



Saliva As a Diagnostic Fluid A Review

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DOI: 10.31080/ASDS.2022.06.1360

Received: February 07, 2022

Published: April 19, 2022

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Abstract

Saliva like other body fluids has its own functions and importance in daily life of an organism. Humans can manage without saliva it is not life threatening but it results in variety of difficulties and miseries. The analysis of saliva, like blood – based analyses has two purposes 1) Identify individuals with disease 2) Follow the progress of affected individuals under treatment. The study and use of saliva-based diagnostics have increased during past 10yrs. This has been proved to be very useful in large scale screening and epidemiological studies. The highly sensitive test procedures that are now common place makes it practical to quantitate, despite very low conc.

Keywords: Saliva; Diagnostic; Fluid; Flow Rate

Introduction

According to Meudel 1980 saliva lacks trauma of blood, sincerely of sweat and emotional appeal of tears but saliva is a wonderful marvelously equipped fluid to patient and preserve oral tissue.

Saliva like other body fluids has its own functions and importance in daily life of an organism.

Humans can manage without saliva it is not life threatening but it results in variety of difficulties and miseries.

The diagnostic value of saliva lies in its components, flow and structure of the glands. Hence, firstly, it is important to know its normal components, formation, flow and structure of salivary glands [1].

Composition of saliva [1,2]

Water: 99.5%

Solids: 0.5%

Organic constituents: 0.3%

Proteins

Enzymes and Hormones

Amino acids

Immunoglobulin (IgA, IgG, IgM)

- Glycoprotein (Mucins)
- Glucose
- Urea, ammonia, uric acid
- Blood group substances
- Miscellaneous – cholesterol, Creatinine, Vitamins AMP etc.

Inorganic Constituents: 0.2%

- Sodium,
- Potassium
- Thiocyanate
- Calcium
- Phosphate
- Chloride
- Fluoride

Cellular constituents

Yeast cells, bacteria, protozoa, polymorphonuclear leucocytes desquamated epithelial cells etc.

Gases

Oxygen - 1ml/100ml

Nitrogen: 2.5 ml

CO₂: 50 ml

Factors influencing the composition of saliva [1-3]**Flow rate**

- Increase in flow rate with increase in concentration of total protein, amylase, sodium and bicarbonates.
- Decrease in flow rate with decrease in concentration of phosphate, urea amino acids, uric acid, ammonia serum albumin magnesium
- Increase in flow rate with decrease in concentration of chloride calcium protein.
- No change in flow rate with concentration of substances like – potassium and possibly fluoride.

Differential gland contribution

The concentration of substance is different in different glands change in concentration of particular constituents in mixed saliva will stimulate the particular gland to secrete more Ex: Even increase in Ca concentration particular gland the concentration of Ca in mixed saliva is reduced due to higher portion of parotid saliva in mixed secretion.

Circadian rhythm

Rhythmic variations in concentration of many constituents are seen Ex. Calcium and phosphate levels are low in early morning.

This intern causes variation in flow rate.

Duration of stimulus

At a constant rate of flow, the composition may vary with duration of stimulus.

Nature of stimulus

If the flow rate is held constant, salt stimulates a higher protein content sugar stimuli give rise to high amylase content in saliva.

Diet

- High protein diet increases the salivary urea levels.
- Fluoride in saliva increases transiently after a dose of fluoride.

Major salivary glands [3]**Parotid gland**

The parotid gland is the largest of the salivary glands and is composed almost entirely of serous acini. It is situated below the external auditory meatus and lies in a deep hollow behind the ramus of the mandible and in front of the sternocleidomastoid.

Shape and lobes of the gland

As seen from the superficial surface, the parotid gland is roughly wedge-shaped, with its base above and its apex behind the angle of the mandible. If cut across in a horizontal plane, it would also be found to be wedge - shaped, with its base in the lateral position and its apex against the pharyngeal wall.

During development, the parotid gland covers the lateral surface of the facial nerve. As development proceeds, the deep part of the gland extends medially between the branches of the facial nerve. In the fully formed gland, the facial nerve may be said to divide the parotid gland into superficial and deep parts, or lobes.

The superior margin of the gland extends upward behind the temporomandibular joint into the posterior part of the mandibular fossa. This part of the gland is called the glenoid process.

The anterior margin of the gland extends forward superficial to the masseter muscle to form the facial process. A small part of the facial process may be separate from the main gland and is called the accessory part of the gland.

