Utility of a New Caries Activity Test

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Introduction

The detection of caries activity is greatly important in order to understand the susceptibility of the host to caries and further make an effective plan for prevention and treatment of caries according to the results detected^{1,2)}. It is a cost-effective example to make a special program of the prevention and treatment of caries after screening and grouping the results detected and will strengthen the education of oral hygiene especially in kindergartens and primary schools³⁻⁷⁾.

There have been no very ideal methods for detection up to date and few studies in this field in China. The purpose of this study is to investigate a convincing, simple and inexpensive detecting method which is comparable to the methods well known in the world, and to enhance the efficiency in the prevention and treatment of caries on a large scale. The method was named as Shanghai Tiedao University Caries Activity Test (SCAT). Its usefulness was evaluated in terms of the relationship of the test results with caries status obtained from dental examination as well as the comparison with other two conventional caries activity tests.

Materials and Methods

I. Materials

1. SCAT reagent preparation of standard solution for colorimetry

The contents in 100 ml SCAT reagent are shown in Table 1. The reagent was adjusted to pH 6.10 with 10 % lactic acid, and 1.5 ml each was poured into tube and sterilized for use.

The standard solution for colorimetry containing bromocresol green 0.0015 g and bromocresol purple 0.003 g per 100 ml was prepared by adjusting pH with 10 % lactic acid and divided into four degrees: Degree 0, dark-blue, pH 6.10; Degree 1, green pH 5.40; Degree 2, yellow-green, pH 4.70; Degree 3, yellow, pH 4.00. The caries activities were expressed by these degrees according to the color changes. Degree 3 means the most caries risk condition.

2. Dentocult LB detection materials

The materials used were special paraffin for chewing, thin layer of selected medium

specialized for *lactobacillus* and the standard map for measurement. The map was divided into four degrees according to the number of bacterial colonies: Degree 0, 10^3 CFU/ml; Degree 1, 10^4 CFU/ml; Degree 2, 10^5 CFU/ml; Degree 3, 10^6 CFU/ml. They were used to represent the caries activity. Degree 3 means the highest caries risk condition.

3. Dentobuff strip detection materials

In this test, pipettes for drawing up saliva, detective paper and standard map were used. The buffer capacity of saliva was expressed as follows: Degree 1, yellow, $\text{pH} \leq 4.0$; Degree 2, green, $\text{pH} 4.5-5.5$; and Degree 3, dark-blue, $\text{pH} \geq 6.0$. Degree 3 means the highest buffer capacity or lowest caries activity.

Dentocult LB and Dentobuff strip materials were from Orion Diagnostica, Finland.

II. Methods

1. Dental examination

The examiners and recorders were selected and fixed throughout the study. Type TK dental chair and cold light source (Yoshida, Japan) were used for dental examination. The grades of caries were commented as follows: 0, no caries; 0.5, filled tooth; 1, secondary caries, enamel caries or dentin caries; and 2, pulp exposed deep caries, remaining root.

2. SCAT detection

Three times of scrubbing on buccal cervical one third region of E+E with a sterilized cotton swab were made for each examined child. The swab was inserted into a test tube containing SCAT reagent and mixed for 1 min on a vortex. The test tubes were incubated at 37°C for 48 hr in type III incubator (Sankin, Japan). The color of SCAT reagent was checked and the caries activity was determined by comparing with the standards. The children were examined at 2:00 pm without eating, oral cavity cleaning or tooth brushing for more than two hours.

3. Dentocult LB and Dentobuff strip tests

The special paraffin was chewed for 5 min by each examined child. Then 3 to 5 ml saliva was collected and both the two tests were performed by specialists respectively.

1) Dentocult LB test

Saliva was poured slowly on both sides of the medium layer and incubated at 37°C for 96 hr. The number and the density of bacterial colonies were checked and the degrees were determined by comparing with the standards.

2) Dentobuff strip test

After piping saliva on the test paper to cover the yellow area for 5 min, the color changing was checked and the Dentobuff strip degrees were determined by comparing with standards.

