

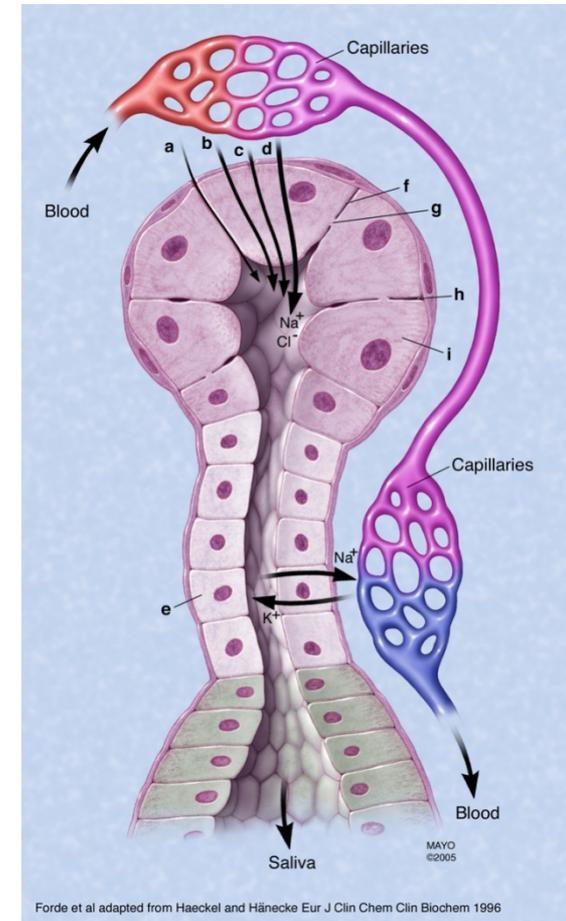
Biomarkers into saliva



Saliva used as biomarker for risk for caries

Saliva composition ?

- Saliva comes from blood serum associated with oral microflora and viruses :
- Hormones: cortisol, melatonin...
- Chemical: drugs, ethanol...
- Immunoglobulins: IgA
- Sugar: glucose
- Bacteria: caries, periodontal, helicobacter pylori
- Virus: HBV, HPV
- Free mRNA and cells



Saliva hormones

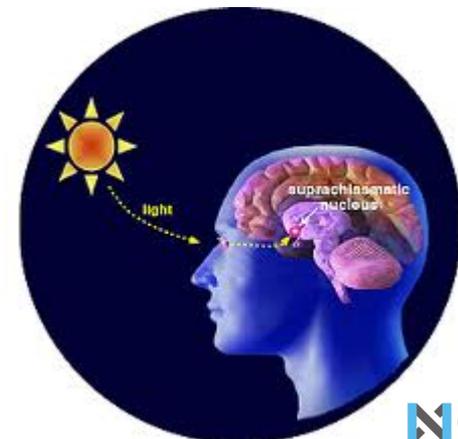
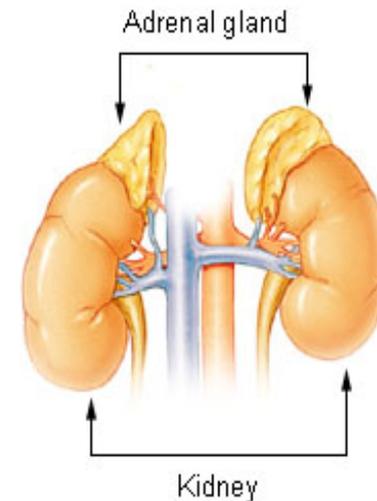
- Cortisol, melatonin, testosterone, DHEA...

2008, Restituto, et al., : cortisol dosage into saliva is equivalent to serum (Addison disease screening).

2010 Sakihara, et al., : cortisol saliva dosage is equivalent to serum and urinea (Cushing syndrome diagnostic)

2010 Bagcim, et al., : melatonin level into saliva is Equivalent to serum

Adrenal Gland



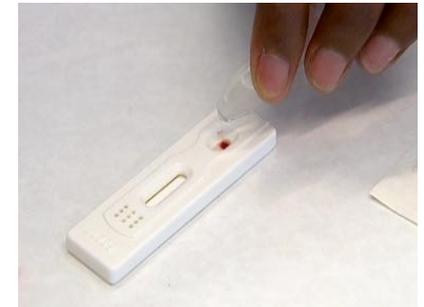
Chemical in saliva

- Alcohol, drugs, organic molecules, pesticides, solvents

Ethanol, methanol, ethylene glycol are all detected in saliva & urine (Skin, et al., 2008) and exhaled breath

2011 Vindenes, et al., shows that morphine, amphetamine, methamphetamine, N-desmethyldiazepam, benzodiazepines, cannabis & cocaine are very well detected into saliva

(PCP, pesticides, solvents...)



