CARIES ACTIVITY TESTS

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INTRODUCTION:

Dental caries can be defined as a carbohydrate modified, transmissible local infection, with saliva as a critical regulator. The diagnosis is most often based on clinical examination. Although clinical examination is certainly important, the modern dentistry diagnosis should be extended with identification and evaluation of factors related to, or the causative agents of the disease. The multifactorial etiology of dental caries is well known and the disease is therefore not only a treatable, but in most aspects also a preventable infection. Evaluation of etiologic factors can be made before clinical

signs occur. Subsequently, measures can be taken to reduce risk factors considered to create problem in the future.

Or Dental caries is defined as “a progressive irreversible microbial disease affecting hard part of tooth exposed to oral environment, resulting in demineralization of the inorganic constituents and dissolution of the organic constituents, there by leading to a cavity formation”.

The word caries is derived from Latin, meaning ‘rot’ or decay. It is similar to the Greek word ‘ker’ means death.

Benjamin Franklin said that “hot things, sharp things, sweet things, cold things, all rot the teeth and make them look like old things”.

"To run a caries preventive program without using microbiological methods is like running a weight control program without a scale."

Caries Activity:

Refers to the increment of active lesions (new and recurrent lesions), over a stated period of time.

“Measure of the speed of progression of a carious lesion”

Caries Susceptibility/Risk:

Refers to the inherent tendency of the host and target tissue, the tooth, to be afflicted by the caries process.

“Susceptibility of a tooth to a caries producing environment”

Difference between caries risk and caries activity:

From → To	**Means**
Sound → Carious	Caries risk assessment: before onset of disease.
Carious → More decay	Caries activity assessment: before progression of lesion

Caries Activity Tests:

Measure the degrees to which the local environment challenge (e.g. dietary effect on microbial growth and metabolism) favors the probability of carious lesion.

Caries Activity Test facilitates the clinical management of patients for the following reasons:

1. To determine the need and extent of personalized preventive measures.
2. To serve as an index of the success of therapeutic measures.
3. To motivate and to monitor the effectiveness of education progress relating to dietary and oral hygiene procedures.
4. To manage the progress of restorative procedures.
5. To identify high-risk groups and individuals.

Currently, no single caries activity test is sufficiently accurate or reliable.

According to Snyder are:

1. Should have maximum correlation between predicted and actual caries development.

2. Should have reliability and validity i.e. them test must be consistently accurate and reproducible.

3. Should have simplicity with regard to technical procedures and skills required.

4. The results should be obtained rapidly

5. Should have measurement of mechanism involved in caries process.

6. Should be inexpensive, non–invasive, easy to evaluate, and applicable to any clinical setting.

OBJECTIVE OF CARIES ACTIVITY TESTING:

To identify some parameter(s) related to the trial of challenge, defense and repair that will indicate impending or existent caries activity/ inactivity, which to formulate strategies for the prevention of disease.

Various caries activity tests are:

1. LACTOBACILLUS COLONY COUNT TEST (Hadley 1933):

Principle involved:

Estimation of the number of acidogenic and aciduric bacteria in the patient saliva by counting the number of colonies appearing on Tomato peptone agar plates (pH 5.0) after inoculation with a sample of saliva.

Procedure:

1. Immediately after arising, the patient chews a small piece of paraffin.

2. The saliva that accumulates in the following 3-minute period is collected in a sterile container, and shaken well.

3. The saliva sample is diluted to 1:10 dilution by sterile saline solution, and then 1:100 dilutions.

4. 0.4 ml of each dilution is spread on the surface of an agar plate containing

20 ml of cooled liquefied agar (Rogosa's SL agar plates, better then

Tomato Peptone agar).

5. Incubation at 37°C for 3-4 days.

6. Counting of colonies using colony counter equipped with bright light and a large magnifying glass.

7. The number of lactobacilli per mm saliva is calculated by multiplying the number of colonies on the plate by the dilution factor.

Interpretation:

No. of organisms/cc	Symbolic designation	Degree of caries activity
0-1000	+/-	Little or none

1000-5000	+	Slight
5000-10,000	+ +	Moderate
>10,000	+ + + or + + + +	Marked

Advantages:

1. Useful for monitoring the effectiveness of restorative dentistry.

2. Simple to carry out.

3. Useful as a screening test for caries activity in large groups.

Disadvantages:

1. Inaccurate for predicting the onset of caries.

2. Does not completely exclude the growth of other acid uric organisms.

3. Counts involving single individuals are not as reliable.

4. Takes few minutes to do the test, but the results takes several days.

5. Counting is a tedious procedure.

2. CALORIMETRIC SNYDER TEST (Snyder 1951):

Principle involved:

Measures the ability of salivary microorganisms to form organic acid from a carbohydrate medium. The medium contains an indicator dye “Bromocresol green”, changes color from green to yellow when pH changes from 5.4 to 3.8. Indirectly measures the number of both aciduric and acidogenic organisms in saliva.

