Research Article
ISSN: 0974-6943

Priyanka Kapooret al. / Journal of Pharmacy Research 2012,5(2),963-967

Available online through
http://jprsolutions.info

Influence of the blood group reactive substances in saliva on the aggregation of *Streptococcus mutans*

Priyanka Kapoor*, Narayan patel, Leena Parihar, Vipin Gupta

Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara, Punjab (INDIA) -144402)

Received on:09-12-2011; Revised on: 15-12-2011; Accepted on:10-02-2012

ABSTRACT

Streptococcus mutans is one of the most dominant species of oral cavity that also initiates the dental plague formation. An attempt has been made to establish the correlation between the secretor status and aggregation of *Streptococcus mutans* with the salivary blood group reactive substances. The level of aggregation of *S. mutans* with salivary mucin was studied spectrophotometrically (630 nm) and has been greatly influenced by the blood group reactive substances. A person can be either a secretor or a non-secretor. This is completely independent of whether one's blood type is A, B, AB or O. In the present study it was observed that out of the 98 individuals 94 were secretors. The secretor status was determined by absorption elution technique. The presence of *Streptococcus mutans* in the saliva was detected by the colony morphology grown on Nutrient agar followed by Gram's staining and then the presence of *S. mutans* in the saliva were confirmed by their biochemical test of the same samples. The aggregation in the non-secretors is almost constant whereas the level of aggregation in blood group A, B, AB and O are found to be reducing with respective dilution.

Key words: *Streptococcus mutans*, aggregation, Infection immunity, Secretor.

INTRODUCTION

Interactions between bacteria and salivary components are of profound interest in oral biology. Salivary components facilitate and influence the adherence of micro-organisms on the teeth and oral epithelia and thus in dental plaque formation (1). Substances in saliva can interact both with mucosal-cell surfaces and with invading bacteria and these interactions may have a profound influence on the fate of bacteria entering the oral cavity. Dental caries is a complex infectious and transmissible disease (2), resulting from the interaction of several factors including the host, agent, substrate and time. Modern concepts consider dental caries as an interaction between genetic and environmental factors, in which social, biological, psychological and behavioral factors are expressed in a complex interactive manner (3). *Streptococcus mutans* was isolated for the first time from the dental plaque by Clarke in 1924. The name '*mutans*' was chosen because of its tendency to exhibit both coccal and rod shaped (mutant) cell morphology. It is a primary aetiological agent in dental caries and is reported to colonize orthodontic patients in significant numbers (4). *Streptococcus mutans* is a Gram-positive, facultatively anaerobic bacterium commonly found in the human oral cavity and is a significant contributor to tooth decay (5, 6). *S. mutans* is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth. Sucrose is used by *S. mutans* to produce a sticky, extracellular, dextran-based polysaccharide that allows them to co-here to each other, forming plaque. *S. mutans* produces dextran via the enzyme dextransucrase (a hexosyltransferase) using sucrose as a substrate in the following reaction:

n sucrose → (glucose)n + n fructose

Sucrose is the only sugar *S. mutans* can use to form this sticky polysaccharide (5). Conversely, many other sugars—glucose, fructose, lactose can be digested by *S. mutans*, but they produce lactic acid as an end product. It is the combination of plaque and acid that leads to dental decay (7). In saliva there are various substances able to aggregate bacteria like a-amylase (8), lysozyme (9), secretory IgA (10) and blood group reactive glycoproteins (11). Blood group antigens are oligosaccharides found on the surface of erythrocytes and can be detected in other tissue cells and in body fluids like saliva, sweat, semen, milk etc. It has been established that, secretion of group specific substances in body fluids is controlled by a pair of alleles Se and se. Thus, a person can be homozygous [SeSe], heterozygous [Sese] or homozygous [sese]. The first 2 classes are called secretors and third class, non-secretor (12).

The aim of the research work is to determine the connection between secretor status in saliva and aggregation of *S. mutans* with salivary blood-group reactive substances.

***Corresponding author.**
Priyanka kapoor
Assistant Professor,
Department of Biotechnology,
Lovely Professional University,
Jalandhar-Delhi G.T. Road (NH-1),
Phagwara, Punjab (INDIA) -144402

MATERIALS AND METHODS

1). Collection of saliva sample: The saliva samples were collected randomly after taking consent from 98 subjects (irrespective of gender, caste, diet) for blood group A (23), B (18), O (13) and AB (9). (13)

2). Bacterial strain: The standard strain of *Streptococcus mutans* MTCC 890 was procured from IMTECH, Chandigarh, India.