The two tests were performed at 10:00 am and the examined children were without eating, oral cavity cleaning or tooth brushing for more than two hours.

Table 1 Contents of SCAT reagent (g/100 ml)

Composition	Weight
Tryptose	1.0
NaN_3	0.02
NaCl	0.4
Bromocresol green	0.0015
Bromocresol purple	0.003
Sucrose	10.0

4. Statistics

CSI (Caries Severity Index) was calculated as follows:

$$\text{CSI} = \frac{\text{Total grades of caries}}{\text{Total number of teeth} \times 2} \times 100$$

The data were analyzed by X^2 , ANOVA and SNK method. Correlation coefficients between the factors were also calculated.

III. Examining children

398 two to six-year-old children from the kindergarten of Shanghai Medical University were examined randomly for caries status and SCAT detection. For comparison of the tests, 96 two to six-year-old children were examined randomly for caries status, SCAT, Dentocult LB and Dentobuff strip tests.

Results

I. Caries incidence and SCAT detections for children

1. Caries incidence of children

The caries incidence of 398 children is shown in Table 2. Among 2 to 6-year-old children, the caries prevalence, dft and CSI rapidly increased with age up to 5 while their increment

Table 2 Caries incidence in 398 preschooling children

Age	Sex	Number of children	Caries prevalence		dft		CSI	
			Number	%	Examinees	Sufferers	Examinees	Sufferers
2	Male	25	3	12.00	0.28±0.16	2.33±0.33	0.77±0.43	6.44±0.57
	Female	24	5	20.83	0.33±0.18	1.60±0.60	0.86±0.45	4.14±1.47
	Subtotal	49	8	16.33	0.31±0.13	1.87±0.40	0.82±0.31	5.00±0.99
3	Male	42	15	35.71	1.10±0.28	3.07±0.48	2.98±0.82	8.33±1.52
	Female	31	13	41.94	2.23±0.79	5.31±1.53	6.72±2.64	16.03±5.40
	Subtotal	73	28	38.36	1.58±0.37	4.11±0.77	4.57±1.23	11.91±2.68
4	Male	59	34	57.63	2.68±0.46	4.65±0.62	7.63±1.47	13.24±2.08
	Female	58	34	58.62	2.67±0.50	4.56±0.68	8.13±1.68	13.86±2.43
	Subtotal	117	68	58.12	2.68±0.34	4.60±0.46	7.88±1.11	13.55±1.59
5	Male	65	46	70.77	3.32±0.40	4.70±0.43	9.97±1.33	14.09±1.50
	Female	58	38	65.52	3.41±0.50	5.21±0.59	9.26±1.54	14.14±1.92
	Subtotal	123	84	68.29	3.37±0.32	4.93±0.35	9.64±1.00	14.11±1.19
6	Male	18	14	72.22	2.94±0.65	3.79±0.69	7.92±2.11	10.19±2.39
	Female	18	13	77.78	4.50±1.07	6.23±1.16	11.65±3.03	16.13±3.45
	Subtotal	36	27	75.00	3.72±0.63	4.96±0.69	9.79±1.84	13.05±2.11
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	Male	209	112	52.11	2.30±0.21	4.29±0.28	6.63±0.67	12.37±0.97
	Female	189	103	52.97	2.70±0.28	4.96±0.40	7.66±0.89	14.05±1.34
	Total	398	215	52.52	2.49±0.17	4.61±0.24	7.12±0.55	13.18±0.82

