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Comparative analysis of CRT Buffer, GC Saliva Check Buffer tests and laboratory titration to evaluate saliva buffering capacity

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SUMMARY

Objective. The purpose of this study is to evaluate the ability of two commercial strip tests and laboratory titration to detect saliva buffer capacity.

Materials and methods. Sixty-four patients were examined. Stimulated saliva was collected and buffer capacity was determined with two different chair-side strip tests in addition to immediate transportation to the laboratory to check the buffering ability by titrating with 0.005 M HCl and measuring pH by digital pH/Ion meter, used as a gold standart. The correlation were analyzed using the Spearman Rank Correlation Test, Cohen's Kappa coefficient and Pearson's Correlation test, $p<0.01$. Sensitivity and specificity were used to measure precision of these tests.

Results. The response rate was 80%. High buffer capacity was found in 23.4% of cases, medium in 62.5%, and low in 14.1%. The Spearman Rank Correlation coefficient between the titration method and CRT Buffer test was 0.685 and the GC Saliva Check Buffer was 0.837. The Kappa coefficient for the CRT Buffer test was 0.508, while the coefficient for the GC Saliva Check Buffer was 0.752. The Pearson Correlation for the GC Saliva Check was 0.675. The difference is found in the buffer capacity at initial pH and at pH value 3.

Conclusions. Both colorimetric tests correlate with the acid titration method in laboratory and are usable for saliva buffer capacity detection in dental offices. Buffer capacity detected in laboratory at different pH values can provide more information regarding caries risk.

Key words: saliva, caries risk, buffer capacity.

INTRODUCTION

Caries risk assessment has become a commonly used term both among researchers and in practical dentistry. Due to the fact is that caries is a multifactorial disease, to determine caries risk detailed information about patient should be collected, including saliva properties [1].

Saliva is essential for the lifelong conservation of dentition. There are various functions of saliva: tooth surface protection through a film of salivary mucins and proline-rich glycoprotein; a re-mineralisation function by attracting calcium, phosphate and fluoride ions; IgA, IgM, IgG, enzymes and other proteins ensure antibacterial function; and with bicarbonate, phosphate and protein buffer systems in saliva it also has a buffering ability [2].

Ericsson in 1959 wrote a review of 21 reports that appeared prior to 1956 and found caries experience connectivity to saliva buffer capacity [3]. Additionally, the Vipeholm study showed that caries-active patients had a lower buffer capacity than caries-inactive patient [4].

Buffer Capacity is the mmoles of NaOH or HCl per mL of buffer solution needed to produce a unit change in pH [5]. In saliva, we are interested in its buffering ability against acids. Salivary glands produce buffer systems in saliva, from which the most important bicarbonate system is produced from CO2. The bicarbonate level in plasma is about 20 – 25 mmol / l, in un-stimulated saliva it is about

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ten times lower – during stimulation, however, glandular cells increase CO2 production, increasing bicarbonate concentration and thereby making it closer to plasma concentration. With very high stimulation the bicarbonate concentration may even exceed the plasma level [6, 7]. It is also important to know that a reduction of the bicarbonate level in saliva reduces the saliva secretion rate [8], thus the buffer capacity is considered to be one of the most important factors for reducing secretion rate. Since the bicarbonate concentration is lower and the saliva flow rate can't provide buffer systems penetration in dental plaque, the plaque buffering ability is also reduced and the pH value falls to more acidic. That means there are more H+ ions in an individual's plaque that cause demineralization. Such plaque has a reduced ability to accumulate Ca and P [9, 10], so also re-mineralization possibilities are decreased.

In 1959, Ericsson developed a test [3] that could be used in laboratories or by researchers, but is too complicated and time-consuming a procedure for clinical practice [11]. There are several commercial buffer capacity diagnostic tests now available in the world market, but unfortunately there is no ideal test and the tests have varying prices and usage simplicity.

Therefore, the aim of the study was to evaluate the ability of two commercial strip tests and titration in laboratory to detect saliva buffer capacity.

MATERIALS AND METHODS

Study subjects

The study population involved 64 individuals aged from 24 to 62 years-old. This research received ethical approval from the Riga Stradins University Ethics Committee. All patients signed an agreement prior to entry into the study. There were 80 individuals invited, of which 64 agreed to the terms and were involved in study.

A questionnaire about age, gender, general diseases, drug use, diet and hygiene habits [1] was filled out for all participants. The survey was in the form of an interview focusing on general diseases and drug use, which could influence saliva flow rate and buffer capacity. Diet and drinking habits were also registered in detail.

