Evaluation of a new caries risk test

The cariogenic significance of Streptococci mutans (SM) and Lactobacilli (LB) has led to the development of various methods of detection. Today, these methods range from simple culturing (Dentocult SM Strip Mutans, Dentocult LB, Orion Diagnostica, Finland; CarioCheck SM and LB, Hain Diagnostika, Germany; CRT, Vivadent, Schaan, Liechtenstein) to immunoassays (Streptococcus-mutans-Elisa, Autoimmung GmbH Diagnostika, Germany), and molecular-biological techniques (Streptococcus-mutans-PCR).

Among these methods, the culturing of bacteria groups in conjunction with a chairside test is currently the easiest, most reliable, and least expensive method for dental practices. The most popular culture systems are Dentocult SM Strip Mutans and Dentocult LB. The Caries Risk Test (CRT) is a new culturing device for the simultaneous detection of both bacteria groups.

**CULTURE MEDIA FOR THE DETECTION OF SM: A BRIEF HISTORY**

Mitis-salivarius agar was introduced in clinical microbiology to differentiate between faecal and alpha-haemolytic Streptococci (Chapman, 1944). It was then modified by adding sucrose and bacitracin (Gold et al, 1973). Today, it is the most frequently used medium for the detection of MS, and is known as MSB-agar. S mutans (Figure 1) grows in typical muruloid to star-shaped colonies in the depth of the agar, while S sobrinus (Figure 2) forms an exudate droplet of extracellular polysaccharides.

Previously, trypticase-yeast-extract-cystine-agar (TYC) was recommended for the quick macroscopic determination of S sobrinus. On this medium, a white halo develops around the S sobrinus colony (De Stopelaar et
TABLE 1: ACID FORMATION OF ORAL AND MUTANS STREPTOCOCCI FROM MANNITOL AS A MEANS OF DIFFERENTIATION

<table>
<thead>
<tr>
<th>Oral Streptococci</th>
<th>Mannitol positive</th>
<th>Mannitol negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutans Streptococci</td>
<td>S sanguis</td>
<td>S salivarius</td>
</tr>
<tr>
<td></td>
<td>S gordonii</td>
<td>S oralis</td>
</tr>
<tr>
<td></td>
<td>S milleri</td>
<td></td>
</tr>
<tr>
<td>Mutans Streptococci</td>
<td>S mutans (c, e, f), S sobrinus (d, g)</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S ferus (c), S cricetus (a), S rattus (b)</td>
<td>(Human), hamster, rat</td>
<td></td>
</tr>
</tbody>
</table>

Kimmel and Tiano (1991) also focused their efforts on the MSB-agar. Their aim was to further suppress the concomitant oral flora for the S mutans detection in plaque and saliva samples. They added canamycin. The concomitant flora were indeed reduced, but the bacterial yield of S mutans decreased by 13% at the same time.

On the whole, the aim of all attempts that led to the development of the above selective culture media was to improve the quantitative culturing of S mutans with simultaneous suppression of the concomitant flora in plaque and saliva samples. On the other hand, the aim was also to enable quick, reliable identification on a macroscopic basis alone.

**PERFORMANCE EVALUATION OF THE SELECTIVE MEDIA FOR SM**

On the basis of incomprehensive performance evaluations, a certain selective medium is preferred over any other, for one reason or another. Usually, reference strains were used exclusively, or only a limited number of plaque and saliva samples were consulted for.

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**Figure 3: S sobrinus colony with a glucane halo on trypicase-yeast-extract-cystine-sucrose-agar with bacitracin**

**Figure 4: Growth of reference strains on Mitis-salivarius agar with bacitracin and modified sucrose content according to Lautensch (1997) plaque and saliva (log CFU). Bacteria count classes 0 to 3 (from right to left)**

**Figure 5: Comparative determination of Lactobacilli using Dentocult LB and CRT**
control purposes. Gold et al (1973) examined five plaque samples. Van Palenstei
Heldermann et al (1983) used plaque and saliva samples of six pa-
tients, while Little et al (1977) only investigated the growth of refer-
ence strains.

Only Schaecken et al (1986) were able to express their preference of one agar (TSY20B)
over another one (TYCSB, MS and MSB agar) after examining 185 plaque and
saliva samples, which were taken from 37 test subjects. TYCSB-
agar, which is equivalent to the TSY20B-agar, was used together
with the MSB-agar for the basic examina-
tion of plaque and saliva samples of 60 children in the Erfurt caries risk study (Kneist et
The results were unsatisfactory in so far as not
only S sobrinus, but also gram-
negative germs in particular (Figure 3), developed gluconate halos. Therefore, reliable
identification of S sobrinus with
TYCSB-agar is not possible.
MSB-agar proved to be more reliable.

