*Advanced Diagnostic Aids

Dr Parichaya batra

*Contents

- Introduction
- General purposes of diagnostic procedures
- Conventional periodontal diagnosis- limitations
- Advances in clinical diagnosis
  - Periodontal probes
  - Gingival bleeding
  - Gingival temperature
  - Tooth mobility
- Other diagnostic techniques
- Limitations of conventional radiographs
- Advances in radiographic techniques

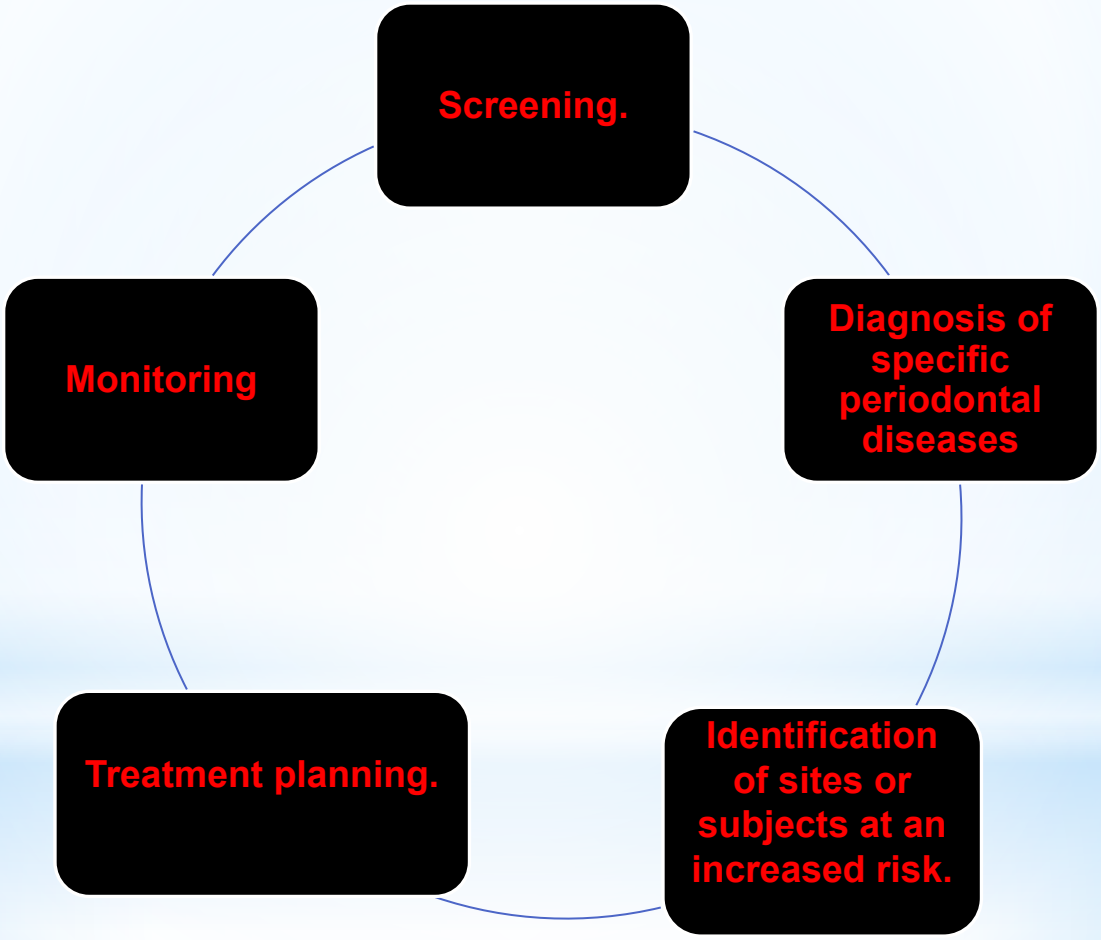
- Advanced microbiological diagnostic aids
- Enzymatic methods
- New molecular technologies-DNA probes & PCR
- Advances in genetic assessment
- Advances in characterising the host response
- Saliva & GCF as a source of biomarkers
- Various potential markers of host response
  - Inflammatory mediators and products
  - Host-derived enzymes
  - Tissue-breakdown products
- Conclusion
- Bibliography

*Introduction

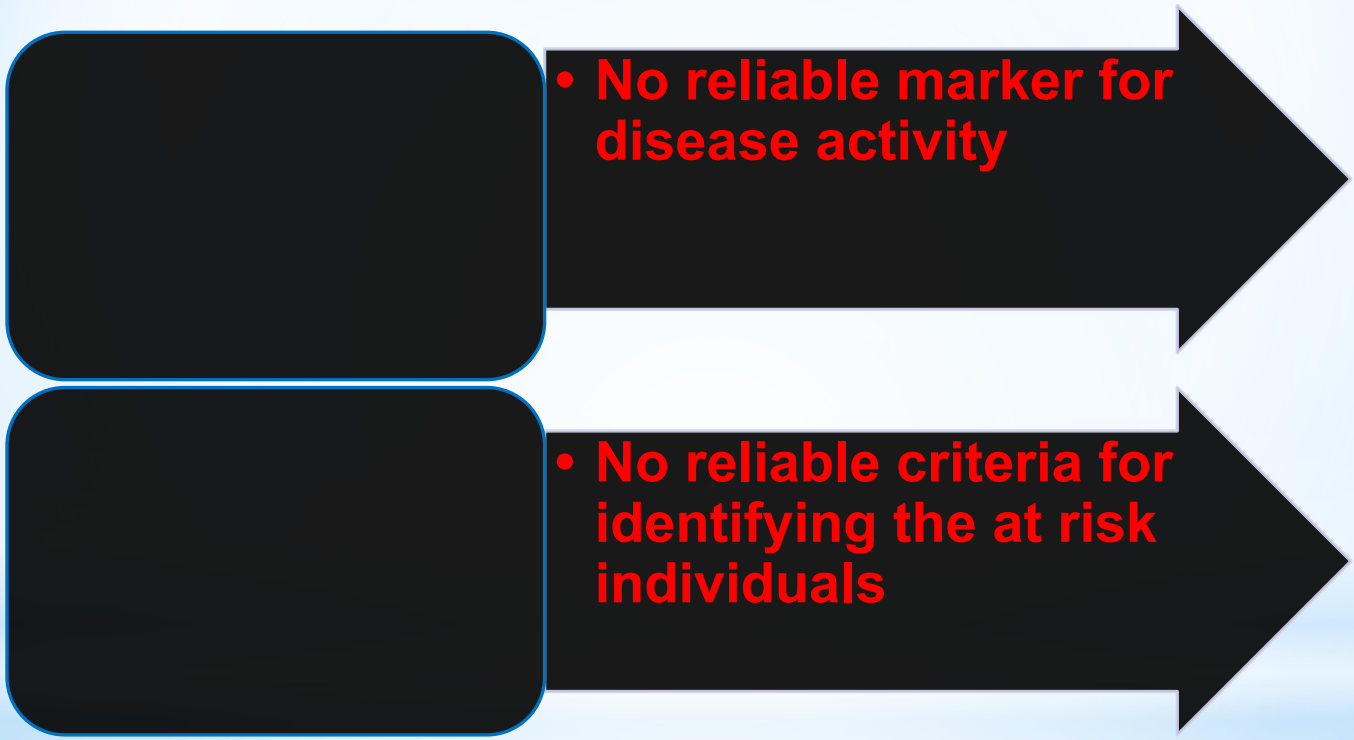
* Diagnosis of a disease is mainly based on the etiology, pathogenesis and the clinical symptoms associated with the specific condition (as in case of mono infecitous conditions like tuberculosis) but it is not so with the periodontal diseases because of the multifactorial etiology and polymicrobial nature of periodontal infections.

* As we know that the correct diagnosis of the disease forms the strong foundation for accurate treatment and favorable patient outcome it is our duty to consider and choose a proper diagnostic aid that are useful for particular patient.

* General Purposes Of Periodontal Diagnostic Procedures



* At present we are handicapped in making precise diagnosis & prognosis by 2 important limitations:



*Traditional Diagnostic Procedures

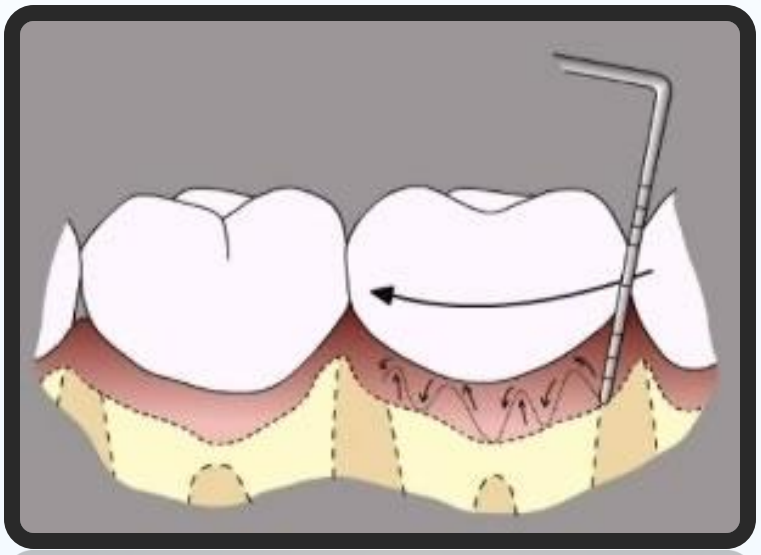
- suffer from a number of drawbacks such as:

  - Not precisely accurate
  - Provide only retrospective information
  - Not reproducible
  - Cannot reliably identify sites with ongoing destruction.

Therefore not entirely suitable for monitoring the progression of periodontal disease.

* Advances In Clinical Diagnosis

*Periodontal Probing



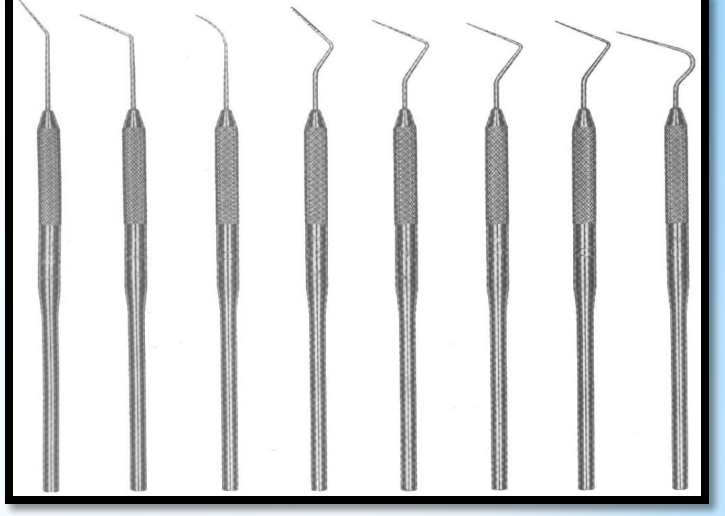
- G.V Black- First to describe use of the periodontal probe to explore periodontal pockets.

* Generations Of Probes

Philstrom [1992]

- ✔Gen I: Conventional probes.
- ✔Gen II: Pressure sensitive.
- ✔Gen III: Computerized.
- ✔Gen IV: Aim at recording sequential probing positions along the gingival sulcus.
- ✔Gen V: Ultrasonic device attached to the 4th generation probe.

*Gen I:Manual Probes



Diadvantages:

- Errors in manual recording
- Pain provoked by probing
- Variability in probing force, diameter
- Lack of stable reference point

* Criteria Defining Conventional Probes [NIDCR]

Precision =1 mm

Range =12 mm

Probing force = Not standardized

Applicability = Non Invasive. Easy to use

Reach = Easy to access any location around all teeth

Angulation = Subjective

Security = Simple stainless steel instrument-Easily sterilizable

Read out = On voice dictation

Recording = In writing

*Criteria For Automated Probes

Precision = 0. 1 millimeter
Range = 10 millimeter
Probing force = Constant & standardized
Applicability = Non Invasive, but easy to use
Reach = Easy to access any location
Angulations' = Guidance system for proper angulations
Security = Complete sterilization of all portions entering the mouth
Read out = Digital
Recorded = Digital

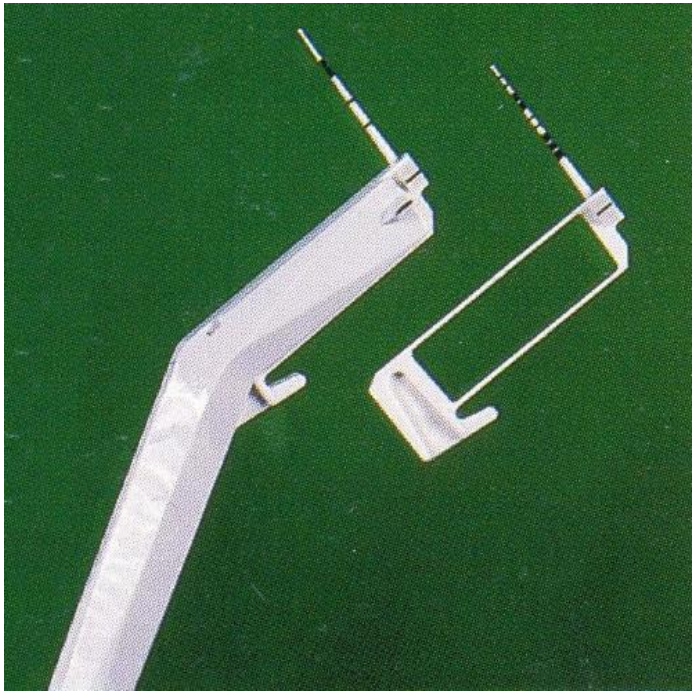
*Gen II: Pressure Sensitive Probes

- These are introduced by Gabathuler & Hassel in 1971.-probe with piezoelectric pressure sensor

a pressure sensitive probe holder to standardize the insertion forces is designed.