Clinical examination involved the DNFT index according to the WHO criteria.

Saliva sampling

Saliva was stimulated by chewing paraffin wax and then collected from individuals who were instructed not to drink, eat, smoke, or chew chewing gum for at least 2 h and to rinse their mouths at least 1 h before examination [3, 12, 13]. Patients sat upright in a relaxed position with their heads tilted down [14] and started to chew paraffin wax. The first portion of saliva was swallowed, then time was taken for 5 minutes [15, 16], during which patients were instructed not to swallow, but collect all saliva in a container. Saliva was divided into two parts: 1) 5 ml of saliva was collected in plastic containers and within one hour transported to the laboratory; 2) the rest of the saliva was used for buffer capacity determination by the GC Saliva-Check Buffer test and the CRT Buffer test.

CRT Buffer test

With a pipette a saliva sample was taken from the collection cup, and 1 drop was dispensed onto a test pad. After waiting the 5 minutes recommended by the manufacturers, the final color was detected and buffering ability was assessed. Blue color means high buffer capacity, green means medium and yellow means low.

GC Saliva-Check Buffer

Using a pipette, a saliva sample was taken and 1 drop was placed on each of the 3 test pads. Test pads began to change color immediately, but the final color was only detected after 2 minutes. Then the result was calculated by adding the points according to the final color of each pad: green – 4 points; green/blue – 3 points, blue – 2 points, red/blue – 1 point, red – 0 points. All points were counted and result was determined: 0 – 5 points as very low buffering ability, 6 – 9 points as low, 10 – 12 points as normal/high.

Acid titration

In the laboratory 5 ml of saliva was collected into a special cup to maximize the contact area of the pH-sensitive electrode with saliva. The initial pH was measured by using a pH-sensitive electrode [17]. Then, using a precise burette, 0,5 ml of 0,005 M HCl was added to the test saliva and allowed to stabilize for a few seconds, after which the pH was measured again. The saliva sample was constantly stirred using a magnetic stirrer and glass rod [18]. The process was continued until the pH decreased by 1 unit. Then the buffer capacity was calculated using the formula $C_{HCl} \times V_{HCl} / \Delta_{pH} \times V_{saliva}$, where C_{HCl} means concentration of HCl, V_{HCl} means volume of HCl, Δ_{pH} means pH changes from initial, and Vsaliva represents volume of saliva sample [5].

The same process was continued until the pH decreased to a value of 3, and again buffering ability was calculated using the same formula.

Data analysis

The unit of measurement for buffering ability is mmol HCl / 1 liter saliva to decrease pH by 1 unit [5]. GC company includes these values in its colorimetric scale, and just after detecting the buffering ability value it is possible to detect whether the buffering ability is very low (buffer capacity 0 – 5), low (6 – 9) or high (10 – 12). The same values were calculated after titrating with 0,005 M HCl, and results were divided into 3 groups according to the previous mentioned system.

The manufacturers had interpreted test results in different ways. CRT Buffer test has been designed to detect high, medium and low buffer capacity, while GC Saliva Check Buffer divided results into normal/high, low and very low. As both tests use 3 groups, it was decided to divide all results into high, medium and low buffer capacity [19].

The correlation of ranking results of CRT Buffer, GC Saliva Check Buffer tests and titration were analyzed using the Spearman Rank Correlation Test and Cohen's Kappa coefficient, while the correlation of scale data, which was possible for GC Saliva Check Buffer and titration, were detected by Pearson's Correlation test, $p<0.01$.

To analyze colorimetric methods, results from the titration method with HCl were defined as "true" scores. Medium and low buffering ability were defined as "positive" cases (as the test showed a deviation from normal buffer capacity), while high cases were defined as "negative" (as the test indicated a normal buffer capacity). After measuring the buffer capacity with colorimetric tests, true positive cases (TP) were defined as medium and low scores when titration also detected as medium and low. False positive cases (FP), by contrast, were when colorimetric test indicated medium or low score, but titration indicated a high buffering ability. True negative cases (TN) were defined as when colorimetric tests indicated high capacity, as did titration method. False negative cases (FN) were where chair-side tests indicated high capacity, but titration with acid revealed medium or low buffering ability. Sensitivity was calculated by TP / (TP + FN). Specificity: TN / (TN + FP) [20].

To detect whether there are significant differences between different tests and the buffering ability in neutral saliva and at pH 3, the Paired samples Student T – test was used. We considered as significant any value of $p<0.05$.