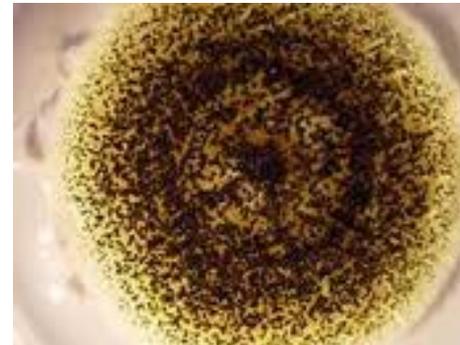
Immunoglobulins in saliva

- Allergies

IgA ratio in saliva changed for population having immuno-sensitivity to peanuts
(2011 Peeters, et al.,)



Saliva IgA increased in presence of fungus mycotoxins (2003 Vojdani, et al.,)



Sugar in saliva and diabetic patient

- Diabetic situation

Sugar quantity in saliva increases for diabetic patients.

2009 Rao, et al., 65 proteins in saliva are specific biomarkers for type-2 diabetic.

2010 Soell, et al., overexpression of saliva chromogranin A for diabetic patients.



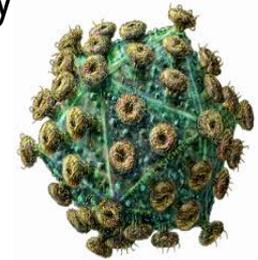
Bacteria and Virus

- Infectious diseases

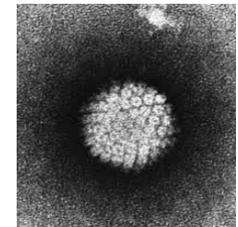
Caries bacteria (*Streptococcus mutans*...) cause dental plaque



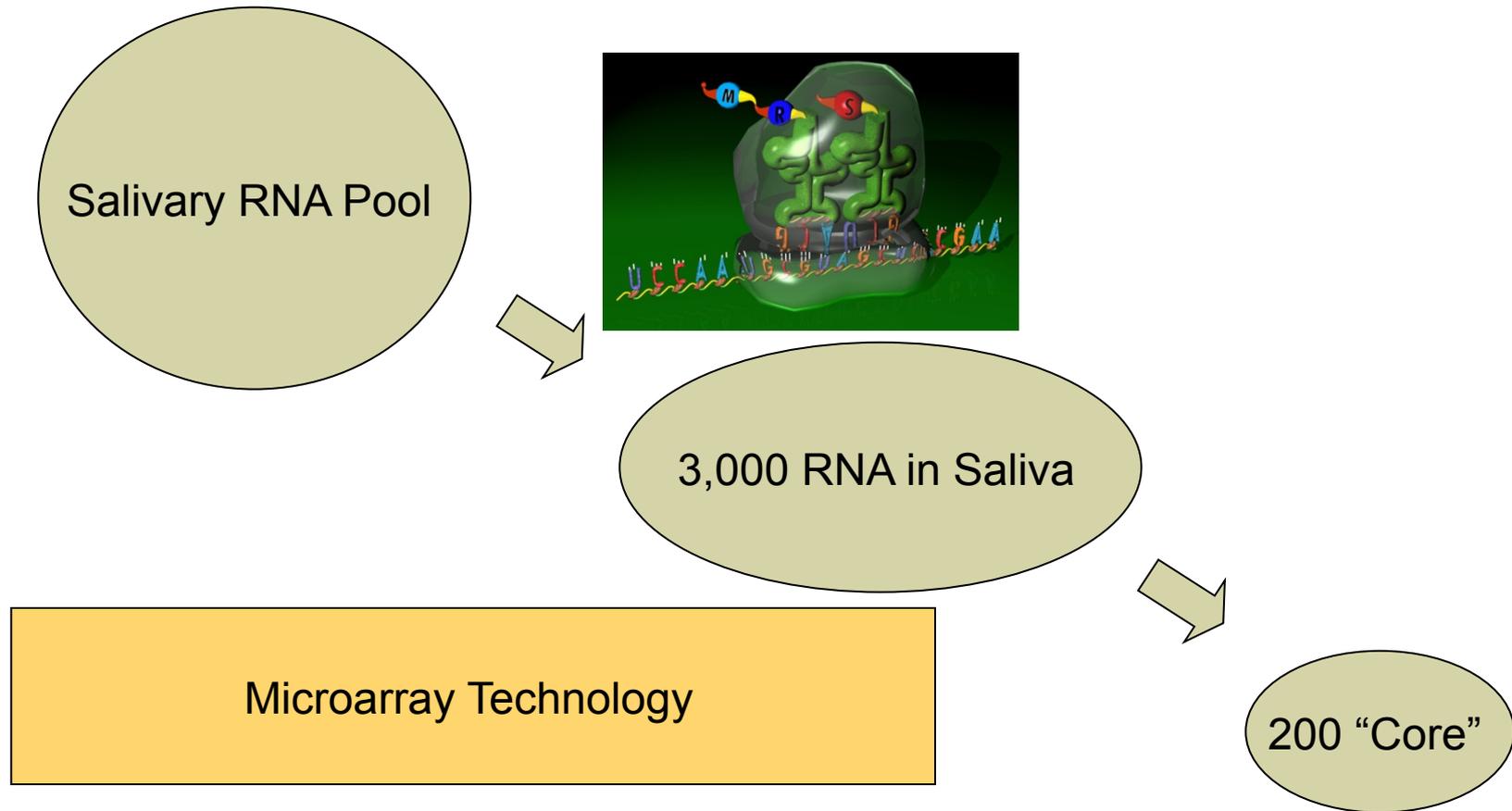
HIV: anti-HIV antibodies are present into saliva with 100% specificity (2008 by Zelin, et al., Pascoe, et al.,)