- At force of 25pounds is used .

EG: Vine valley probe
Hunters probe



- ✔ Neither intra or inter examiner variability improved with a controlled force of 0.75N.
- ✔ No difference in reproducibility between a controlled force probe & a manual probe in shallow or deep pockets.

Thus,

"The failure of constant force probes to dramatically improve exact reproducibility is a clear indication that sources of error other than probing force variation are involved."

ADVANTAGES:

The pressure sensitive probe yield more reproducible probing depth measurements than a manual probe.

* Gen III: Automated & Computerized Periodontal Probes

Third generation probes combine -

- ✔ Controlled force,
- ✔ Automated and computerized data recording.

Advantage:

- ✔ Automated data capture,
- ✔ Thus facilitates data entry into patient records & eliminates error in data.

* Florida Probe

- ❑ Introduced by **Gibbs et al in 1989.**
- ✔ Combined advantage of constant probing force
- ✔ with precise electronic measurement
- ✔ computer storage of data
- ✔ and also has a guidance system that ensures reproducible pathway.

Probe tip similar to common Michigan ‘o’ probe with williams markings.

- ❑ Two models: **The stent & disk models**

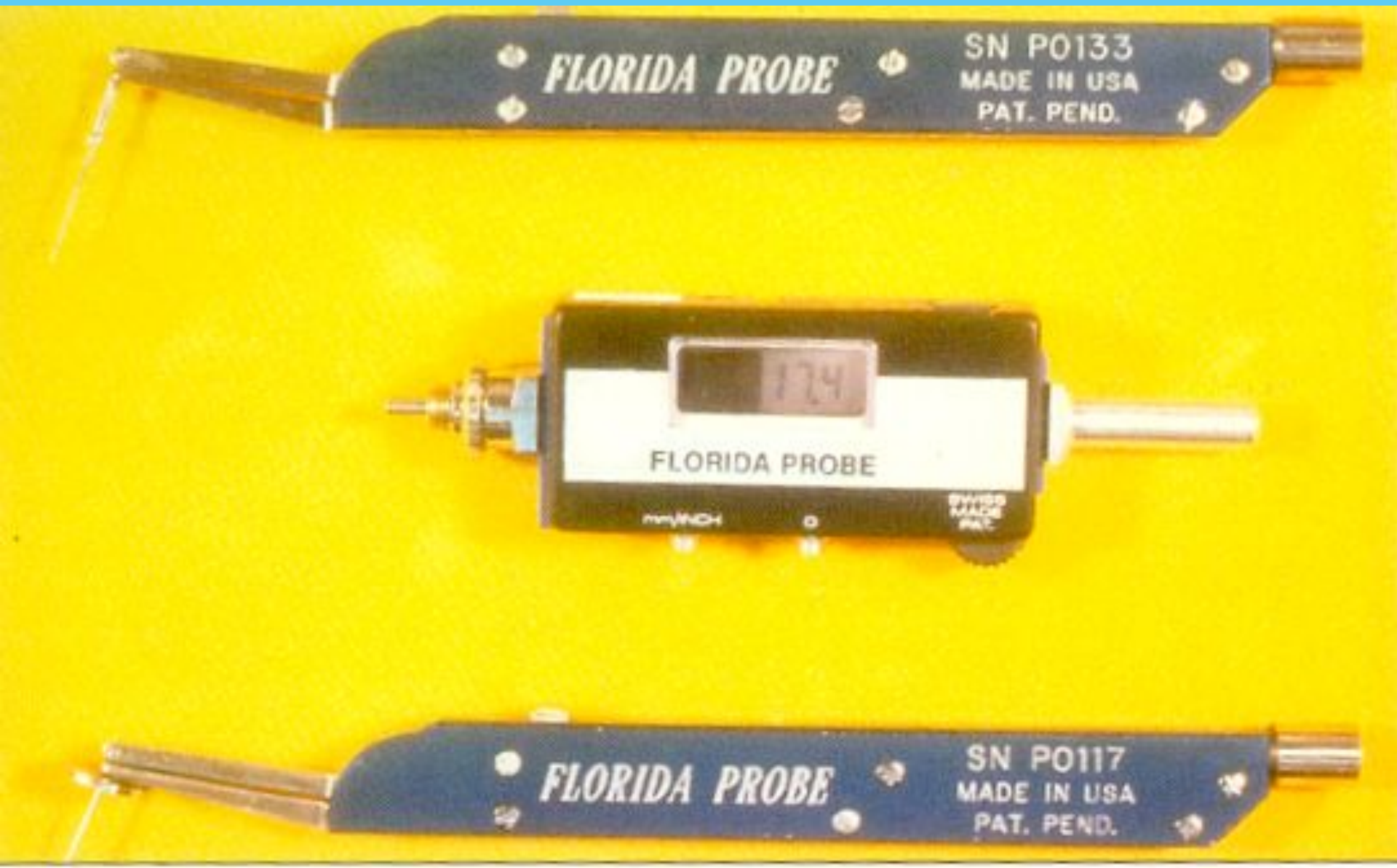
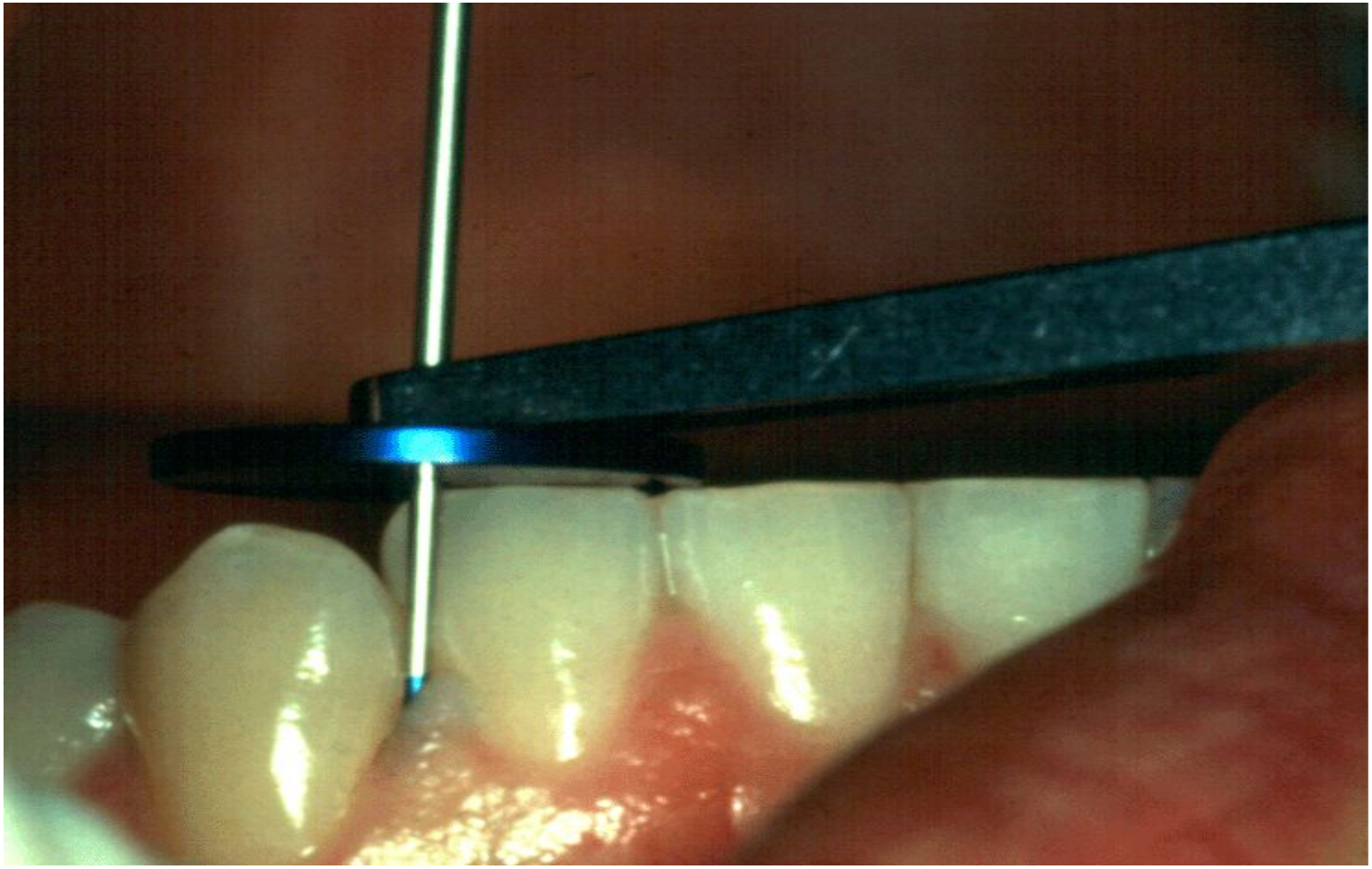
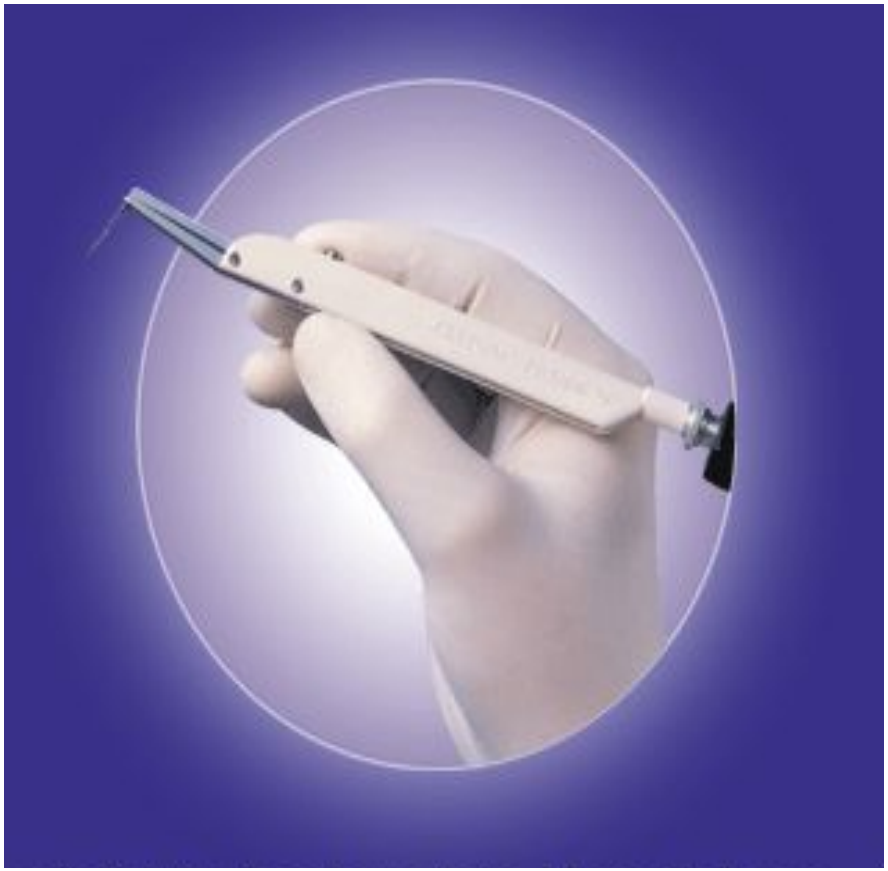


Fig 4-1a The Florida Probe (Florida Probe Co, Gainesville, FL) is an accurate pressure-sensitive device used to measure probing depth. Two models are available; *(top)* the regular probe and *(bottom)* the disc probe. (From

*Stent Model

*Disk Model





Florida Probe with a

Titanium tip for the implant.

Reproducibility is superior to manual probes with SD of 0.21-0.28mm

Advantages

- Light weight autoclavable handpiece.
- New titanium tips for implants
- Standardized probing force-0.2mm resolution
- Override button on hand piece to walk the sulcus

Limitations

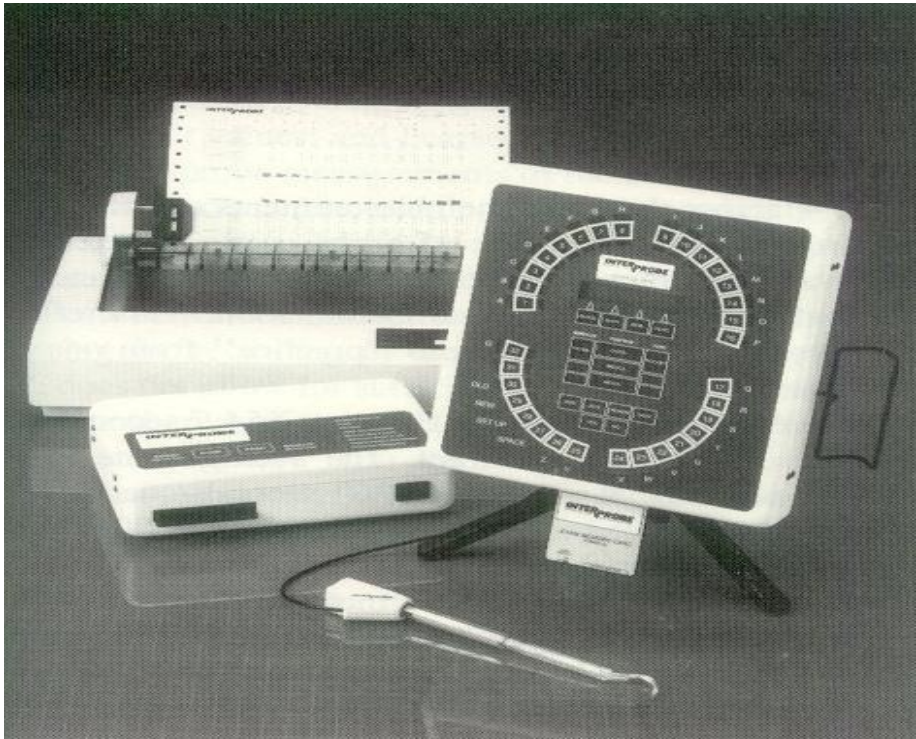
- Lacks tactile sensitivity
- Uses a fixed force setting
- Underestimation of PD and CAL.

