Laboratory Examination of Saliva

MBS 240 – Lab Science

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Objectives

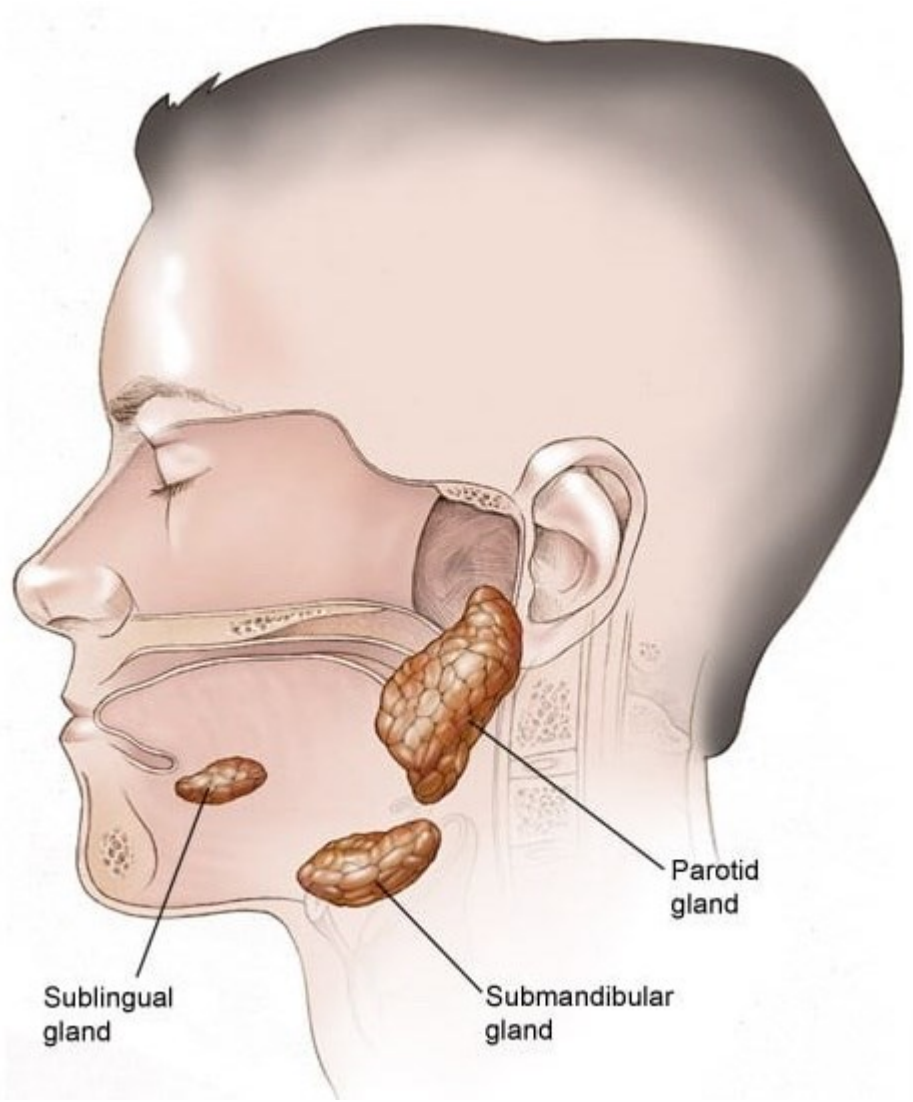
- To define saliva and understand the general properties and composition
- to list the functions of saliva
- Differentiate between total and partial saliva
- To briefly discuss the different saliva collection methods.
- To introduce various assays used to analyse the saliva samples.

What is Saliva?

A clear, tasteless, hypotonic, odourless, slightly acidic (pH 6.8) viscous fluid, consisting of the secretion from the parotid, sublingual, submandibular salivary glands and the mucous glands of the oral cavity.

