

Applied Aspects of Salivary Analysis**Sourab Kumar, Abhishek Jadhav, Payoshnee Bhalinge¹, Nitesh Perla², Shilpi Suman³**

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ABSTRACT:

There exists a hierarchical model for disease-identifying technology test/evaluation (Fryback and Thornbury, 1991) which consists of five basic levels of analysis at which the effectiveness of any disease-identifying test should be (deciphered the worth, amount, or quality of): (1) the (related to careful studying or deep thinking) ((high) quality and (quality of being very close to the truth or true number)); (2) disease-identifying (sensitivity and level of detail); (3) patient result effectiveness (medical decision making); (4) operational (describe a possible future event) value and (wasting very little while working or producing something)); (5) cost/benefit ((related to social pressure, how people act toward each other, etc.) effectiveness). The role of saliva, the number of oral dryness and the resulting consequentiality of salivary flow as well as the relationship between xerostomia and salivary gland hypofunction among the causes of oral dryness. Other parts of oral conditions connected with saliva are also including Sjogren's Disease and Oesophageal function. Determinately, erudition, and the current utilization of salivary tests and the utilization of saliva as a disease-identifying fluid must be surveyed. This report provides a well-thought-out review of the (medical information that proves something) beginning and building on the role of saliva in protecting people against tooth disease.

Keywords: Diagnostic, Saliva, Analysis, Outcome.

INTRODUCTION

Saliva has different functions in the oral (surrounding conditions). It contributes to clearing of the oral cavity of the food debris and bacteria, buffering capacity on tissue damaging the strong bases and acids. It provides a saturated solution of calcium, which is needed in the mineralization of teeth along with antibacterial, antifungal and antiviral capacity.¹ Saliva is essential for lifelong conservation of dentition.² Saliva is composed of variety of electrolytes including sodium, potassium, calcium, magnesium, bicarbonate, phosphates, immunoglobulins, proteins, enzymes, mucins.³ Serving or acting to prevent harm properties of saliva which increase on stimulation include salivary clearance, buffering power and degree of saturation with respect to tooth mineral. Saliva

is very important to the preservation and maintenance of oral health, and any changes in its amount/quality may change the oral health status. Oral fluids often called 'the mirror of the body' or 'window on health status' is the perfect medium to be explored for health and disease.⁴

ANALYSIS OF SALIVA

Step 1: Questionnaires with detailed case history and consent form taken. Resting saliva of patient collected between 9 to 10.30am asking the patients to chew paraffin tablets for 15min and amount of saliva collected within 5min.

Step 2: Stimulated saliva of patient collected between 1.30pm to 3pm (after food), asking the patient to chew paraffin tablets for 15min and saliva collected in sterile container for 5min

Step 3: Analysis of amount of saliva and hydration status done by visual inspection and metal scale.

Step 4: Analysis of quality of saliva done by visual inspection.

Step 5: Analysis of consistency of saliva done by visual inspection.

Step 6: Analysis of temperature of saliva done by clinical thermometer.

Step 7: Analysis of pH of saliva done by pH indicator dip sticks and matching the result with the corresponding reading on the chart.

Step 8: Analysis of specific gravity of saliva done by hydrometer by diluting the saliva obtained from the patient up to 1 liter in a beaker.

Step 9: The sucrose level measured with the readings obtained from hand held refractometer. Bimetallic strip of refractometer indicates the glucose level of saliva sample.

METHODS OF RECORDING SALIVARY AND DENTAL CARIES PARAMETERS

TEST FOR HYDRATION STATUS OF SALIVA⁵

Everting the lower lip and recording the small drops of saliva for 1min and tested/evaluated with water status of un-stimulated saliva.

TEST FOR SALIVARY SECRETION RATE⁶

Flow rate of saliva can be measured by using salivary tests. It is recommended that the tests are performed at least one hour after the person has eaten something (imbibing dihydrogen monoxide is sanctioned). It is paramount that the person is relaxed and placid.