Other examples of third generation probe:

Interprobe

Foster miller probe

Toronto automated probe



* Generation V: Ultrasonic Probes

- A non invasive ultrasound technique to detect, image & map the pdl
- One of the key technical obstacles- Designing an ultrasonic probe that would be small enough to be useful, but yet transmit & receive sufficient signal strength.

- Ultrasound gives more information- as secondary echoes are recorded from tissues at various depths.

- This may provide valuable data to aid the clinician in the diagnosis & treatment charting of the disease.

* Advantages

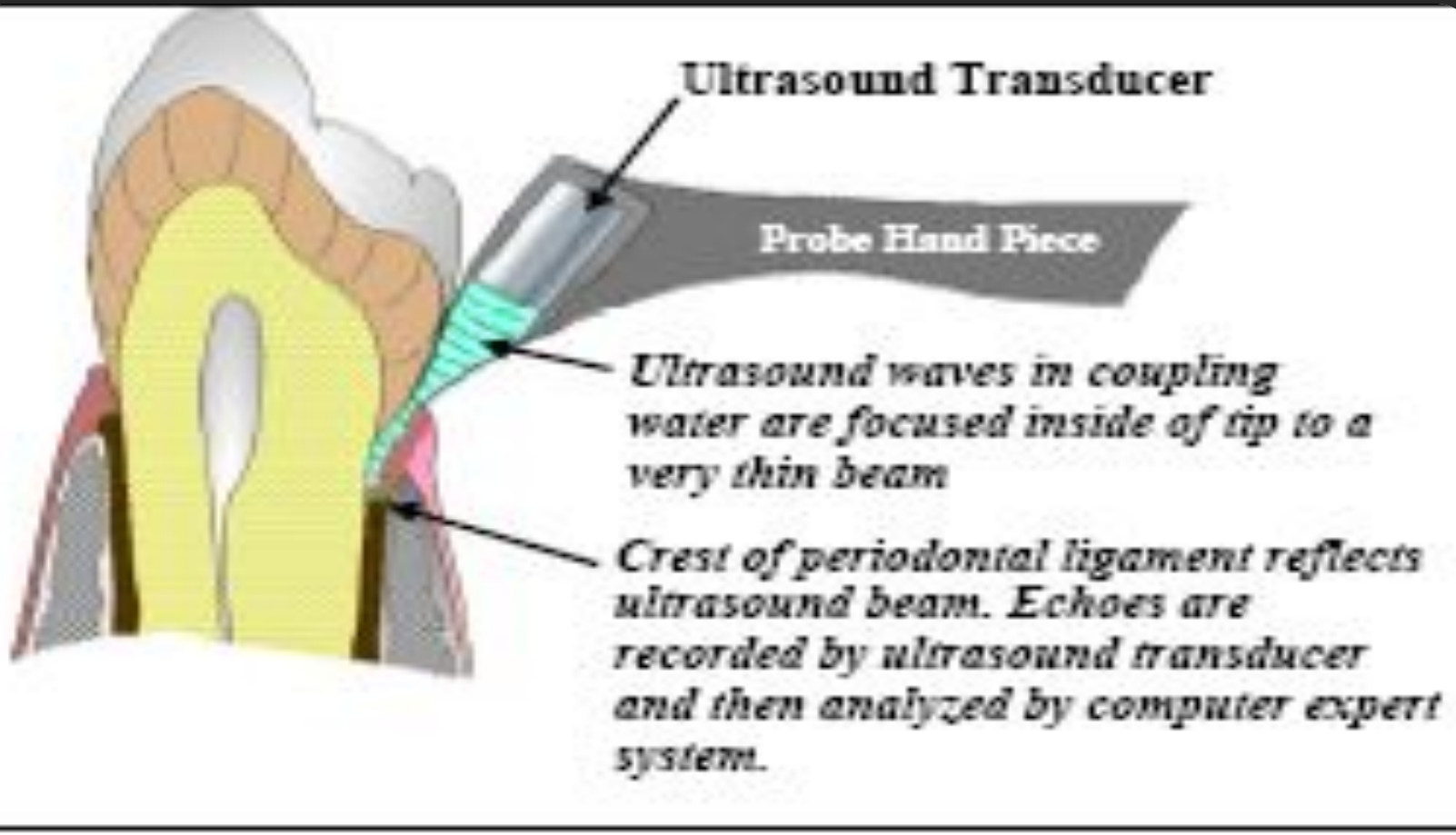


Figure 3. Schematic showing the concept for the US Probe. Ultrasound is projected down between the gum and tooth. The ultrasound reflects from the ligament attachment and returns to the transducer. Analyzing the return signal characterizes the periodontal condition.

*Gingival Bleeding

- Gingival bleeding is a sensitive clinical indicator of early gingival inflammation
- Clinical advantage of being more objective.
- Good indicator of the presence of an inflammatory lesion in the connective tissue

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.

- Limited predictive value for disease progression
- Absence indicates periodontal stability with high probability

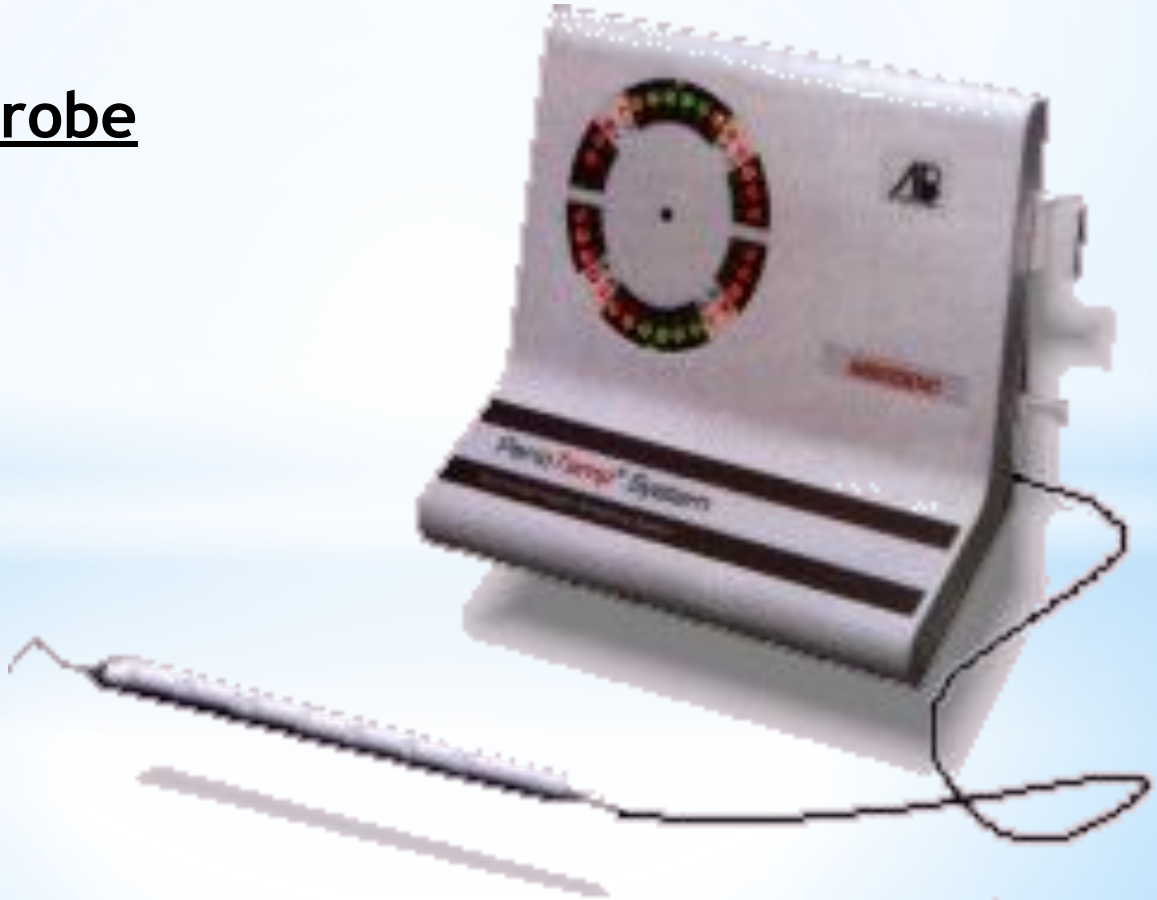
Limitation: Healthy sites may bleed on probing if force is greater than 0.25 N

*Gingival Temperature

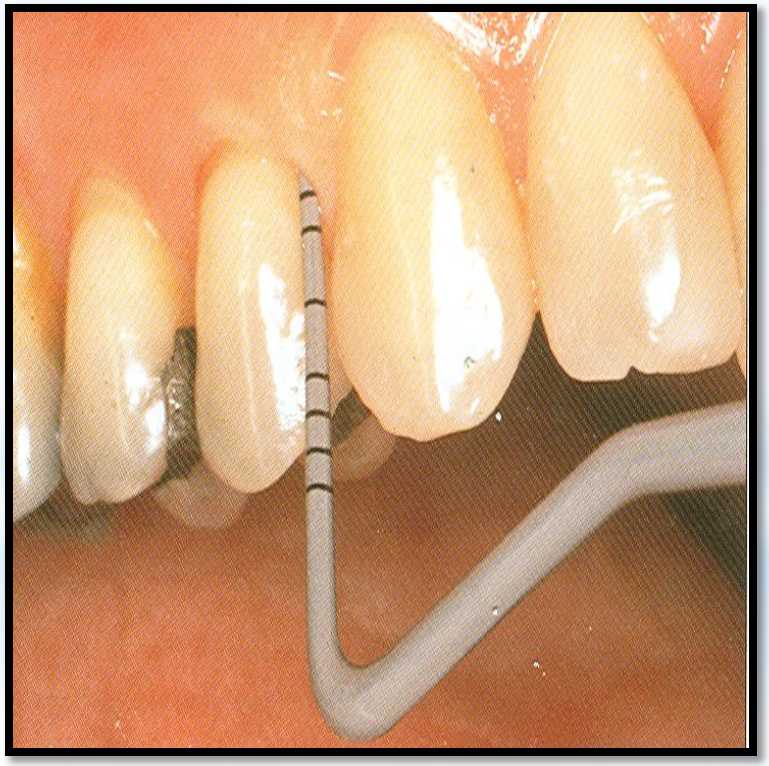
Extensive study to assess subgingival temp is done by:

PERIO TEMP probe

[Abiodent]



Subgingival temperature- Has good specificity but poor Sensitivity when considered alone as a marker for progressive periodontitis.



❑ Rationale for ▫ in temp with ▫ in pocket depth:

- ✔ Endotoxins of infecting bacteria, especially lipopolysaccrides from Gram –ve organisms, exogenous pyrogens, that stimulate macrophages to release endogenous pyrogens, producing fever.

- ✔ Alteration in the cellular and molecular activity of bacteria

*Tooth Mobility

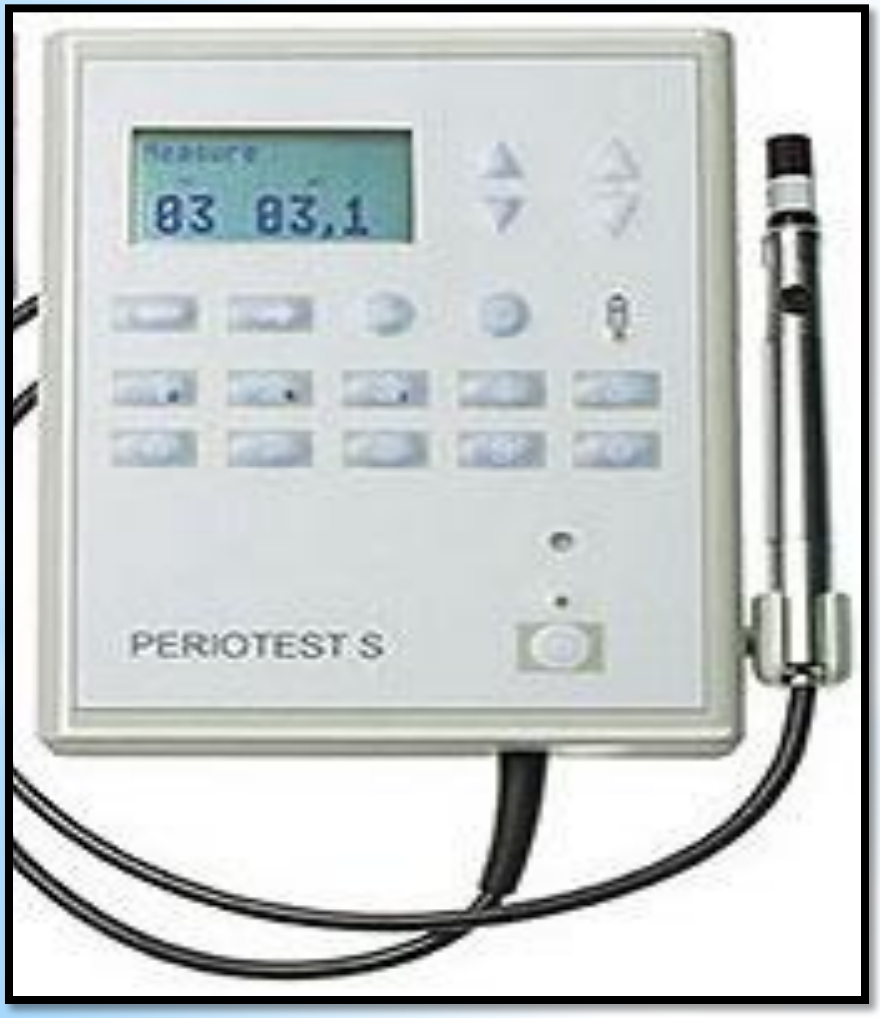
- Tooth mobility is a clinical expression of periodontitis.

