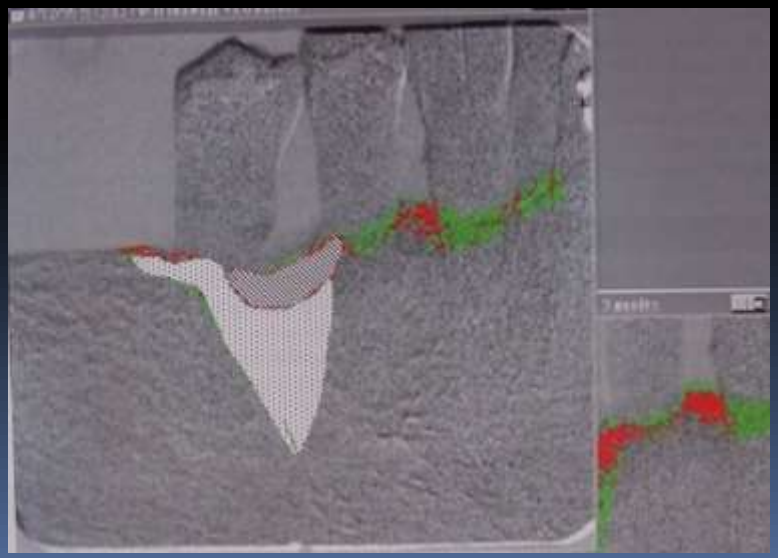
- The ***indirect method (Digora System®)*** - phosphor luminescence plate, which is a flexible film like radiation energy sensor placed intraorally and exposed to conventional x – ray tubes.
- A laser scanner reads the exposed plates offline and reveals digital image data
- Advantage - the plate size and flexibility

Substraction Radiography

- Serial radiographs→ converted to digital images→ superimposed→ composite image– Quantitative changes
- Changes in the density and/or volume of bone can be detected as lighter areas (bone gain) or dark areas (bone loss)
- Computer-assisted subtraction radiography

- Limitations: needs paralleling technique and accurate superimposition
- Recently, new image subtraction methods ***diagnostic subtraction radiography (DSR®)*** have been introduced combining the use of a positioning device during film exposure with specialized software designed for digital image subtraction using conventional personal computers in dental offices





Computer Assisted Densitometric Image Analysis. (CADIA)

Video camera measures the light transmitted through radiograph and the signals from the camera is converted to gray scale image.

Advantage:

→Measures quantitative changes in bone density overtime.

→Higher sensitivity, reproducibility and accuracy as compared to DSR.

Computed Tomography (CT)

- Advanced – Cone Beam Computed Tomography (CBCT)
- Specialized radiographic technique that allows visualization of planes or slices of interest
- Since CT equipment is expensive and the procedure carries a relatively high radiation burden for the patient - use should be reserved for questions that cannot be answered via clinical examination and / or conventional transmission radiography

Magnetic Resonance Imaging (MRI)

- Does not use ionizing radiation
- To acquire an MRI image the patient is placed in a strong magnetic field
- Unlike conventional radiographic techniques, the hard tissues, bone, and teeth are not strongly imaged
- whereas soft tissue, such as salivary glands, dental pulp, and the disc of the temporomandibular joint, may be easily observed if the correct scanning parameters are used

Nuclear Medicine Bone Scans

- Branch of radiology that uses radiolabeled pharmaceuticals
- For the diagnosis of periodontal disease, bone scans have been used to detect sites of active bone loss. The radiopharmaceutical is a ***technetium*** – labeled disphosphonate called 99m –Tc – methylene diphosphonate

- To perform a bone scan, the radiopharmaceutical is injected intravenously.
- Following a period to allow for bony uptake of the agent, uptake is either imaged using a gamma camera or measured using specially designed detectors for intraoral use.
- Areas of active bone loss appear as ***hot spots*** in the image
- Sensitivity more than 90%

Advances In Microbiologic Analysis

- Since subgingival oral bacteria are the main initiating agents in the development of periodontal disease, it makes sense to look for specific bacteria in the subgingival microflora of patients with disease.
- Subgingival microenvironment has 350+ species
- Only few organisms are thought to be involved with periodontal disease.
- Strong evidence for Aa, Pg, and Tf

Uses of microbiologic analysis

- Support diagnosis.
- Treatment planning
- Indicator for disease activity (absence is a better indicator)

Bacterial culturing

Plaque samples are cultivated under anaerobic conditions using selective and nonselective media.