Procedure:

1. 0.2ml stimulated saliva collected by chewing paraffin before breakfast is thoroughly mixed with 10 ml melted agar containing medium in a test tube (cooled to 50°C).

2. Allowed to solidify and then incubated at 37°C.

3. Amount of acid produced by acidogenic organisms is detected by changes in pH indicator, and is compared to an inoculated control tube after 24, 48, and 72 hours.

4. The rate of color, change from green to yellow is indicative of the degree of caries activity.

Interpretation:

If the color is yellow-

Time	**Color**	**Caries susceptibility**
24 hours	Yellow	Marked
48 hours	Yellow	Definite
72 hours	Yellow	Limited

If the color is green-

Time	**Color**	**Caries susceptibility**
24 hours	Green	Continue the test
48 hours	Green	Continue the test
72 hours	Green	Caries inactive

Advantages:

1. Relatively simple to carry out.

2. Tests are of value in assessing the cariogenic Challenge.

3. Only one tube, and no serial dilutions are required.

Disadvantages:

1. Time consuming.

2. Sometimes the color changes are not so clear.

3. THE SWAB TEST (Grainger et.al. 1965):

Principle involved:

Same as Snyder's test.

Procedure:

1. The oral flora is sampled by swabbing the buccal surfaces of teeth with a cotton applicator, which is subsequently incubated in the medium.

2. The change in the pH following 48 hour incubation is read on a pH meter or colour change is read by the use of a colour comparator.

Interpretation:

pH	**Caries activity**
4.1 or <4.1	Marked
4.2-4.4	Active
4.5-4.6	Slightly active
4.6 or >4.6	Caries inactive

Advantage:

Useful in predicting caries increments or changes, particularly in children, as no collection of saliva is required.

4. STREPTOCOCCUS MUTANS LEVEL IN SALIVA:

Principle involved:

Measures the number of S.mutans colony forming units per unit volume of saliva and plaque samples from discrete sites, such as occlusal fissures and proximal areas.

Incubation is done on Mitis Salivarius Agar(MSA), selective streptococcal medium with addition of high concentration of sucrose(20%), and 0.2 U Bacitracin (MSB), suppress the growth of most non-S.mutans colonies.

Procedure:

1. Sample collection by the use of tongue blades (wooden spatulas).

2. Tongue blades then pressed against MSB Agar.

3. Incubation at 37°C for 48 hours at 95:5 % CO2 gas mixture.

Interpretation:

Levels of Streptococcus mutans > 10^5/ml of saliva is unacceptable.

Advantage:

Useful in caries management as S.mutans are main causative agents.

Disadvantages:

1. Difficulty of distinguishing between a carrier state and cariogenic infection.

2. S.mutans may constitute less than 1 % of total flora of plaque.

3. S.mutans tends to be located at specific site only.

5. DIP-SLIDE (DENTOCULT-SM) METHOD FOR S.MUTANS COUNT:

Principle involved:

Estimation of Streptococcus mutans levels in saliva.

Procedure:

1. Undiluted paraffin- stimulated saliva is poured on a special plastic slide,coated with MSA(Mitis Salivarius Agar) containing 20% sucrose. The agar surface is thoroughly moistened and excess saliva is allowed to drain off.

2. Two discs containing 5 mg of bacitracin are placed on the agar 20 mm apart.

3. The slide is tightly screwed into a cover tube and incubated at 37°c for 48 hours in a sealed candle jar.

Interpretation:

Score 1 =Low:

The colonies are discrete and could be readily counted at 15X magnification with the total count of CFU inside the inhibitions zones less than 200.

Score 2 =Medium:

The colonies are discrete and the number in the zone of inhibition is more than 200 and 32X magnification.

Score 3 = High:

The colonies are tiny and almost completely or totally cover the inhibition zone with the number of colonies uncontrollable even with 32X magnification.

6. SALIVARY BUFFER CAPACITY TEST:

Principle involved:

Buffer capacity can be Quantities using either a pH meter or colour indicators.

