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Review Article

Caries Activity Indicators: Guide for Dental Practitioners

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Abstract

An objective evaluation of caries activity requires clinical examination of quantification of factors associated with the pathogenesis of caries. Caries activity tests have been in use in dental research and clinical dentistry for years. They are useful in establishing categories of risk for caries, and for targeting specific preventive measures to these groups. They are even more useful in situations of limited resource availability. The limited resources can be concentrated on those identified by caries activity indicators as in most need of help. In recent years a number of techniques to diagnose caries have emerged. It is important for the clinical practitioner to utilize cost-effective measures in diagnosing those at risk. The objective of this paper is to review the current types of caries activity indicators and their relative efficacy for dental practitioners.

Keywords: Dental Caries; Caries Activity Indicators; Dental Practitioners.

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Introduction

Caries because of its uniqueness as a disease, its ubiquitous nature, remains one of man's most common, oldest ailments. Caries activity test have been in use in dental research and clinical dentistry for years.[1]They are useful in establishing the risk for caries and for targeting specific preventive measures to these groups. They are even more useful in situations of limited resource availability.[2]

Caries activity can be defined as the speed with which teeth are destroyed by caries; in other words the sum of new caries lesions and the enlargement of existing cavities during a certain time. Endogenous factors, such as the salivary fluoride buffer capacity of saliva, activity of muscles involved in masticatory function and salivary factors which may reduce bacterial retention in oral cavity. An objective evaluation of caries activity requires clinical examination and quantification of factors associated with the pathogenesis of caries like the host, micro flora and diet. They are useful in establishing the risk for caries and for targeting specific preventive measures to these groups. There is no ideal test in existence at the present time, although caries activity tests are a valuable adjunct for patient motivation in a plaque control program. Saliva serves as a major component in most caries activity tests, and aids in the categorization patients into high, medium and low caries activity. Although a multiplicity tests have been described in the literature, none the currently available methods are completely satisfactory.[3-6.]

Various Caries Activity Indicators:[3,7]

I: The testing defence factors
- a) Teeth based tests
- b) Saliva based tests

II: Miscellaneous Host factors
- a) Medications
- b) Age
- c) Socio-economic conditions

III: Testing challenge factors
- a) Microflora tests
- b) Plaque index
- c) Diet based tests

IV: Various Caries activity tests
- a. Lactobacillus colony count test
- b. Snyder's test
- c. Alban's test
- d. reductase test
- e. Buffer capacity test
- f. Fosdick calcium dissolution test (Enamel solubility test)
- g. Dewar test
- h. Streptococcus mutans screening test
  1. Plaque/toothpick method
  2. Saliva/tongue blade method
  3. Streptococcus mutans adherence test
- i. Prediction future caries activity based on previous experience
- j. Dip's slide test
- k. Plaque and saliva ph measurement test

l. Electronic caries detector
m. Ora test

V: DMF Index
VI: CLR Index

1) The testing defence factors[8]

a) Teeth based tests:

i. **Fluoride content enamel:** Teeth with high surface fluoride content are more **r**esistant to acid dissolution.
ii. **Crowding teeth and morphology:** crowded teeth are prone to caries.
iii. **Past caries experience:** Is an indirect measure in which host resistance correlates well with future caries activity.

b) Saliva based test:

i. **Secretion rate saliva:** Give the patient a piece paraffin wax (1.5 gm) to chew for 5minutes.Any subject with a secretion rate below 0.7 ml per minute should be considered a caries risk.
ii. **Buffer capacity saliva:** Bicarbonate is the major salivary buffer.

II: Miscellaneous host factors:

i. **Medications :**example- Penicillin by children with rheumatic heart disease
   1) Reduce caries activity.
ii. **Age:** Fissures in newly erupted teeth are more susceptible to caries.
iii. **Socio economic conditions:** Children with high socioeconomic group in some societies have less caries risk than children with lower socio economic group.