Advantage:

Relative and Absolute count of the cultured species.

Disadvantage:

- Strict sampling conditions
- Difficulty in culturing most organisms
- Low sensitivity : organisms lesser then 10^3 is difficult to detect
- Time consuming
- Expensive equipment and experienced personnel

Direct Microscopy

- Darkfield or phase contrast microscopy
- Most of the periodontal pathogens are nonmotile so it is difficult to identify

Immunodiagnostic Methods

Immunological assays employ antibodies that recognize specific bacterial antigens to detect target microorganisms.

1. Direct and indirect immunofluorescent microscopy assays (IFA)
2. Flow cytometry
3. Enzyme –linked immunoabsorbent assay (ELISA)
4. Membrane assay
5. Latex agglutination

Immunofloresence Assay (IFA):

- **Direct IFA:** AB conjugated with Fluorescein marker + Bacteria (Antigen) = Fluoroscent Immuno complex
- **Indirect IFA:** Primary AB + Bacteria (Antigen) = Immune Complex+ Secondary Fluorescein conjugated AB
- IFA has been used mainly to detect A. actinomycetemocomitans and P. gingivalis

Cytofluorography or flow cytometry

- Rapid identification of oral bacteria

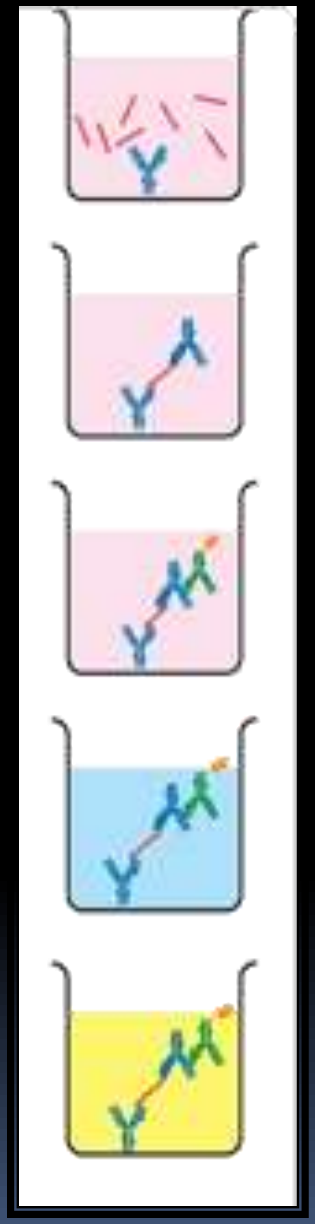
Bacterial cells (Plaque)+ species specific AB + Secondary FL Conjugated AB→ Introduced in flowcytometer

Bacterial cells is separated into single cell suspension-→ passes through the tube→ Cells identified by lasers.

ELISA= Enzyme Linked Immunosorbent Assay

- Similar AB and Antigen reaction, but the fluorescence is read using a photometer.
- The intensity of the color depends on the concentration of the antigen


250 ml
MicroGeneSys, Inc.
Stopping



→ Well with precoated antibody + Sample to be tested= immune complex

→Specific antigen bind to the antibody + Secondary antibody added.

→Immunofloresence dye bound to secondary antibody

→ Substrate added which changes the color of the solution

→Amount of florescence checked by photometer (450nm)


CLASSIC

Latex Agglutination Test

Latex beads coated with species specific AB→ when beads come in contact with specific species in sample they bind and agglutination occurs→ clumping of beads is visible→ test positive.

Advantages:

Simple and Rapid testing

Higher sensitivity and specificity

Membrane immunoassay

- Marketed as ***(Evalusite®)***
- It involves linkage between the antigen and a membrane – bound antibody to form an immunocomplex that is later revealed through a colorimetric reaction.
- Evalusite - designed to detect A. actinomycetemcomitans, P. gingivalis, and P. intermedia
- Disadv: Cross reactivity leading to detection of false positives

Enzymatic Methods

- *T. forsythus*, *P. gingivalis*, *T. denticola*, and *Capnocytophaga* species share a common enzymatic profile, since all have in common a ***trypsin-like enzyme.***
- The activity of this enzyme can be measured with the hydrolysis of the colorless substrate N – benzoyl – 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 is added to the solution.

- Diagnostic Kit – ***Perioscan***
- *Loesche et al* showed that shallow pockets exhibited only 10% positive BANA reactions, whereas deep pockets (7 mm) exhibited 80% to 90% positive BANA reactions.