Perio test

- It utilizes dynamic forces of short duration of low millisecond range.

- Evaluates the damping characteristics of the tooth.

*Perio Test



Ranges:

-8 to +9 : Clinically firm tooth

10-19 : Palpable mobility

20-29: Visible mobility

30-50 : Mobility in response to lip & tongue movements

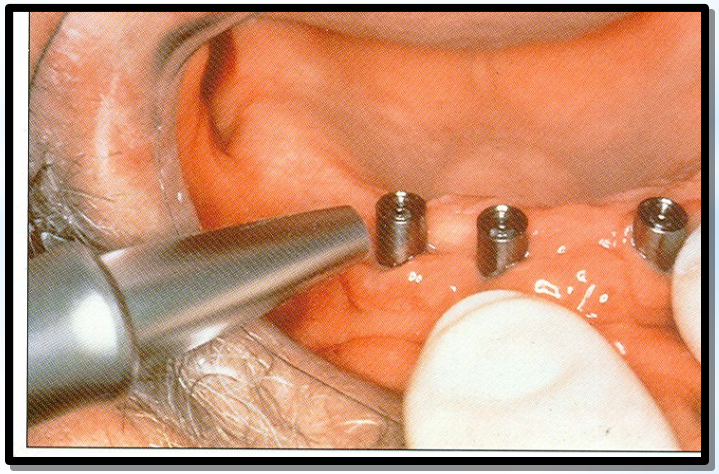
Periotest values for implants

- 08 to -01: Implant is well osseointegrated

00 to +09: Clinical examination is necessary

+10 & higher: Suspicious alarming. Implant is not sufficiently osseointegrated.

Errors- Due to variation in duration, point & mode of application, manner, & time of force applied.



CALCULUS

The Detectar™ system [Ultradent, Salt Lake City] subgingival calculus diagnosis by evaluating the root surfaces

- Light is emitted onto the root surface through a flexible fiber.
- Reflections of the this light are also sensed by the optical fiber & converted into an electrical signal for analysis.
- A computer processing algorithm determines whether the Detectar probe has detected calculus, and activates an auditory & light signal thus notifying the clinician of the presence of calculus **[Felix Krausse Nad Andreus Braun 2004]**

*Detectar ™

Halitosis

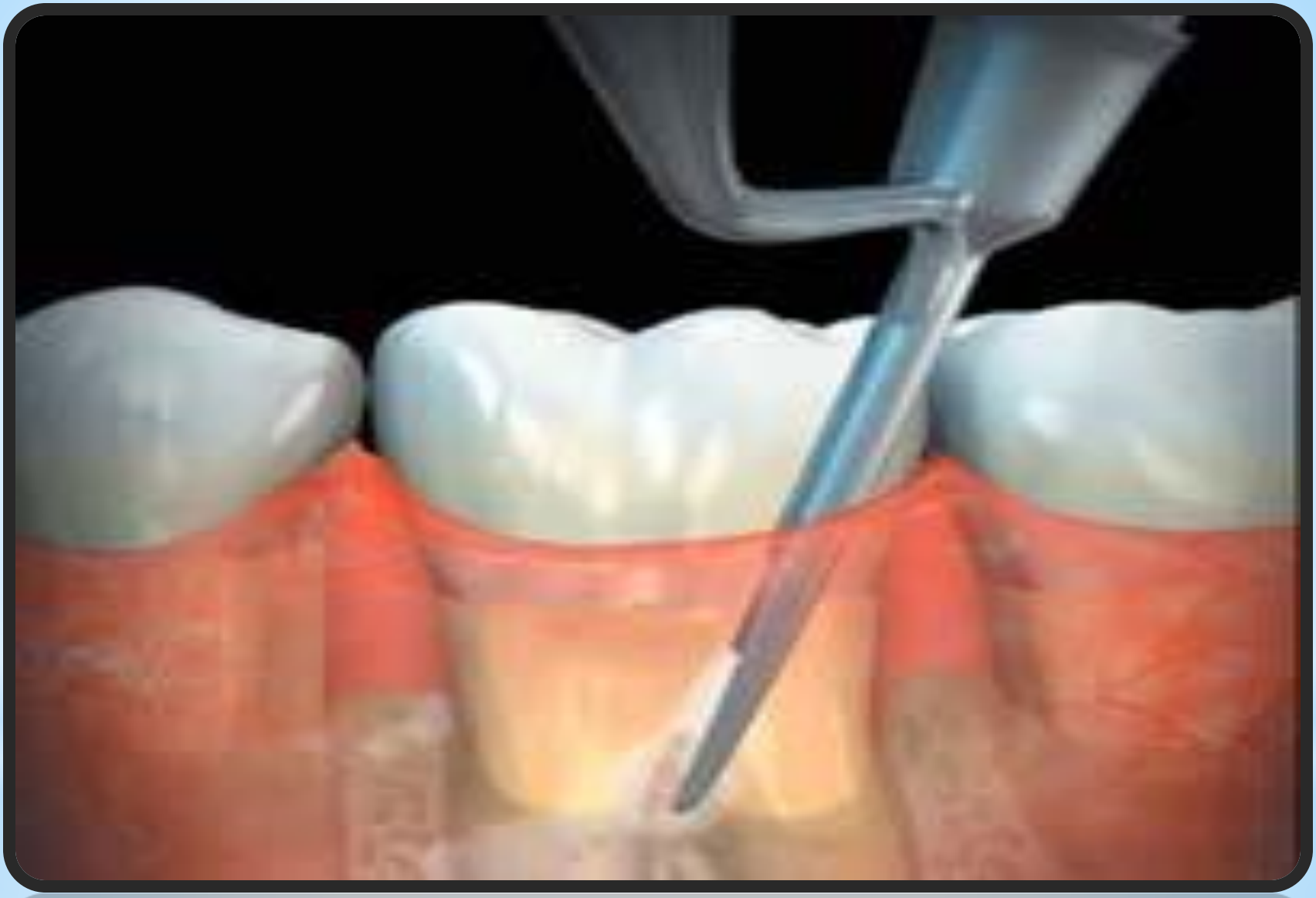
- Its performance lacks specificity in the analysis of the different components of mouth air in comparison to the gold standard gas chromatography.

* Diamond Probe/ Perio 2000 system

It has been designed so that it combines the features of a periodontal probe with the detection of volatile sulphur compounds in the periodontal pocket.

* Dental Endoscope

- It is introduced for use, subgingivally in the diagnosis & treatment of periodontal disease.
- Produced by Dental View, Inc.- called as Perioscopy system.
- It consists of 0.99mm diameter reusable fibroptic endoscope over which is fitted a disposable, sterile sheath.
- The fibroptic endoscope fits on to the periodontal Probes and ultrasonic instruments that have been designed to accept it.



Dental endoscope viewing furcation

Uses:

1. Allows clear visualization of deep subgingival pockets & furcations.

2. Enables operator to detect the presence & location of subgingival deposits and guides the operator in their removal.

3. Possible to achieve levels of root debridement & cleanliness that are much more difficult to produce without it.

* Advances In Radiographic Techniques

* Limitations Of Conventional Radiographs

1. It is a 2 dimensional representation of a 3 dimensional structure.

2. Only interproximal alveolar bone levels can be assessed with some level of certainty.

3. They do not reflect the current disease activity.

4. Sufficient bone should be destroyed to be detected.

5. Radiographs are specific but not sensitive.

6. Misdirection of the central ray of the X-ray beam+ exposure and processing errors further limit accuracy.

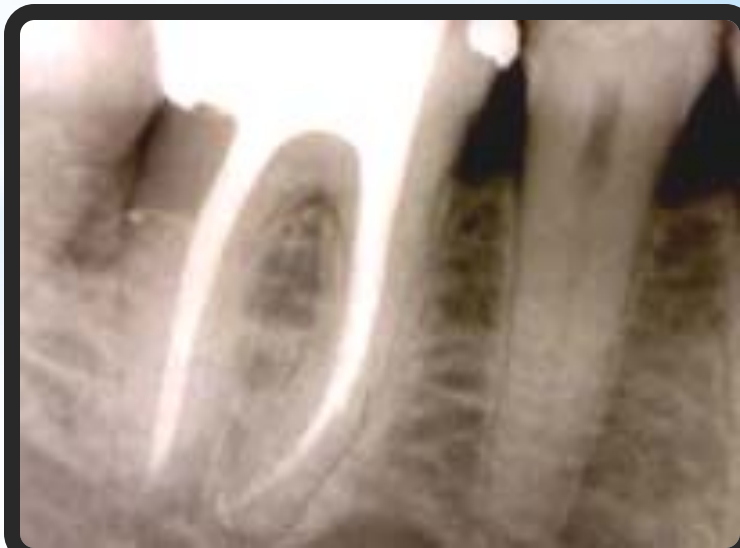
7. Most importantly morphologic or pathologic aspects of the alveolar bone may go undetected as a result of superimposition of teeth and other anatomic structures.

* Radio Visio Graphy [RVG]

- RVG consists of 3 components:
  - Radio Component
  - Detector / Image receptor
  - Graphy component

Advantages:

- Immediate image display
- Ability to manipulate the image
- Patient dose reduction of 60%



* Digital Subtraction Radiography

- 1st introduced to medical literature by Zeidses Des Plantes in 1935.
- Later Grondhal & Grondhal [1983] introduced this technique in Periodontal diagnosis.
- Jeffcoat et al [1992] used to determine periodontal disease

- This technique is very sensitive and it can detect 0.12mm change [Rudolph 1987]

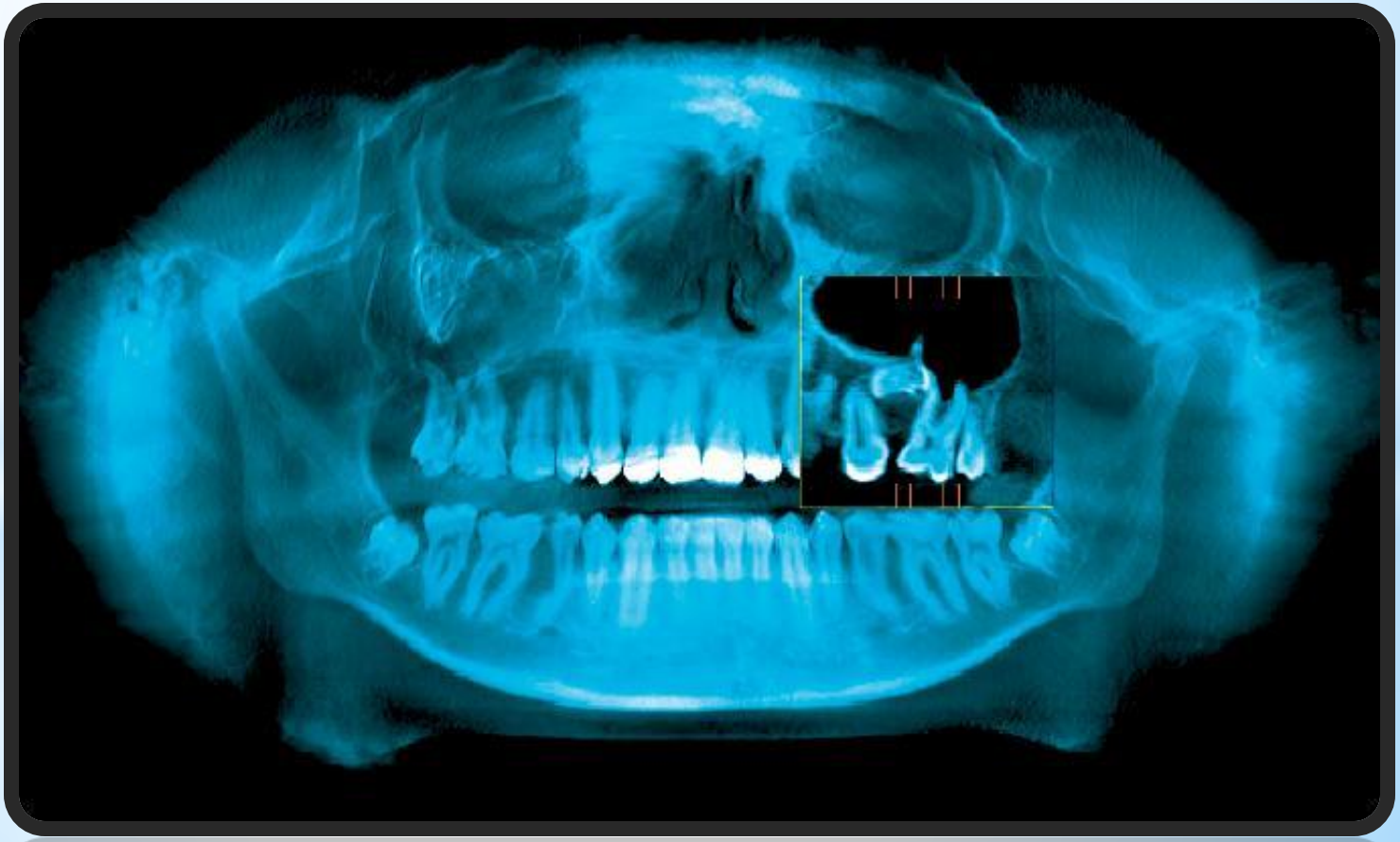
- The image i.e. obtained is an isolated structure that have undergone the change.
- Once the subtracted image is formed, it is electronically contrast enhanced to display the final image.
- Color coding of images:

  Bone gain: Shades of green

  Bone loss: shades of red

* Cone Beam CT

- Cone Beam CT like the conventional units, can also be used to generate 3-D CT images at a much lower radiation dose.