The deep part of the gland may extend forward between the medial pterygoid muscle and the ramus of the mandible to form the pterygoid process.

Submandibular gland

The submandibular gland is a large salivary gland and is composed of a mixture of serous and mucous acini, the former predominating. It lies partly under cover of the body of the mandible and is made up of a large superficial part and a small deep part, which are continuous with each other around the posterior border of the mylohyoid muscle.

The superficial part of the gland lies in the digastric triangle, reaching upward under cover of the body of the mandible. Posteriorly, it is separated from the parotid gland by the stylomandibular ligament.

Sublingual gland

The sublingual gland is the smallest of the three main salivary glands. It lies beneath the mucous membrane of the floor of the mouth, close to the midline. It contains both serous and mucous acini, the latter predominating.

Structure and function of salivary gland cells :

The terminal secretory units are composed of serous, mucous and myoepithelial cells arranged into acini/secretory tubules. The secretions of these units are collected by the intercalated ducts.

Serous cells

It is specialized for the synthesis storage and secretion of proteins. The typical serous cell is pyramidal in shape, with its broad base resting as a thin basal lamina and its narrow apex bordering on the lumen. The spherical nucleus is located in the basal region of the cell occasionally binucleated cells are observed. The most prominent feature of the serous cell is the accumulation of secretory granules in apical cytoplasm.

Mucous cells

Its structure differs from that of the serous cell. They have little/no enzymatic activity and probably serve mainly for lubrication and protection of the oral tissues. The ratio of carbohydrate to protein is greater and larger amounts of sialic acid and occasionally sulfated sugar residues are present.

Myoepithelial cells:

Myoepithelial cells are closely related to the secretory and intercalated duct cells. Lying between the basal lamina and the basal membranes of the parenchymal cells. Myoepithelial cells are diffi-

cult to identify in routine histologic preparations but their stellate shape can be observed in sections stained by immunofluorescent techniques or sp. Histol chemical techs.

Ducts

The duct system of the salivary glands is formed by the confluence of small ducts into ones of progressively larger caliber. Within a lobule, the smallest ducts are the intercalated ducts, they are thin branching tubes of variable length that connect the terminal secretory ducts. In the interlobular connective tissue the ducts continue to join due another, increasing in size until the main excretory duct is formed.

Minor salivary glands

The minor salivary glands are located beneath the epithelium in almost all parts of the oral cavity. These glands usually consist of several small groups of secretory units opening via short duct in to the mouth they lack a distinct capsule. Instead mixing with the connective tissue of the submucosa or muscle fibers of the tongue/cheek.

Labial and buccal glands

- These are described as mixed consisting of mucous tubules with serous dewilunes.
- Ultra structural studies of labial glands have revealed the presence of mucous cells only.

Glossopalatine glands

These are pure mucous glands. They are principally localized to the region of isthmus in the glossopalatine fold but many extend from the post extension of the sublingual gland to the gland of the soft palate.

Palatine glands

These are also pure mucous variety present in posterolateral region of the hard palate and in submucosa of the soft palate and uvula. The opening of the ducts on the palatal mucosa are often large and easily recognizable.

Lingual glands

Glands of tongue can be divided into several groups 1) the anterior lingual glands (glands of blandin and nohn) are located near the apex of the tongue. These ducts open on the ventral surface of the tongue near the lingual frenum.

The posterior lingual glands are located lateral and posterior to the vallate papillae and in association with lingual tonsil. These post. lingual glands (van Ebner's glands) ducts open into the trough of the vallate papillae and at the rudimentary foliate papillae on the sides of the tongue.

Of all the minor salivary glands, the posterior lingual serous glands are among the most interesting. Classically their secretions have been described as serving to wash out the trough of the papilla and ready the taste receptors for a new stimulus.

Recent studies suggested that these glands have significant protective and digestive function.

Formation of saliva [4]

Saliva is formed in two stages

- The pri. Secretion occurs in the acini, then modified as it passes through the ducts.

The pri. Secretion is formed activity by movement of sodium and chloride ions into the lumen, this great an osmotic gradient which leads to passive movement of water.

Other acinar components are added here before the fluid enters the duct, where sodium ions are actively reabsorbed and potassium and bicarbonate ions are secreted.