III: Testing challenge factors:

i. **Microflora test:** The lactobacillius count therefore can be used for both estimation caries risk and control dietary changes.
ii. **Plaque index**: It has been demonstrated that personal teeth cleaning can reduce caries activity significantly. Therefore plaque index is of value in predicting caries activity. Biochemical activity is more important than the microbiological content.
iii. **Diet (Substrate) based test**: Frequent intake sugar between meals is the key factor in the development of caries. To determine food intake and food habits different kinds of nutritional analysis are in use such as balance Sheet computer assisted analysis, weighing methods and interviews. A diet history of 24hrs can be obtained. [8]

IV) CARIES ACTIVITY TEST

Definition: Caries activity can generally be identified as the occurrence and rate at which teeth are destroyed by acid produced by plaque bacteria .It is also a sum of new caries lesions and the enlargement of the existing cavities during a certain time. Some of the proposed uses of an accurate caries susceptibility tests are**:**

1) For the clinician:

i) To determine the need for caries control measures

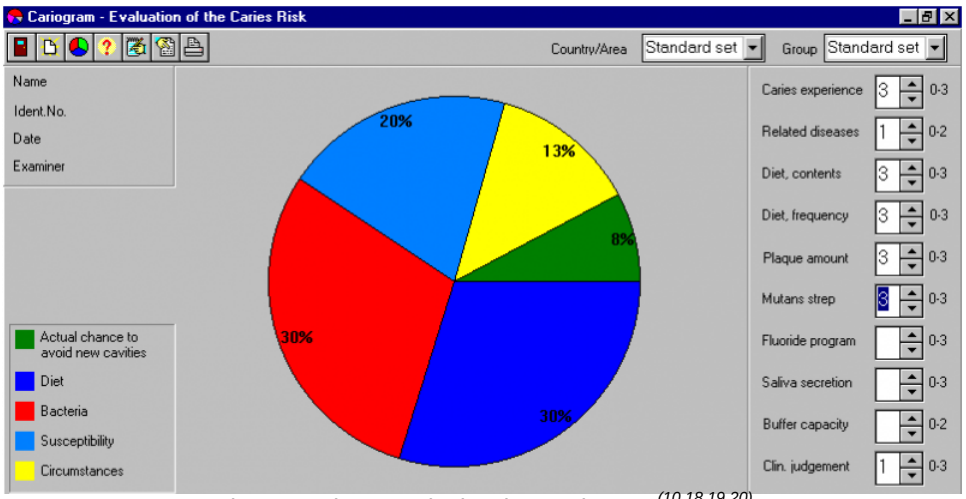

Figure 1: Photograph showing Cariogram[10,18,19,20]

ii) As an indicator of patient cooperation

iii) To act as an aid in timing recall appointments.

iv) As a guide to insertion expensive restoration.
v) To aid in the determination of prognosis.
vi) As a precautionary signal to the orthodontist in placing bands.

2) For the research worker:
i) As an aid in the selection of patients for caries study.
ii) To help in the screening of potential therapeutic agents.
iii) To serve as an indicator periods exacerbation and remission [9]

Some of the more widely used tests are:

1. Lactobacillus Colony Count Test: (Table 1)

Principle : This test, first introduced by Hadley in 1933 and popularised by Jay, is quantitative test, which 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 (pH 5.0) or Ragusa's SL Agar plates, after inoculation with a sample saliva .

Procedure: Saliva is collected before breakfast by chewing paraffin and collecting the saliva in the following three minute period in a sterile bottle. The specimen is shaken to mix it. A 1:10 dilution is prepared by pipetting out 1ml the saliva sample into a 9ml tube or sterile saline solution .This is shaken and a 1:100 solution is made by pipetting 1ml the 1:10m dilution into another 9ml tube sterile salt solution. The 1:100 dilutions are mixed thoroughly and 0.4ml each dilution is spread on the surface the agar plate with a bend glass rod. The plates are labelled and incubated at 37^0C for 3 - 4 days. A count of the number of colonies is then made by using the Quebec counter.

Advantages
i) It takes only a few minutes to do the test.
ii) Correlation with clinical caries activity has been demonstrated.
iii) It is still used as reference test for new caries activity test.

Disadvantages
i) The results are not available for several days.
ii) Counting colonies is a tedious process.
iii) Test is not simple; requires personnel with bacteriological training.
iv) Cost is relatively high. [4,9,10,11]

No lactobacillus per ml saline	Symbolic designation	Caries activity
0-1000	-	Little or non
1000-5000	+	Slight
5000-10,000	++	Moderate
>Than 10,000	+++ or ++++	Marked

Table 1: Results lactobacillus count. [4,10,11]

2. Snyder Test: (Table 2) [12.13]

Principle: The Snyder test measures the rapidity acid formation in sample stimulated saliva which is inoculated into glucose agar, adjusted to pH 4.7 – 5, and with bromocresol green as colour indicator. Indirectly the test is the measure acidogenic and aciduric bacteria.