General properties

- **Volume**: 500 to 1500 mL of saliva is secreted per day and, it is approximately about 1 ml/ minute.
- **Contribution by each major salivary gland is:**
  - Parotid glands: 25%
  - Submandibular glands: 70%
  - Sublingual glands: 5%

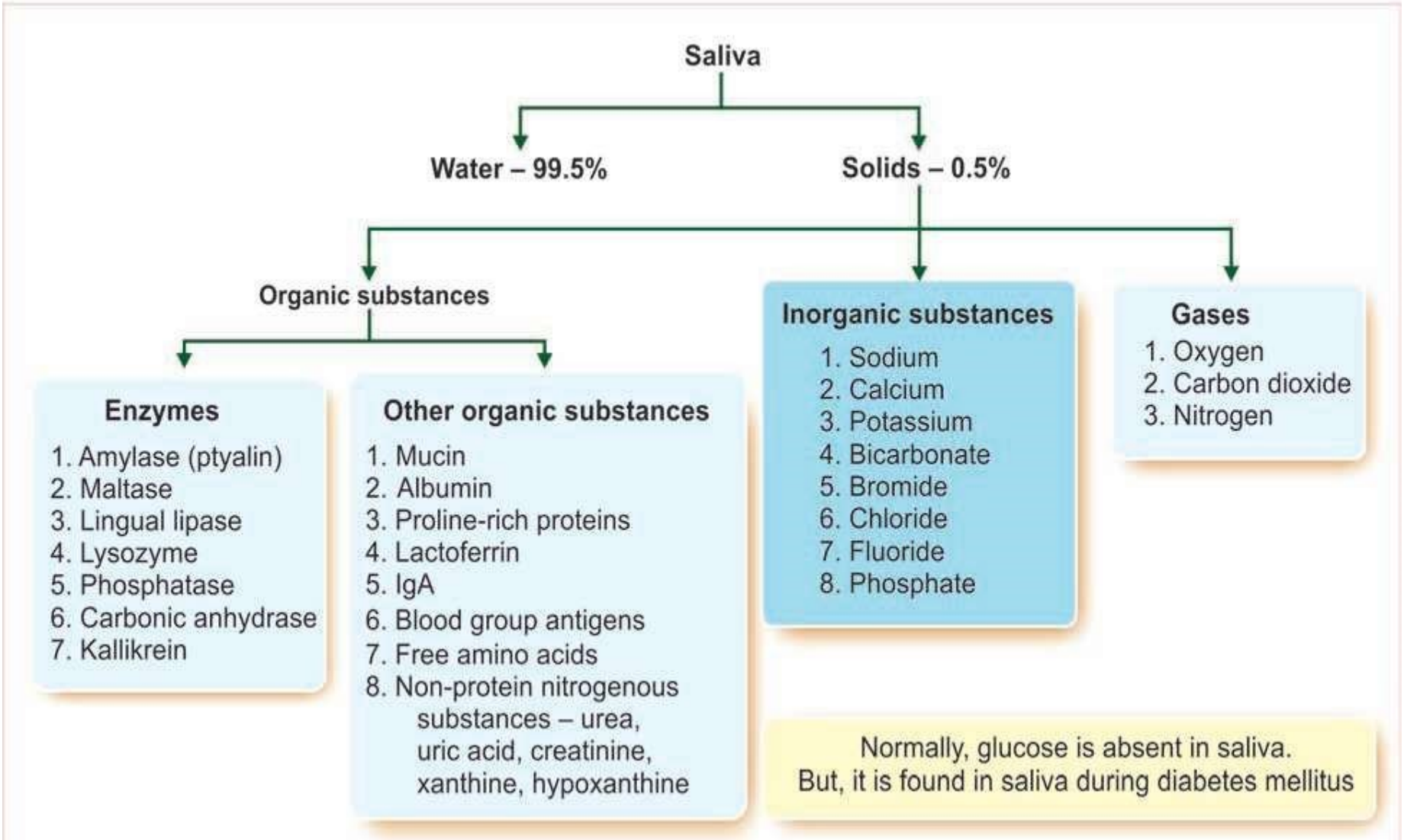


General properties

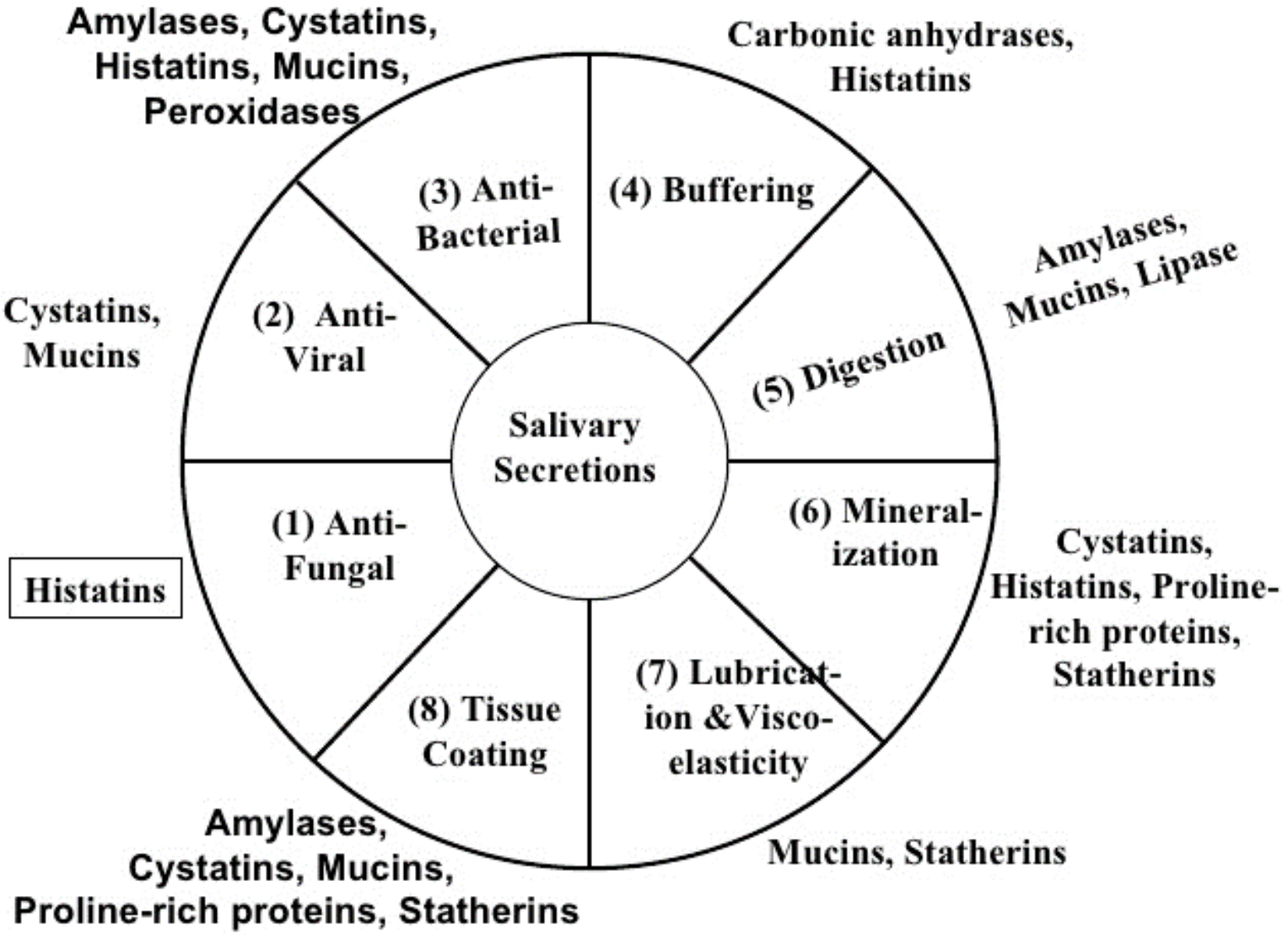
- **D**uring Unstimulated Flow (resting salivary flow—no external stimulus) there is typically 0.2 mL – 0.3 mL per minute.
- Stimulated Flow (response to a stimulus, usually taste, chewing, or medication [e.g., at meal-time]) increases to typically 1.5 mL – 2 mL per minute

Composition

- Saliva contains 99.5% water and 0.5%solids.



