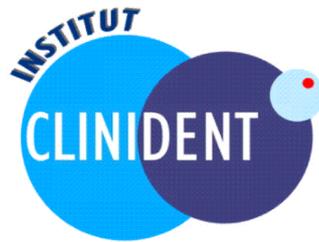


CARIO-ANALYSE



How to measure risk of caries and prevent disease using Cario-ANALYSE testing



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Caries disease is the consequence of prolonged presence of specific bacteria biofilm (plaque). Bacterial identities and quantities can be associated with caries risk measurements and oral preventive actions.

Dental caries has been an important public health problem worldwide. Information about an individual's caries risk and state of carious lesions should be used in making a personalised treatment plan. Recent studies have successfully identified patients with a high or low risk of primary caries by using Classification and Regression Trees (CART) according to the number of specific cariogenic bacteria, as well as those at risk of secondary caries according to the number of particular bacteria and previous caries experience. The same study also provides information regarding how long it takes to develop a new carious lesion for each high and low risk group. This information assists in planning a personalised regular preventive program with an interval appropriate to the individual's risk of caries. The origin of caries disease is the formation of bacterial biofilm with specific pathogenic bacteria on the teeth occlusal surface. The pathogenic bacteria are present in the saliva of patients before plaque formation. The colonization of the mouth by caries bacteria can take place at an early age of the patient but can also take place after dental prosthetic installation or orthodontic treatment for patients with poor oral hygiene and non-adapted diet.

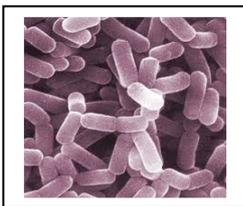
- ✓ **The non-existence of a certain bacteria strain (*Streptococcus mutans*) in the oral cavity will prevent oral pathologies and will guarantee the absence of caries biofilm and disease development.**
- ✓ **The growth of certain bacteria strains (*Lactobacillus spp.* and *Streptococcus mutans*) at a certain level during reduced period (approximately 3 months) is a significant biological signal of increased risk of caries.**
- ✓ **Abnormal salivary function (limited saliva buffer capacity, Sjoreen syndrome, hyposialia following head and neck irradiation, genetic diseases) will increase the risk by increasing the pathogenic bacteria plaque quantity.**
- ✓ **A fixed installation of retentive elements (orthodontic devices) that remain in the mouth for at least 1 or 2 years' results in a multiplication of cariogenic microorganisms and caries risk increase. cariogenic microorganisms (*Streptococcus mutans* and *Lactobacillus spp*) have a significant increase at 6 months following placement of orthodontic devices due to the irregular nature of their surfaces, which promote the growth of these aciduric and acidogenic bacteria that prefer hard surfaces to grow on.**

A great number of cases of caries disease patients can be maintained under control for years by dentists and/or dental hygienists according to the use of the biological risk assessment measures to guide clinical decision making.

Assessment tools definition:

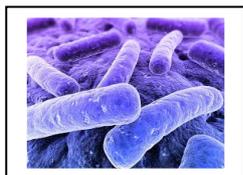
- patient history (primary or secondary caries)
- clinical examination
- diet analysis
- radiographic assessment
- salivary and plaque analysis including bacterial identification and ratio evolution.

The list of pathogenic bacteria strain to be at high risk for caries and need specific treatment by a specialized dentist:



Lactobacillus spp.

The genus *Lactobacillus* currently contains over 180 species and encompasses a wide variety of organisms. Major species are *L. casei* group, and the species *L. acidophilus*, *L. salivarius*, and *L. reuteri*. They are a major part of the lactic acid bacteria group (i.e. they convert sugars to lactic acid). In humans, they constitute a significant component of the microbiota of saliva and are responsible for acid production and low pH situation.



Streptococcus mutans

Streptococcus mutans is a cariogenic microorganism that breaks down sugar for energy and produces an acidic environment, which demineralizes the superficial structure of the tooth. Transmission of *S. mutans* can be found in people of all ages although it is more common in infants and children. There is transmission of *S. mutans* from mothers to their children.