TEST FOR UNSTIMULATED SALIVA

Materials needed:

1. Graduated test tube or a measure-cup
2. A funnel
3. A watch or timer.

Procedure:

1. The individual sits in a straight position with his head tilted forward so that the

engenderment of saliva is amassed in the floor of the mouth and then flows out over the lip.

2. Saliva composed is let to drip into the graduated test tube or a quantification-cup for 15 min.

3. The result of this accumulation is expressed as milliliters minutely.[Table 1]

Table 1: Reference Values: (For Unstimulated Saliva)

>0.25ml/min	Normal
0.1-0.25ml/min	Low
Less than 0.1ml/min	Very Low

TEST FOR STIMULATED SALIVA

Materials needed:

1. A piece of paraffin for masticating to stimulate saliva secretion.
2. Graduated test tube or a measure-cup.
3. A funnel.
4. A timer.

Procedure:

1. The person masticates a piece of paraffin until it becomes soft.
2. Before the collection is started the first portion of saliva is swallowed.
3. Start timer and the masticating is perpetuated for another 5min (for subjects with high secretion rate, 3min may be enough).
4. The saliva is expectorated out at short intervals in a graduated test tube or a quantification-cup during the amassment period.
5. The amassed saliva is then quantified. The quantification should not include the foam which is composed during the amassment. The result is expressed as milliliters/min.[Table 2]

Table 2: Reference Values (For Stimulated Saliva)

>1.0ml/min	Normal
0.7-1.0ml/min	Low
<0.7ml.min	Very Low

TEST FOR EVALUATION OF PH⁷

Dentobuff Strip System

For chair-side use, a simplified method has been developed under the denomination

Dentobuff divest. A test pad contains dry acids and color indicated strips. When saliva is integrated, the acids are dissolved and pH drops. If saliva can buffer, pH will raise. The indicators show the final pH.

Materials Needed:

Dentobuff divest (paraffin tablet, a test divest containing acid and pH – bespeaker, a disposable pipette, a timer, a standard color chart, a cup or tube)

1. Saliva is amassed in the same way as described in Tests for stimulated saliva.
2. Customarily buffer capacity is taken simultaneously with secretion rate.

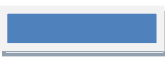

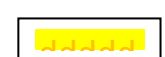
3. The pipette is utilized and one droplet of this stimulated saliva is placed on a minuscule test pad of a test divest.

4. Wait precisely 5 min, as color will transmute with time, for the reaction of saliva and designator.

5. Results: Thus compare the chart of the test pad with the standard color chart. Color of this designator reflects the pH on the divest. [Table 3]

From a cariological perspective, it is good to have a high buffer capacity – blue color betokens a good buffer effect of the saliva.

Table 3: Reference values and Color Chart:

Dentobuff strip			
		Final pH value	Buffer capacity
	blue	6.0 or more	High
	green	4.5 – 5.5	Medium
	yellow	4.0 or less	Low

TEST FOR MEASURING BUFFER VALUE OF SALIVA⁸

Method of Ericsson's:

The classical method for salivary capacity determination.

1. Collect saliva, either unstimulated for 15 min or stimulated for 5 min.
2. The amassed saliva is commixed by inverting the tube twice.
3. 1ml of the saliva is transferred to 3ml HCl (0.0033mol/l for unstimulated saliva, 0.0005mol/l for stimulated saliva).
4. For obviating the foaming, one drop of 2-octanol is integrated.
5. Mixing for 20min to abstract CO₂.
6. Electrometrically evaluation of Final salivary pH.[Table 4]

SALIVARY SUCROSE RATE (% BRIX)

{Using a refractometer to test the quality of fruits and vegetables}. Rex Harill, 1998. 'Perfect organic blends' journal.