Disadvantage:

→May be positive in clinically healthy site

→Cannot detect disease activity

→Limited organisms detected

→Other pathogens may be present if it's negative

TOPAS I Kit

- TOPAS I (Toxicity prescreening assay) is a chairside ; calorimetric assay designed to detect 2 markers of bacterial infection in gingival crevicular fluid i.e. toxins and proteins.
- Toxins/Proteins + Colourless reagent = Colour

Molecular Biology Techniques

- Basic Principle: Analysis of DNA, RNA and protein
- **Hybridization:** Pairing of complimentary strands of DNA to produce a double stranded DNA structure
- **Nucleic acid probe:** is a known DNA/RNA probe
- To prepare the probe, specific pathogens used as marker organisms are lysed to remove their DNA.
- Their double helix is denatured, creating single strands that are labeled with a radioactive isotope or enzyme for detection when placed in a plaque sample.

- The assay can rapidly test for multiple bacteria, including *A.actinomycetemcomitans*, *P. gingivalis*, *B. intermedius*, *C. rectus*, *E. corrodens*, *F. nucleatum*, and *T. denticola* in multiple clinical plaque samples.

Two types of DNA probes

- **Whole genomic** - Targets the whole DNA strand - Lower sensitivity and specificity
- **Oligonucleotide probes** - targets variable region of 16sRNA or a specific sequence in the DNA strand - Higher sensitivity and specificity

Checkerboard DNA-DNA Hybridization Technology:

- Developed by *Socransky et.al*
- Large number of samples can be tested and upto 40 oral species detected with a single test

Advantages of DNA probes

1. the sensitivity and specificity are not affected by the presence of unrelated bacteria in mixed culture samples
2. Able to detect as few as 10^2 to 10^4 bacteria
3. Multiple species detected with a single test
4. Does not require viable bacteria
5. Large number of samples can be assessed.

Disadvantage

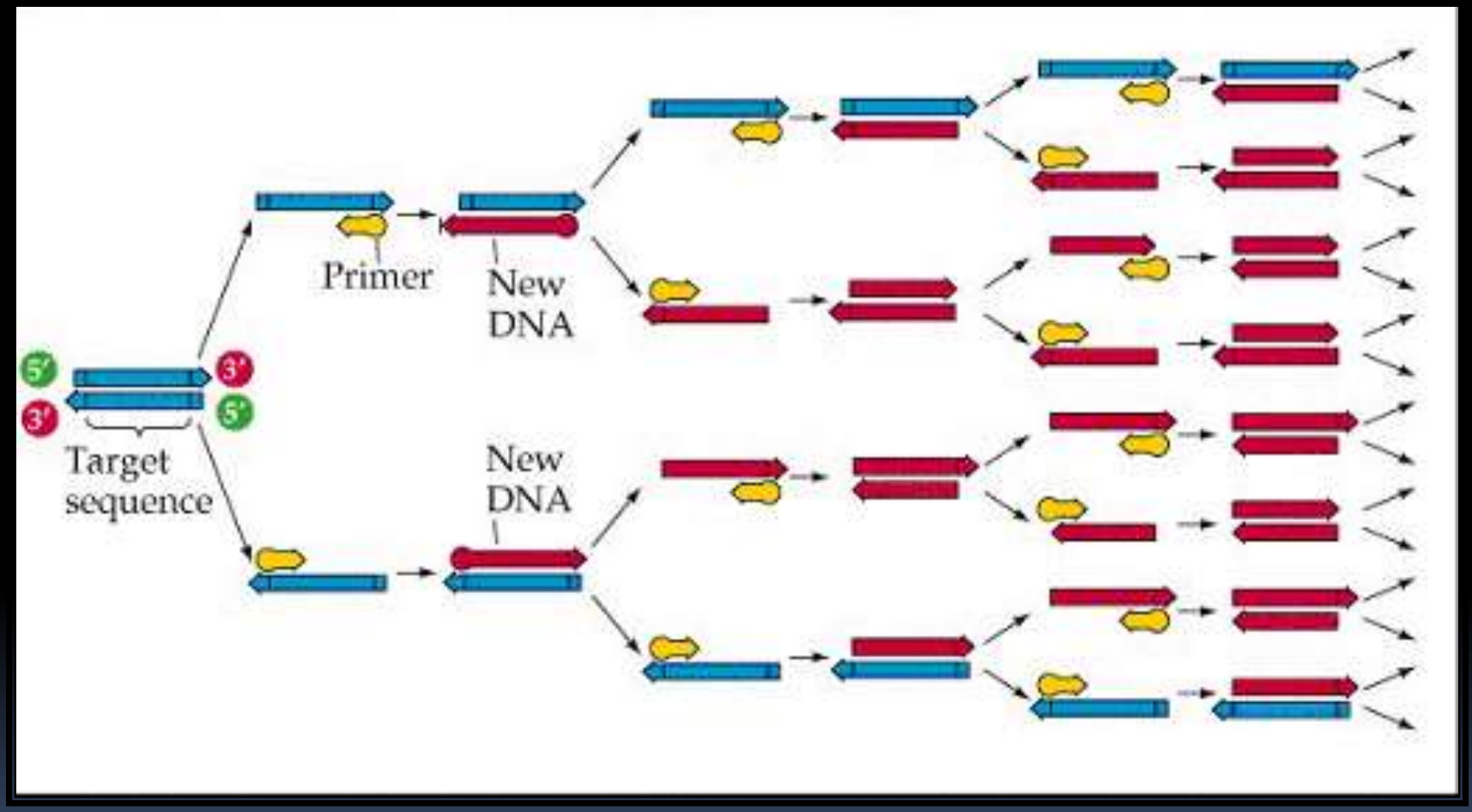
1. Expensive
2. Expert personnel to carry out the test
3. Not easily available

