SUBJECT	FORENSIC SCIENCE
Paper No. and Title	PAPER No. 12: Forensic Biology and Serology
Module No. and Title	MODULE No. 9: Biological characteristics, Identification and Tests of Saliva
Module Tag	FSC_P12_M9

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1. Learning Outcomes

After studying this module, you shall be able to-

- Understand the mechanism of secretion of saliva
- Know the components of human saliva
- Learn about the various tests for its identification

2. Introduction

On average, a human being secretes 1.0 to 1.5 liters of saliva every day. It is a fluid largely composed of water with little amounts of electrolytes and enzymes. It is secreted by parotid and submandibular salivary glands in the mouth. Forensically, it is often seen in sexual assault cases.

Saliva tests can reveal certain disease markers, viral infections, and the presence of therapeutic as well as illicit drugs in the body.

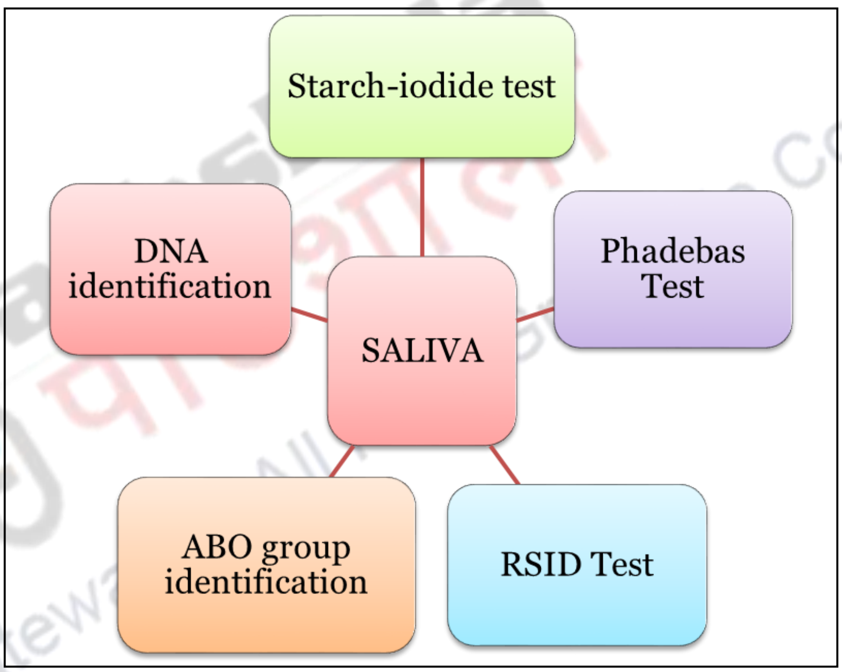
Saliva samples can be analyzed from various types of surfaces such as body parts, paper, envelopes, cigarette butts, plastic and glass bottles, and metal cans, etc. Saliva is an important evidence which can provide useful information about the personal contact of victim and perpetrator.

The presence of saliva can be ascertained by Starch-iodide and Phadebas tests.

Starch-iodide and Phadebas tests, however, do not confirm the presence of Human saliva. These are merely the test for amylase activity regardless of whether that amylase has come from human or any other source.

An advanced, monoclonal antibody based test kit is used to identify human specific salivary amylase. With RSID method, if a stain gives positive reaction, then it is confirmed that human saliva is present. In addition, ABO group antigens can also be detected in saliva, if the person is secretor. A secretor is an individual whose saliva and other body fluids contain ABO antigens. Approximately 80% individuals are known to be secretors.

Finally, since saliva may also contain buccal mucosa cells, it is possible to identify the DNA profile of the person in question using advanced DNA profiling techniques.



3. Composition of Saliva

It comprises the following components:

1. Water;
2. Electrolytes: sodium (lesser than blood plasma), potassium (greater than plasma), calcium (similar to plasma), magnesium, chloride (lesser than plasma), bicarbonate (greater than plasma), phosphate;
3. Iodine (mmol/L commonly greater than plasma, but reliant on variable according to nutritive iodine consumption);
4. Mucus- Mucus in saliva chiefly contains of mucopolysaccharides and glycoproteins;
5. Antibacterial combinations (thiocyanate, hydrogen peroxide, and secretory immunoglobulin A);
6. Epidermal growth factor or EGF;
7. Several enzymes- There are 3 main enzymes present in saliva.
8. α-Amylase, or ptyalin, produced by the acinar cells of the parotid and submandibular glands, initates the absorption of starch. It has an ideal pH of 7.4.
9. Lingual lipase produced by the acinar cells of the sublingual gland, have a pH optimum ~4.0 so it is not triggered till arriving the acidic situation of the stomach.
10. Kallikrein- an enzyme that proteolytically splits high-molecular-weight kininogen to form bradykinin, which is a vasodilator. It is released by the acinar cells of all three major salivary glands.
11. Antimicrobial enzymes: Lysozyme, Salivary lactoperoxidase, Lactoferrin, Immunoglobulin A.
12. Proline-rich proteins (role in enamel creation, Ca^{2+}-binding, microbe death and lubrication).

