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## Diagnostic aids to detect caries-A review

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### Abstract

The main objective of caries risk assessment in dentistry is to deliver preventive and restorative care specific to an individual patient. Many factors may affect the caries susceptibility of different individuals. Caries risk assessment of a person can be done by caries activity tests. Assessment of a caries risk at screening or initiation of therapy allows better appraisal of caries activity and refinement of the treatment planning. This review explains recent advances to detect caries and their outcomes.

**Keywords:** diagnostic aids, detect caries.

### Introduction

Diagnosis is often derived from personal and cognitive experiences. Good diagnosticians use past experience, based on knowledge and diagnostic tools. To become a successful diagnostician, one must develop a number of assets. The most important of these are knowledge, interest, intuition, curiosity, and patience [1].

The clinician must systematically gather all of the necessary information to make a “probable” diagnosis. When taking the medical and dental history, the clinician should already be formulating in his or her mind a preliminary but logical diagnosis, especially if there is a chief complaint. The clinical and radiographic examinations in combination with a thorough periodontal evaluation and clinical testing (pulp and periapical tests) are then used to confirm the preliminary diagnosis. In some cases, the clinical and radiographic examinations are inconclusive or give conflicting results and as a result, definitive pulp and periapical diagnoses cannot be made. It is also important to recognize that treatment should not be rendered without a diagnosis [2].

The oldest diagnostic tests were the simple percussion and palpation. In the early 1900s, the following tests were considered essential- roentgenograms (in particular, bitewings for children and adolescents), trans illumination (noting the color changes between a tooth with a vital and nonvital pulp), percussion and palpation, thermal vitality tests (use of ice or hot water), electric pulp tester, mobility tests, test cavity and the anesthetic test. Although these have served the dental clinician well over the many decades, these come with their share of limitations- putting the patient through any more pain than already is in or invading the irreplaceable tissues, being not free from doctor and patient bias, along with the lack of correlation with the histological status of the pulp and dubious accuracy and therefore the need arose for newer methods to detect pulp vitality [3].

### Caries Diagnostic Advances

#### 1) Cariostat Test (Caries Risk Test)

- A new, quick and effective caries activity test.
- CRT has two components:
  - a. **CRT bacteria** – which allows estimation of a number of cariogenic bacteria in the patient’s saliva.
  - b. **CRT buffer** – which determines the buffering capacity of the same.

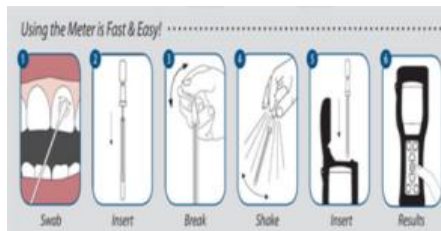
### CRT bacteria is a two-in-one dip-in-slide test which identifies counts of

- a. Mutans Streptococci
- b. Lactobacilli

Stimulated saliva is collected and applied to both the sides of the dip-in-slide. Then it is incubated for hrs at 37 °C. The CRT buffer is available in a strip form, which changes color to indicate whether the patient has a high, medium or low buffering capacity. This occurs in five minutes.



**Fig 1:** Caries susceptibility testing meter.



**Fig 2:** Caries Screen Meter

#### ➤ **CariScreen an easy 1 minute test (Fig 2)**

The quick and easy CariScreen test determines the level of decay-causing bacteria. It takes only one minute to determine if a patient is at low, moderate, or high risk of developing cavities in the future.

##### • **Features**

When adenosine triphosphate (ATP) is brought into contact with the unique liquid-stable luciferase/luciferin reagent within the CariScreen Swab sampling device, light is emitted in direct proportion to the amount of ATP present. The CariScreen meter measures the amount of light generated and provides information as to the level of contamination within seconds.

- CariFree (Oral BioTech, Albany, OR) is a caries risk assessment and treatment model based on the CAMBRA approach. Caries risk is determined based on a questionnaire and a chair-side measurement of the adenosine triphosphate (ATP) bioluminescence (CariScreen Caries Susceptibility Testing) from the plaque present on specific sites within the oral cavity. The ATP-driven bioluminescence assays have long been used as a quantitative measure of microbial numbers in the packaged food industry and more recently for measuring total bacterial mass in dental plaque. Based on the level of caries risk, the patient is placed on a treatment regimen. This do-at-home treatment includes oral rinses, toothpaste substitutes and chewing gum containing the benefits of xylitol, fluoride and pH neutralizing agents. These specific agents help to modify the salivary environment and build resistance against acid

attack. The CariScreen Caries Susceptibility Testing meter can be used chair side and is a validated tool measured instead of the amount of calcium dissolved<sup>4,5]</sup>

