Saliva diagnostics and the Expanded ecological plaque hypothesis - An assessment of the current situation

Lutz Laurisch

Indices

Extended ecological plaque hypothesis, saliva test, caries risk, Streptococcus mutans, lactobacilli, diagnosis-based individual prophylaxis, functional saliva parameters, caries risk diagnostics

Summary

Which factors play a relevant role in the development of caries and how can they be identified for the benefit of dental care? The hypotheses on the aetiology and pathogenesis of caries (plaque hypotheses) have evolved over the past decades. In parallel, there has been a continuous search for correspondingly suitable risk parameters to be able to predict the occurrence of disease in clinical practice. Microbial and functional saliva parameters are intensively discussed in this context as a component of caries risk diagnostics. The further development of plaque hypotheses has inevitably led to changes in the interpretation of the findings obtained by saliva diagnostics. The article summarises the developments and interpretations of salivary diagnostics in the last decades and gives hints on how to interpret the findings obtained in the concept of the extended ecological plaque hypothesis. The presence of plaque alone cannot be the sole diagnosis for the indication and implementation of preventive measures. A comprehensive risk diagnosis and inclusion of all important parameters should always form the basis for the treatment measures to be planned.

Introduction

Individual prophylaxis is historically distinct from group prophylaxis. Since the latter was first established in dentistry, many elements of group prophylaxis were adopted in dental practice with the introduction of individual prophylaxis. One of these adopted strategies is the determination of the current caries risk on the basis of the existing caries prevalence - an inference from the past caries experience to the current or future risk. This can be done with a relatively high degree of reliability. This is because the patient who has clinically visible carious lesions is also very likely to develop new caries.

However, it is precisely the development of a new carious lesion that is to be prevented by individual prophylaxis. This is about treating the risk of disease and not the disease itself[16] (Table 1). In this respect, the criteria of the Deutsche Arbeitsgemeinschaft für Jugendzahnpflege e. V. (DAJ) are not helpful. The definitions of the American Dental Association (ADA)[88] are somewhat more detailed (Table 2).

Tab. 1 Caries risk classification of the German Working Group for Youth Dental Care (DAJ).

Age	dmf(t) resp. DMF(S)
until 3 years	dmf(t) > 0
until 4 years	dmf(t) > 2
until 5 years	dmf(t) > 4
6 to 7 years	dmf(t) > 5, D(T) > 0
8 to 9 years	dmf(t) > 7, D(T) > 2
10 to 12 years	DMF(S) at approximal/smooth surfaces > 0

Tab. 2 Caries risk definitions of the "American Dental Association" (ADA).

Low caries risk in adolescents	Medium caries risk	High caries risk
• No carious lesions in the last year • Morphologically favourable shape of the dimples and fissures • good oral hygiene • appropriate fluoride application • Regular visit to the dentist	• 1 carious lesion in the last year • Deep dimples and fissures • mediocre oral hygiene • inadequate fluoride application • White spots and/or approximal radiolucencies • irregular visit to the dentist • orthodontic treatment	• more than 2 carious lesions in the last year • former smooth surface caries • increased *Streptoccocus mutans*-Values • Deep dimples and fissures • poor oral hygiene • No or hardly any fluoride application • White spots and/or approximal radiolucency • frequent consumption of sweets • irregular visit to the dentist • insufficient salivation • Bottle-feeding or breastfeeding for too long (infants)

Tab. 3 Caries risk definitions in very young children according to the current guideline of the Joint Federal Committee [G-BA] on early detection examinations for tooth, mouth and jaw diseases (FU-RL).

Age until	dmf(t)
3 years	dmf(t) > 0
4 years	dmf(t) > 2
5 years	dmf(t) > 4
6 years	dmf(t) > 5

The current guideline of the Joint Federal Committee on Early Dental, Oral and Maxillofacial Screening (FURL) defines the caries risk in very young children as shown in Table [324]. At the same time, however, the S2k guideline on caries prophylaxis for permanent teeth confirms that "especially patients with a higher caries risk should be recommended to participate in structured prophylaxis programmes".

But what should such structured prophylaxis programmes look like and on what diagnostic basis should they be based? Especially if they "aim to reduce the number of germs by reducing the consumption of sugary foods "[24]. In principle, it should also be questioned whether it makes sense for a preventive dental practice to determine the caries risk on the basis of caries incidence in the [past18].

Fig. 1 Application of a saliva sample with a wooden spatula onto an agar (here CRT bacteria, Ivoclar Vivadent, Ellwangen).

An exact diagnosis - the basis of all dental treatments

After Willoughby Dayton Miller presented his chemo-parasitic theory of caries development, scientific research focused on plaque or biofilm and the bacteria found in [it60]. This led to the development of the non-specific plaque theory, which postulated that plaque is cariogenic per se. The non-specific plaque hypothesis saw the disease as an interaction of all plaque microorganisms, whereby more biofilm also meant more disease. Caries prevention strategies therefore aimed at regular plaque removal. This concept is still reflected today in the fact that in many places patients are encouraged to come to the practice regularly for a professional dental cleaning - as the sole service.

This non-specific plaque theory was later by the specific plaque hypothesis. It postulated that the bacterial composition of the plaque is decisive for the development of caries and not the plaque per se[55,83,82]. Lactobacilli (LB) and *Streptococcus mutans* (SM) were identified as the decisive and most important germs. As a result, bacteriological tests - commonly referred to as "saliva tests" - were developed with the aim of detecting the presence and quantity of the germs in a simple way.