13. Minor enzymes comprise salivary acid phosphatases A+B, N-acetylmuramoyl-L-alanine amidase, NAD(P)H dehydrogenase (quinone), superoxide dismutase, glutathione transferase, class 3 aldehyde dehydrogenase, glucose-6-phosphate isomerase, and tissue kallikrein (function unidentified).
14. Cells: Perhaps as numerous as 8 million human and 500 million bacterial cells per mL. The existence of bacterial products (small organic acids, amines, and thiols) causes saliva to occasionally display foul odor.
15. Opiorphin, a recently investigated pain-killing element established in human saliva.
16. Haptocorrin, a protein which fixes to Vitamin B12 to safeguard it besides deprivation in the stomach, previously it bind to Intrinsic Factor.

In humans, the submandibular gland comprises nearby 70–75% of secretion, though the parotid gland discharges about 20–25% and small quantities are released from the other salivary glands.

4. Secretion of Saliva

The formation of saliva is done both by the sympathetic nervous system and the parasympathetic.

The saliva secreated by sympathetic innervation is denser, and saliva secreated parasympathetically is further watery. Sympathetic stimulation of saliva is to assist respiration, but parasympathetic stimulation is to assist digestion.

Parasympathetic stimulation pointers to acetylcholine (ACh) discharge onto the salivary acinar cells. ACh fixes to muscarinic receptors, precisely M3, and create an improved intracellular calcium ion concentration (by the IP3/DAG second messenger system). Amplified calcium causes vesicles inside the cells to fuse with the apical cell membrane prominent to secretion. ACh also make the salivary gland to discharge kallikrein, an enzyme that transforms kininogen to lysyl-bradykinin. Lysyl-bradykinin turns upons blood vessels and capillaries of the salivary gland to produce vasodilation and enlarged capillary permeability individually.

The resultant amplified blood flow to the acini allows making of more saliva. Further, Constituent P can fix to Tachykinin NK-1 receptors forming to increased intracellular calcium concentrations and then amplified saliva secretion. At the end, both parasympathetic and sympathetic nervous secretion can lead to myoepithelium contraction which forms the expulsion of simulation from the secretory acinus into the ducts and eventually to the oral cavity.

Sympathetic stimulation results in the release of norepinephrine. Norepinephrine binding to α-adrenergic receptors will cause an upsurge in intracellular calcium levels leading to more fluid vs. protein secretion. If norepinephrine binds to β-adrenergic receptors, it would result in additional protein or enzyme simulaion vs. fluid secretion. Secretion by norepinephrine originally decreases blood flow to the salivary glands due to constriction of blood vessels but this effect is overtaken by vasodilation caused by various local vasodilators.

5. Starch-Iodide Test

It is a fact that iodine reacts with starch (complex carbohydrate) and develops a dark blue color. On the other hand, monosaccharides or disaccharides do not react with iodine to develop color. These facts are utilized in this trick to identify the presence of saliva. Saliva contains amylase which breaks down starch into monosaccharides or disaccharides. The test can be carried out in a starch-containing agarose gel with sample wells. The questioned sample is then loaded into the sample well. The gel is incubated and then stained with an iodine solution. The starch-containing gel is stained blue. If amylase is present in the sample (due to saliva!), it diffuses out from the sample well and cleaves starch in the gel as it diffuses. A clear area around the sample well indicates amylase activity.

6. Phadebas Test

This test is based on the fact that amylase digests starch. Phadebas reagent consists of a dye cross-linked with starch. The presence of saliva digests the starch and releases the dye from the complex. The solution thus becomes blue in color. This indicates the presence of saliva.

The test can be used as a quantitative test by measuring the intensity of the developed color at 620nm wavelength. A standard concentration curve of known concentration of colored dye may be prepared and used for quantitative data.

7. Immunochromatographic Test

Commercially developed immunochromatographic kits are available for testing saliva samples. The method is based on antigen-antibody interaction principle. One such kit is known as RSID-saliva kit. The presence of saliva can be ascertained by the appearance of pink line in the RSID-saliva kit. This technique can detect saliva as low as 1 µL.

8. RNA based Test

Mouth contains cells that are specific to it. These cells contain specific protein coding genes that render them to be exclusively identified. Two such genes are HTN3 and STATH. HTN3 gene encodes histan-3 protein which is involved in defense mechanism in oral cavity. STATH encodes statherin which is prevents the precipitation of calcium phosphate salts in salivary glands.

9. Summary

1. It is a fluid largely composed of water with little amounts of electrolytes and enzymes.
2. Saliva is a fluid largely composed of water with little amounts of electrolytes and enzymes.
3. Saliva tests can reveal certain disease markers, viral infections, and the presence of therapeutic as well as illicit drugs in the body.
4. Saliva may also contain buccal mucosa cells, it is possible to identify the DNA profile of the person in question using advanced DNA profiling techniques.

5. Saliva contains amylase which breaks down starch into monosaccharides or disaccharides.

6. α-Amylase, or ptyalin, produced by the acinar cells of the parotid and submandibular glands, initiate the absorption of starch previously the food is even believed.

7. Kallikrein is an enzyme that proteolytically splits high-molecular-weight kininogen to release bradykinin, which is a vasodilator.

8. Haptocorrin, a protein which fixes to Vitamin B12 to safeguard it beside degradation in the stomach, earlier it fixes to Intrinsic Factor.

9. Both parasympathetic and sympathetic nervous stimulation can result in myoepithelium contraction which creates the exclusion of simulation from the secretory acinus into the ducts and eventually to the oral cavity.

10. Phadebas reagent consists of a dye cross-linked with starch.

11. The presence of saliva can be ascertained by the appearance of pink line in the RSID-saliva kit.

12. Two such genes are HTN3 and STATH. HTN3 gene encodes histan-3 protein which is involved in defense mechanism in oral cavity.