<table>
<thead>
<tr>
<th>Homofermentative</th>
<th>Facultativ</th>
<th>Heterofermentative</th>
</tr>
</thead>
<tbody>
<tr>
<td>L salivarius</td>
<td>L alimentarius</td>
<td>L fermentum</td>
</tr>
<tr>
<td>L delbrueckii</td>
<td>L casei</td>
<td>L brevis</td>
</tr>
<tr>
<td>ss lactis</td>
<td>L paracasei</td>
<td>L buchneri</td>
</tr>
<tr>
<td>ss delbrueckii</td>
<td>ss paracasei</td>
<td></td>
</tr>
<tr>
<td>ss bulgaricus</td>
<td>ss tolerans</td>
<td></td>
</tr>
<tr>
<td>L acidophilus</td>
<td>L pseudoplanarum</td>
<td></td>
</tr>
<tr>
<td>L gasseri</td>
<td>L planarum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L rhamnosus</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2: FREQUENTLY OCCURRING ORAL LACTOBACILLI**

**THE NEW CRT**

The development of CRT also aimed at enhancing the selective
medium for culturing S mutans. Mitis-salivarius-agar (Chapman,
1944) was used as the basic agar. Various quantities of sucrose
were added. High concentrations of sucrose have a conservative
effect and thus suppress the growth of germs. S mutans,
however, tolerate high concentrations and are able to
use sucrose as a nutrient. It goes
without saying that Gold et al (1973) also investigated the
concentrations. The sucrose
content was eventually increased to 41%, which resulted in a
higher S mutans yield (Laurisch,
1997).

However, the increased S
mutans yield is not sufficient for
the everyday routine in the dental
practice, as the diagnosis must
often be made by an individual
who has not studied
microbiology and who cannot be
expected to identify the type of
bacteria with certainty.

Therefore, it was eventually
decided to add bacitracin to
reduce the concomitant flora,
which mainly consists of S
salivarius and S sanguis (Kneist
et al, 1998e). In contrast to performance
evaluations of selective media
for S mutans known to date, the
modified agar was examined
using reference strains and
subsequently subjected to a
clinically relevant investigation
involving saliva samples (Kneist
et al, 1998e). As a reference,
there were also plaque and saliva
findings determined with MSB-
agar for the same children.

**Figure 4** clearly shows that
particularly S salivarius and S
sanguis, the types of Streptococci
usually found in saliva, were
suppressed by the bacitracin. The
higher bacterial yield compared to
MSB is shown in **Figure 5**.
The S mutans count cultured on
MSB and MS41B-agar was
classified according to the
bacterial count categories of the
Dentocult SM Strip Mutans
culturing device.

The modified agar was
combined with a practicable
culturing device – the CRT. S
mutans macrocolonies can no
longer fall from the plastic
spatula and represent an
interfering factor (Kneist, 1998).
Furthermore, the CRT is suitable
for the simultaneous
identification of Lactobacilli in
saliva (Figures 6 and 7), since the
reverse side of the carrier is
covered with Rogosa-agar.

**ON THE GOLD STANDARD – DENTOCULT SM STRIP MUTANS**

Up until today, only culturing
devices for the identification of
either S mutans or LB alone have

**Figure 6:** Detection of S mutans using CRT. Bacteria count classes
0 to 3 (from right to left)

**Figure 7:** Detection of Lactobacilli using CRT. Bacteria count classes
1 to 4 (from right to left)
TABLE 3: CARIES PROGNOSIS FOR 7-8- AND 12-13-YEAR-OLD CHILDREN FROM ERFURT ON THE BASIS OF THE S MUTANS AND LACTOBACILLUS COUNTS IN SALIVA ACQUIRED BY MEANS OF MICROBIOLOGICAL CHAIRSIDE TESTS

<table>
<thead>
<tr>
<th>Caries risk</th>
<th>Bacteria count class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>LB 0 and SM 0</td>
</tr>
<tr>
<td>Medium</td>
<td>LB &gt; 0 and ≤ 2 and/or SM &gt; 0 and ≤ 1</td>
</tr>
<tr>
<td>High</td>
<td>LB ≥ 3 and/or SM ≥ 2; [mixed dentition LB and/or SM ≥ 2</td>
</tr>
</tbody>
</table>

been commercially available. Aalhusua et al (1984) developed a dip-slide test on the basis of S mutans agar in 1984. During the incubation of the saliva sample, bacitracin disks are placed on the agar. Subsequently, the S mutans count is semi-quantitatively determined on the basis of the S mutans growing in the area inhibited by the bacitracin. Later on, Matsukubu et al (1981) recommended the adherence test of a bouillon that contained sucrose and that was inoculated with saliva on the wall of the culture vial for quick S mutans identification and to determine the caries activity.