Table 4: Buffer for unstimulated saliva

Final pH value	Evaluation
>4.75	High
4.25 – 4.75	Normal
3.50 – 4.24	Low
<3.50	Very Low
>6.50	High
5.75 – 6.50	Normal
4.00 – 5.74	Low
<4.00	Very Low

1. First Calibrate your instrument.
 - Place a drop of distilled dihydrogen monoxide over prism and tilt the plate down (if you have model plate). Shut off the flip hinged prism if there is a double prism model.
 - View instrument towards the source of light (a clean firmament is best).
 - Adjust the focusing ring until you visually perceive a clear fine picture of the scale in Brix. The demarcation line of dark and light fields must strike at zero level.

• Models of ATC (Automatic Temperature compensated) are scaled with the screw of adjustment to read zero. Adjustment of this is infrequently needed. Standard (non-ATC) models may require temperature rectification.⁹

Normal – 0.5-1.00mg/100ml
 High - >1.00mg/100ml
 Low - <0.5mg/100ml (Table 5)

Table 5: Criteria for Visual examination of Carious tooth.

Score	Criteria
0	No or only a slight change in enamel translucency after prolonged air drying (>5s)
1	Opacity or discoloration hardly visible on the wet surface, but distinctly visible after air drying.
2	Opacity or discoloration distinctly visible without air drying.
3	Localized enamel breakdown in opaque or discolored enamel and/or grayish discolored from underlying dentine.
4	Cavitation in opaque or discolored enamel exposing the dentine beneath.

METHOD OF CALCULATION OF DMF INDEX. (WHO 1986)¹⁰

The largest number for a single DMFT score is 28 or 32; when teeth are included. For example, a DMFT score of 3+5+5=13 for an individual betokens that 3 teeth are decayed, 5 teeth are missing and 5 teeth have fillings. Furthermore, it additionally betokens that 15(i.e. 28-13=15) teeth are intact.

PRINCIPLE:

Refractometer

A handheld refractometer is best instrument for quantifying a liquid's refractive index. It works on the critical angle principle by which lenses and prisms project a shadow line onto a minuscule glass reticle inside the instrument, which is then viewed by the utilizer through a magnifying eyepiece.

In utilization, a sample is placed between a quantifying prism and a minute cover plate. Light peregrinating through the sample is either passed through to the reticle or consummately reflected internally. The effect is that a line of shadow forms between the dark and illuminated area. This line of shadow crosses the scale that a reading is taken. Because refractive index is very temperature dependent, it is paramount to utilize a refractometer with automatic temperature emolument. Emolument is accomplished through the utilization of a minuscule bi-metallic divest that moves a lens or prism in replication to temperature changes.

DISCUSSION

The general term “saliva” refers to the fluid that circumvents all oral hard and soft tissues. This oral fluid (that is, whole saliva) represents an amalgamation of individual fluids and components derived from several sources. Major and minor salivary glands make the bulk contribution to whole saliva, with minor contributions from nonglandular sources such as crevicular fluid, oral microorganisms, host-derived cells, and cellular constituents, as well as diet-cognate components. Rudimental salivary research pertinent to the development of caries has provided considerable understanding of sundry salivary anti-cariogenic mechanism in vitro. Despite this erudition, the relative paramountcy of these mechanisms in vivo remains obscure. This study provides a link of clinical evidence establishing the role of saliva in bulwarking individuals against caries.¹¹

Comparing other studies, it was found that 50 extracted human teeth were culled by examination to study enamel lamellae by B.N.Walker, O.F.Makinson, M.C.R.B. Peters in 2011.¹² Data extraction by Rebecca Harris et al, studied four types of study design: cross-sectional, cohort, case-control and interventional studies. Heterogeneity among the studies especially with deference to varying quality and presentation of results, precluded utilization of statistical methods of pooling data such as meta-analysis. A secondary objective was to describe the extent

to which relationships between risk factors are expounded by ethnic group and material deprivation.¹³ Later on 40 subjects between the ages of 18 and 40 years old were culled which underwent five tests (hydration status in unstimulated saliva, salivary viscosity, unstimulated salivary pH, stimulated salivary flow rate, buffering capacity in stimulated saliva) by utilizing a saliva testing kit (GC Asia Dental private Circumscribed, Japan).¹⁴ Similarly, a total of 14 sound teeth, premolars and third molars extracted due to orthodontic reasons and impaction were accumulated and stored in thymol solution.¹⁵ Further, 196 children were assessed for the possible relationship between salivary cariogenic microflora, buffer capacity, secretion rate and caries experience by B. Sakeenabi, S.S. Hiremath.¹⁶