- The patients will know their test results from the Cari Screen meter within 1 minute. Should their test suggest a moderate to high risk of caries caused by cariogenic biofilm, the dental assistant or hygienist can explain the consequences of a biofilm infection — and the ease and cost-effectiveness of treatment.
- Since risk factors change over time, it is recommended that patients take the Cari Screen test once per year. For the most accurate test results, ask your patients to not eat or brush their teeth for at least one hour prior to the test.<sup>5</sup>

## 2. Caries detecting dyes

- If an object is hard to distinguish from its background, the color induced by the dye can make it easier to visualize, or, if several objects have a similar appearance, coloring a dye may discriminate between them and allow identification. The observation of the coloring can be either qualitative or quantitative.
- For a qualitative assessment it is sufficient to observe a color change or differentiate the colored objects from the uncolored ones. For a quantitative assessment, either the amount of staining or the intensity of the colour has to be measured. The amount of staining can be determined for instance by counting the number of stained cells and comparing this with the number of unstained cells, or a measurement of the area of staining compared with the unstained area.
- The intensity of the color can be determined by measurement of absorption or fluorescence, which in way is opposite quantities. Absorption can be measured by quantitating the decrease of light intensity at a particular wavelength, and fluorescence by quantitating the increase in light intensity at a particular wavelength.
- In cariology most often the visual appearance of the dyes is observed because traditionally, for diagnosis identification is far more important than quantification. Quantification of carious lesions has been recognized as an important tool for evaluating the level of mineralization. Quantification of lesions enables the status of the lesions to be monitored as arrested or progressing.
  - a. Dyes should fulfil the following criteria before being recommended for clinical use.
  - b. Dyes should be absolutely safe for intraoral use.
  - c. Dyes should be specific and stain only the tissue it is intended to stain.
  - d. Dyes should be easily removed and not lead to permanent staining.
- **Dyes for detection of carious enamel (Fig 3, 4)**
- Early dental caries is characterized by demineralization through increase porosity. It is possible to identify more porous areas by selective dye penetration. Several studies with the use of common dyes demonstrate a differential penetration of these dyes through enamel.
- Dye is considered not only as a marker of extensive overt lesions. Dyes are useful as a means of identifying circumscribed areas of demineralized enamel areas that are readily observed without staining.

### 1. Procion Dyes

- To stain enamel lesions
- Staining is irreversible because the stain reacts with -OH and -NH<sub>2</sub> groups and acts as a fixative.
- Also used in neurology as vital stain

### 2. Calcein

- To measure the infiltration into carious enamel
- Complexes with calcium and remains bound in the lesion.
- Penetration in vitro is better than *in vivo*

### 3. Zyglo ZL – 22

- Unsuitable for intra oral use
- Penetrates into the microspores of lesions and can be made visible by ultraviolet illumination.
- Also penetrates into the areas where not white spots are visible.
- Carrier solvents. Ethanol, water and propylene glycol were used, of which the ethanolic dye solution produced the most promising results.

### 4. Zyglo ZL – 30A

In vitro demineralisation can be quantified by the amount of dye measured. Another way of quantification is by measurement of fluorescence by means of a photo diode and fiber -optics while the dye is still present in the lesion.

### 5. Fluorol 7GA

- The fluorescence intensity can be correlated well with mineral loss as measured by microradiography. This method can be applied *in vivo*.
- To enhance the diagnostic quality of fiberoptic trans illumination, Brilliant Blue was used. A better control was the only observation made from photographs that were analysed with a colorimeter for colour differences.

### • Dyes used for detection of carious dentine

-In human carious dentine two layers of decalcification can be identified: one layer of decalcified dentine which is soft and cannot be remineralized; and a second decalcified layer which is hard, with intermediate decalcification and can be remineralised.



Fig 3: Caries Detector Dyes-Several dyes have been tested



Fig 4: Representing Areas of stained areas.