Only S mutans are capable of adhering. Köhler and Brathall (1979) had developed the spatula method preceding this test. A wooden spatula was swabbed over the tongue and subsequently 'stamped' on MSB agar. After incubation of the petri dish, the bacterial count of S mutans was semi-quantitatively determined on the basis of the density of the colonies that developed on the impression left in the agar by the spatula. Jensen and Brathall (1989) then introduced Dentocult SM Strip Mutans, which has become the most frequently used test in the meantime. This test makes use of the pronounced adhering properties of S mutans (Matsukubu et al, 1981) and the selective culture-promoting capacities of Mitis-salivarius bouillon (Gold et al, 1973) with bacitracin. Irrespective of the growth of other oral germs present in the bouillon, only S mutans can settle on the plastic spatula. This fact was confirmed by our own investigations (Kneist, 1998).

ON THE DETECTION OF LACTOBACILLI

As a counterpart to the S mutans culturing device, Dentocult LB is commercially available for the detection of Lactobacilli (Larmas, 1975). The test consists of a dip-slide covered with Rogosa-agar. In the thirties, Rodriguez (1931) used a selective serum agar for L acidophilus-odontolyticus. Until the development of the first synthetic agar by Rogosa (Rogosa et al, 1951), tomato juice agar was usually used. With its acid pH-value (5.4) Rogosa-agar promoted the culturing of Lactobacilli. Westergreen and Krasse (1978) and Köhler et al (1984) introduced the first semi-quantitative micromethod for the detection of Lactobacilli. Today's widely used Dentocult LB semi-quantitative culturing device is closely associated with the name of Larmas (1975).

From a microbiological point of view, Dentocult SM Strip Mutans and Lactobacilli culture systems and the CRT are of equal value in comparison to conventional tests, however, the new CRT offers the advantage that both S mutans and Lactobacilli can be detected using the same culture system, due to the fact that the two sides of the test device are covered with different culture media. In other words, the two sides of the test device, each featuring a different culture medium, are inoculated with paraffin-stimulated saliva. The results are available after the usual incubation period of two days. In the process, the combined bacterial count classes are interpreted as caries risk (S mutans 2 and 3 and/or Lactobacilli 3 and 4) or non-caries risk (S mutans <2 and Lactobacilli <3) respectively (Table 3, Figures 6 and 7).

After all, high S mutans and/or Lactobacilli counts led to at least six new carious tooth surfaces in children aged 12 to 13 years over the observation period of four years (Heinrich-Weltzien et al, 1998a; Heinrich-Weltzien et al, 1998b; Kneist et al, 1998d; Kneist et al, 1998a; Kneist et al, 1998b) (Figure 8).

For the microbiological performance evaluation, the CRT was compared with the gold standard, i.e. Dentocult SM Strip Mutans and LB. The saliva of 150 children from Erfurt, aged between seven and eight, was examined. For that purpose, salivation was stimulated by means of chewing paraffin pellets. Saliva samples were taken and cultured. The mutants determination was carried out using the plastic spatula. 84%, of the children showed identical high (27%) or low (57%) mutans counts in

Figure 8: Caries incidence in adolescents from Erfurt with microbiologically different caries risk over a period of four years.
their saliva in both test methods. In 11% of the cases, the CRT was superior to the Dentocult SM Strip Mutans culture system (Figure 9). Both tests resulted in more or less equally high or low Lactobacillus counts (Figure 10). After combining the S mutans and Lactobacilli figures (Table 3), both test methods provided almost identical risk prognoses (Figure 11). Given these findings, the Dentocult culturing devices and CRT have proved to be of equal value, while the latter even further facilitates the already very easy procedures by offering the possibility of simultaneous determination of both S mutans and Lactobacilli (Figures 12a-f).

**DISPOSAL OF THE SALIVA TESTS**

Saliva tests are not only simple as regards their handling, but also as regards their disposal. Disinfecting with a customary solution is as efficient as autoclaving (Kneist and Heinrich-Weltzien, 1997).

**REFERENCES**


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Figure 12a-f: Application of the CRT in the dental practice. From saliva stimulation to evaluation


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