Table 3 Caries incidence under different SCAT degrees

SCAT degree	Sex	Number of children	Caries prevalence		dft		CSI	
			Number	%	Examinees	Sufferers	Examinees	Sufferers
0	Male	81	34	41.98	1.32 ± 0.24	3.15 ± 0.41	3.37 ± 0.76	8.02 ± 1.47
	Female	67	33	49.25	1.63 ± 0.30	3.30 ± 0.45	4.22 ± 0.87	8.56 ± 1.41
	Subtotal	148	67	45.27	1.46 ± 0.19	3.22 ± 0.30	3.75 ± 0.57	8.29 ± 1.01
1	Male	73	35	47.95	2.18 ± 0.38	4.54 ± 0.57	6.31 ± 1.20	13.16 ± 1.91
	Female	69	27	39.13	1.62 ± 0.41	4.15 ± 0.85	4.86 ± 1.47	12.43 ± 3.30
	Subtotal	142	62	43.66	1.91 ± 0.28	4.37 ± 0.49	5.61 ± 0.94	12.48 ± 1.78
2	Male	33	24	72.73	3.85 ± 0.61	5.29 ± 0.62	11.31 ± 1.83	15.54 ± 1.87
	Female	34	26	76.47	4.76 ± 0.83	6.23 ± 0.90	14.13 ± 2.63	18.48 ± 2.95
	Subtotal	67	50	74.63	4.31 ± 0.52	5.78 ± 0.55	12.74 ± 1.61	17.07 ± 1.78
3	Male	22	19	86.36	3.95 ± 0.63	4.58 ± 0.61	12.69 ± 2.29	14.69 ± 2.34
	Female	19	17	89.47	6.74 ± 0.88	7.53 ± 0.77	18.35 ± 2.37	20.51 ± 2.08
	Subtotal	41	36	87.80	5.24 ± 0.57	5.97 ± 0.54	15.31 ± 1.69	17.44 ± 1.63

became small and CSI of cases rather decreased in 6-year-old children. There were no significant differences between male and female in caries prevalence ($p=0.86$), dft and CSI of examinees or of sufferers ($p=0.24, 0.35, 0.16, 0.30$, respectively).

2. Caries incidence classified by SCAT degree

Table 3 shows the caries incidence of 398 children classified by different SCAT degrees. Significant differences were found among caries prevalences of different SCAT degrees ($p=0.001$). There were highly significant differences in dft ($p=0.0001$) and CSI ($p=0.0001$) for different SCAT degrees of examinees. The SNK method distinctly showed significant differences in dft or CSI of examinees between Degree 0 and 2 or 3 and between Degree 1 and 2 or 3. There were also significant differences in dft ($p=0.0001$) and CSI ($p=0.0001$) for different SCAT degrees of sufferer. The SNK method revealed distinct differences between Degree 0 and 1, 2 or 3 in dft and between Degree 0 and 2 or 3 and between Degree 1 and 2 or 3 in CSI of sufferer.

3. Correlation between SCAT degree and caries incidence

The results of SCAT showed close correlations with caries prevalence ($r=0.26, p=0.0001$), dft of examinees and sufferers ($r=0.34, p=0.0001$, for both), and CSI of examinees and sufferers ($r=0.35, p=0.0001$, and $r=0.38, p=0.0001$, respectively).

II. Comparison between the three caries activity tests

Figs. 1 to 3 show the results of linear regression analyses for dft and CSI versus SCAT degree, Dentocult LB degree and Dentobuff strip degree, respectively, for 96 three to six-year-old children. Both of dft and CSI showed significant linear correlations with SCAT degree ($p=0.005, p=0.006$). In Dentocult LB test, similar increases in mean values of dft and CSI with increased degree were found as in SCAT although the standard deviations were extremely

Fig. 1 Distribution of dft or CSI in relation to SCAT degree. Positive correlation existed between SCAT degree and dft or CSI.

Fig. 2 Distribution of dft or CSI in relation to Dentocult LB degree. Positive correlation existed only between Dentocult LB degree and dft

large. The linearity between dft and the degree was significant ($p=0.026$) but not between CSI and the degree ($p=0.066$). There was no significant linear correlation between dft or CSI and Dentobuff strip degree ($p=0.405$ or 0.440).

Discussion

I. The significance of caries activity detection

The caries activity indicates the sensitivity of the examinee to caries. The more active the reaction in the test, the higher the caries prevalence^{8 10)}. The caries activity is usually detected from saliva or dental plaque, and the prevention and treatment plan can be made scientifically according to the test results. In order to utilize the test effectively and economically, it should be performed in kindergartens and primary schools and adequate prevention and/or treatment plans should be made for children grouped by different levels of activity. It is essential, therefore, to get understanding and cooperation of the parents.