3). Spectrophotometric Analysis of Bacterial aggregation

0.1ml saliva was taken in appendorf (collection vials, 1.5ml.) and serially diluted in 10mM potassium phosphate buffer (pH-7.0) with 0.5 mM calcium chloride. Subsequently, 0.2 ml of bacterial cell suspension (fresh bacterial culture of *Streptococcus mutans* grown in nutrient broth) was added to it. By aggregation the absorbance of a cell suspension decreases, which can be measured spectrophotometrically (630nm) (14). A non-aggregating cell suspension with buffer instead of saliva was used as a control. Two parameters were chosen to quantify the aggregation process:

- the decrease in absorbance after one hour (6Abs), which was a measure for the rapidity of the aggregation process, and
- the bacterial aggregating titer, which was a measure for the minimum saliva concentration inducing aggregation. For aggregation inhibition studies the bacterial cell suspensions were pre-incubated at 7° C for 10 to 30 min and were observed at 1000 x magnification.

4). Determination of secretor status

The secretor status of an individual was determined using Absorption Elution technique and was found to yield the most accurate results as compared to Absorption Inhibition technique and Mixed Agglutination technique. In this technique strong antiserum is used directly instead of its titre

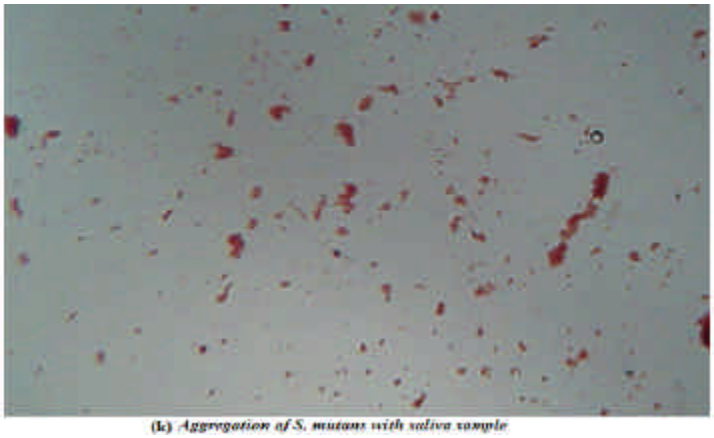
because it takes less time to determine the secretor status of the individual. (15, 16)

5). Biochemical test

Biochemical test for identification of unknown bacterial samples was done including Arginine Hydrolysis, 6.5% NaCl Tolerance Test, Starch Hydrolysis Test, Urea Hydrolysis test, Voges-Proskauer Test, (17), Manitol test, MR-VP (18)