- 0.5% Basic fuchsin in propylene glycol is successful. A distinct boundary separates the stained and unstained zones. Bacterial invasion apparently coincides with staining and no bacteria are found when all stained dentine has been removed. The dye solution is recommended as a clinical guide for complete removal of bacterially infected dentine. Basic fuchsin has been replaced by acid red because of its carcinogenic potential.
- Acid red is specific and more reliable than clinical judgement for complete removal of bacterially infected and soft carious dentine.
- Methylene blue is used but it is slightly toxic
- Acid reacting dyes have also been used but they are non-specific.
- The staining by the dye is the result of denaturation of collagen but not the result of loss of mineral.
- The advantages of using fluorescent dyes over absorbing dyes are that the fluorescence can be observed more clearly than absorbance. Fluorescent dyes are hardly visible after application unless illuminated with a special light source. Thus they are less obtrusive than absorbing dyes.

### • Accuracy of Caries-Detector Dyes

- A diagnostic aid should show a very low level of false positives to avoid unnecessary treatment. Yet in one study, when the level of infection of dye-stained and unstained dentin at the enamel-dentinal junction was measured at the completion of cavity preparation, it was discovered that not all dye stainable dentin was infected. Fifty-two per cent of the completed cavities showed stain in some part of the enamel-dentin junction, but subsequent microbiological analysis of dye-stained and non-stained sites resulted in the recovery of very light levels of infection, with no differences between sites. Such bacterial levels were considered clinically insignificant. On the other hand, it has also been demonstrated that absence of stain does not ensure elimination of bacteria. It is now clearly established that these dyes do not stain bacteria but instead stain the organic matrix of less mineralized dentin. The lack of specificity of caries-detector dyes was confirmed in 1994 by Yip and others, who correlated the location of dye-stainable dentin with mineral density. The dyes neither stained bacteria nor delineated the bacterial front but did stain collagen associated with less mineralized organic matrix. Of even greater significance was the fact that when these authors utilized the dyes on caries-free, freshly extracted human primary and permanent teeth, they discovered that sound circum-pulpal dentin and sound dentin at the dentino-enamel junction took up the stain because of the higher proportion of organic matrix normally present in these sites [6].

### • Some new compounds

1. Carbolan green
2. Coomasie blue
3. Lissamine blue [7]

- **Cari-D-Tect** - contains 2% combination of red and blue disodium salts which stains dark blue to bluish green [5].

### 3) Caries Activity Tests

- Caries activity tests have been used in dental research for

many years, and some tests have been adapted for routine use in the dental office. There is no ideal test in existence at the present time, although caries cavity tests are a valuable adjunct for patient motivation in a plaque control program.

#### A. Lactobacillus Colony Count (Fig 5, 6)

- **Action:** This test, first introduced by Hadley in 1953, estimates the number of acidogenic and aciduric bacteria in the patient's saliva by counting the number of colonies appearing on tomato peptone agar plates after inoculation with a sample of saliva.
- **Equipment:** The necessary equipment includes saliva collecting bottles, paraffin, 9 ml tubes of saline, 2 agar plates 2 bent glass rods, facilities for incubating, and a Quebec. By having the subject chew paraffin before breakfast and then collecting the saliva in a bottle. The specimen is shaken to mix it. A 1:10 dilution is prepared by pipetting 1 ml of the saliva sample into a 9 ml tube of sterile saline solution. This is shaken and 1:100 dilutions is made by pipetting 1 ml of the 1:10 dilution another 9 ml tube of sterile salt solution. The 1:100 dilutions is mixed thoroughly and 0.4 ml of each dilution is spread on the surface of an agar plate with a bent glass rod. The plates are labelled and incubated at 37 °C for 3 to 4 days. A count of the number of colonies is then made by using the Quebec counter.

#### B. Snyder Test (Fig 7)

- **Action:** The Snyder test measures the rapidity of acid formation when a sample of stimulated saliva is inoculated into glucose agar adjusted to pH 4.7 to 5 and with bromocresol green as colour indicator. Indirectly the test is also a measure of acidogenic and aciduric bacteria.
- **Equipment:** The equipment includes saliva collecting bottles, paraffin, a tube of Snyder glucose agar containing bromocresol green and adjusted to pH 4.7 to 5, pipettes, and incubating facilities.
- **Procedure:** Saliva is collected before breakfast by having the subject chew paraffin. A tube of Snyder glucose agar is melted and then cooled to 50°C. The saliva specimen is shaken vigorously for 3 min. The 0.2 ml of saliva is pipetted into the tube of agar and immediately mixed rotating the tube. The agar is allowed to solidify in the tube and is incubated at 37°. The colour change of the indicator is observed after 24, 48 and 72 hours of incubation by comparison with an incubated tube against a white background.
- This test meets some of the "ideal test" characteristics. Snyder and others have found a high correlation between the synder did acid production test and lactobacillus plate count. Also, Snyder and others have found a high correlation between clinical caries activity and +ve. Snyder test results on a group basis. The best agreement was between a -ve Snyder test and the absence of caries activity.