- The macromolecular components like amylase, mucous glycoprotein etc. are formed in acinar cell endoplasmic reticulum processed into secretory vesicles in the Golgi apparatus and are exported from cell by exocytosis.

Functions of saliva [1,4]

Digestive function

Salivary amylase a major component of parotid secretion and submandibular secretion initiates the digestion of cooked starch.

Salivary lipase secreted bilingual serous glands initiates digestion of fat.

Lubrication

Saliva mucous glycoprotein which helps in mastication bolus formation, swallowing and speech.

Maintenance of mucous membrane integrity

Mucins control the permeability of mucosal surfaces and it limits the penetration of variety of potential irritants and toxin of food, beverages tobacco and other sources.

Soft tissue repair

The nerve growth factor and epidermal growth factor in submandibular saliva may accelerate wound healing.

Maintenance of ecological balance

Debridement

The flow of saliva augmented by muscular activity of lips and tongue to remove large no. of harmful bacteria from teeth and mucosal surface.

Aggregation

Mucins function by clumping or aggregating bacteria causes loss of adherence to hard and soft tissue and are expectorated or swallowed.

Direct Antibacterial

Enzymes like lysozymes, histidine etc antibodies has antibacterial action

Maintenance of pH

Bicarbonate, phosphate and histidine rich peptides act as buffers and maintain neural pH.

Maintenance of tooth integrity

- Tooth immediately after eruption interacts with saliva provides a post eruptive maturation via diffusion of ions such as calcium phosphorus, magnesium fluoride increases surface hardness decreases permeability and shows increase resistance to caries.
- It provides protective barrier and lubricating film against wear, and diffusion barrier against acid.

Water balance

Maintain levels of hydration thirst and need for fluids usually signaled by a dry mouth.

Methods for collection of human saliva [5]

Whole saliva

- The fluid most frequently employed for salivary diagnostic purpose is expectorated whole saliva.
- It includes secretions of major salivary glands + secretion of minor salivary glands + GCF + exfoliated cells.
- Whole saliva requires centrifugation to provide clear sample but some times bacteria or cells have diagnostic value.

Parotid gland

- Collected readily by duct by cannulating directly with polythene tubing or by lashley cannula

Lashley cannula (Carlson – Crittenden Cannula)

It consists 2 concentric rings of metal or plastic attached to a disc about ½ an inch diameter. Tubes are inserted.

- So that the outer space between the 2 rings may be evaluated
- From the inner space which is placed over the parotid duct to lead the saliva away into a receptacle placed outside the mouth.
- Cups made from Perspex, aluminium other material
- The cup is held against the cheek by applying suction to outer wall through a small syringe which can be conveniently placed in a top pocket or attached by adhesive tape to a collar.

Curby cup (1953)

It is a modification of lashley cannula, whose 3rd tube through which air bubbles can be released from the control chamber of suction of air from the outer space is affected by a rubber pipette.

Submandibular and sublingual saliva

For collection of saliva from submandibular and sublingual glands by schneyer's apparatus (1955)

- It consists of an acrylic appliance with 3 chambers. The central one is connected to the duct of submandibular gland
- Two lateral ones covers the numerous ducts of sublingual gland. Polythene tubing leads the secretion to collecting vessels outside the mouth.

Minor salivary gland secretions

Using micropipette

- **The un stimulated whole saliva:** Can be collected by allowing saliva to drip from the lower lip into a funnel and then into a graduated tube.
- **Stimulated whole saliva:** by the subject is allowed to chew on some inert material such as paraffin wax or on rubber band.

Saliva as a diagnostic fluid

The analysis of saliva, like blood – based analyses has two purposes

- Identify individuals with disease
- Follow the progress of affected individuals under treatment

It consists of 5 basic levels of analysis at which the effectiveness of any diagnostic test should be evaluated

- The analytic (precision and accuracy)
- Diagnostic (sensitivity and specificity)
- Patient outcome efficacy (medical decision making)
- Operational (predictive value and efficiency) b
- Cost/benefit

Autoimmune disorders [6-8]**Sjogren's syndrome**

Is a chronic, autoimmune disorder described as a triad consisting of keratoconjunctivitis sicca, Xerostomia and Rheumatoid arthritis.

Pri S.S. (Sicca complex) = Dry eyes + Dry mouth

Sec S S = Systemic lupus erythematosus, polyarthritis nodosa, polymyositis or sclerodermas and Rheumatoid arthritis.