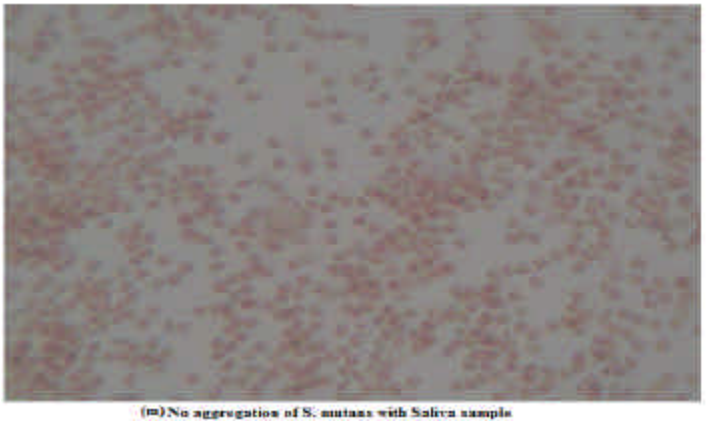
RESULTS

It was observed that out of 98 individuals participated in the study for determining the secretor status, 4 were found to be non secretor and remaining were secretors. The secretors showed good level of aggregation (Figure-1)as compared to non secretors in which there was no aggregation (Figure-2) or less aggregation (Figure-3) seen of *S. mutans* with saliva sample. The absorption (630nm) was calculated for 70 individuals as shown in Table-1 and Table-2 interprets that the level of aggregation in the non-secretors remains nearly constant whereas the level of aggregation in secretors were found to be reducing up to 32-fold dilution. It is seen that at all the dilutions the bacterial aggregation is maximum in saliva samples that secrete Antigen O, followed by AB, B and the minimum in A. The aggregation in saliva samples of the non-secretors is negligible (Figure-4). Out of 98 saliva samples when 40 were employed for the biochemical characterization test, almost all the samples were found to be showing positive result for the presence of *S. mutans* (Table 3). All the samples were found to be Gram positive, and showing alike colony morphology when grown on the nutrient agar media. They were absent when grown in 6.5% NaCl and not utilizing Arginine but there is a fluctuation in the utilization of Mannitol. 15 samples out of 40 exhibited the utilization of Mannitol whereas 25 did not exhibit its utilization. They are also found to be utilizing starch.



(k) *Aggregation of S. mutans with saliva sample*

Fig: 1. The aggregation of *Streptococcus mutans* mutans with saliva sample



(m) No aggregation of S. mutans with Saliva sample

Figure-2.No aggregation of *S. mutans* with saliva sample

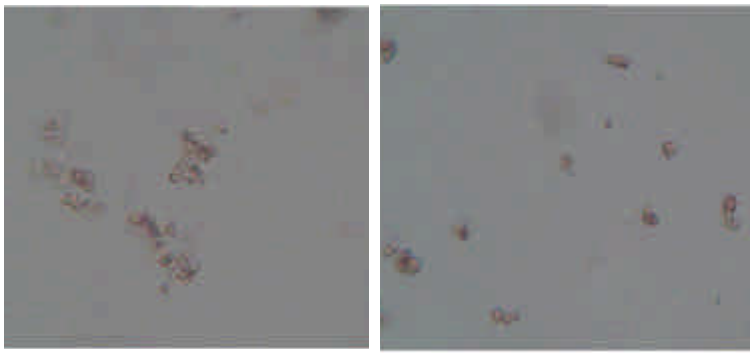


Fig: 3 less level of aggregation of *Streptococcus mutans* with saliva sample.

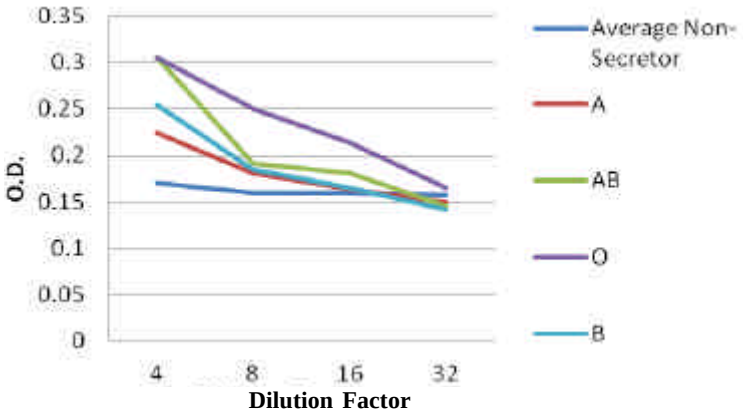


Fig:-4 Variation of optical density(cell concentration) with dilution

Reading interpretation for Biochemical Test:

1. Arginine Hydrolysis- Out of 40 samples only 3 samples showed positive result as the broth shows a deep purple colour indicating an alkaline reaction due to release of ammonia. .
2. 6.5% NaCl Tolerance Test- All samples showed negative results as no turbidity appeared.
3. Starch Hydrolysis Test- A clear zone surrounding the growth is observed in all samples showing positive test
4. Urea Hydrolysis test- No colour change in the slants indicates a negative test in urea hydrolysis test for all samples.
5. Voges-Proskauer Test (VP)- Out of 40 samples, red colouration was observed only in 4 samples showing positive result.
6. Mannitol Test- The positive results for Mannitol Test were seen for 36 samples out of 40 samples.

DISCUSSION

Presence of microorganisms in saliva is based on the findings that there is an association between the type and number of bacteria in plaque and saliva. The total counts and the *Actinomyces viscosus/naeslundii* and *Streptococcus sanguis* counts in dental plaque had increased by approximately two log units, while the *Streptococcus mutans* counts had increased by more than one log unit.(19). Evaluation of patients for Sjogren's syndrome and radiation-induced xerostomia had the lowest salivary gland performance but displayed a mucosal status similar to denture-wearing healthy patients with normal salivary flow who had idiopathic xerostomia. However, those patients with a total lack of salivary flow rarely had normal-appearing oral mucosa. These results confirm a role for saliva in oral mucosal preservation and also suggest that other factors may act to maintain oral mucosal integrity (20). There is a strong correlation between salivary blood group antigen or secretor status and caries susceptibility. Authors have studied lower caries prevalence in secretors than in non-secretors (21) and among blood

Table: 1 Showing the result of secretor status determination with absorbance.