This test measures the number of milliliters of acid required to lower the pH of saliva through an arbitrary pH interval (6 to 7) or the amount of acid or base necessary to bring color indicators to their end point.

Equipments:

Titration equipment

0.05 N lactic acid

0.05 N base, paraffin

Sterile glass jars containing a small amount of oil.

Procedure:

1. 10 ml of stimulated saliva is collected under oil at least I hour after eating.

2. 5 ml of this is measured into a beaker.

3. After correcting the pH meter to room temperature, the pH of saliva is adjusted to 7.0 by addition of lactic acid or base.

4. Lactic acid is then added to the sample until a pH of 6.0 is reached.

5. The number of ml of lactic acid needed to reduce pH from 7.0 to 6.0 is a measure of buffer capacity.(can be converted to mill equivalents per liter)

Interpretation:

“Inverse relationship between buffering capacity of saliva and caries activity”.

The saliva of individuals whose mouths contain a considerable number of carious lesions frequently has a lower acid-buffering capacity than the saliva of those who are relatively caries free.

Advantage:

Simple to carry out.

Disadvantage:

Doesn't correlate adequately with caries activity.

7. SALIVARY REDUCTASE TEST (SUSCEPTIBILITY TEST):

Principle involved:

Measures the activity of the reductase enzyme present in salivary bacteria, using a dye Diazo-resorcinol.

Procedure:

1. Saliva is collected in a plastic container.

2. The sample is then mixed with the dye.

3. The caries conduciveness is measured by color change, seen after 15 minutes.

(A kit is availably under the trade name Treatex.)

Interpretation:

The evaluation is based on the color change:

Color	Time	Score	Caries activity
Blue	15 min.	1	Non conducive
Orchid	15 min.	2	Slightly conducive

Red	15 min.	3	Moderately conducive
Red	Immediately	4	Highly conducive
Pink or White	Immediately	5	Extremely conducive

Advantages:

Quick results, as no incubation period is required.

Disadvantage:

Test results vary with time after food intake and after brushing.

8. ALBANS TEST:

It is a simplified substitute for the Snyder test.

Principle involved:

Same as Snyder test.

Procedure:

To prepare the Alban test medium:

Materials required-

. Snyder test agar

. A small scale, to measure 60 grams.

. A 2 liter Pyrex glass, to melt the medium.

. A funnel, to dispense the medium into test tubes.

. 100, 16 mm test tubes with screw caps.

60 grams of Snyder test agar is placed in 1 liter of water and the suspension is brought to a boil over a low flame. When thoroughly melted, the agar is distributed about 5 ml per tube. These tubes should be autoclaved for 15 min. Then cooled and stored in a refrigerator.

Steps:

1. 2 tubes of Alban medium are taken from the refrigerator.

2. The patient is asked to expectorate a small amount of saliva directly into the tubes.

3. The tubes are labeled and incubated at 98.6°F (37°C) for up to 4 days.

4. The tubes are observed daily for;

   a. Change of color from bluish green (pH 5) to definite yellow (pH 4 or below)

   b. The depth in the medium to which the change has occurred.

5. The daily results collected for a 4 day period recorded on the patient chart.

Interpretation:

Level of color change	**Scoring**
No change	¾
Beginning of color change (from top)	+
One half color change (from top)	+ +
Three forth color change (from top)	+ + +
Total color change to yellow	+ + + +

Inferences:

1. Readings negative for the entire incubation period are labeled- Negative.

2. All other readings are labeled- Positive (+, + +, + + + or + + + + +)

3. Slower change or less color change (compared to previous test) is labeled- Improved.

4. Faster or more pronounced color change (compared to previous test) is labeled- Worse.

5. When consecutive readings are nearly identical, labeled as- No change.

Advantages:

1. Use of a somewhat softer medium that permits the diffusion of saliva and acids without the necessity of melting the medium.

2. Use of a simpler sampling procedure in which the patient expectorates directly into tubes that contain the medium.

3. Low cost.

4. Diagnostic value even when negative results are obtained.

5. Motivational value (ideal for education).

6. Good for indicating caries inactivity.

Disadvantages:

1. More armamentaria required.

2. Based on subjective evaluation of a color change that is often not clear cut.

9. STREPTOCOCCUS MUTANS SCREENING TEST:

A. Plaque / tooth pick method:

Principle involved:

Simple screening of dilutes plaque sample streaked on a selective culture media.

"Semi quantitative screening of dental plaque for S.mutans"

Equipments:

Sterile tooth picks.