Equipment: Saliva collecting bottles, paraffin, a tube Snyder glucose agar containing bromocresol green and adjusted to pH 4.5 - 5, pipettes and incubating facilities.

Procedure: Saliva is collected before breakfast by chewing paraffin. A tube Snyder glucose agar is melted and cooled to 50^0C saliva specimen is shaken vigorously for 3 minutes. Then 0.2ml saliva is pipetted into the tube agar and immediately mixed by rotating the tube. The agar is allowed to solidify in the tube and incubated at 37^0C. The colour change the indicator is observed after 24, 48 and 72 hrs incubation by comparing with an uninoculated tube against white background.

The Snyder test is simple, takes 24-48 hrs and requires only simple equipments; some training is needed and the cost is relatively low. This test meets some of the "ideal test" characteristics, Snyder and others have found a high correlation between clinical caries cavity and positive Snyder test results on a group basis. The best agreement was between a negative Snyder test and the absence caries activity. [4, 9, 10, 11]

	24 hours	48 hours	72 hours
Colour	Yellow	Yellow	Yellow
Caries activity	Marked	Definite	Limited
Colour	Green	Green	Green
Caries activity	Continue test	Continue test	Inactive

Table 2: Results Snyder Test. [4,10,11]

3. Alban's Test: (Table 3)

The Alban's test is a simplified substitute for the Snyder test. Because its simplicity, its low cost, its diagnostic value when negative results are obtained, and most all, its motivational value, the Alban's test is recommended, for all patients prone to caries, especially children undergoing

orthodontic treatment. The main features of Alban's test are as follows;

i) Use fewer agars in the medium so that the tubes do not require melting and cooling.
ii) Use a simpler sampling procedure in which the patient expectorates directly into the tube containing the medium.
iii) Instead the degree colour change as in the classical Snyder test, the Alban's test measures the depth to which the medium has turned yellow.
iv) A new method scoring provides a calibrated result.

Procedure: The Alban test medium is prepared by placing 60 gm Snyder test agar in 1L water ,and the suspension is brought to a boil over a low flame or a hot plate at medium heat. When thoroughly melted, the agar is distributed, using about 5ml per tube. The tubes should be autoclaved for 15 minutes, allowed to cool and stored in a refrigerator. Two tubes Alban medium are taken from the refrigerator and the patient is asked to expectorate small amount saliva directly into the tubes. The use of a sterilised glass funnel simplifies the collection. The volume saliva should be sufficient to cover the surface of the test medium. The tubes are labelled and incubated at 98.6/F (37/C) for 4 days. The tubes are observed daily for;

i) Change colour from bluish green (pH around 5) to Definite yellow (pH 4 or below).
ii) The depth in the medium to which the change had occurred.
iii) The results are recorded on the patients chart.

The following method is used for the final recordings (after 72 or 96hrs of incubation).

1) Readings negative for the entire incubation period are labelled "Negative".
2) All other readings are labelled "positive" whether +, ++, +++, or ++++.
3) Slower change or less colour change (when compared with previous test) is labelled "improved".
4) Faster change or more pronounced colour change when compared with previous test is labelled "worse".
5) When consecutive readings are nearly identical, they are labelled "no change".[10]

No colour change (negative)	-
Beginning colour change (from top medium down)	+
One-half colour change (from top down)	++
Three-fourths colour change (from top down)	+++
Total colour change to yellow	++++

Table 3: *Alban test suggests the following scale for scoring purposes (fig 1 & 2)* [10]

4. Reductase Test: (Table 4)

Principle: The test measures the rate at which an indicator molecule, diazoresorcinol, changes from blue to red and to colourless on reduction by the mixed salivary flora. Rapp claims that the test measures the activity a single enzyme, reductase. This enzyme is involved in some very definitive and limiting reactions in the formation of products dangerous to the tooth surface."

Equipment: The Reductase test comes in a kit ("Treate") which includes calibrated saliva collection tubes with the reagent on the inside of the tubes 'cap, plus flavoured paraffin.