The null hypothesis is that there is no correlation between commercial tests and the precise titration method in laboratory.

Table 1. Titration method and CRT Buffer test crosstabulation

		CRT Buffer			
		high buffer capacity	medium buffer capacity	low buffer capacity	Total
Titration method	high buffer capacity	14	1	0	15
	medium buffer capacity	11	25	4	40
	low buffer capacity	0	3	6	9
	Total	25	29	10	64

Table 2. Titration method and GC Saliva Check Buffer Crosstabulation

		GC Saliva Check Buffer			
		high buffer capacity	medium buffer capacity	low buffer capacity	Total
Titration method	high buffer capacity	15	0	0	15
	medium buffer capacity	4	33	3	40
	low buffer capacity	0	2	7	9
	Total	19	35	10	64

RESULTS

From an invited 80 patients, 64 (80%) agreed to take part in the study. The mean age of participants was 36 years.

From 64 saliva samples titration showed a high buffer capacity in 15 cases (23.4%), medium in 40 cases (62.5%), and the remaining 9 (14.1%) subjects were classified as having a low buffering ability. Both colorimetric tests had different results – for the CRT Buffer test, 19 from 64 cases had results divergent from laboratory measurements. For the GC Saliva Check Buffer just 9 cases showed divergent results (Table 1, 2).

The Spearman Rank Correlation indicated a positive coefficient between the titration method and both the CRT Buffer test ($r_s=0.685$) and the GC Saliva Check Buffer ($r_s=0.837$). The Kappa coefficient, however, showed a positive correlation for the CRT Buffer test ($k=0.508$) and strong correlation for the GC Saliva Check Buffer ($k=0.752$).

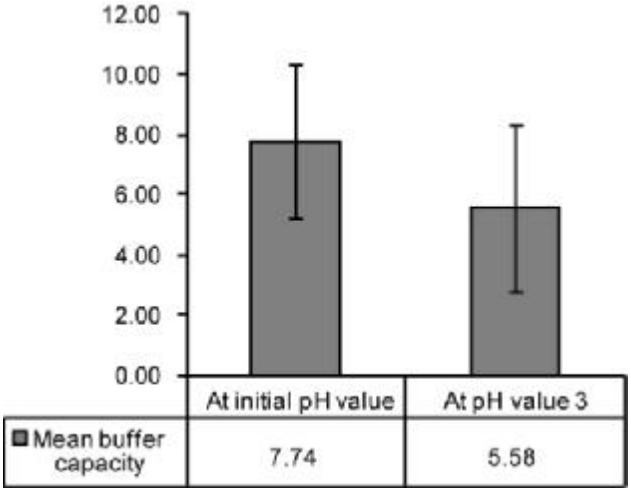

Fig. 1. Mean buffer capacity detected by using acid titration method at initial pH value and at pH value 3

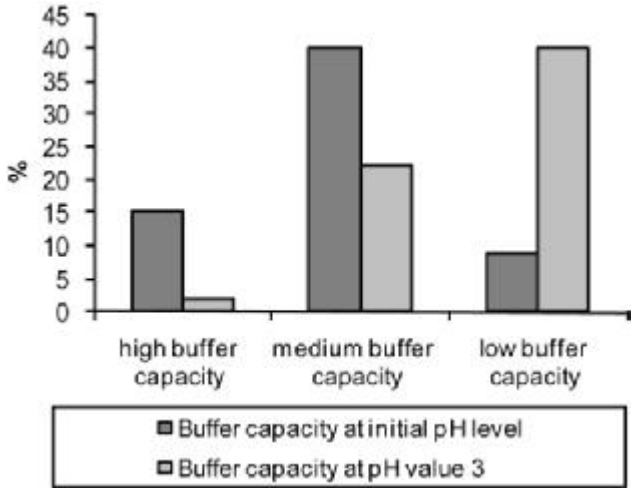

Fig. 2. Buffer capacity changes from initial pH to pH value 3

It is possible to get parametric variables for titration method and GC Saliva Check Buffer test, though the Pearson Correlation was detected (r=0.675), which showed a high correlation as well.

Both colorimetric tests had acceptable accuracy, but the GC Saliva Check Buffer test has a higher sensitivity (0.87) and specificity (0.83) than the CRT Buffer test (sensitivity – 0.69, specificity – 0.74) (Table 3).

The difference is found between buffer capacity at initial pH (lowering pH by 1 unit from initial pH, mean buffering ability was 7.74 ml HCl / l saliva) and at pH value 3 (lowering pH from initial pH till pH value 3, mean buffering ability was 5.58 ml HCl / l saliva) (Figure 1). Paired samples T test showed a significant difference between these two buffer capacity mean values.