HPV is directly detected into saliva and represents a risk factor for Oral Cancer development comparable to HPV in cervical cancer



Free mRNA are detected into saliva



Yang Li, et al. *Journal of Dental Research*, 83(3), 199-203, 2004

Cario-analyse
Leveraging saliva components to
improve prognosis and treatment of
caries

« Early diagnosis ensures better prognosis »

Dr Franck Chaubron (PhD)
CEO
Institut Clinident – Aix en Provence- France

Saliva Biomarkers for caries risks

CARIO-ANALYSE



How to measure caries risks and prevent caries disease using Cario-ANALYSE testing procedure

Caries associated with biofilm (plaque formation)

Caries is the consequence of specific bacteria biofilm (plaque) presence.

Bacterial identities and quantities could be associated with caries risk measurements and oral preventive actions.

The origin of caries disease is the formation of bacterial biofilm with specific pathogenic bacteria on teeth.

Pathogenic bacterias are present in the saliva of patients before plaque formation.

The colonization of the mouth by caries bacterias can take place at an early age of the patient life but can also take place after dental prosthetic installation or orthodontic treatment for patient with poor oral hygiene and non adapted diet.

Bacteria identities

The absence in the oral cavity of a certain bacteria strain (***Streptococcus mutans***) will prevent the caries biofilm formation.

The growth of certain bacteria strain (***Lactobacillus spp.*** and ***Streptococcus mutans***) at a certain level during reduced period (about 3 months) is a significant biological signal of caries risk increasing.

Abnormal salivary function (limited saliva buffer capacity, Sjoreen syndrome, hyposialy following head and neck irradiation, genetic diseases) will increase the risk by increasing the pathogenic bacteria plaque quantity.

Streptococcus mutans and ***Lactobacillus spp*** have a significant increase at 6 months following placement of orthodontic devices explained by the irregular nature of their surfaces, which promote the growth of these aciduric and acidogenic bacterias that prefer rough surfaces to grow on.

Lactobacillus & Streptococcus mutans

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). For human beings, they constitute a significant component of the microbiota of saliva and are responsible of acid production and low pH situation.

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 for infants and children. There is transmission of *S. mutans* from mothers to their children .

Pathogenic action & quantities in saliva

After adherence to the tooth, *S. mutans* begins to divide and produce microcolonies to construct a biofilm producing lactic acid by decalcifying the enamel, and leads to dental caries. The combination of acid and plaque results in the causative agent of caries.

It has been estimated that there are over 100 millions in every milliliter of saliva.

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

Cario-Analyse® kit composition

Cario-Analyse® sampling kit :

- 1 sampling green envelope with mark identification (Cario-Analyse ®)
- 1 sterile 4ml tube (Simport Cryovial steril)
- 1 sterile single-use syringe (B-Braun model Injekt)
- 1 Tyvek envelope with the NOVEO and/or its affiliates address



Pathogenic Threshold

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 : $\geq 1E+06$ 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 : $\geq 1E+04$ LB /ml of saliva and $< 1E+06$ SM /ml of saliva

High risk : $\geq 1E+04$ LB /ml of saliva and $\geq 1E+06$ SM/ml of saliva

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

All these risk 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.

Cario-Analyse® report



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

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

Definitions

Analysis 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.

References

- Arino M. et al. Multicenter study on caries risk assessment in adults using survival Classification and Regression Trees. Sci. Rep. 6, 29190; doi: 10.1038/srep29190 (2016).
- Edelstein BL, Ureles SD, Smaildone A. Very High Salivary Streptococcus Mutans Predicts Caries Progression in Young Children. Pediatr Dent. 2016;38(4):325-30.
- Nishihara T, Suzuki N, Yoneda M, Hirofujii T. Effects of Lactobacillus salivarius-containing tablets on caries risk factors: a randomized open-label clinical trial. BMC Oral Health. 2014 Sep 2;14:110. doi: 10.1186/1472-6831-14-110.

Real Time PCR Method and References

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Recommendation and objectives of the test

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

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

Risk reduction

For dentists and hygienists, treatment for risk reduction should be

- Dietary changes (reduction of acidification)
- Hygiene protocols to reduce plaque
- Increase saliva quantity and turnover by stimulation to reduce acids
- Increase microbial competition (probiotics)
- Use of varnish protocols
- Regular controls of patient with orthodontic appliances
- Regular visit and controls for at risk patient

Thank you