According to Rebecca Harris et al³⁰ cohort studies and chance of his or her remaining caries free until three years of age is highest, if good oral hygiene habits subsist and no visible plaque is present at 2 years age. Karjalainen et al(2001) additionally found the coalescence of unsuitable dietary habits and poor oral hygiene to be paramount, for whilst saccharine intake of more than weekly and presence of visible plaque did not increment caries by themselves, the two amalgamated gave a 17 fold caries risk as compared to children with neither habit. Schwartz et al (1998) showed an effect above the amendments that might be achieved through dental health edification, by introducing daily toothbrushing in kindergartens. Some may suppose that the amelioration cognate to toothbrushing is on account of the utilization of fluoride toothpaste rather than abstraction of plaque, but the paramount effect of visible plaque in the final regression model betokens that atleast in a population with circumscribed oral hygiene, the mechanical cleaning may integrate to the effect of fluoride. There is evidence in more studies that toothbrushing circadianly or more as opposed to less than once daily and the presence of visible plaque is paramount.¹³

Relatively Weerheijm KM et al in 1992 reported that the mundane salivary flow rate (hydration status and stimulated saliva flow rate) imparts a vigorous protective effect against dental caries. It was observed in his study that 90% subjects of control group had taken less than 30 second to hydrate their mucosa and had a stimulated salivary flow rate more preponderant than 1ml/minute. Significantly lower salivary flow rate in other group may be associated with a number of predisposing factors such as lack of raw material (dihydrogen monoxide), lack of stimulus to the salivary gland or could be a quandary with salivary gland itself. The patient with stimulated saliva flow rate less than 1.0 ml / min are considered to be in peril to develop dental caries where as a stimulated salivary flow rate more preponderant than 1.0 ml/min is considered to be mundane.¹⁷ According to N.P. Walsh et al dehydration induced by a circadian period without victuals and dihydrogen monoxide was shown to reduce parotid saliva flow rate in both adolescent and older adults. Collectively, this evidence suggests that there is a relationship between whole body hydration status and saliva.¹⁷ According to Lumikari et al, saliva secretion and salivary components secreted in saliva are consequential for dental health. The lubricating and antimicrobial functions of saliva are thus maintained mainly by reposing saliva. Stimulation of saliva results in a flushing effect and the clearance of oral debris and noxious agents. In general, the higher the flow rate the more expeditious the clearance (Mura et al 1991) and the higher the buffer capacity (Birkhed and Heintze, 1989). Reduced salivary flow rate and the concomitant reduction of oral bulwark systems may cause rigorous caries and mucosal inflammations. (Daniels et al, 1975; Van der Reijden et al, 1996).¹⁹

As studied by Johanston et al, 1992 secretion rate of stimulated saliva decreases as the degree increases, the buffer effect additionally increases. A paramount correlation was reported between degree of salivary secretion

rate and rigor of caries.²⁰ Logerlof and Oliverby, 1994¹⁹ designated a low flow rate coalesced with a low or moderate buffer effect proving poor salivary resistance against microbial attack. Dawes, 1987 studied patients with stimulated salivary flow rate less than 1.0ml/minute and considered it as risk to develop dental caries whereas a stimulated salivary flow rate more preponderant than 1.0 ml/minute was considered to be mundane.