Restriction Endonuclease Analysis

- Restriction endonucleases recognize and cleave double-stranded DNA at specific base pair sequences.
- The DNA fragments generated are separated by electrophoresis, stained with ethidium bromide, and visualized with ultraviolet light.
- These DNA fragment patterns constitute a specific ***"fingerprint"*** to characterize each strain.
- Powerful tool for determining a specific pathogenic strain

Polymerase Chain Reaction (PCR):

- Involves amplification of a region of DNA by a primer specific to the target species.
- If there is amplification then it indicates the presence of the target species in the sample.


5'
3'
3'
5'
Target sequence
Primer
New DNA
New DNA

Advantages:

1. High detection limit. As less as 5- 10 cells can be amplified and detected.
2. Less cross reactivity under optimal conditions
3. Many species can be detected simultaneously

Disadvantage:

1. Small quantity needed for reaction may not contain the necessary target DNA
2. Plaque may contain enzymes which may inhibit these reactions.

Advances in Characterizing Host Response

- Assessment of host response by studying mediators as a response to specific bacteria or local release of inflammatory mediators or enzymes as response to infection.
- Source of samples may be; GCF, Saliva, or Blood.

GCF

- Most well studied - host-derived enzymes, tissue breakdown products, and inflammatory mediators.
- Collected with paper strips, micro capillary tubes, micropipettes, microsyringes, plastic strips.
- Paper strips commonly used and volume is measured using various methods e.g.Periotron.

Periotron 8ooo

Perio Paper Strips

Saliva

- Next most commonly used after GCF
- Easily collected
- Contain both local and systemic derived markers for periodontal disease
- Collected from parotid, sub-mand or sub lingual or as ‘Whole saliva’
- Whole saliva contains secretions of major and minor salivary glands, desaqumated cells, and GCF

Cytokines:

- Are substances released by cells of the immune system
- Cytokines in GCF are: TNF-alpha, IL-1, IL-6, and IL-8
- Have actions on immune cells and release of enzymes, including bone resorption.
- Can be used to determine the disease activity

Host derived enzymes:

- Aspartate aminotrasnferase(AST),Alkaline phosphatase, B-glucoronidase, elastase, cathespins, and MMPs
- Aspartate aminotrasnferase(AST) : derived from dead cells
- Elevated in periodontal disease
- ***Periogard*** is a commercially available colorimetric test
- Cannot differentiate active and inactive sites

- Alkaline Phosphatase: released from osteoblast, neutrophils, fibroblast. Levels increases in disease
- β-Glucuronidase and Elastase: found in Neutophils.
- β-Glucuronidase, Elastase, Neutral protease, and cathepsins - all shown to be higher in diseased sites. May be used to predict severity of disease or to predict disease activity.

- A rapid chairside test kit ***(Periocheck®)*** has been developed to detect neutral proteases in GCF.
- ***Prognostik (Dentsply)*** - This system detects the presence of the serine proteinase, elastase, in GCF samples.

- **Matrix metalloprotienases**

- **Halimeter**
- **Diamond Probe**

THANK YOU