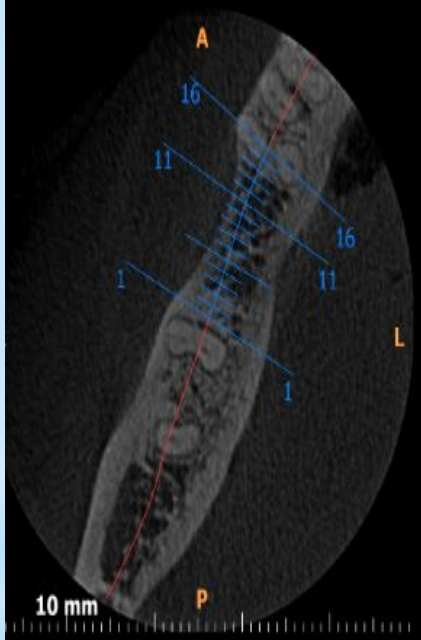
- The simplified design of the Cone Beam CT unit also allows for considerable cost saving as compared to medical CT units.

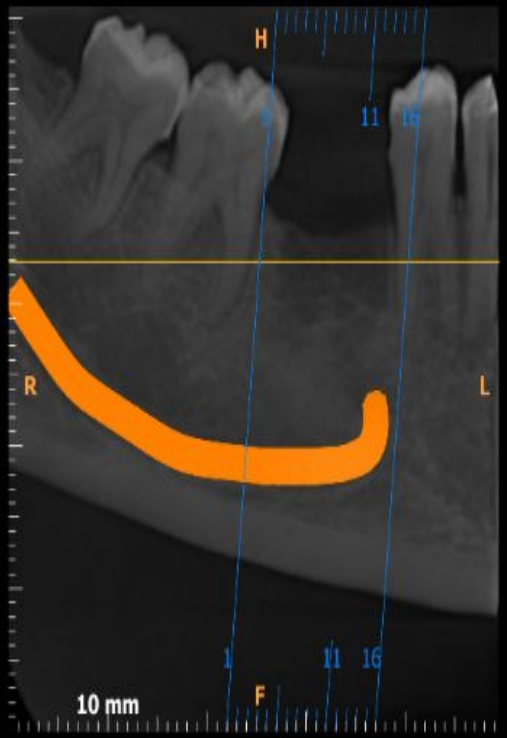
- Disadvantages: Is the increased effect of scattered radiation on the imaging quality.