S.No	Sample No	Sex M/F	Diet Veg/Nveg	se/nse	Blood Group tested from blood	Reaction with Anti- A	B	D	H	Blood group from saliva	Dilution Factor 4-fold	8-fold	16-fold	32-fold
1	29	F	Veg	se	B+	-	+	+	-	B+	-0.03	-0.081	-0.09	-0.09
2	30	F	Veg	nse	B+	-	-	-	-	-	-0.06	-0.09	-0.09	-0.09
3	31	M	N.vg	se	A+	+	-	+	-	A+	-0.08	-0.084	-0.08	-0.09
4	32	M	Veg	se	B+	-	+	-	+	B+	-0.05	-0.09	-0.09	-0.09
5	33	F	N.vg	se	A+	+	-	-	-	A+	-0.02	-0.01	-0.01	-0.02
6	34	M	N.vg	Se	O+	+	+	-	+	O+	-0.08	-0.076	-0.08	-0.08
7	35	M	N.vg	Se	AB+	+	+	+	-	AB+	-0.08	-0.073	-0.07	-0.07
8	36	F	N.vg	Se	AB+	+	+	+	-	AB+	0.481	0.116	0.17	0.042
9	37	M	N.vg	se	B+	-	+	+	-	B+	0.344	0.155	0.12	0.085
10	38	F	N.vg	se	B+	-	+	+	-	B+	0.215	0.084	0.08	0.045
11	39	M	N.vg	se	AB+	+	+	+	-	AB+	0.424	0.182	0.18	0.179
12	40	M	Veg	se	B+	-	+	-	-	B+	0.176	0.13	0.1	0.084
13	41	M	N.vg	se	O+	-	-	+	+	O+	0.292	0.201	0.16	0.093
14	42	M	N.vg	se	A+	+	-	+	-	A+	0.31	0.217	0.1	0.064
15	43	M	N.vg	se	A+	+	-	+	-	A+	0.301	0.207	0.1	0.094
16	44	M	N.vg	se	B+	-	+	-	-	B+	0.349	0.142	0.11	0.104
17	45	M	N.vg	se	O+	+	+	-	+	O+	0.582	0.44	0.38	0.3.76
18	46	M	N.vg	se	B+	-	+	+	+	B+	0.52	0.387	0.38	0.379
19	47	M	N.vg	se	B+	-	+	+	-	B+	0.321	0.296	0.28	0.178
20	48	M	N.vg	se	B+	-	+	+	-	B+	0.512	0.323	0.19	0.101
21	49	M	N.vg	se	B+	-	+	-	-	B+	0.362	0.228	0.19	0.106
22	50	M	N.vg	se	B+	-	+	+	-	B+	0.316	0.238	0.18	0.153
23	51	M	N.vg	se	A+	+	-	+	+	A+	0.359	0.341	0.3	0.212
24	52	M	N.vg	se	O+	-	-	+	+	O+	0.455	0.383	0.35	0.307
25	53	M	N.vg	se	AB+	+	+	+	-	AB+	0.618	0.403	0.36	0.188
26	54	M	N.vg	se	O+	-	-	+	+	O+	0.509	0.415	0.35	0.211
27	55	M	N.vg	se	A+	+	-	-	-	A+	0.241	0.193	0.19	0.177
28	56	M	N.vg	se	AB+	+	+	-	-	AB+	0.367	0.269	0.2	0.19
29	57	M	N.vg	se	A+	+	-	-	-	A+	0.2	0.187	0.18	0.175
30	58	M	N.vg	nse	A+	-	-	-	-	-	0.18	0.17	0.17	0.165
31	59	M	N.vg	se	B+	-	+	-	-	B+	0.204	0.197	0.18	0.179
32	60	M	N.vg	se	A+	+	-	-	-	A+	0.239	0.183	0.18	0.177
33	61	M	N.vg	se	A+	+	-	-	-	A+	0.276	0.189	0.18	0.164