Recently such works have been more developed and much progress was made in the prevention and treatment of caries. Anyhow, the caries status has not been improved. The caries prevalence and caries are still high in children. In 1995, the caries prevalence of primary teeth in five-year-old children was 76.55 % in China (75.69 % in cities, 78.28 % in rural area). The dft was 4.48 (4.32 in cities, 4.80 in rural area). Compared with those in

Fig. 3 Distribution of dft or CSI in relation to Dentobuff strip degree. No correlation existed between Dentobuff strip degree and dft or CSI

1980's, the caries incidence of primary teeth in children has changed. Higher caries prevalence is in rural area instead of cities^{11, 12)}.

In this study, the caries prevalence was 68.29 % and dft was 3.37 in five-year-old children and 52.52 % and 3.86 in two to six-year-old children, respectively, which are lower than those in 1980's and early 1990's in Shanghai, and also lower than those in China in 1995^{11, 13, 14)}. The better results in this study may be related to the examined children from the kindergarten with dental health care room and health education work for six years. The caries prevalence and dft or CSI in six-year-old children group were not always higher than those in five-year-old children group. It may be due to the increased shedding of primary teeth and treatment of the caries in the former as described in the literatures^{15, 16)}.

Facing with the severe caries status in more than three hundred million children¹⁷⁾ and with the small number of dentists, especially pediatric dentists against the big population in China, the newly developed SCAT has its scientific, social and economic extension and significance in the prevention and treatment of caries as an economic, simple and sensitive method for detecting caries activity.

II. Characteristics of SCAT

The examination by SCAT is based on the acid-producing ability of dental plaque. During incubation the growth of bacteria dissociates sucrose into lactic acid, which reacts with the indicator and results in the color changes. The amount of bacteria and caries activity was determined according to lactic acid produced. The main composition and selected principles of SCAT reagent are as follows:

1. Selection of carbon source

Sufficient carbon is necessary for caries-induced bacteria. When compared with glucose, sucrose which is commonly used in our life has bigger molecular weight and is less inhibitory to the growth of microorganisms according to its lower osmotic pressure. As one kind of disaccharides it is more used by caries-induced bacteria than monosaccharide and will exclude the growth of other bacteria using monosaccharide only. The selection of sucrose will therefore make the test more accurate^{18, 19)}.

The concentration of sucrose is also important. With less than 1 % sucrose the caries-induced bacteria could not get enough carbon for growing and no color reactions appeared because of lack of lactic acid. More than 40 % sucrose, under high osmotic pressure circumstance on the other hand, inhibited the metabolism of bacteria and led them to die, especially for the caries-induced bacteria and Gram's positive bacteria. Also under high temperature without bacteria, high concentration of sucrose reacted with amino acid from peptone and caused brown color, which resulted in the increase of pigment and the decrease of nutrient elements. From the experiments with various concentrations of sucrose, we found that 10 % was the best. Under this condition, there will not any increase of pigment and the color reaction will not be delayed.

2. Selection of nitrogen source

Nitrogen is also necessary for growth of bacteria. Tryptose was selected as the source of

nitrogen in SCAT reagent. The concentration of tryptose is also important. If it is less than 0.1 %, caries-induced bacteria can not get enough nitrogen for growing and the detection may lead to mistake. When it exceeds 5 %, the balance between carbon and nitrogen will be broken and metabolism will be disrupted¹⁸⁾. The use of NaN_3 inhibited the growth of Gram's negative bacteria in SCAT reagent and it will save more nitrogen.

The detection method is an acid producing fermented culture. The main element in produced organic acid is carbon with no or little nitrogen. Usually the bacteria need more carbon and less nitrogen and the ideal ratio of carbon to nitrogen is 100 : 0.2–2.0²⁰⁾. In SCAT reagent, therefore, the concentration of tryptose was selected as 1 % because it almost satisfied the ideal ratio. As comparing with the expensive tryptose used abroad, the native tryptose produced in China is cheaper and good enough for use.