Functions of Saliva



Saliva-
Collection methods

1. **Total saliva**: composed of the mixture of saliva derived from all glands
2. **Partial saliva**: composed by the secretion of only one gland.

Total saliva is preferred over partial saliva for biomarkers determination due to its easy collection

Saliva as a non-invasive sample

Methods of obtaining Total unstimulated saliva

Method		Collection tool	Advantages	Disadvantages
Drainage		Salivary flow into a funnel that will be connected to a graduated sterile tube	Lower risk of sample contamination, increased reliability of analytes by not stimulated saliva	Analytes alteration due to the presence of lipstick or other chemical substances on the lips
				Difficult in patients with dementia, severe disability, or any disease that prevents proper collection of the sample
Spitting		Saliva flow is accumulated for a certain time and then spat out in the collection vessel	Lower risk of sample contamination, increased reliability of analytes by not stimulated saliva	Analytes alteration due to the presence of lipstick or other chemical substances
				Difficult in patients with dementia, severe disability, or any disease that prevents proper collection of the sample
Ejector		An ejector (plastic or glass) connected to a vacuum pump stores saliva into a sterile graduated tube	Greater patient comfort because does not need collaboration, it is fast and not painful	Greater risk of contamination and analyte alteration by interposing more materials until the collection container is reached and the degree of stimulation of glands
			It can be performed in patients with disabilities, dementia, etc	More expensive equipment

Saliva as a non-invasive sample

Methods of obtaining Total unstimulated saliva

Method		Collection tool	Advantages	Disadvantages
Syringe collection		It is collected by sterile syringe and needle between the patient's lips	Little alteration of analytes	Difficult in patients with a low amount of saliva
Cotton		Cotton rolls are placed in the vestibular and sublingual area for 2 min	Easy. Useful in patients with difficulty to cooperate	Alteration of analytes absorbed by the cotton

Saliva as a non-invasive sample

Methods of obtaining Total stimulated saliva

Allow assessing of the gland's response to a stimulus.

1. Mechanical stimulation
    1. Chewing a paraffin
    2. Saxon test
2. Chemical stimulation
3. Stimulation with pharmacological substance e.g. pilocarpine
4. Electrostimulation
5. Acupuncture

Saliva as a non-invasive sample

Methods of obtaining partial saliva

1. **Cannulas**: Thin polyethylene tubes with diameters ranging from 0.5 to 1.5 mm are introduced directly into the excretory duct of the target gland.
2. **Lashley capsule**: obtain saliva principally from the parotid gland using disk with two completely separate concentric chambers generating two compartments.
3. **Schneyer segregator device**: developed for specific collection of the submandibular and sublingual glands secretions

Saliva as a non-invasive sample

Methods of obtaining partial saliva

4. **Periotron absorbent strips**. These strips are mainly used for saliva collection from minor salivary glands located above all on the lips.

Advantages of saliva compared to other samples

	Blood	Feces	Urine
SALIVA ADVANTAGES	Not painful	Less uncomfortable	
	No need of specific training		
	Ready for its immediately use		
	Easier transport and storage		
	Not invasive		
	No stress		
	No fear		
	Less risk of infections		

Disadvantages of saliva compared to other samples

- Inter- and intra- subject variability
- Inter-laboratory variability
- Blood contamination
- Low concentration of target analyte
- No assays or reference materials available for saliva measurements
- Not consistent results

Methodology assays for the salivary biomarkers' identification and measurement

A biomarker is a measurable indicator of a specific biological state, which reflects the interaction between a biological system and a potential hazard or a positive situation.

Methodology assays for the salivary biomarkers' identification and measurement

Examples include;

1. Antibodies
2. DNA
3. RNA
4. Lipids
5. Metabolites
6. Microorganisms
7. Proteins

Methodology assays

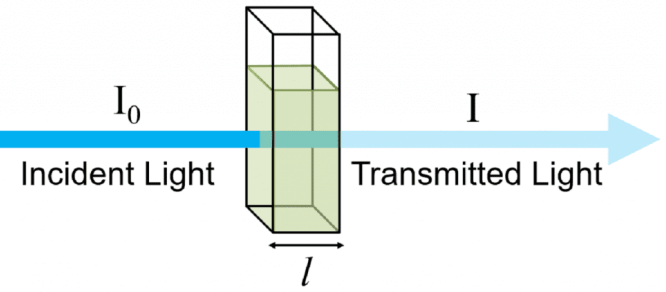
1. Spectrophotometric assays

Spectrophotometry is the quantitative measurement of the interaction of light with a material at a selected wavelength and is one of the most used methods of analysis.