#### C. Reductase Test

- **Action:** The test measures the rate at which an indicator molecule, diazoresorcinol, changes from blue to colorless or leukoform on reduction by the mixed salivary flora. Rapp claims the test "measures the activity of a single enzyme, reductase. This enzyme is involved in

some very definite and limiting reactions in the formation of products dangerous to the tooth surface.

- **Equipment:** The reductase test comes in a kit that includes calibrated saliva collection tubes with the reagent on the inside of the tubes cap, plus flavoured paraffin.
- **Procedure:** Saliva is collected by chewing special flavoured paraffin and expectorating directly into the collection tube. When the saliva reaches the calibration mark (5ml) the reagent cap is replaced. The sample is mixed with a fixed amount of diazoresorcinol, the reagent upon which the reductase enzyme is to react. To change in color after 30 seconds and after 15 minutes is taken as a measure of caries activity.

#### D. Buffer Capacity Test

- **Action:** Buffer capacity can be quantitated using either a pH meter or color indicators. The test measures the number of millimetres of acid required to lower the pH of saliva through an arbitrary pH interval, such as from pH 7.0 to 6.0 or the amount of acid or base necessary to bring color indicators to their end point.
- **Equipment:** Needle equipment includes a pH meter and titration equipment, 0.05n lactic acid, 0.05 N base, paraffin and sterile glass jars containing a small amount of oil.
- **Procedure:** 10 millimetres of stimulated saliva are collected under oil at least 1 hour after eating; 5ml of this are measured into a breaker. After correcting the pH meter to room temperature, the pH of the saliva is adjusted to by addition of lactic acid or base. The level of lactic acid in the graduated cylinder is re-recorded. Lactic acid is then added to the sample until a pH of 6.0 is reached. The number of millimetres of lactic acid needed to reduce pH from 7.0 to 6.0 is a measure of buffer capacity.

#### E. Fosdick calcium dissolution test:

- **Action:** The test measures the milligrams of powdered enamel dissolved in 4 hours by acid formed when the patients saliva is mixed with glucose and powdered enamel.
- **Equipment:** Powdered human enamel, saliva collection bottles, sterile test tubes, test tube agitation equipment and equipment for determining the calcium content of the saliva. Saliva is stimulated by moving the subject chew gum or paraffin, in which case a 5% solution of glucose needed. Two procedures can be used;
- A. **Procedure:** 25 millimetres of gum stimulated saliva are collected. Part of this is analysed for calcium content. The test is placed in an 8 inch sterile test tube with about 0.1 gms of powdered human enamel. The tube is sealed and shaken for 4 hours at body temperature after which it is again analysed for calcium content. The chewing of gum to stimulate the saliva produces sugar, if the paraffin is used; a concentration of about 5% glucose is added. The amount of enamel dissolution increases as the caries activity increases.
- B. **Procedure:** Saliva is collected before breakfast by having the subject chew paraffin. A tube Snyder glucose agar is melted and then cooled to 50°C. The saliva specimen is shaken vigorously into the tube of agar and immediately mixed by rotating the tube. The agar is



allowed to solidify in the tube and is incubated at 37°C. The color change of the indicator is observed after 24, 48 and 72 hours of incubation by comparison with an uninoculated tube against a white background.

#### F. Dewar test

- **Action:** This test is similar to the Fosdick calcium dissolution test except that the final pH after 4 hours is measured instead of the amount of calcium dissolved [6]



Fig 5: Showing Snyder test.



Fig 6: Lactobacillus Colony Count Test.

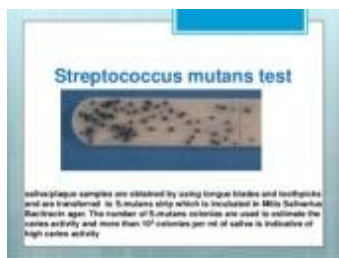


Fig 7: Streptococcus mutans test.

#### Conclusion

An accurate assessment of tooth vitality is of paramount importance in clinical practice. Although sensitivity testing is the de facto standard employed by the majority of clinicians, it has acknowledged limitations. Careful attention to diagnostic aids and an understanding of both their usefulness and limitations is essential if they are to be employed most effectively in clinical dentistry.

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