3. The use of NaN_3

NaN_3 is an inhibitor for the growth of microorganisms by restricting biologic respiration. It shows different effects on different kinds of bacteria. The growth of Gram's negative bacteria is more strongly inhibited than that of Gram's positive one^{18, 21)}. We took advantage of this feature, using NaN_3 in SCAT reagent to inhibit the growth of non-caries-induced bacteria such as Gram's negative bacteria effectively and to less inhibit the growth of Gram's positive bacteria such as *Streptococci mutans* or *Lactobacilli* for decreasing the unfavorable influence on the test.

The concentration of NaN_3 is also important. The optimum concentration for good detection with this test was 0.02 % NaN_3 , which preferably inhibited the growth of most of the non-caries-induced bacteria. At this concentration NaN_3 will not affect the color reaction of bromocresol green and reaction rate can be controlled to ensure the accuracy of the test.

4. Indicators

We used two color indicators, bromocresol green and bromocresol purple here to avoid the color concentration on the top of the cotton swab when bromocresol green was employed alone. By adding 0.0015 % bromocresol green and 0.003 % bromocresol purple, the color reaction occurred homogeneously and the change was easily and clearly evaluated.

III. The application of SCAT detection

1. Caries incidence

The analyses of the caries incidence for different degrees of SCAT showed highly significant differences in caries prevalence, dft and CSI of examinee or sufferer. There was also high close correlation between SCAT degrees and caries prevalence, dft or CSI, indicating that SCAT could be a good measure of the caries status and its severity and caries sensitivity.

The similarity of SCAT and Cariostat method used for detecting caries activity is that both of them detect the capability of producing acid from dental plaque to determine the caries sensitivity of the host^{22, 23)}. Tsubouchi et al. (1994)²⁴⁾ examined 637 eighteen-month-old children with Cariostat method and found the correlation between the results and caries incidence, and the children with high caries risk could be determined. Koroluk et al. (1994)²⁵⁾

gave dental examination and Cariostat test for 153 three to five-year-old children and found that there was significant difference between Cariostat degrees in dft, dfs or CSI. It was shown from these references that the detection of capability of producing acid from dental plaque could well represent the caries sensitivity of the host indeed.

The author previously detected the caries activity for 391 three to five-year-old children with Cariostat method and analyzed if the amount of sample had any effect on the results, and revealed that there was highly significant correlation between the results and caries prevalence, dft or CSI, and sample amount did not affect the results²⁶⁾. It means that no attention needs to be paid to sample amount in SCAT detection.

2. Comparisons among SCAT, Dentocult LB and Dentobuff strip detections

Dentocult LB and Dentobuff strip tests are new methods for detecting caries activity by examining the amount of *Lactobacilli* in saliva in the former and the buffering capability of saliva against acid in the latter.

Among the bacteria related to caries, *Streptococcus mutans* is considered to be the most active caries inducer. Fitzgerald et al. (1966)²⁷⁾ thought that there was no direct relation between *Lactobacilli* and caries, and not a few reports stressed that *Lactobacilli* could indirectly participate in caries mainly induced by *Streptococcus mutans*²⁸⁾. Oshikane (1970)²⁹⁾ indicated that during pH 4.5–5.0 of saliva both *Streptococcus mutans* and *Lactobacilli* were active while only *Lactobacilli* could be active under pH 4.0. The method for detecting the capability of producing acid by *Lactobacilli* for confirming caries activity of the host is still progressing now. The author³⁰⁾ detected the amount of *Lactobacilli* in saliva with Dentocult LB test in 164 three to five-year-old children and found that there were close correlation between the amount of *Lactobacilli* and caries prevalence of primary teeth, dft or CSI and that the amount of *Lactobacilli* could reflect the caries incidence of primary teeth.