Procedure: Saliva is collected by chewing a specially 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 diazoresorcinol, which colours the saliva blue. The change in colour after 30seconds and 15minutes is taken as a measure caries activity. Rapp has claimed a good correlation the results this test with clinical caries experience. Other investigators have reported a correlation between Reductase activity and the number salivary anaerobes.[4]

Colour	Time	Score	Caries Activity
Blue	15min	1	Non conducive
Orchid	15min	2	Slightly conducive
Red	15min	3	Moderately conducive
Red	Immediately	4	Highly conducive
Pink or white	Immediately	5	Extremely conducive

Table 4: Results the Reductase Test [4,11]

5. Buffer Capacity Test

Principle: The buffer capacity can be quantitated using either a pH meter or colour indicators. The test measures the number millilitres acid required to lower the pH saliva through an arbitrary pH interval, such as from pH 7.0 to 6.0 or the amount acid or base necessary to bring colour indicators to their end point.

Equipment: Needed equipment include a pH meter a titration equipment, 0.05 N lactic acid, 0.05 N base, paraffin and sterile glass jars containing small amount of oil.

Procedure: Ten ml stimulated saliva is collected under oil at least 1hr after eating; 5ml this are measured into a beaker. After correcting the pH meter to room temperature, the pH the saliva is adjusted to 7.0 by addition lactic acid or base. The level lactic acid in the graduated cylinder is re-recorded. Lactic acid is then added to the sample until a pH 6.0 is reached. The number millilitres lactic acid needed to reduce pH from 7.0 to 6.0 is a measure buffer capacity. This number can be converted to milli-equivalents per litre. There is a trend an inverse relationship between buffering capacity saliva and caries activity. This test however, does not correlate adequately with caries activity. [4]

6. Fosdick Calcium Dissolution Test (The Enamel Solubility Test)

Principle: The test measures the milligrams powdered enamel, dissolved in 4hours by acids formed when the patient's saliva is mixed with glucose and powdered enamel.

Procedure: The 25ml gum stimulated saliva is collected. Part this is analysed for calcium content. The rest is placed in a sterile tube with about 0.1gm powdered human enamel. The tube is sealed and agitated for 4hrs at body temperature after which it is again analysed for calcium content. The chewing gum to stimulate the saliva produces sugar, if paraffin is used in concentration 5% glucose is added. The amount enamel dissolution increases as the caries activity increases. In limited studies, the correlation is reported to be good. However, this test is not simple, the equipment is complex, personnel must be trained and the cost is high.[4, 10, 11]

7. Dewar Test:

Principle: This test is similar to the Fosdick calcium dissolution test, except that the final pH after 4hrs is measured instead the amount calcium dissolved. This procedure has not been adequately tested for clinical correlation. [4, 10]

8. Streptococcus Mutans Screening Test

A. Plaque / Toothpick Method: (Table 5)

Principle: The test involves a simple screening diluted plaque sample streaked on a culture media.

Procedure: Plaque samples are collected from the gingival third the buccal tooth surfaces (1from each quadrant) and placed in Ringer's solution. The sample is shaken and homogenized. The plaque suspension is streaked across a mitis-salivarius agar plate. After aerobic incubation at 37/C for 72 hrs, the cultures are examined under a low power microscope and the total colonies in 10 fields are recorded.

This test is an attempt to semi-quantitatively screen the dental plaque for a specific group caries inducing streptococci, S Mutans. The presence S Mutans in plaque and subsequent dental caries experience correlates best for patients in Grade 3 i.e., with large number of colonies.

Grades	Colonies / 10 fields
1	None
2	Less Than 8
3	More than or Equal to 8

Table 5: Results the Streptococcus Mutans Screening Test[(10)]

B. **Saliva /Tongue Blade Method :**

Principle: The test estimates the number S Mutans in mixed paraffin stimulated saliva when cultured on mitis-salivarious bacitracin (MSB) agar.

Procedure: This subject chews a piece of paraffin wax for 1minute to displace plaque microorganisms, thereby increasing the properties of plaque micro-organisms in the saliva. The subject's then are given a sterile tongue blade which they rotate in their mouth 10 times, so that both sides the blade are thoroughly inoculated by the subject's flora. Excess saliva is removed by withdrawing the tongue blade through closed lips. Both sides the tongue blade are then pressed into an MSB agar, which is then incubated at 37/C for 48hrs. Counts more than 100 colony-forming units (CFU) by this method are proportional to greater than 100 CFU S Mutans per ml saliva by conventional method. This simplified and practical method for field studies requires no transport media or dilution steps.