It was calculated that just 13.3% (2) of the subjects with high buffer capacity also had high buffering ability at pH value 3, but for 80% (12) of the high capacity patients it changed to medium – and even to low buffering ability for 6.7% (1) of participants. Also, 65% (26) of medium capacity patients observed a lowering of buffering capacity (Table 4) (Figure 2).

In the current study there no correlation was found between buffer capacities, detected either by the two colorimetric tests or by the acid titration method at initial pH value and DMFT. There was also no correlation between buffer capacity at different pH values and caries prevalence at confidence level $p<0.01$, but there was a negative correlation between caries prevalence and buffer capacity changes from initial pH and at pH value 3 at confidence value $p<0.05$ ($r=-0.310$) (Table 5).

DISCUSSION

This study compared two different colorimetric saliva buffer capacity tests and the acid titration method in laboratory. It is important that the real buffering ability of the saliva was detected, which gives more information than just a relative ranking of high, medium or low buffer capacity.

Ericsson in 1959 reported that there were differences in the saliva buffering capacity of caries free and caries active patients [3]. He developed a saliva buffer capacity test, which showed that the final pH is an acceptable measure of saliva buffering ability. In more recent decades different companies have developed test strips that gave possibilities to detect buffer capacity in a dental office, as it is economical and time-effective. Different studies have been made to measure precision of chair-side, int. al. colorimetric tests, detecting correlation with the Ericsson method [11, 19, 21]. Kitasako et. al. found the highest correlation for a quantitative test using a hand-held pH meter [11]. In another publication the same researchers concluded that the Adapted Checkbuf test is also useful for measuring buffer capacity in resting saliva, as this test displayed the highest agreement with Ericsson method [19].

In the present study, the correlation of colorimetric tests and titration was calculated using different statistical methods. From the CRT Buffer it is possible just to get ranking results and it is also possible to convert results from other tests into ranks of high, medium and low buffer capacity. For such results the Spearman Rank Correlation Test and Kappa coefficient showed a higher value for the GC Saliva Check Buffer test. Pearson's Correlation coefficient showed weaker, but still acceptable, agreement between laboratory results and the GC Saliva Check Buffer test as a scale data are obtainable from these tests, but it isn't possible to compare with the CRT Buffer as Pearson's Correlation test is just for scale data.

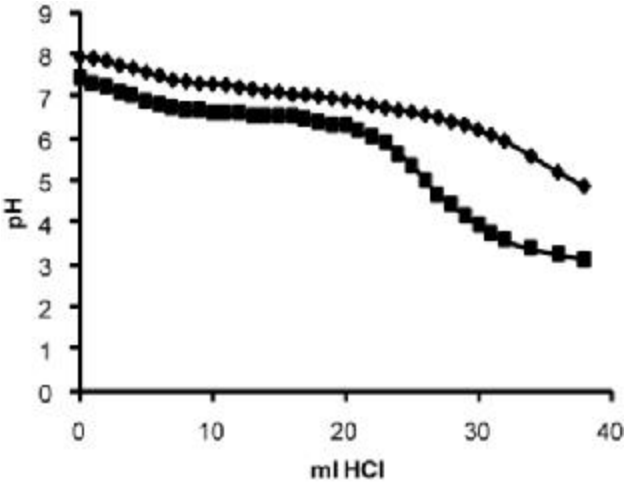

Fig. 3. Buffer curve for two patients, both had high buffer capacity detected at initial pH value

Comparing the Spearman Rank and the Kappa correlation coefficients, as well as the sensitivity and specificity of colorimetric tests, the GC Saliva Check Buffer test showed better results in absolute numbers than the CRT Buffer test, but the Paired samples T test didn't show a significant difference between the results of these tests ($p>0.01$).