34	62	F	Veg	se	A+	+	-	-	-	A+	0.222	0.183	0.2	0.18
35	63	F	N.vg	se	A+	+	-	-	-	A+	0.21	0.184	0.18	0.152
36	64	M	N.vg	se	A+	+	-	-	-	A+	0.23	0.195	0.19	0.164
37	65	M	N.vg	se	A+	+	-	-	-	A+	0.203	0.175	0.16	0.15
38	66	M	N.vg	se	AB+	+	+	-	-	AB+	0.189	0.188	0.18	0.177
39	67	M	N.vg	se	B+	-	+	+	-	B+	0.213	0.204	0.19	0.187
40	68	M	N.vg	se	O+	-	-	+	+	O+	0.341	0.323	0.2	0.147
41	69	F	Veg	se	O+	-	-	+	+	O+	0.228	0.197	0.18	0.179
42	70	M	N.vg	se	B+	-	+	+	-	B+	0.223	0.196	0.19	0.19
43	71	F	Veg	se	B+	-	+	+	-	B+	0.278	0.232	0.2	0.184
44	72	M	N.vg	se	AB+	+	+	-	-	AB+	0.242	0.21	0.19	0.191
45	73	M	N.vg	se	A+	+	-	+	-	A+	0.229	0.198	0.19	0.191
46	74	M	N.vg	se	AB+	+	+	-	-	AB+	0.302	0.211	0.21	0.196
47	75	M	N.vg	se	A+	+	-	+	-	A+	0.202	0.196	0.19	0.191
48	76	M	Veg	se	A+	+	-	-	-	A+	0.371	0.209	0.18	0.159
49	77	M	N.vg	se	A+	+	-	+		A+	0.257	0.21	0.21	0.205
50	78	M	Veg	se	O+	-	-	+	+	O+	0.217	0.197	0.19	0.156
51	79	M	N.vg	se	A+	+	-	+	-	A+	0.253	0.191	0.19	0.185
52	80	M	N.vg	se	A+	+	-	-	-	A+	0.271	0.234	0.19	0.185
53	81	M	N.vg	se	O+	-	-	+	+	O+	0.335	0.237	0.22	0.208
54	82	M	N.vg	se	O+	-	-	+	+	O+	0.26	0.212	0.19	0.156
55	83	M	N.vg	se	B+	-	+	+	-	B+	0.236	0.151	0.15	0.118
56	84	F	N.vg	se	A+	+	-	-	-	A+	0.25	0.206	0.19	0.178
57	85	M	N.vg	se	B+	-	+	+	-	B+	0.223	0.191	0.18	0.156
58	86	M	N.vg	se	O+	-	-	+	+	O+	0.267	0.243	0.2	0.191
59	87	F	Veg	se	A+	+	-	+	-	A+	0.198	0.178	0.17	0.151
60	88	F	Veg	se	B+	-	+	+	-	B+	0.25	0.205	0.21	0.191
61	89	M	N.vg	se	B+	-	+	+	-	B+	0.253	0.222	0.22	0.212
62	90	M	N.vg	se	O+	-	-	+	+	O+	0.238	0.235	0.2	0.191
63	91	M	N.vg	se	O+	-	-	+	+	O+	0.332	0.24	0.23	0.214
64	92	F	N.vg	se	B+	-	+	+	-	B+	0.215	0.211	0.2	0.187
65	93	M	N.vg	se	B+	-	+	+	-	B+	0.222	0.213	0.21	0.191
66	94	F	N.vg	se	B+	-	+	+	-	B+	0.238	0.198	0.19	0.178
67	95	M	N.vg	nse	AB+	-	-	-	-	-	0.211	0.212	0.21	0.209
68	96	M	N.vg	nse	B+	-	-	-	-	-	0.297	0.297	0.3	0.297
69	97	F	Veg	se	A+	+	-	-	-	A+	0.228	0.212	0.21	0.209
70	98	M	N.vg	se	B+	-	+	+	-	B+	0.261	0.222	0.22	0.212