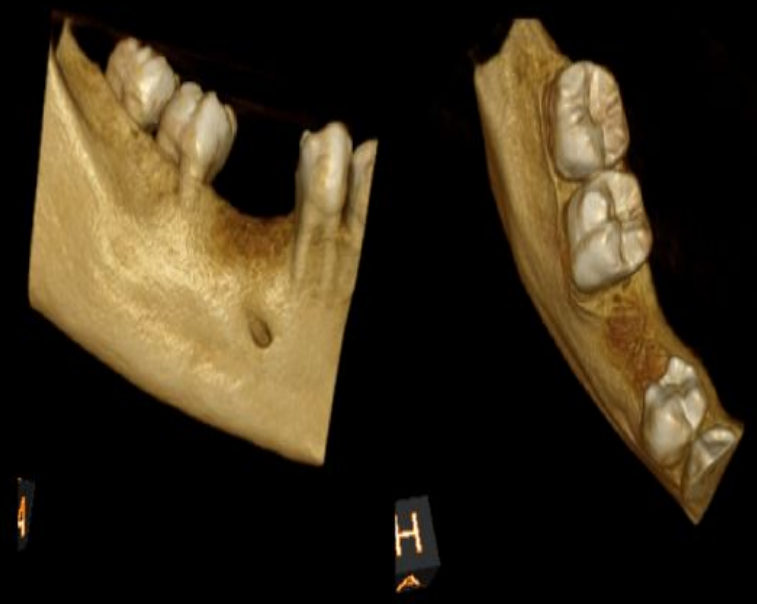
MAGNUS DIAGNOSTIC CENTRE 1

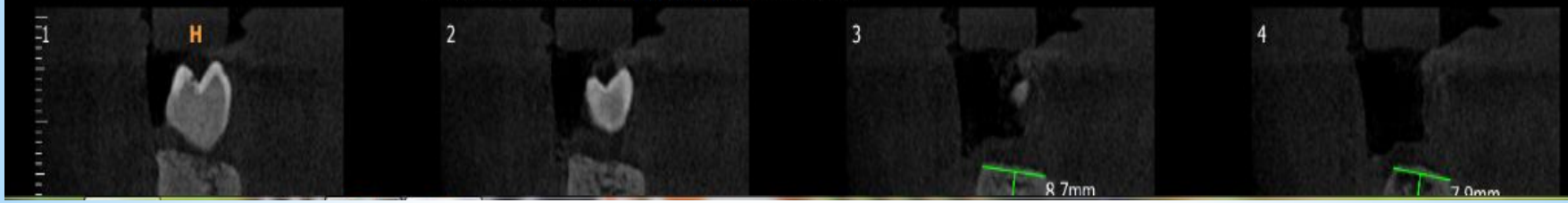
4TH BLOCK KORAMANGALA

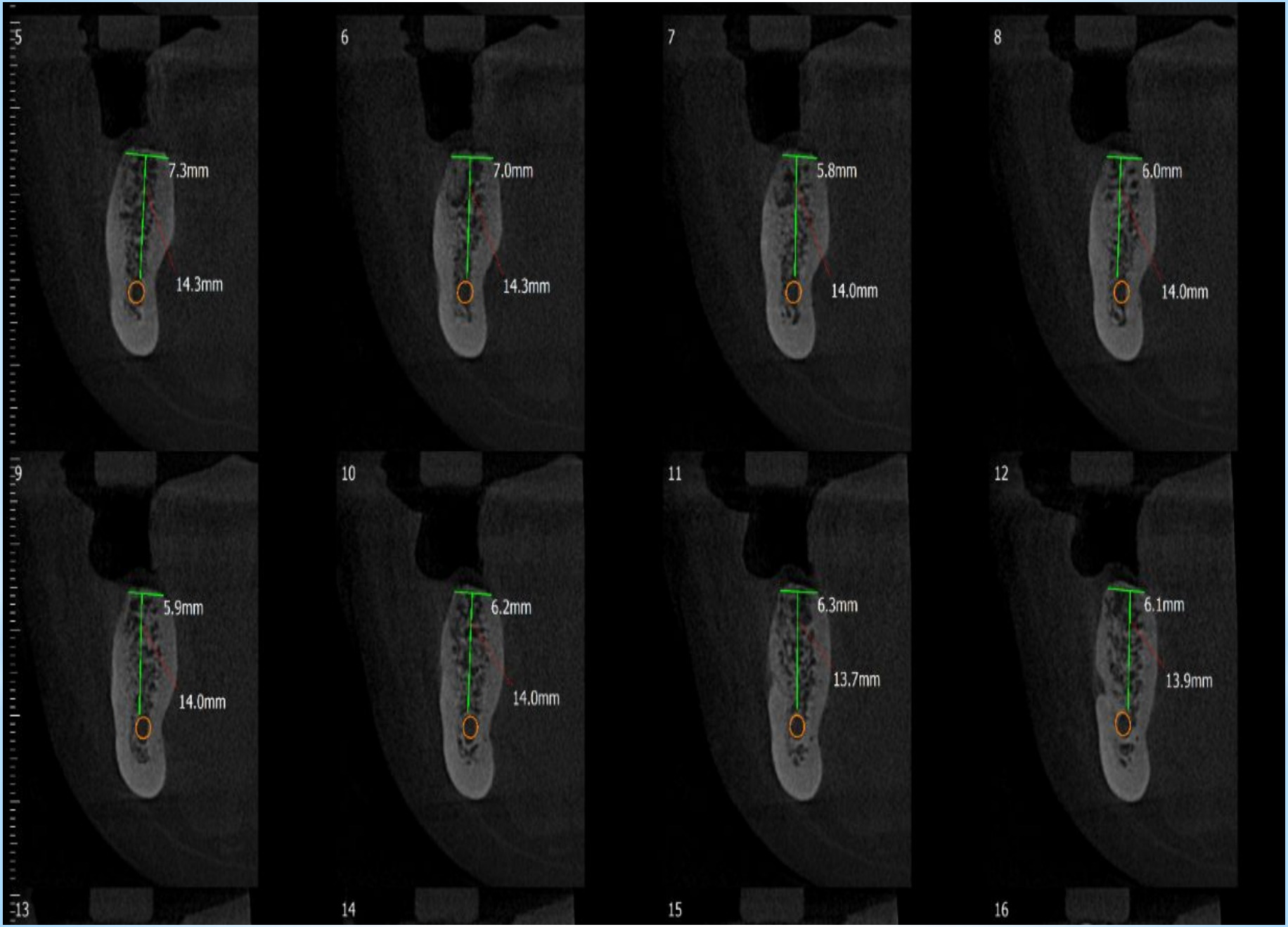
BANGALORE - 560034

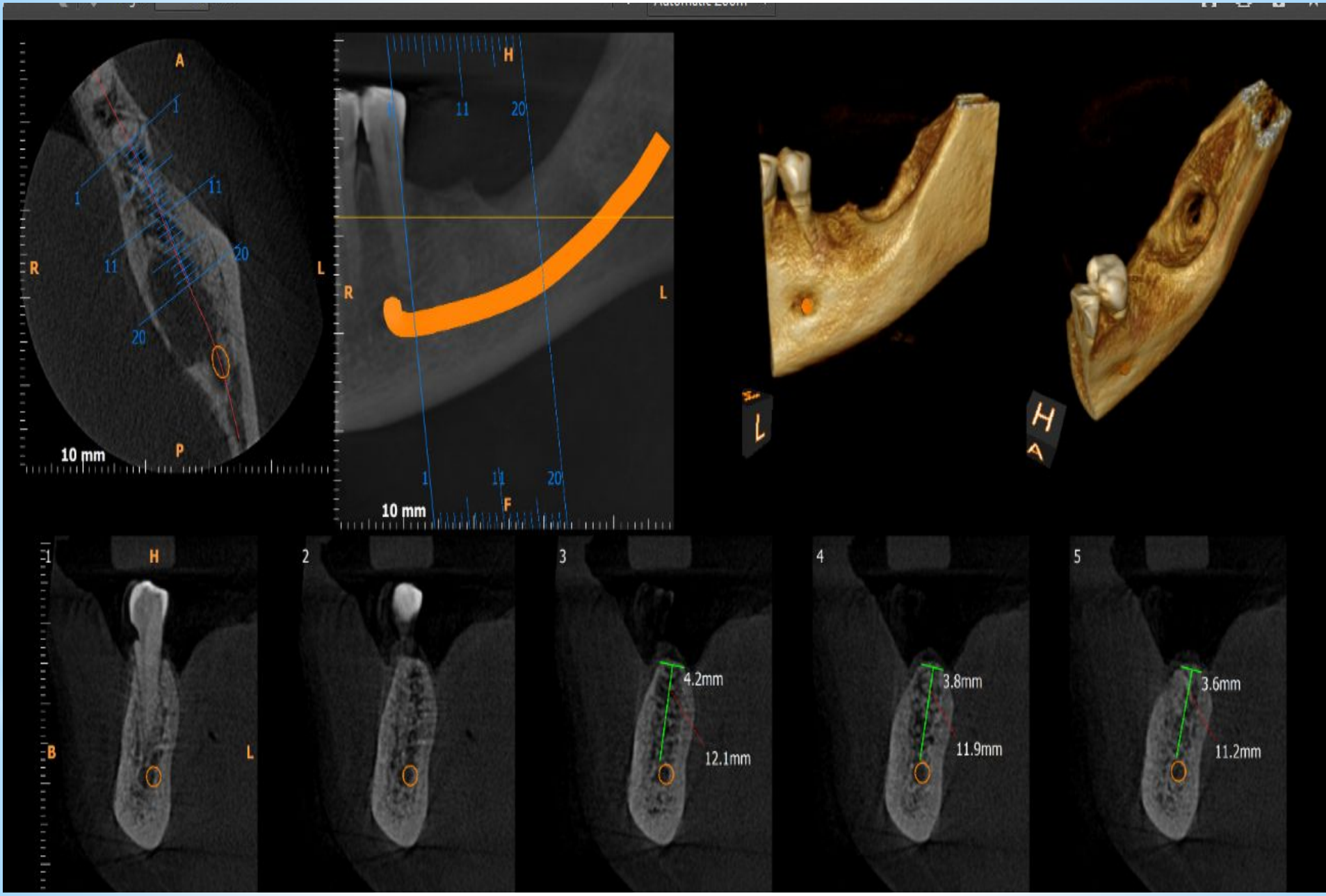


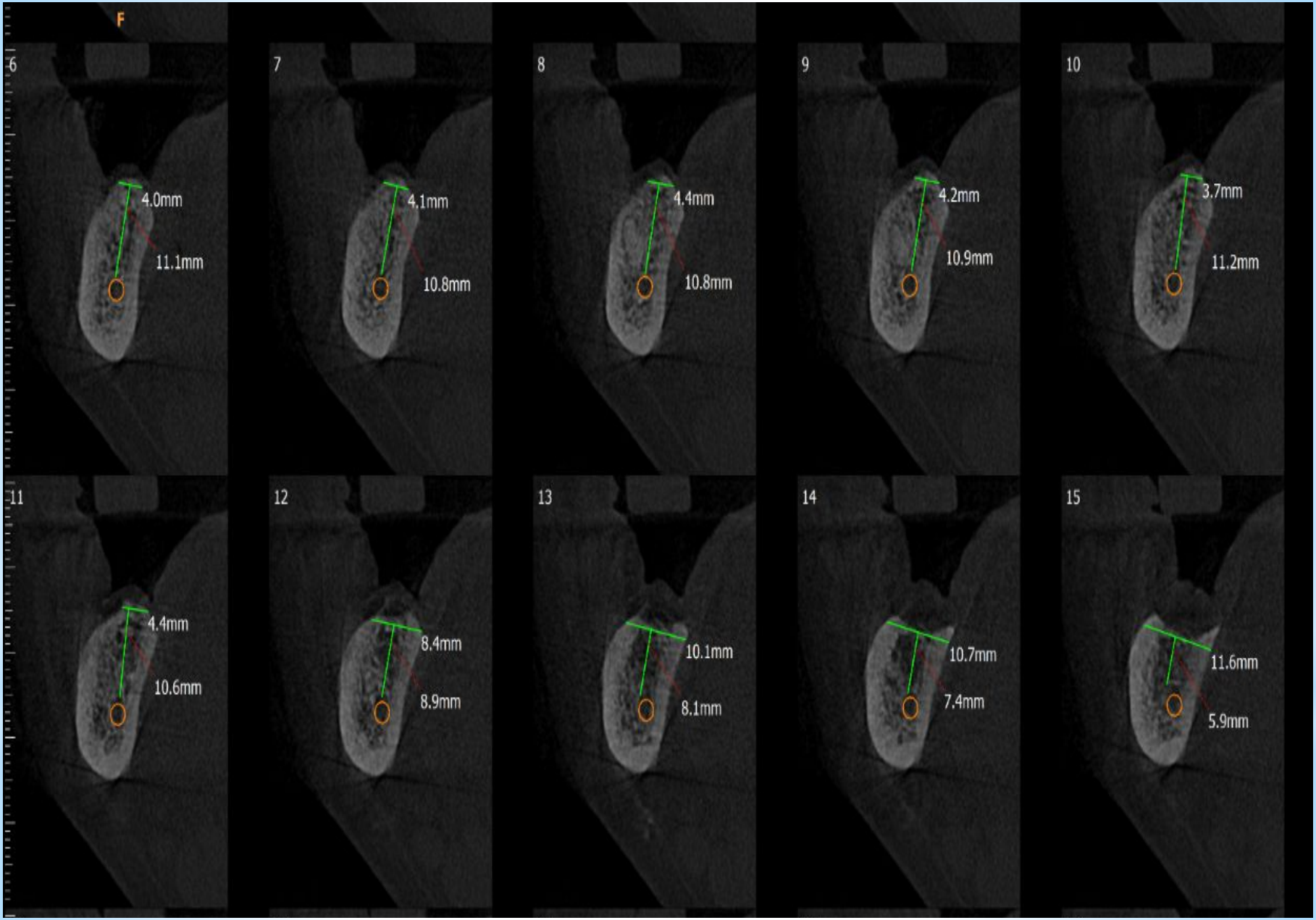


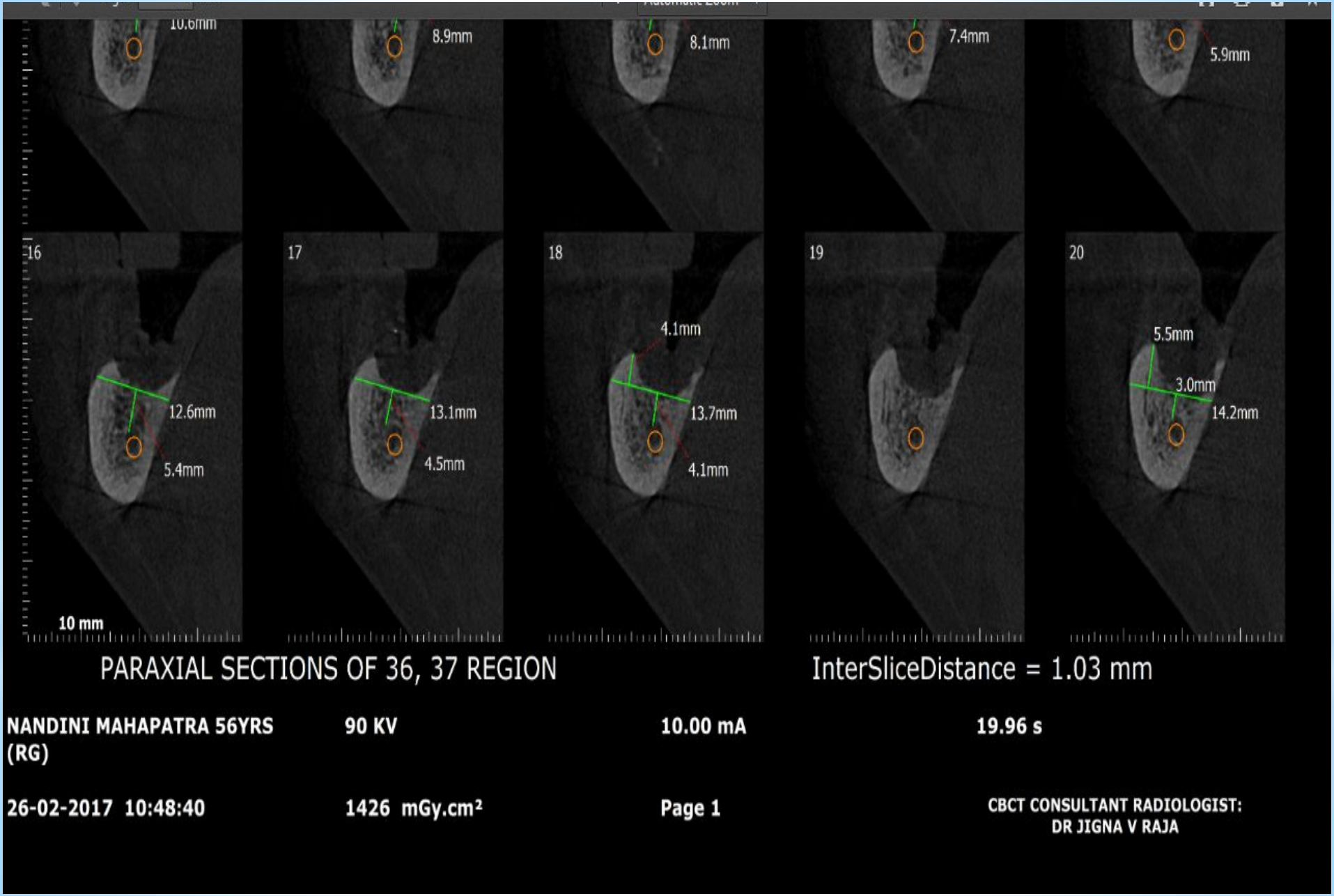





5
7.3mm
14.3mm
6
7.0mm
14.3mm
7
5.8mm
14.0mm
8
6.0mm
14.0mm
9
5.9mm
14.0mm
10
6.2mm
14.0mm
11
6.3mm
13.7mm
12
6.1mm
13.9mm
13
14
15
16


A
1
11
20
R
L
P
10 mm
H
11
20
R
L
1
11
20
F
10 mm
L
H
A
1
H
B
L
2
3
4.2mm
12.1mm
4
3.8mm
11.9mm
5
3.6mm
11.2mm


F
6
4.0mm
11.1mm
7
4.1mm
10.8mm
8
4.4mm
10.8mm
9
4.2mm
10.9mm
10
3.7mm
11.2mm
11
4.4mm
10.6mm
12
8.4mm
8.9mm
13
10.1mm
8.1mm
14
10.7mm
7.4mm
15
11.6mm
5.9mm


10.6mm
8.9mm
8.1mm
7.4mm
5.9mm
16
12.6mm
5.4mm
17
13.1mm
4.5mm
18
4.1mm
13.7mm
4.1mm
19
20
5.5mm
3.0mm
14.2mm
10 mm
PARAXIAL SECTIONS OF 36, 37 REGION
InterSliceDistance = 1.03 mm
NANDINI MAHAPATRA 56YRS (RG)
90 KV
10.00 mA
19.96 s
26-02-2017 10:48:40
1426 mGy.cm²
Page 1
CBCT CONSULTANT RADIOLOGIST:
DR JIGNA V RAJA

* Magnetic Resonance Imaging [MRI]

- MRI uses non ionizing radiation
- It essentially involves the behavior of protons -positively charged nuclear particles in a magnetic field.
- MR images are obtained by measuring changes in low frequency radio signals in the magnetic field.
- The resulting data can be used to create images of the structures examined or chemical profiles of the tissues.

- This technology gives better soft tissue images than CT and the patient is not exposed to radiation.

- MR imaging is mainly used in the study of TMJ and the soft tissue lesion of gingiva and other oral structures.

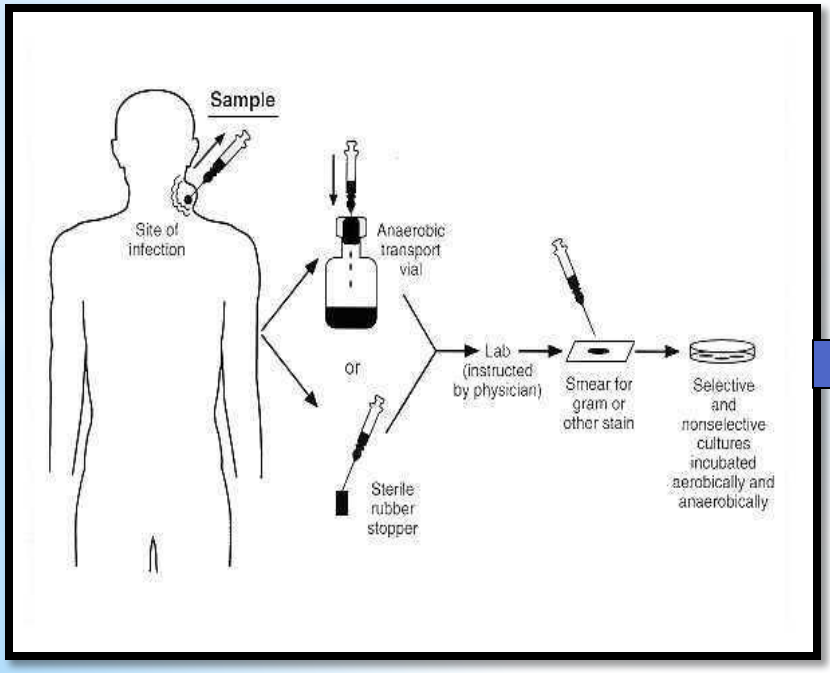
Advantages over conventional:

- ✔ Offers the best resolution of tissues of low inherent contrast.
- ✔ No ionizing radiation is involved.
- ✔ Because the region of the body imaged in MRI is controlled electronically, direct multiplanar imaging is possible without re-orienting the patient

* Advanced Microbiological Diagnostic Aids

*Bacterial Culturing

- It is considered as a "Reference method /Gold standard" when determining the performance of other new microbiological diagnostic aids



* Direct Microscopy

- Alternative to culture methods
- Simple, non invasive & non expensive
- Ability to count all the bacteria in the plaque sample.