Bartram and Halber (1964) Meeswan, *et al.* (1967), Anderson, *et al.* 1973 found increased levels of β_2 – microglobulin and IgG, IgM or IgA autoantibodies to salivary duct antigens with sjogrens syndrome

Stuchell RN, Mandel ID and Baurdash (1983) performed sialometry and compared concentration of electrolytes urea and various proteins.

Results

- There was a reduced flow rate, increased Na and Cl and reduced phosphate level compared to normal controls.
- Significant increase in IgA, IgG and lactoferrin was seen
- Albumin concentration was also elevated compared to normal.
- Total protein and amylase were not significantly different but amylase level was low in low flow rate subgroup maybe due to acinar damage in low flow rate group.

Salivary antibodies to gliadin in celiac disease:

Celiac disease is a form of malabsorption characterized by diarrhea weight loss abdominal distension, anemia and in children shunted growth.

- This condition is thought to be due to intolerance to the gliadin fraction of dietary gluten found in wheat and rye flour.

- Antibodies to what gliadin is found in saliva, serum and in intestinal secretions.
- Al - Bayaty, *et al.* 1989 has reported that increase in IgA antibody levels to both crude gliadin and α - gliadin in compared to controls.

Salivary immunoglobulins in Asthma childrens

Allergic asthma is associated with IgE antibody response to various allergens. Tula Hyypa (1980) reported IgE levels were increased to significant levels in asthmatic childrens when compared to normal.

Systemic lupus erythematosus

- It is an autoimmune disease characterized by multi systemic involvement.

Clinical feature

- Earliest manifestations involve joints, fever and skin lesions cardiovascular and central nervous system lesions
- SLE is frequently the collagen disease in sec sjogrens syndrome
- Hannah Bin Aryeh, *et al.* (1993) reported increase in salivary composition and flow rate compared to normal people.

They have also reported elevation in saliva sodium Mg concentration. IgM, IgA conc also elevated and IgG levels show no difference from controls

Granulomatous conditions:

Crohn's disease

- Crohn's disease is a granulomatous inflammatory condition affecting parts of gastrointestinal track continuously from mouth to anus.
- Its peak incidence is in young adults.

Studies of salivary changes in Crohn's disease have conflicting reports. Basu, Asquith, Thompson and Cooke (1975) reported reduced levels of IgA.

Croma - Bohbouth, *et al.* 1984 reported increased levels in saliva, which did not reflect the disease activity.

Cardiovascular disease

Determination of total serum amylase and salivary amylase activity have been made before and 6hr after cardiovascular surgery.

If salivary amylase levels were low in preoperative patients with ruptured aortic aneurysm there was an associated increase in mortality (Adam, *et al.* 99).

Needs further research to determine their clinical utility.

Endocrinology

Diabetes mellitus

Marder, Abelson, Mandel (1975) reported that flow rates did not differ significantly with that of controls but the calcium ion content and salivary IgG levels were increased.

Tenovuo, *et al.* (1986) reported increased levels of IgA and salivary peroxidase compared to normal controls.

Thorstensson, *et al.* (1989) showed increased salivary glucose compared to non-diabetics.

Yavuzylmez, *et al.* (1996) reported increase in mean level of salivary potassium, salivary total protein and amylase in diabetic patients.

Aldosteronism

Lauler, Hickler and Thorn in 1962 reported that the salivary Na/K conc ratio is decreased in aldosteronism seen in the range of 0.21 and 0.60 and normal range being 0.61 to 3.37.

Hormone monitoring

Saliva levels of steroid hormones reflect the free and thus active, level of these hormones while most blood measurements reflect the total level i.e., for both free and bound.

All steroids of diagnostic significance in routine clinical endocrinology can be measured in saliva.

Hormones whose salivary levels reflect serum levels are

Cortisol:17 estradiol

Aldosterone: Estriol

Dehydroepiandrosterone: Insulin

Testosterone: Melatonin

Progesterone

Salivary progesterone is being used for

- Assessing the functional capacity of corpus luteum in both normal women and those with defects in the hypothalamic pituitary ovarian axis.

- Studies of sub fertile women
- Studies of pregnant women
- Examining the effect of contraceptive steroids on ovarian activity
- Assessing hormonal changes during adolescence.

Some investigators have found that salivary cortisol is a better measure of adrenal cortical function than serum cortisol especially in children.