Out of 98 samples 4 found to be non secretor and The O.D was taken at 630 nm interprets that the level of aggregation in the non-secretors remains nearly constant whereas the level of aggregation in secretors were found to be reducing up to 32-fold dilution.

Table: -2 O.D. of *S.mutans* in Saliva samples of secretors and non-secretors at given dilutions.

O.D. of *S.mutans* in Saliva samples at given dilutions.					
Saliva samples	**Dilution Factor (folds)**	**4**	**8**	**16**	**32**
Secretors(66)	**Avearge O.D. of blood group**				
	A	0.224565	0.181043	0.163478	0.148609
	B	0.254391	0.184957	0.164783	0.14087
	AB	0.306	0.190889	0.18125	0.144667
	O	0.305846	0.249769	0.213077	0.164417
Non-Secretors(4)	Average O.D.	0.17075	0.15975	0.15925	0.15775

The Optical density of *S. mutans* in Saliva samples of secretors and non-secretors interprets that the level of aggregation in the non-secretors remains nearly constant whereas the level of aggregation in secretors were found to be reducing up to 32-fold dilution. From the table it is seen that at all the dilutions the bacterial aggregation is maximum in saliva samples that secrete Antigen O, followed by AB, B and the minimum in A.

Table: 3 Biochemical characterization of salivary organism

Sample .no.	Lactose	Mannitol	MR	VP	6.5% NaCl	Arginine	Starch	Urea
1	+	-	+	-	-	-	+	-
2	+	-	+	-	-	-	+	-
3	+	-	-	+	-	-	+	-
4	+	-	+	-	-	-	+	-
5	+	-	+	-	-	-	+	-
6	+	-	+	-	-	-	+	-
7	+	-	+	-	-	-	+	-
8	+	-	+	-	-	-	+	-
9	+	-	+	-	-	-	+	-
10	+	-	+	-	-	-	+	-
11	+	-	+	-	-	-	+	-
12	+	-	+	-	-	-	+	-
13	+	-	+	-	-	-	+	-
14	+	+	+	-	-	-	+	-
15	+	+	+	-	-	-	+	-
16	+	+	+	-	-	-	+	-
17	+	-	+	-	-	-	+	-
18	+	-	+	-	-	+	+	-
19	+	-	+	-	-	-	+	-
20	+	-	+	-	-	-	+	-
21	+	-	+	-	-	+	+	-
22	+	-	+	-	-	+	+	-
23	+	-	+	-	-	-	+	-
24	+	-	+	-	-	-	+	-
25	+	-	+	-	-	-	+	-
26	+	+	+	-	-	-	+	-
27	+	+	+	-	-	-	+	-
28	+	+	+	-	-	-	+	-
29	+	+	+	-	-	-	+	-
30	+	+	+	-	-	-	+	-
31	+	+	+	-	-	-	+	-
32	+	+	-	+	-	-	+	-
33	+	+	-	+	-	-	+	-
34	+	+	-	+	-	-	+	-
35	+	+	+	-	-	-	+	-
36	+	+	+	-	-	-	+	-
37	+	+	+	-	-	-	+	-
38	+	-	+	-	-	-	+	-
39	+	-	+	-	-	-	+	-
40	+	-	+	-	-	-	+	-

Biochemical characterization of salivary organism from all the samples were found to be showing positive result for the presence of *S. mutans*.

group A saliva samples (22) but found to aggregate *S. rattus* very well, even according to our study four non-secretors were unable to aggregate *S. mutans* which gives the basis to this study. In previous study, aggregations between selected strains of oral streptococci and saliva were studied spectrophotometrically. A model for the aggregation reaction was developed which explained the shape of these parabolic curves. The parameters of the model can be calculated from the experimental data and can be used to estimate the concentration of aggregation-inducing substances in undiluted saliva (23).In conclusion, the results presented in this paper strongly suggest that blood group antigens on salivary glycoproteins may be involved in the interaction with *Streptococcus mutans*.

LIST OF ABBREVIATIONS

O.D- Optical Density
B.S.A- Bovine Serum Albumin
nm- Nanometre
ml- Millilitre
mg- milligram
D/W- Distilled water
veg- Vegetarian
N.vg- Non- Vegetarian

ACKNOWLEGEMENT: Authors are thankful to Lovely Professional University, Punjab, India for providing necessary laboratory facilities for carrying research work.

DISCLOSURE: There is no interest to disclose author's financial arrangements with any organization.