Limitations

1. Inability to determine the relative susceptibility to AMA.
2. Most of the pathogens are non motile, hence unable to detect them

Dark field / Phase contrast microscopy
Electron microscope,
Confocal scanning electron microscopy

* Immunodiagnostic Methods

- Antibody-Antigen reaction can be revealed by:

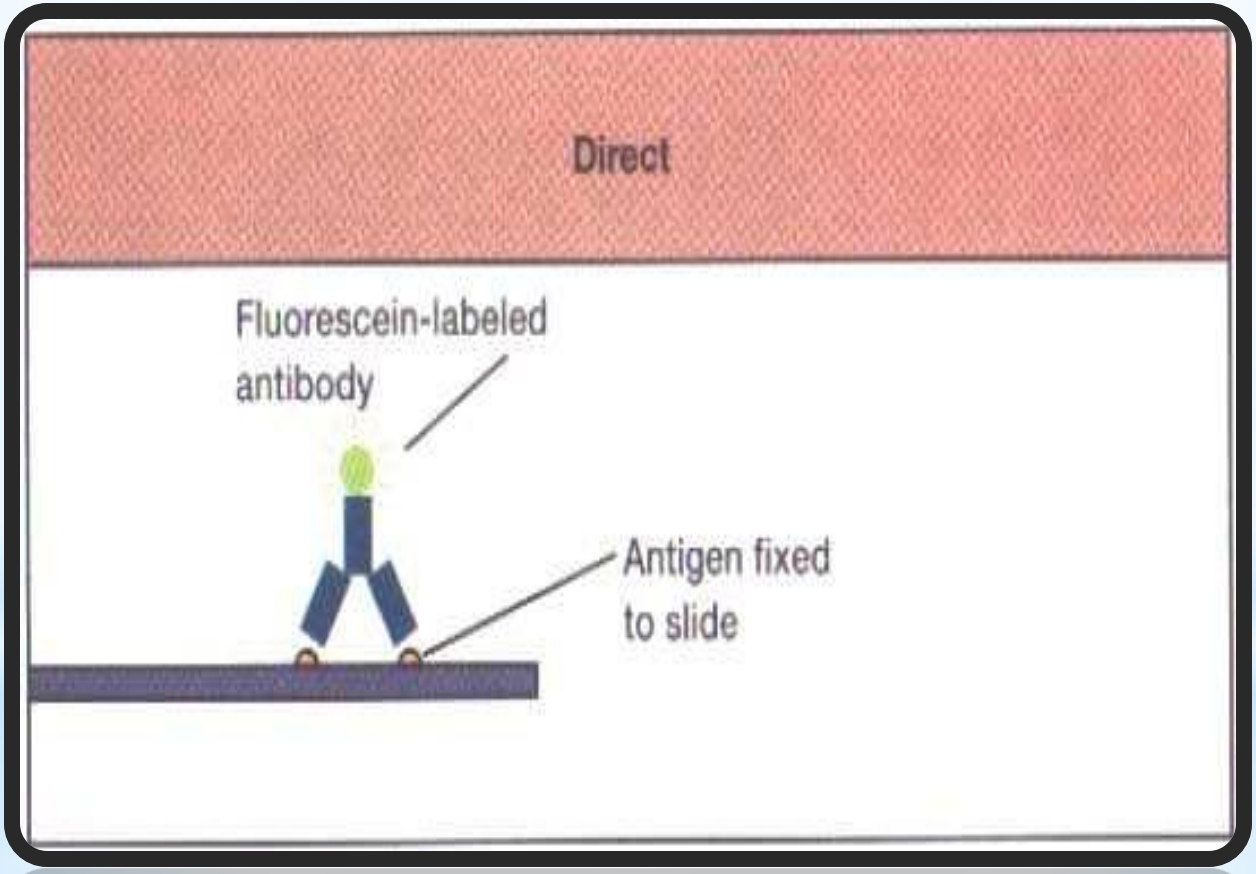
- Immunofluorescent assay- direct and indirect
- Flow cytometry
- Radio immunoassay
- ELISA
- Western blot
- Latex agglutination.

Immunofluoroscence

- Immuno fluoresence is a process in which dyes called fluorochromes are exposed to UV, violet, or blue light to make them fluorescence or emit visible light.

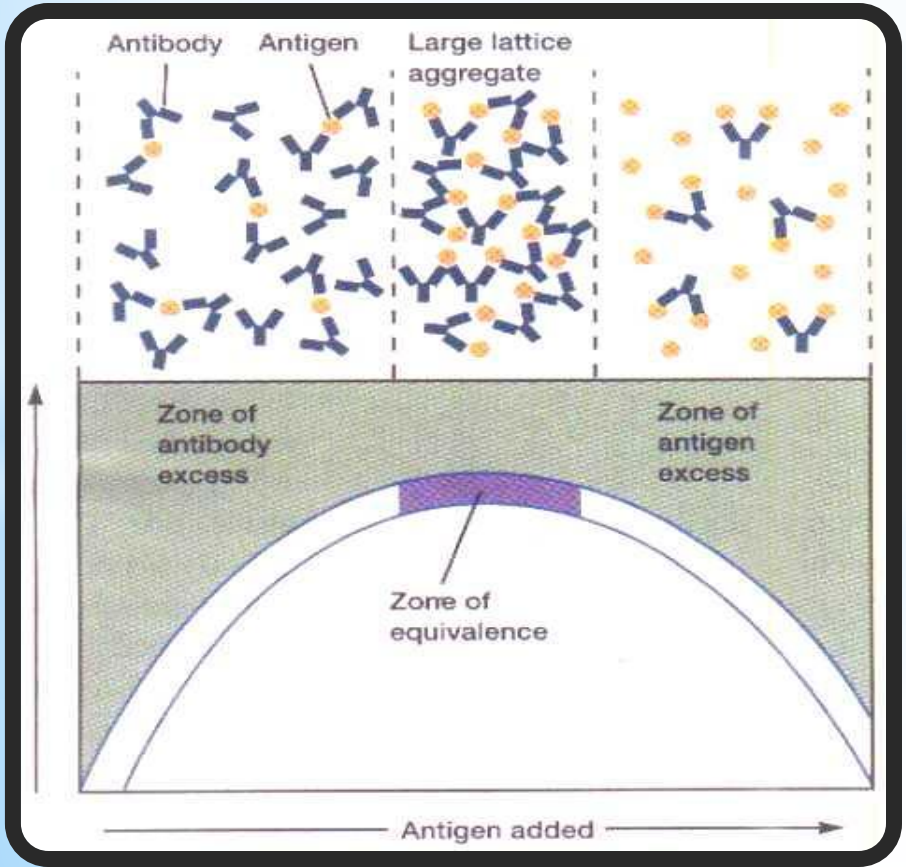
- Eg; **Rhodine B or fluoresecin iso thiocyanate (FITC)**

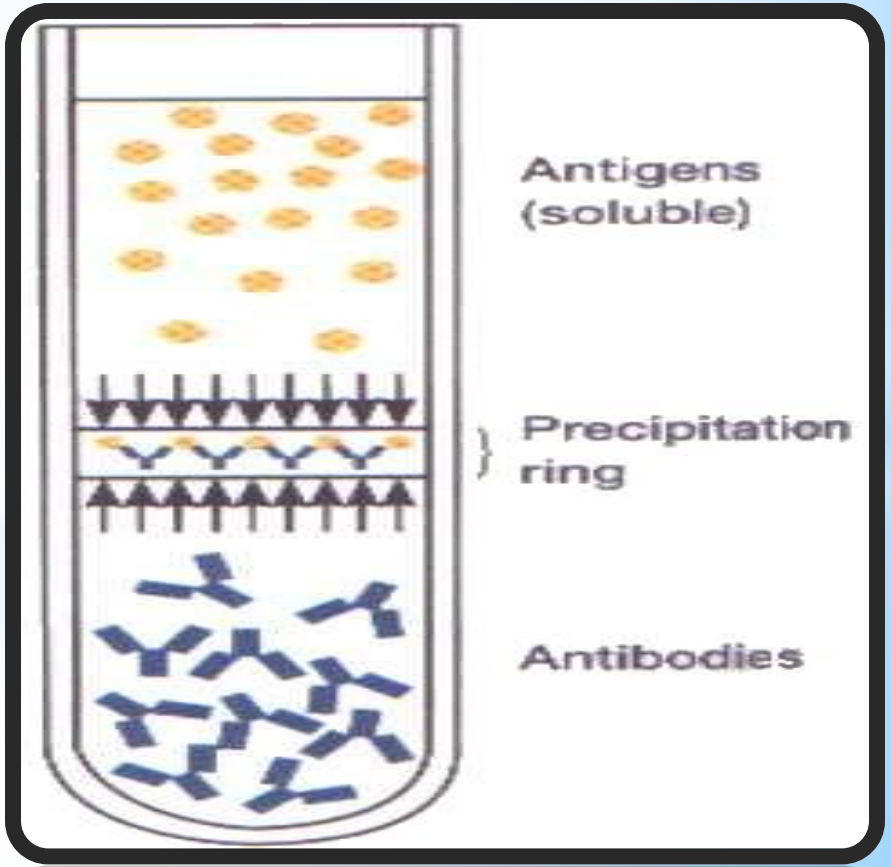
*Direct Immunofluoroscence



* Immuno Precipitation

- The immuno precipitation technique detects soluble antigens that react with antibodies called **precipitins.**

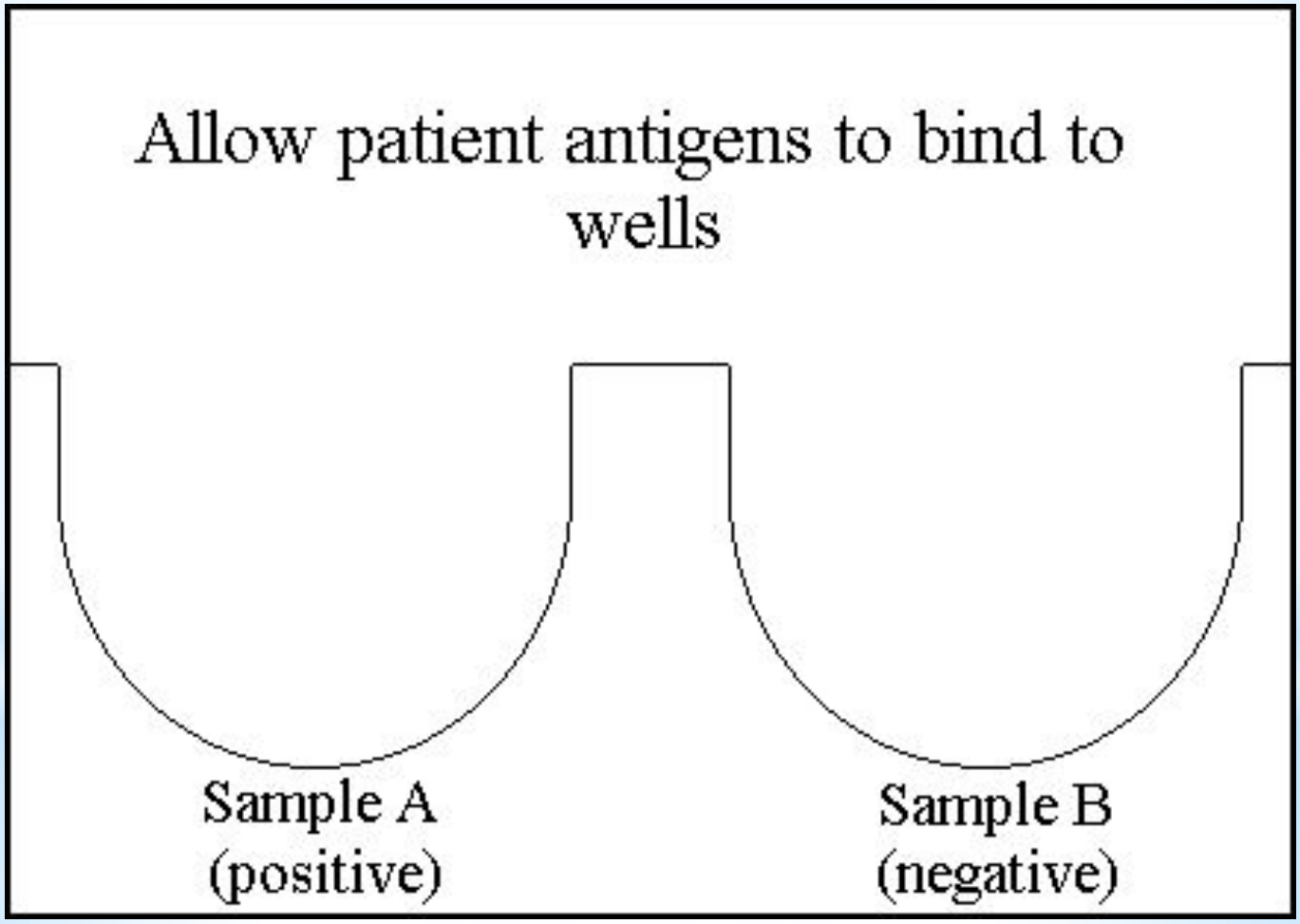




* ELISA

- It is the most widely used serological tests for antibody or antigen detection.
- This test involves the linking of various label enzymes to either antigens or antibodies.
- 2 basic methods are used
  - Double antibody sandwich assay
  - Indirect immunosorbant assay.

ELISA


Allow patient antigens to bind to wells
Sample A (positive)
Sample B (negative)

Advantages

1. Very **specific** & frequently used for detection of periodontal pathogens

2. Used to monitor antibody levels as they are **2.3-4.7** times as sensitive as other immunological assays

Disadvantages

1.Not all pts affected with microbes demonstrate increased Ig levels

2. Organisms like Capnocytophaga & Treponema either don't induce or may suppress immune reactions

* Enzymatic Methods

- BANA: It is an enzymatic assay for the identification of trypsin like proteases

- The activity of this enzyme is measured by the hydrolysis of the colourless substrate N-Benzyl-Arginine-DL- 2 Napthylamide.