Methodology assays

1. Spectrophotometric assays

The relationship between absorbance and concentrations is given by the Bouguer-Lambert-Beer law, which states that the concentration of a solute is proportional to the absorbance



$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

Methodology assays

1. Spectrophotometric assays

a) **Ultraviolet and Visible (UV-Vis) Absorption Spectroscopy.** Biomarkers determined include:

- Adenosine deaminase, aspartate aminotransferase, arginase, total esterase, creatine kinase, albumin, uric acid, hydrogen peroxide, protein carbonyls

Methodology assays

1. Spectrophotometric assays

b) Atomic Spectrophotometry. Biomarkers analysed include:

- Calcium, Magnesium, Chromium, Cadmium, Manganese, Nickel, Lead, Sodium, Potassium

Methodology assays

1. Spectrophotometric assays

c) Near Infrared Spectrophotometry. Biomarkers analysed include:

- α-amylase (sAA), cortisol, secretory IgA, urea, total protein and phosphates.

Methodology assays

1. Spectrophotometric assays

d) Flow Injection Spectrophotometric Analysis. Biomarkers analysed include:

- α-amylase (sAA) and Lead.

Methodology assays

2. Immunoassays (IA*s*)

IAs are methods that measure the presence or **concentration of biomolecules** (from small molecules to macromolecules) in a solution, by the use of a **specific antibody** or an **antigen** as biorecognition agent which produce an analytical signal.

Methodology assays
2. Immunoassays (IA*s*)

a) Enzyme-Linked Immunoassays (EIAs). Exists in two main formats;

   i. Enzyme-linked immunosorbent assay (ELISA)

   ii. Competitive EIAs

   Biomarkers include: Lactoferrin, Immunoglobulin A, M, G, E (IgA, IgM, IgG, IgE), C-Reactive Protein (CRP), cortisol, adiponectin, cortisone

Methodology assays
2. Immunoassays (IA*s*)

b) Chemiluminescence immunoassays

Biomarkers analysed include:

- Cortisol,
- testosterone,
- lactate

Methodology assays
2. Immunoassays (IA*s*)

c) Fluoroimmunoassays

Biomarkers analysed include:

- Chromogranin,
- Salivary α-amylase,
- CRP,
- Haptoglobin

Methodology assays
2. Immunoassays (IA*s*)

d) Radioimmunoassays

Biomarkers analysed include:

- Cortisol
- Estradiol
- Oxytocin

Methodology assays
2. Immunoassays (IA*s*)

e) Non-labelled immunoassays

Biomarkers analysed include:

- IgA, IgG
- Albumin
- Cortisol
- Interleukin 8 (IL-8)
- Prostate specific antigen

Methodology assays
2. Immunoassays (IA*s*)

f) Paper based-immunoassays

Biomarkers analysed include:

- Cortisol

Methodology assays
2. Immunoassays (IA*s*)

g) Multiplex immunoassays: multianalyte detection of biomarkers allows to improve assay efficiency, low sample and reagent consumption, and reduce overall cost per assay compared with parallel single-analyte IAs methods

Biomarkers analysed include:

- IL-8, IL1β, CRP, Myoglobin, myeloperoxidase

Methodology assays
2. Immunoassays (IA*s*)