After adherence to the tooth, *S. mutans* begin to divide and produce microcolonies within the slime layer to construct a biofilm. *S. mutans* begin to grow and synthesize dextran with the enzyme dextransucrase. Dextran contains a capsule that binds to the enamel and forms a biofilm. The biofilm produce fructose fermented as an energy source for bacterial growth while glucose is polymerized to an extracellular dextran polymer matrix of dental plaque. The dextrin can also depolymerize to glucose and use the monosaccharide as a carbon source, which produces lactic acid in the biofilm by decalcifying the enamel, and leads to dental caries. The combination of acid and plaque results in the causative agent of decay. It has been estimated that there are over 100 million in every milliliter of saliva from more than 600 different species. In order to decrease caries risk, it is encouraged for people to have less than 10,000 CFU per ml of saliva *Streptococcus mutans* in their mouth.

Pathogenic threshold for *Streptococcus mutans*

Colony Forming Units CFU *S. mutans* /ml saliva

class 0-1	<100,000
class 2	100,000< CFU/ml <1,000,000
class 3	>1,000,000 CFU/ml

In the table, class 0-3 is referring to how many *Streptococcus mutans* reside in the mouth with class 0-1 acting as best case with good oral hygiene, while class 3 acts as the worst case. Several investigations presented a significant increase in the cariogenic microorganisms *Streptococcus mutans* and *Lactobacillus* spp in saliva after commencing fixed orthodontic therapy. A recent report from Arino et al, in Osaka, aims to prove how preventive therapies reduce the onset of caries in adult patients, and to identify patients with high or low risk of caries.

Technical recommendation and objectives:

For dentists and hygienist, it is advised to carry out an analysis in the following situations:

- first visit
- patient with primary or secondary caries
- diabetic patient
- patient that smoke
- patient with poor oral hygiene and/or poor diet
- patient with orthodontic prosthesis
- rapid progressive caries patients
- children to be motivated or controlled
- 3 months after the first Cario-Analyse test
- every year for all patients during annual visit

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Cario-Analyse

Analytical report

Cario-Analyse - Microbial Test for Evaluation of Caries Risk

Dentist information

Name : Sophie COUTURIER
Address : 3 Cours Mirabeau
13100 AIX-EN-PROVENCE
e-mail : cabinetsc@gmail.com

Sample information

Patient : WU Fei
Saliva sample
Accession number : FCAR170116-001
Date of collection : 12/01/2017
Date of analysis : 16/01/2017

Measurement of the buffer capacity of saliva

The buffer capacity of saliva is low

Bacterial Quantification with real-time Polymerase Chain Reaction technology

Bacteria	Number of bacteria / ml of saliva	% bacteria / total bacteria
Total bacteria	3,2E+07	100%
Lactobacillus spp.	3,0E+05	0,96%
Streptococcus mutans	0,0E+00	0,00%

Microbiological situations/Treatment considerations

If primary caries lesion:

Low risk patient Second test in 12 months during annual check-up

If secondary caries lesion:

Moderate risk patient Second test in 3 months for new risk evaluation after curative therapy and preventive program

Risk & threshold definition

Caries risk levels for Lactobacillus spp. (LB) and Streptococcus mutans (SM) for primary and secondary lesions :

Primary caries lesion

Low risk : < 1E+06 SM /ml of saliva

High risk : ≥ 1E+06 SM /ml of saliva without preventive program

Secondary caries lesion

Low risk : < 1E+04 LB /ml of saliva and < 1E+06 SM /ml of saliva with good compliance with a preventive program

Moderate risk : ≥ 1E+04 LB /ml of saliva and < 1E+06 SM /ml of saliva

High risk : ≥ 1E+04 LB /ml of saliva and ≥ 1E+06 SM/ml of saliva

All these risks could be increased by low saliva buffer capacity.

All these risks are increased by number of caries experience > 15.

All these risks are reduced by personal intensive preventive care (fluoride, plaque control, dietary plan).

Lactobacillus salivarius-containing tablets were suggested to increase resistance to caries risk factors.

Definitions

Analyse is performed on saliva sample. Minimal volume required for complete analysis is 600 µl of saliva.

Genomic DNA is extracted from the submitted sample and tested for micro-organisms associated with caries risk.

The bacterial DNA is tested by quantitative Polymerase Chain Reaction (PCR) amplification method.

Secondary carious lesion referred to a substantial tooth decay at a margin of an existing restoration.

Preventive program : education, plaque control, diet, no smoking, scaling, polishing, fluoride application.

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