Sterile ringer's solution (5ml).

Platinum Loop.

Mitis Salivarius Agar plates (MSA) containing sulphadimetine.

Procedure:

1. Plaque samples are collected from the gingival thirds of buccal tooth surfaces one from each quadrant and placed in Ringer's solution.

2. The sample is shaken until homogenized.

3. The plaque suspension is stretched across MSA plates.

4. Aerobic incubation at 37°C for 72 hours.

5. Cultures are examined and total colonies in 10 fields are recorded.

B. SALIVA / TONGUE BLADE METHOD:

Principle involved:

Estimation of the number of S.mutans in paraffin-stimulated saliva when cultured in Mutans Salivarius Bacitracin (MSB) agar.

Equipments:

Paraffin wax.

Sterile Tongue Blades.

Disposable Petri Dish containing MSB agar.

Procedure:

1. The subjects chew a piece of paraffin wax for one min to displace plaque microorganisms, to increase their proportion in saliva.

2. Sterile tongue blades are rotated in the mouth 10 times so that both the sides are thoroughly inoculated with the subject's flora.

3. Tongue blades are then pressed into MSB agar.

4. Incubation is done at 37°C.

5. Numbers of colonies are counted.

Advantages:

1. Simple, practical method for field studies as there is no requirement of transport media/dilution.

2. Suitable for use in the studies involving school children.

10. FOSDICK CALCIUM DISSOLUTION TEST:

Principle involved:

Measurement of amount of powdered enamel dissolved in 4 hours by acid formed, when the patient's saliva is mixed with glucose and powdered enamel.

Procedure:

1. Saliva is stimulated by having the patient to chew gum or paraffin.

2. 2.5 ml of saliva is collected.

3. One part of this is used to analyze the calcium content.

4. The remaining is taken into a 8 inch sterile test tube, in which 0.1 gm of powdered enamel is added.

5. Test tube is sealed and shaken for 4 hours at body temperature.

6. Then calcium content is analyzed.

7. When paraffin is used to stimulate saliva, 5% glucose is added.

(Chewing gum contains sugar).

Interpretation:

Amount of calcium increases, as the caries activity increases.

Advantage:

In the limited studies, correlation reported is good.

Disadvantages:

1. Requires complex equipment and trained personnel

2. Expensive.

11. DEWAR TEST:

Principle involved:

Same as Fosdick Calcium Dissolution test.

Procedure:

Same as Fosdick Calcium Dissolution test, difference is in Dewar test *final pH after 4 hours is measured,* instead of the amount of calcium dissolved.

This procedure is not commonly used, as it has not been adequately tested for clinical correlation.

LIMITATIONS OF CURRENT CARIES ACTIVITY TESTS:

1. Since caries activity tests measure a single parameter like acid production or colony count of bacterial species, none of these tests are highly reliable indicators of expected caries increments. It is better to use combination of tests to solve this problem.

2. Most of the tests are time consuming.

3. There is need to develop chair side tests.

FUTURE METHODS:

A serious concern with the culturing methods of today is the time span from sampling until the results are available for the professionals and their patients. It is necessary to improve today's methods to make them suitable for chair side use or for field condition.

New tests, measuring, for example, bacterial adhesion and bacterial binding saliva ligands as genetically determining factors for caries, might be developed.

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REFERENCES

1. Klock B, Krasse B. A comparison between different methods for prediction of caries activity. European Journal of Oral Sciences. 1979 Apr;87(2):129-39.
2. Nishimura M, Oda T, Kariya N, Matsumura S, Shimono T. Using a caries activity test to predict caries risk in early childhood. The Journal of the American Dental Association. 2008 Jan 1;139(1):63-71.
3. Wyne AH, Guile EE. Caries activity indicators. A review. Indian Journal of Dental Research: Official Publication of Indian Society for Dental Research. 1993 Apr 1;4(2):39-46.
4. Wyne AH, Guile EE. Caries activity indicators. A review. Indian Journal of Dental Research: Official Publication of Indian Society for Dental Research. 1993 Apr 1;4(2):39-46.
5. Shi S, Deng Q, Hayashi Y, Yakushiji M, Machida Y, Liang Q. A follow-up study on three caries activity tests. Journal of Clinical Pediatric Dentistry. 2003 Jul 1;27(4):359-64.
6. Tenovuo J. Salivary parameters of relevance for assessing caries activity in individuals and populations. Community dentistry and oral epidemiology. 1997 Feb;25(1):82-6.