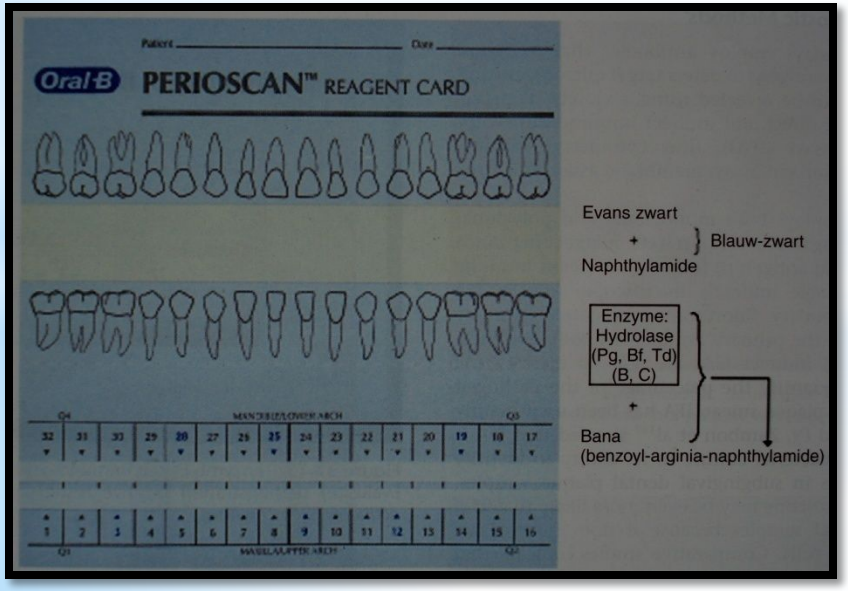
- When the hydrolysis takes place it releases the chromophore-β-Naphthylamide which turns orange red when fast garnet is added to the solution.

- **Loeshe et al in 1986**-proposed the use of BANA in subgingival samples

- Reported that shallow pockets exhibited **10% BANA positive reaction** , where as deeper pocket [7mm] were **70-90% BANA positive.**

Perioscan

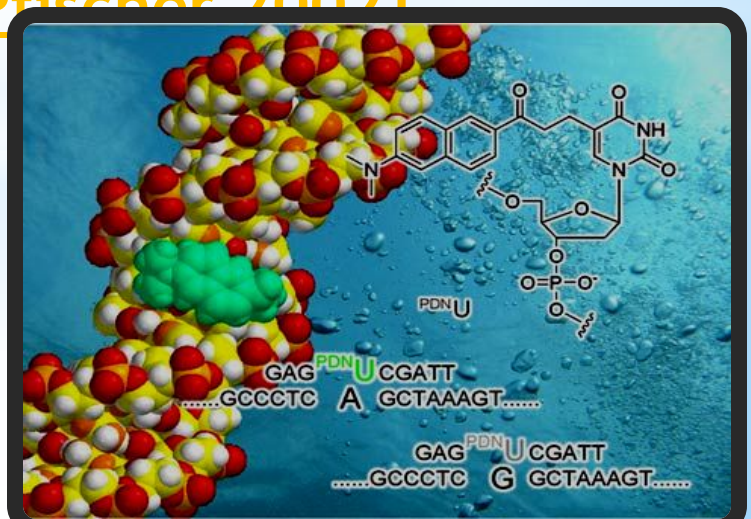

BANA
Reagent Strips


Oral-B
PERIOSCAN™ REAGENT CARD
Evans zwart
+
Naphthylamide
Blauw-zwart
Enzyme:
Hydrolase
(Pg, Bf, Td)
(B, C)
+
Bana
(benzoyl-arginia-naphthylamide)

* Nucleic Acid Probes

- These probes consists of nucleic acid sequences that are labeled with a radioactive & enzymatic calorimetric marker that bind to complimentary nucleic acid sequences on corresponding micro organisms

- Commercially available kit **"MicroDent"** employs probes for **Pg, Aa, Tf, Td, &Pi.** [Eick S Pfischer 2002]



The probes may be

1. Whole genomic probes
2. Randomly cloned probes
3. Oligonucleotide probes.(16SrRNA)

* Advanced Diagnostic Aids In Characterizing The Host Response

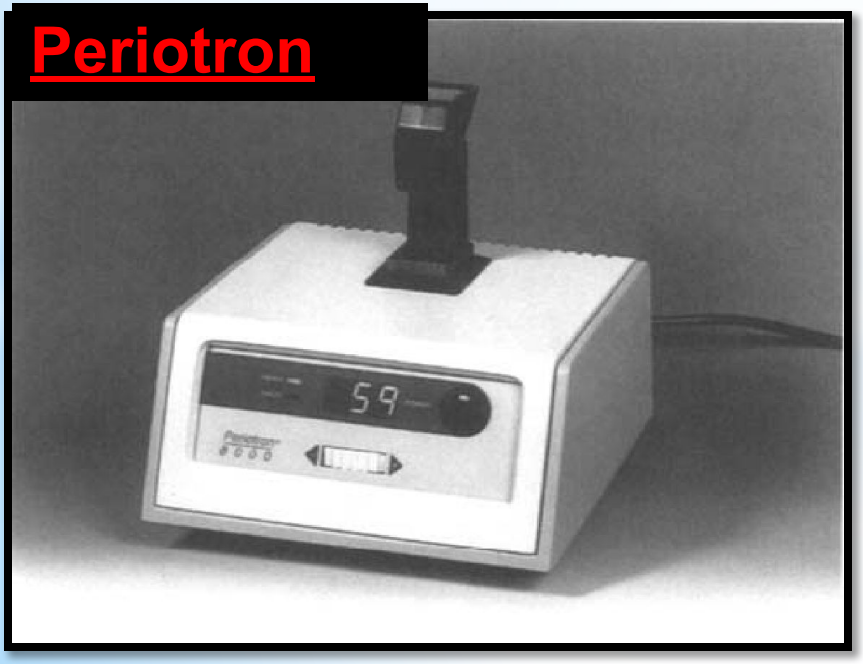
❑Assessment of host response refers to the study of mediators by immunologic or biochemical methods that are recognized as part of the individual response to the periodontal infection.

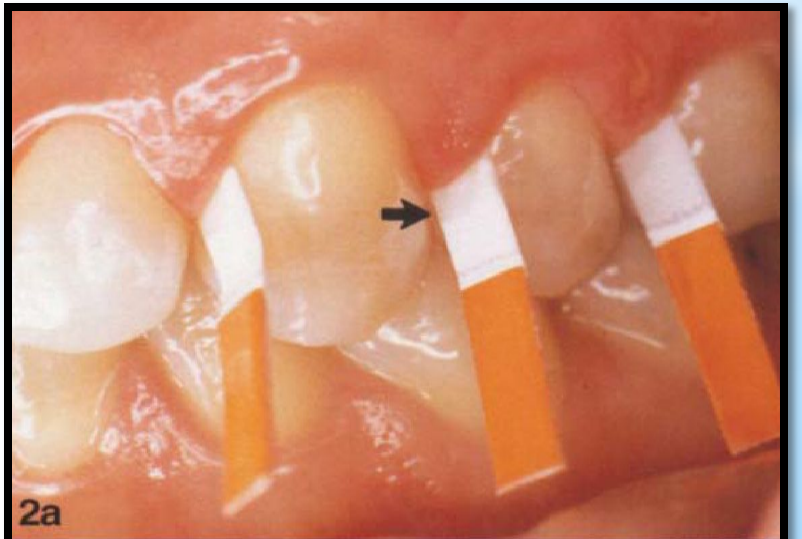
- ✔ **To monitor & identify pts at risk for periodontitis.**
- ✔ **Early detection of pts at risk for disease**
- ✔ **Proper treatment intervention**
- ✔ **Decrease the need for aggressive treatment &**
- ✔ **Improve the response to periodontal therapy**

*GCF

Electronic device, measures the change in the capacitance Across the wetted strips & this change is converted into a digital read out correlated with GCF volume.

Periotron





*Saliva

❑Proposed diagnostic markers in saliva-

✔**Proteins,**

✔**Enzymes of host origin,**

✔**Host cells,**

✔**Hormones,**

✔**Volatile compounds,**

✔**Bacteria & its products, ions etc**

Enzymes & inhibitors	Tissue break down products	Inflammatory mediators
•AST •Alkaline phosphatase •β-glucoronidase •Elastase •Elastase inhibitors α1- macroglobulin α2- protinease inhibitor •Cathepsins Cysteine & Serine proteinase •Trpsin like enzyme •Ig degrading enzymes •Glycosidases •Collagenases [MMP-1,3,8] •Gelatinases [MMP 2,9] •Stromyelysins	•Glycosaminoglycans Hyaluronic acid Chondroitin 4sulphate Chondroitin 6sulphate •Hydoxyproline •Fibronectin fragments •Keratin •Laminin •Calprotectin	•Cytokines Interleukin 1α Interleukin 1β Interleukin 6 & 8 •PgE2 •Acute phase proteins Lactoferrin Transferrin α2- macroglobulin •Autoantibodies •Plasminogen activator •Substance P •Antibacterial antibodies

* Complement

- The complement proteins are present in GCF from sites with inflammation.

* Cytokines

Saliva: Potential cytokine marker in saliva is **PAF (platelet activation factor).** Salivary PAF is significantly higher in untreated chronic periodontitis pts, correlate with clinical indices of disease severity & also reduces following treatment. (Rasch et al,Garito et al 1995)

GCF: **IL-1β & TNF- α** in GCF causes stimulation of prostaglandin E2 & collagenase production -thus most important in the pathogenesis of periodontitis

- **IL-1α & β** are present in inflamed gingiva

- Levels of IL-6 and IL1 high in patients with refractory periodontitis.
- TNFα in GCF does not correlate with probing depth/ gingival inflammation

* Prostaglandins

- GCF PGE_2 levels are low in health.
- GCF PGE_2 is predictive for periodontal disease activity.

*Host Derived Enzymes

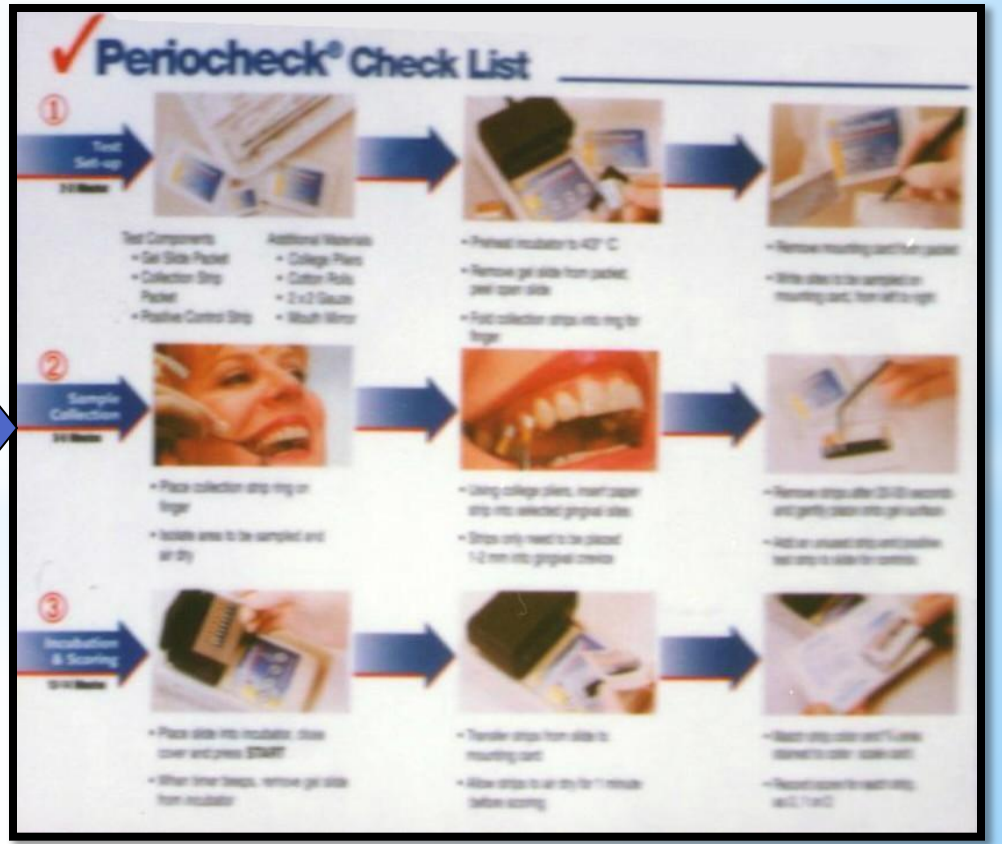
* Collagenases & Related MMPs [Neutral Proteinases]

- These are part of family of MMPs that degrade the collagen

- Collagenase [MMP8 & MMP1] - present in GCF, saliva, gingival tissue
- GCF collagenase activity is shown to increase with -gingival inflammation & pocket depth & alveolar bone loss & decreased post treatment. **(Golub etal)**

* Periocheck [AC tech]

- This system (Pro Dentec Bates ville) detects the presence of neutral proteinases such as collagenase in GCF



MARKERS OF BONE RESORPTION

The components of bone that could be released during bone resorption & are present in GCF are known as *bone specific proteins*.

They are

1. Osteonectin
2. Bone phosphoprotein (N-propeptide)
3. Osteocalcin
4. Telopeptides of type1 collagen.

Sampling & detection

- Osteonectin in GCF – Nitrocellulose strip
- Osteocalcin - conventional paper strip for 30 secs. or multiple collection for 1min at same site

Diagnostic tests:

- Detection by specific monoclonal or polyclonal antibodies - ELISA
- Osteocalcin - ELISA (Nakashima1994) & radioimmunoassay (Giannobile., 1995)
- Osteonectin & N-propeptide – ELISA (Bowers.et.al 1989)

Thank you