In this study, it was shown that dft and CSI significantly increased with larger Dentocult LB degrees. Enomoto (1994)⁸⁾ also reported that the larger the Dentocult LB degree, the more the number of the caries surfaces. The results of Dentobuff strip test in this study, on the other hand, showed neither significant difference nor significant correlation between caries prevalence, dft or CSI and Dentobuff strip degree. Tahara (1995)²²⁾ found in 210 six-month to six-year-old children that there was no difference in dfs between different Dentobuff strip degrees. Enomoto (1994)⁸⁾ reported that there was correlation between caries incidence of permanent teeth but not of primary teeth and Dentobuff strip degree. Saliva buffer is often affected by secreting amount, varying with collecting time and method. It may be one of the reasons why the results of this test could not be well related to the caries incidence. Such disadvantages with the use of saliva may also occasionally cause unstable results in Dentocult LB test as seen in the larger standard deviations of the caries incidence classified for each degree.

The results of SCAT were well correlated with the caries activity of the host, almost parallel to those of Dentocult LB test. Judging from the statistical *p* value and correlation coefficient, however, the linear relationship between the test results and the real dft or CSI

rather appeared more distinct in SCAT than in Dentocult LB test.

In consideration of the severe caries status and lack of professional dentists in China, it is practical and significant to detect caries activity of the children for prevention and earlier treatment of caries. In addition to the usefulness of the results, the newly developed SCAT detection is simple and inexpensive to operate and it is expected to come into wide use.

Conclusion

The present study evaluated the utility of a new caries activity test named SCAT (Shanghai Tiedao University Caries Activity Test) for predicting the caries incidence of primary teeth. The caries activities of 398 preschooling children were classified into 4 degrees according to the color changes of SCAT reagent in association with the capability of producing acid from dental plaque. The conventional Dentocult LB and Dentobuff strip tests were also performed for comparison. Their results were then compared with the real caries status of the children obtained by stomatic examination.

Highly significant differences in caries prevalence, dft and CSI were found between different SCAT degrees with significant positive correlations. The results of SCAT showed almost parallel relationship with those of Dentocult LB test. No significant correlation between Dentobuff strip test and stomatic examination was recognized in this study.

Together with the advantages of SCAT such as simple operation and low cost, foregoing results suggest that SCAT may be an excellent measure of caries incidence of primary teeth for earlier prediction, prevention and treatment of caries.

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新しい齲蝕活動性試験法の有用性

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齲蝕罹患率の減少や予防のために様々な研究が行われている。齲蝕活動性試験はその中の一つであり、現在は数種類の齲蝕活動性試験法が使われている。永久歯よりも乳歯の時点から着手することが最も有効な手段であるが、いずれの方法も高価なものであるから、広範囲（特に中国の場合）に普及することが困難だと思われる。そのため、上海鉄道大学式齲蝕活動性試験（以下 SCAT と略）という新しい齲蝕活動性試験方法を開発した。本研究は、この試験法の乳歯齲蝕状況を予測する手段としての有用性を調べたものである。SCAT は歯垢からの酸生成能による試薬の変色を利用し、標準色溶液との比較から齲蝕活動を 4 段階に分類している。就学前の小児 398 名について SCAT による試験を行い、その結果を口腔内診査から得られた齲蝕状況と対比し、検討を加えた。

また、従来からある Dentocult LB 試験および Dento-buff strip 試験も同時に行い、これらの結果と齲蝕状況との関連についても比較検討した。

4 段階の SCAT 齲蝕活動度の間で、齲蝕罹患率、dft および CSI（齲蝕重度指数）に高度な有意差がみられ、齲蝕活動度と各々の間には正の相関が認められた。本研究の結果では、Dentocult LB 試験では口腔内診査と一部に相関がみられたが、Dentobuff strip 試験では相関は認められず、SCAT 齲蝕活動度が齲蝕状況と最も密接な関連性を示した。

以上の結果は、SCAT が乳歯の齲蝕罹患を予測できるすぐれた手段であることを示しており、操作が容易であること、経済的であることなどの利点とともに有用な試験方法であることを示唆している。