Saliva as a preterm labour predictor

Preterm delivery is a main cause of neonatal morbidity and mortality. The mechanism for which is not known.

Darne., *et al.* reported that the saliva oestriol to progesterone ratio was greater than one for preterm labour with intact membrane and it was less than 1 for preterm labour after prolonged rupture membrane.

- The preterm labour without prior rupture of memb. is preceded by rise in saliva oestriol to progesterone ratio.
- So it may be possible to use this ratio to predict preterm labour.
- The increase in oestriol in late pregnancy may be due to increased fetal adrenal activity.

Infectious diseases [9]

Bacterial

Dental caries and gingivitis

Saliva based diagnosis are useful in identifying the bacteria most frequently associated with dental caries are streptococcus mutans and lactobacillus acidophilus and tests have been developed to conveniently measure porphyomonous gingivitis which is associated with pdl diseases.

Though the saliva based diagnostics appear to be feasible the larmas in 1993 criticized on the following grounds.

- Both Dental caries and gingivitis have not been found always with specific disease. They may be outcome of many individual microbial species or outcome of combination of species.
- Quantification of disease is not possible.
- Cannot differentiate the active and inactive phases of progression of disease.

Periodontitis

Specific oral metabolites like ammonia and urea formed by putrefactive action of microorganisms on protein content of saliva may suggest possible diagnostic and etiologic factor of periodontitis.

Berg, Burrill and forbick 1947 showed the purification is more rapid in patients with severe periodontal disease than in healthy subjects.

It is also found that aromatic nitrogen containing compounds like pyridine and picohines are found in subjects with moderate to severe periodontitis, which could be related to disease process (Kosteletic 1979).

Recurrent aphthous stomatitis

Stress has been postulated as a precipitating factor in recurrent aphthous stomatitis.

Mc Cartan, Lamcy, Wallace (1996) suggested that there is an elevated levels of salivary cortisol in patients with recurrent aphthous stomatitis compared to normal subjects.

Whooping cough

Culture of bordetella pertussis is common method used for diagnosis of whooping cough. Though it is highly specific, it is time consuming hence rapid diagnostic method like IgA to B-pertussis in saliva has been used.

Peptic ulcer

- Helicobacter pylori are the critical pathogen associated with peptic ulcer.
- Nested polymerized chain reaction assay is available to detect H. Pylori DNA in saliva in patients useful in diagnosis of peptic ulcer (Jing., *et al.* 1998).

Viral infections

HIV

The numerous studies have showed the presence of antibodies to HIV-I in oral fluids of seropositive individuals.

- The collecting device consists of specially treated cotton pad attached to nylon stick and vial contains preservative solu.

- After collection of GCF and mucosal transudate, the pad is stored in vial of antimicrobial preservative solution and sent to lab for testing.
- The testing done with an ELISA and confirmed by western blot assay specifically designed for use with oral fluid.
- This helps in early identification, early treatment and prevention of disease transmission.

Hepatitis

- Hepatitis virus is seen in many body secretions including saliva
- Health cote, comeron and Dane (1974) studied hepatitis B antigen in saliva and semen by sensitive radio immunoassay, though it was in minute quantity in saliva compared to serum.

Mumps measles and Rubella

The viruses can be isolated from saliva. The diagnosis of mumps measles and rubella can be diagnosed by testing for virus specific antibodies IgG, IgM.

Fungal diseases

Chronic mucocutaneous candidosis

Study done by Wilton and Ivanyi 1972 said that, salivary IgA antibodies to *Candida* are often reduced in severe grades of chronic mucocutaneous candidosis.

Nephrology

Chronic renal failure

- Chronic renal failure may result from gradual decrease in total number of functioning nephrons.
- In patients with CRF increase in saliva urea, creatinine is found.

Oncology

Because of anatomical proximity of saliva to both premalignant and malignant oral neoplasms, saliva is ideal for screening these lesions.