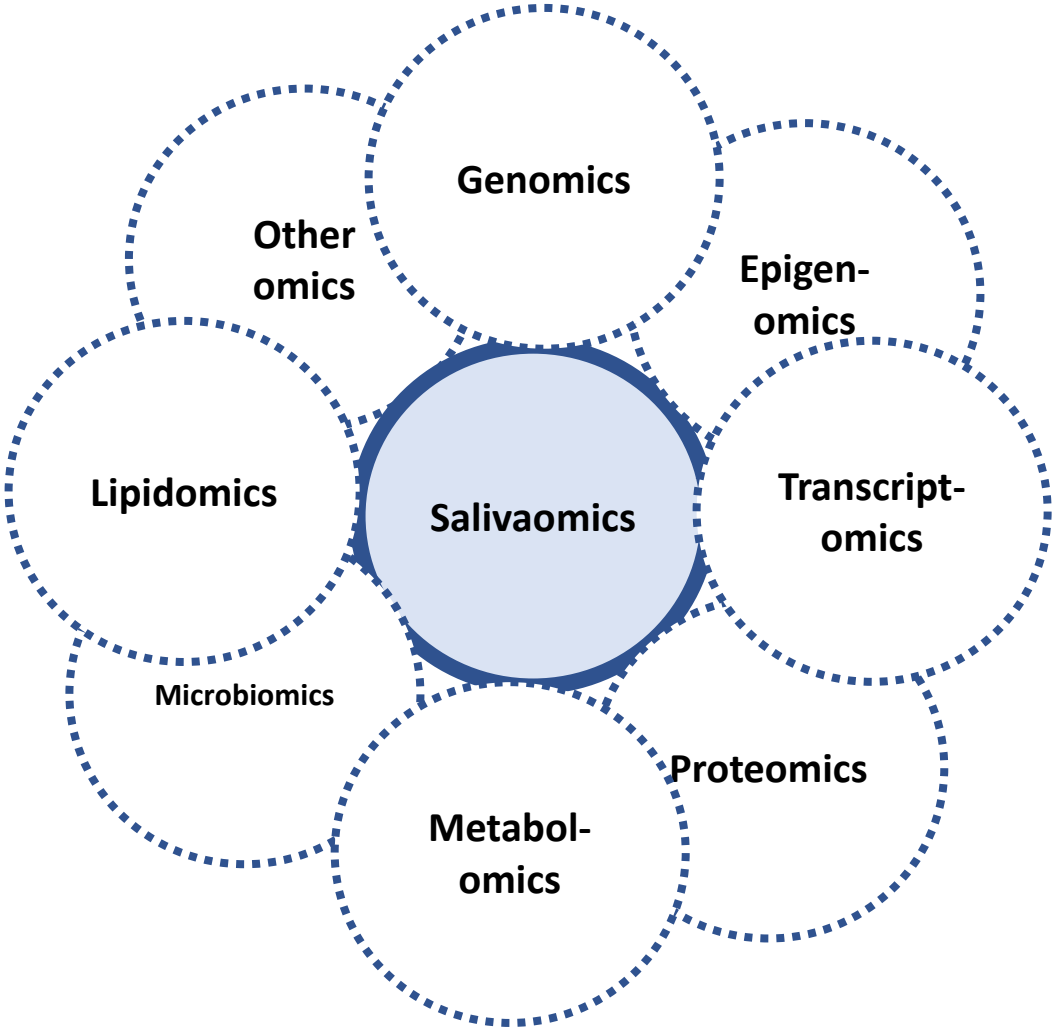
h) Liquid Biopsy: tests that detect circulating tumor cells, exomes, tumor DNA, tumor RNA and proteins that are disseminated into bloodstream or saliva from the primary lesion.

Methodology assays
3. ‘Omics’ Techniques

The term ‘-omics’ englobes different approaches for the study of biological molecules including DNA (genomic and epigenomics), RNA (transcriptomics), proteins (proteomics), metabolites (metabolomics), and others such as lipids and microbiome (lipidomics and microbiomics, respectively).

Methodology assays

3. ‘Omics’ Techniques



Clinical validation

- Comparison of the test results in healthy populations to various groups of diseased subjects should be carried out.
- it is important to keep in mind that is unlike that a single molecular biomarker will be able to discern disease from non-disease states due to the high heterogeneity presented in large populations

Application

1. Dental practice
2. Respiratory diseases
3. Neurologic diseases
4. Diagnosis and monitoring of metabolic and endocrine diseases
5. Inflammatory and autoimmune diseases
6. Kidney diseases
7. Diagnosis of infectious diseases
8. Sports sciences
9. Systemic and oral cancer


End