This test was developed for use with large numbers school children and avoids the necessity of collecting saliva.

C. **Streptococcus Mutans Adherence Method (Table 6)**

Principle: The test categorises salivary samples based on the ability S Mutans to adhere to glass surface when grown in sucrose containing broth.

Procedure: Unstimulated saliva (0.1ml) is inoculated in MSB broth. Inoculated tubes are set at 60^0C angle and incubated aerobically at 37^0C for 24 hrs. After growth has been observed, the supernatant medium

is removed and the cells adhering to the glass surface are examined microscopically and scored as follows. When the adherence score is +++, S Mutans is present at a level higher than 10^5 CFU ml whole saliva. If adherence is scored negative or positive S Mutans is present at less than 10^4 CFU per ml saliva. This method is potentially used for handling many samples in preventive practice and epidemiological studies because of its simplicity. [9]

No growth expressed	-
Few deposits ranging from 1-10	+
Scattered deposits smaller size	++
Numerous minute deposits more than 20 large size deposits	+++

Table 6: Streptococcus mutans adherence method.[10]

9. Prediction Future Caries Activity Based on Previous Caries Experience (Table 7): As an alternative to chemical or bacteriological test for determining caries activity, previous caries experience can be a reasonable indication for future trends. However, it is better to omit occlusal surfaces in such estimates.

Procedure: Koch grouped 9-10 years old children into a high caries active group and a low caries active group on the basis restored;

1) Proximal surfaces incisors and first permanent molars
2) Buccal surfaces upper first permanent molars
3) Lingual surfaces lower first permanent molars.

In addition these restored surfaces provided a type of caries index for that child. Those who had a score 4 or more were considered highly caries active, whereas those who had a score zero were considered low caries active. After one year, those in the highly caries active group developed 9.5 new caries tooth surface as compared to 5.3 in the low caries active group. This method can be used for identifying children with high and low caries activity and in screening children who require extensive preventive therapy. The serious drawbacks for using this method are:

1) Considerable caries will have already occurred in the population.
2) It is not applicable to the very young when preventive intervention is desirable.
3) Requires personal dental examination.
4)

Group	Caries Activity	Number Selected Restored	New Carious Surface	After 1yr
low caries active	Low	Zero	5.32	
High caries active	High	>4	9.52	

Table 7: Prediction Future Caries Activity Based on Previous Caries Experience.[3]

Dip slide test: This is a simple and inexpensive method. A special plastic Dip slide is coated with LBS agar. Undiluted saliva is flowed over the agar surface. This slide is then placed in a sterile tube which is closed tightly and then incubated for four days at 35^{o}C.Then slide is removed. The colour density is compared with a model chart. >10,000 colonies and <1000 colonies [10]

Plaque and saliva pH measurement test: The pH plaque and saliva may be measured directly, intraorally using glass or antimony electrodes.[10] Another method is the use of topical pH indicator. The methyl red has been used for this purpose. The methyl red changed from yellow at pH 6.0 to orange at 5.2 and to red below the pH 5.0. 0.1% solution methyl red is applied topically on the suspected site and the glucose solution is sprayed over the area. The sites that turn red are recorded. This method is very simple, inexpensive and can be used at chair side. Plaque pH can now also be measured by radio-telemetric method. This method involves the use very tiny transmitters filled in the prosthetic devices. A change in the pH noticed by the microelectrodes that contact the proximal surfaces teeth is transmitted to extra oral receivers. With the help this method continuous pH changes that occur in the experimental subject during various activities like eating or sleeping etc.[10] This method is useful for research purposes.[11]

Ora Test: [12]

This test was developed by Rosenberg et al., in 1989 for estimating oral microbial levels.

Principle: It is based on the rate of oxygen depletion by microorganisms in expectorated milk samples. In normal conditions the bacterial enzyme, aerobic dehydrogenase transfers electrons or protons to oxygen. Once oxygen gets utilized by the aerobic organisms, methylene blue acts as an electron acceptor and gets reduced to leuco-methylene blue. This reflects the metabolic activity of the aerobic organisms.

Procedure: Mouth is rinsed vigorously with 10 ml sterile milk for 30 seconds and 3 ml expectorate is collected. This is transferred to the screw cap tube with the help a disposable syringe. To this, 0.12 ml 0.1% methylene blue is added, thoroughly mixed and placed on a stand in a well illuminated area. The tubes are observed every 10 minutes for any colour change at the bottom using a mirror. The time taken for the initiation colour change within 6mm ring is recorded. The higher the infection, lesser the time required for the change in colour the expectorate reflecting higher oral microbial levels.