- Tavassoli., *et al.* 1998 have found that elevated salivary antibodies to p53 were found among the patients with oral carcinomas.
- Saliva tests like conc. of protein in conjunction with imaging may increase the overall diagnostic value in cancer and also reduce the number of false +ve and -ve diagnosis associated with imaging (Kerlikowske., *et al.* 1995)

- Higher levels of kallikrein in patients with diagnosed with malignant tumors compared with those individuals with benign tumors or healthy individuals. But the diagnostic values yet to be determined.
- Di-xia, Schwartz and Fan Qin (1990) found saliva contained CA125, a glycoprotein complex used as marker in ovarian cancer. CA125, conc. is significantly elevated in women with ovarian cancer. Di-Xia., *et al.* also suggested in his study that, the salivary CA-125 assay had better diagnostic value than the comparable serum assay.
- Epidermal growth factor (EGF) conc. is elevated in saliva of women with pri. Brest cancer or recurrence of Brest cancer when compared to women without disease.

Novarro., *et al.* 1997 have stated that EGF is a potential malignancy indicator to be used in postoperative follow-up.

Streck Jus., *et al.* 2000 a showed elevated protein product of oncogene C-erbB-2 also known as HER-2/neu in women with Brest cancer.

Their study also demonstrated that this salivary marker is reliable and may be used in patient postoperative follow-up.

- Salivary sialic acid initially appeared promising as an air for detecting lung cancer. But this has not been substantiated (Koc., *et al.* 1996).

Drug monitoring [10]

Currently, saliva can be used to detect or monitor catinine, cannabinoids, cocaine, phencyclidine, opioids, barbiturates, diazepam, amphetamines and ethanol (schramm., *et al.* 1992b, smolle., *et al.* 1999).

Digitalis toxicity

Digitalis is one of the most widely used and valuable drug in clinical medicine. This drug is thought to be responsible for almost half the drug induced patient death.

Wotman, bigger, mandle and bartlestone 1971 demonstrated that, the salivary potassium and calcium conc. were significantly higher in patients of digitalis toxicity and this could be a reliable rapid and simple method of identifying the patients with digitalis toxicity.

The potassium elevation could be due to intercellular potassium leaking into saliva due to ↑ in permeability in either acinar or striated duct area of salivary gland due to effect of drug. (Watman *et al.* 1971).

Psychiatry

- Saliva may also be useful in providing objective outcome measure during psychiatric therapy.
- Saliva is used to monitor therapeutic responses in the treatment of anxiety, post traumatic stress by measuring salivary levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) (Yamada, *et al.* 1998).

Neurological disorders

The chordatympani can be estimated from a measurement of pH of submandibular saliva.

- Saito, *et al.* 1978), showed that, submandibular salivary pH faithfully reflects the nerve activity of chordatympani and flow.
- The pH raises as the flow increases
- ↑ pH – good prognosis.

Miscellaneous

Smoking

Epidermal growth factor is a polypeptide secreted into saliva which stimulates epithelial proliferation, protects mucosa against acute injury and heals gastric and duodenal ulcer in both animal and humans.

Jones, *et al.* 1992 (concluded in their study that, secretion of EGF was reduced in smokers group compared to nonsmokers group.

Pernicious anaemia

- It is a common megaloblastic anaemia due to deficiency of Vit B₁₂.
- In this type of anaemia, the blocking and binding to intrinsic factor found to be present in saliva and gastric juice (Carmel and Herbert 1967).

Cirrhosis of liver

Abelson, mandel and karmol 1976, have found that the flow of parotid saliva is increased in alcoholic cirrhosis with ↑ in conc. salivary potassium, calcium, total protein and amylase.

Cystic fibrosis

Salivary gland affected by cystic fibrosis shows cystic and fibrotic changes, which has effect on salivary slow and composition.

- The salivary flow of saliva is decreased in patient with cystic fibrosis (Wiesmann, boat and paul 1972).
- Elevated levels of total protein, enzyme, calcium, phosphorous while sodium chloride and potassium levels will be within normal limit (chernick, W.S. Barbero and Parkins E.M (1961).
- The precipitate of calcium and glycoprotein combination, makes saliva turbid, in cystic fibrosis and makes collection of saliva difficult by capillary method, by increasing viscosity or decreasing the flow. This could also be used as a diagnostic aid.

Conclusion

- The study and use of saliva-based diagnostics have increased during past 10yrs.
- This has been proved to be very useful in large scale screening and epidemiological studies.
- The highly sensitive test procedures that are now common place makes it practical to quantitate, despite very low conc.

Despite its uses

- Collection methods should be standardized.
- Cost effective manufacturing methods needs to be developed.
- Convince the medical insurance companies that, saliva based tests are highly accurate and cost effective.

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