According to Pajari 1988; Holbrook et al 1993 showed initial caries found to be the most vigorous prognosticator of caries occurrence in future. They found, carious subjects had low salivary reposing pH level in range of 5.2-6.2.²¹ According to Victorino R et al, determined salivary parameters which included cariogenic bacteria counts, pH, buffer capacity, total protein content and flow rate. Aspects in individual of hygiene and diet habits were considered additionally. Value corresponding to the difference of reposing saliva pH and stimulated saliva pH is positively correlated with index of DMFT. Correlation of Mutans Streptococci with Lactobacilli, as aforetime described for caries diagnostics. However, the results of this study were inconclusive, exhibiting that salivary test parameters either as single test or even in cumulation are incapable of presaging caries accentuating the paramountcy of saliva composition.²¹ According to Veena Shetty et al, saliva is considered as mirror of the body is rarely available and the accumulation process is fairly straight forward. Saliva being the diagnostic implement for detection of dental caries shows lower flow rate, viscosity, pH and buffering capacity. In this study, unstimulated saliva was amassed for the assessment of *S. mutans*, total antioxidant level.²³

Men had a more preponderant buffer affect than women. According to Heintz et al, 1983 studied the buffer effect of saliva in forty men by buffering apparatus and found it was affected by hormonal and metabolic changes, as well as by altered general health.²⁴ According to Sakeenabi et al, 2011 studied the

buffer effects of saliva by chemical analysis and found 75% of participants presented with buffer capacity of >6pH, followed by 23.47% with buffer score of 1 (pH 4.5-5.5) and 1.53% with buffer score of 2(pH <4.5).²⁴ According to Vitorino et al, caries onset and progression is influenced by diverse bacterial, dietary, environmental, socio-economic and physiological hazards. The most sequential markers include the caries experience, the concentrations of lactobacilli and mutans-streptococci, as well as prevential factors, such as the capacity of saliva buffering. Evaluated and compared caries risk factors and found salivary test parameters as single test or even in amalgamation are incapable of presaging caries accentuating the paramountcy of saliva composition.²⁶

According to Cataldo et al studied the effects of the salivary temperature by clinical thermometer and showed no paramount correlation to the effect of dental caries.²⁷

Mean salivary sucrose levels were higher in carious group than the non-carious groups. According to Malvin. E. Ring et al found mundane sucrose levels in saliva are 0.5-1.0% Brix and don't precisely affect health of oral environment or put forth the magnification of micro-organisms. However, higher salivary sucrose levels favor the proliferation of micro-organisms and enhance their colonization on teeth and oral mucous membranes. Proposed likely elevated salivary sucrose levels in carious individuals.²⁸ According to J.C. Hase, D. Birkhed; 1988 studied the effect of different salivary secretion rates on sucrose concentration in saliva and on pH vicissitudes in dental plaque in man. Eighteen dental students, 21-33 years participated. Dry mouth was produced injecting methylscopolamine-nitrate in the submucosa in the labial sulcus. Dry mouth thus was established, then were quantified at 1hour intervals, while the salivary flow was recuperating: secretion rate of reposing and paraffin-wax stimulated whole saliva; sucrose clearance in saliva. A salivary sucrose clearance time was found to be 0.14ml/minute for reposing and 0.62ml/minute

for stimulated whole saliva (mean values). The pH vicissitudes in dental plaque after the mouth rinse with sucrose at astronomically low secretion rate were significantly more pronounced than at mundane flow rate. Thus, salivary secretion rate affects both the sucrose clearance in saliva and pH vicissitudes in dental plaque in man.²⁸

According to Rebecca Harris et al, sodality between caries and consumption of biscuits, cakes, sugar confectionary, chocolate confectionary, soft-drinks, percentage of energy from non-milk extrinsic sugars. According to Lumikari et al, dental caries is probably the most prevalent consequence of hyposalivation (Brown et al 1978; Scully 1986). Caries lesions develop rapidly and withal on tooth surfaces that are not susceptible to caries. Subjects with impaired saliva flow rate often show high caries incidence (Papas et al, 1993; Pak et al 1994) or caries susceptibility (Heintz et al, 1983). It must be accentuated however, that no linear relationship subsists among salivary secretion rate, caries activity and DMFS/DMFT values (Birkhed and Heintze 1989; Russell et al 1990). Only impuissant or no sodality between saliva secretion rates and caries incidence has been stabilised.

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