Advantages: Less time consuming, Economical, Non-toxic vehicle and easily learnt by auxiliary personnel.

Disadvantages: Lack specificity.[12,13]

Electronic Caries Detector: This device works on the principle that intact enamel is resistant to current passage than the enamel which is porous and contains saliva with all its electrolytes. Thus teeth showing less resistance to electronic current have more probability of getting carious.

<250000 ohms – caries susceptible and >60000 ohms – caries resistant. This method is more useful in detecting early pit and fissure lesions than the conventional probe. Plaitr et al., found a positive correlation between the diagnosis made by commercially available electronic caries detector and the histologically determined depth carious lesion.[14]

DMF Index: DMF index is used for assessing the status dental caries. DMF index for an individual is simply calculated as sum numbers decay (D), missing (M) and filled (F) teeth.

Rules for calculating DMF index:

1) Deciduous teeth should have lower letters (dmf)
2) Each tooth is counted only once. "A"coding. means absent for reasons other than caries. e.g. impaction anodontia, periodontal loss)

Shortcomings or drawbacks DMF index:

1) It tends to equate a diseased state with the treated condition.eg-Caries -1,Filled tooth-1
2) DMF score will never reduce even with best possible treatment.
3) Scoring system is equating different stages of caries destruction.
4) It fails to compensate for the prosthetic replacement of lost teeth.
5) Lack provision for arrested caries without structural loss.
6) It is not efficient tool for assessing long term effect preventive technique.

Arrested caries with no structural loss should not be equated to carious teeth. It must be equated with filled teeth.

New Scoring system of DMF Index:

1) Carious tooth-1
2) Missing due to caries-1
3) Correctly restored teeth- ½
4) Arrested caries without structural loss-1/2
5) Prosthesis -1/2

CLR Index (caries, lost and restored): Under the new scoring system DMF, a carious tooth should be given a score 1, a missing (due to caries) tooth that has not been prosthetically replaced should be given a score 1 but a correctly restored tooth is given a score ½. An arrested caries without structural loss is scored ½. If a prosthetic replacement is properly placed, the missing tooth is not scored and the prosthesis is given a score of ½. However any defective restoration or prosthesis needing replacement is scored only as carious or missing tooth and given the score of 1.

It is felt that such modification in scoring pattern the DMF will lead to confusion; the new index can be named as CLR index-i.e. Carious, Lost and Restored index. Restoration can be of any type-fillings, crowns, root canal treatments, bridges, dentures and implants. "C" can be further divided into 'C' and 'I' where 'C' denotes restorable carious tooth and 'I' non restorable teeth indicated for extraction. 'C', 'I' and 'L' are given score 1 each whereas 'R' is given ½.An arrested carious tooth without structural loss is given a score ½ and is treated as restored tooth. This can be termed as CILR index[8.]

Cariogram: The Cariogram is a graphical picture illustrating in an interactive way the patient's risk for developing new dental caries in the future. The dark blue sector 'Diet' is based on a combination diet contents and diet frequency. The Red sector 'Bacteria' - Based on a combination amount plaque and mutans streptococci. The Light blue sector 'Susceptibility' based on a combination fluoride program, saliva secretion and saliva buffer capacity. The Yellow sector 'Circumstances' - Based on a combination past caries experience and

related diseases. The Green sector shows estimation the 'Actual chance to avoid new cavities'. ('what is left' when the other factors have taken their shared) (Fig 1)

Applications: Illustrates the interaction caries related factors, Illustrates the chance to avoid caries, expresses caries risk graphically, recommends targeted preventive actions and can be used in the clinic & as an educational program.[18,19,20]

Summary and Conclusion

No single test can simultaneously measure host susceptibility or resistance, microbial pathogens and the cariogenicity diet. A combination laboratory tests however is required together with sound clinical judgement supported by careful records probability. Current research in developing simplified microbiological method for salivary lactobacillus and S Mutans counts and mechanical devices such as the electronic caries detector will extend the availability such techniques to a wider group dental practitioner. Dental caries is a multi-factorial disease and caries predictive tests do not encompass all those factors involved in determining caries resistance such as fluoride exposure, maturation enamel or immune protection.

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