Table 3. Sensitivity and specificity of CRT Buffer and GC Saliva Check Buffer

	CRT Buffer	GC Saliva Check Buffer
True positive cases	31	40
True negative cases	14	15
False positive cases	5	3
False negative cases	14	6
Sensitivity	0.69	0.87
Specificity	0.74	0.83

Table 4. Buffer capacity changes from initial pH to pH value 3 (acid titration method)

	Frequency	Percent
High - high	2	3,1
High - medium	12	18,8
High - low	1	1,6
Medium - medium	14	21,9
Medium - low	26	40,6
Low - low	9	14,1

Table 5. Correlation between saliva buffer capacity detected by different tests and caries prevalence

	Buffer capacity changes	CRT Buffer	GC Saliva Check Buffer	Titration method at initial pH
Pearson Correlation	-0.310	-0.099	-0.119	-0.198
p value	0.036	0.513	0.432	0.188
Low - low			9	14,1

In the current study buffer capacity is detected by using acid titration. Ability is not calculated according to pH value, but by buffering capacity stated by mmol HCl on 1 liter of saliva to decrease pH by 1 unit. Figure 3 shows how pH decreases by adding acid, and it also shows that in both patients buffer capacity is high, both needed aprox. 20 ml HCl to reach a pH decrease of 1 unit, but for the first case decreasing pH by one more unit it was necessary to add just 5 ml while for second case 10 ml was needed. That means if first patient would have a lower initial pH value, buffering ability would also be lower.

The composition of un-stimulated saliva differs from stimulated. As more saliva is stimulated it becomes more similar to the composition of plasma [13]. For example, an increase in the salivary flow rate, obtained by the stimulation of acidic food, increases the concentration of sodium, chloride and bicarbonate and decreases the concentrations of salivary potassium and phosphate, compared with un-stimulated saliva [22-24]. As in stimulated saliva, as the concentration of bicarbonates becomes higher and closer to plasma concentration, the bicarbonate system plays a major role in buffer capacity [18] - but at lower pH values phosphate and protein systems are also taking part in the buffering process. As these systems have a lower buffering ability, pH also decreases more rapidly at lower pH values [18]. This explains why the buffering ability at the initial pH value is higher than at pH 3, as was shown in this study.

Saliva provides a cleansing of teeth, provides an optimal pH in which all chemical reactions are carried out and moreover saliva has protective functions – one of these is buffering ability, the significance of which is questionable. In most investigations, as well as in this study, buffer capacity tests show weak or negligible correlation to caries experience [6, 25, 26]. The reasons for this could be that colorimetric tests are used in an atmosphere high in carbon dioxide and thus the bicarbonate buffer system could be lost, and remaining is mostly phosphate and protein buffer systems which provide only weak buffering ability [18]. Saliva's buffer capacity role is also discussed because saliva is not always in direct contact with the surface of teeth, as there is peliqula or plaque and the demineralization process take place in the plaque and below the enamel surface, where the buffering mechanisms are different from those in saliva [27]. But Stralfors [18] has verified

that the buffering power in saliva is similar for that in plaque and Malekipour et al [28] have proved in clinical trial that saliva titration curves for patient with active caries differs from the pattern of caries free subjects. In literature more investigations could be found, which have proved a negative correlation between buffering and parameters measuring different aspects of dental caries [3, 29-33].

As saliva buffer capacity has an impact on plaque buffer capacity, it is definitely important to detect it in dental offices as a caries risk factor. In the current study there is only a weak correlation between buffer capacity changes (from buffer capacity at the initial pH level to a pH value 3) and the DMFT index, and no correlation between usual detected buffer capacity at the initial pH level by using either the precise acid titration method in laboratory or by chair-side colorimetric tests – the CRT Buffer and the GC Saliva Check Buffer. The reason could be a small sample size that limits the results of this research. It would be reasonable to get more information about a possible correlation with caries prevalence or caries risk by increasing sample size. Nevertheless it may be a significant finding that there were weak correlations for average buffer capacity detected as pH decreases to pH value 3, as also Driezen in 1946 recommended detecting buffering ability by measuring the amount of 0.01N lactic acid required to reduce the pH from its normal level to pH 4 [34]. And Coogan and MacKeown found significant differences in buffering capacity for groups with no new decays during the past 4 years as compared to groups with higher caries activity, using a modified Dreasen test [35], while other investigators using the colorimetric test GC Saliva Check Buffer found no correlation with caries prevalence scored using DMFT [36] as in the current study. But a weak negative correlation was found with ICDAS (international caries detection and assessment system) scores 3 and 4, as it is a more precise caries detection system [36].

CONCLUSIONS

This study demonstrates that the null hypothesis has been rejected and that both colorimetric tests correlate with the acid titration method in laboratory. This why chair-side diagnostic tests are usable for saliva buffer capacity detection in dental offices. This study shoved that the GC Saliva Check Buffer has higher accuracy than CRT Buffer test.

Saliva buffer capacity gives more information if detected in different pH values as it changes and decreases with lower pH values. Buffer capacity detected at different pH values also showed higher impact on caries prevalence.

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