REFERENCES

1). Gibbons R.J. and Van Houte J. , Dental Caries, *Annu. Rev. Med*., **26**, 1975, 121-136.
2). Seow W.K., Biological mechanisms of early childhood caries, Community Dent Oral Epidemiol., **26(**1),1998, 8-27.
3). Reisine S. and Litt M., Social and psychological theories and their use in dental practice, *Int Dent J*., **43**, 1993, 279-287.
4). Antony B., Rekha B, Shetty A.K., Kuruvilla T. and Ramanathan K., Semiquantitation and characterization of *Streptococcus mutans* from patients Undergoing orthodontic treatment, *Journal of Bioscience Tech.*, **1**, 2010, 59-63.
5). Ryan K.J. and Ray C.G. (editors), Sherris Medical Microbiology, 4th ed. McGraw Hill., (2004)
6). Loesche W.J., Microbiology of Dental Decay and Periodontal Disease. In: Baron's Medical Microbiology (Baron S et al., eds.), 4th ed. University of Texas Medical Branch, (1996) ISBN 0-9631172-1-1.
7). Madigan M. and Martinko J. Brock Biology of Microorganisms, 11th ed. Prentice Hall, (2005)
8). Douglas C.W., The binding of human salivary a-amylase by oral strains of streptococcal bacteria, *Arch. Oral Biol*., **28**, 1983, 567-573.
9). Pollock J. J., Iacono V.J., Bicker H.G., MacKay B. J., Katona L.I. and Taichman L.B. The binding, aggregation and lyric properties of lysozyme. In: Stales HM, Loesche WJ and O'Brien TC (Eds) Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts) 2, 1976, 325-352) Information Retrieval Inc. Washington DC.
10). Bratthall D. and Carlen A., Salivary agglutinin and secretory IgA reactions with oral streptococci, Scand. *J. Dent. Res*., **86,** 1978, 430-443.
11). Gibbons R.J. and Qureshi I.V. Selective binding of blood group-reactive salivary mucins by *Streptococcus mutans* and other oral organisms. *Infect. Immun*. **22**, 1998, 665-671.
12). Karpoor?C.,?Shettar?S. S.,?Jatti?V. B. and?Kulkarni?V.,?(Study of secretors and non-secretors in normal healthy population – Its forensic implication in human identification, *Indian Journal of Forensic Medicine &Toxicology,* **4**(1), 2010, 11-13.
13). Ligtenberg A.J.M., Veerman E.C.I., Graaff J. de and Nieuw Amerongen A.V. Influence of the blood group reactive substances in saliva on the aggregation of *Streptococcus rattus*, *Antonie van Leeuwenhoek* **57,** 1990**,** 97-107
14). Koop H.M., Benz M.V., Amerongen A.V.N., Roukema P.A. and Graaff J.D. (1989) Aggregation of 27 oral bacteria by human whole saliva. Influence of culture medium, calcium, and bacterial cell concentration, and interference by autoaggregation. *Antonie Van Leeuwenhoek,* **55**(3):277-290

15). Kaur G. And Sharma V.K. Comparison of Absorption-inhibition and Absorption-Elution methods in the detection of ABO (H) in sweat stains, *Current Science*, **57**(22), 1988, 1221-1224.
16). http://www.docstoc.com/docs/36391420/PROCEDURE-MANUAL
17). Murray P.R., Baron E.J., Jorgensen J.H., Pfaller M.A. and Yolken R.H. (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C., (2003)
18). Cappuccino, J.G. and Sherman, N. Microbiology- A Laboratory manual. 3rd edition, Cummings publisher, (1992).
19). Schaeken M.J., Creugers T.J. and Van der Hoeven J.S. Relationship between dental plaque indices and bacteria in dental plaque and those in saliva, *J Dent Res.*, **66**, 1987, 1499-502.
20). Wolff A. ,Fox P.C. ,Ship J.A., Jane C. Atkinson, Maeynski A. A. and Baum B.J. Oral mucosal status and major salivary gland function, Oral Surgery, Oral Medicine, *Oral Pathology*, **70**(1), 1990,49-54.
21). Holbrook W.P. and Blackwell C.C. Secretor state and dental caries in Iceland. *FEMS Microbiol. Immunol.* 47, 1989, 397-400.
22). Arneberg P., Kornstad L., Nordb6 H & Gjermo P. Less dental caries among secretors than among non-secretors of blood group substance, *Scand. J. Dent. Res.*, **84**, 1976,362-366.
23). Ericson T, Pruitt K, Wedel H. The reaction of salivary substances with bacteria**,** *J. Oral Pathol.*, **4**(6), 1975, 307-23.

Source of support: Nil, Conflict of interest: None Declared