Development of the detection of *Streptococcus mutans* in saliva

Long after the first description of SM by [Clarke13], Gold developed a selective agar for the detection of SM. He added bacitracin (MSBAgar) to a Mitis salivarius agar and thus exploited the fact that SM is insensitive to bacitracin. However, the shelf life of such agar is limited, as bacitracin can degrade [rapidly25].

In order to transfer the germs from the oral cavity to the agar, Köhler and Bratthall developed the so-called wooden spatula method, in which by means of
"saliva was applied to a culture [medium39] (Fig. 1).

In 1984, Alaluusua and Jorden developed a dipslide test in which a bacitracin tablet was placed on a normal Mitis salivarius agar. Due to the insensitivity to bacitracin, only SM grew in the bacitracin-containing environment around the tablet - the so-called inhibition zone. This circumvented the reduced shelf life of the Mitis Salivarius bacitracin agar of only a few weeks. By means of a chart, the concentration of SM in saliva could be eva [luated2] (Fig. 2 to 5).

Laurisch adapted the generally known 3-eyelet smear for the detection of SM on an agar plate. In the so-called 3-eye streak, saliva is streaked out on an agar with an eyelet (1 µl) according to a special technique. On the basis of the
"Germ carry-over", conclusions could be drawn about the SM content of the saliva [sample48] (Figs. 6 and 7).

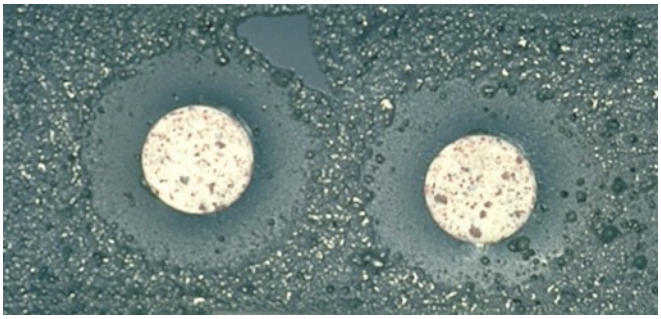


Fig. 2 Clearly visible hemhof.

Fig. 3 No Hemmhof, high density of colonies on *Streptococcus mutans* (SM).

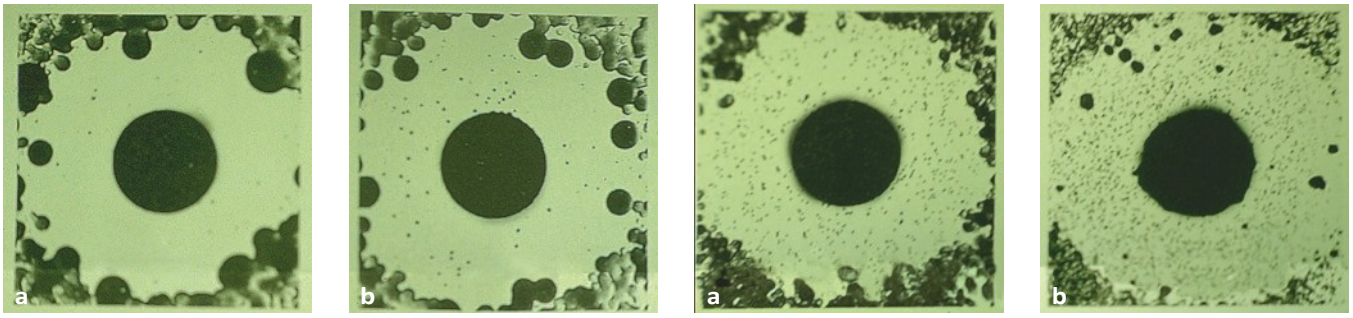


Fig. 4a and b as well as 5a and b evaluation chart.

Fig. 6 Detection of lactobacilli (LB) on Rogosa agar (3-eyelet line).

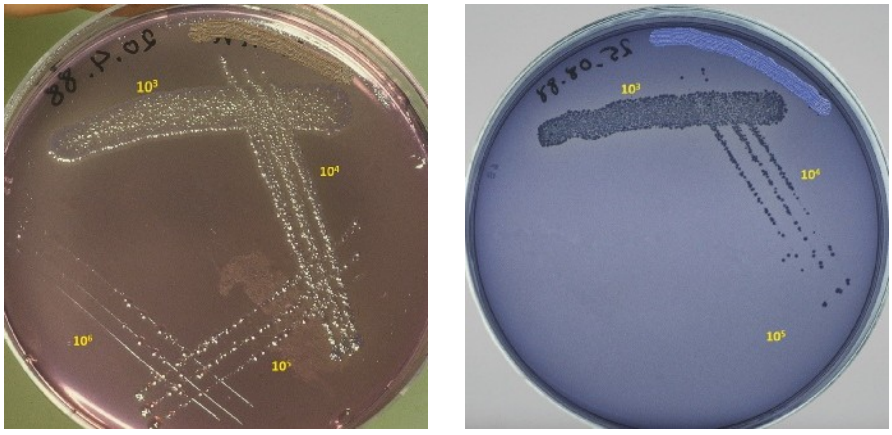


Fig. 7 Evidence of SM on Mitis salivarius bacitracin (MSB) agar (3-eyelet dash).

Based on the adherence of SM to glass tubes and plastic sticks, as demonstrated by Alaluusua et al, Jensen and Bratthall at the University of Malmö developed the Dentocult SM Strip Mutans, which was subsequently produced by Orion Diagnostica in Espoo (Finland). A plastic spatula was spread over the tongue and then incubated in a liquid nutrient medium. Based on the colonies (blue), it was possible to draw conclusions about the amount of SM contained in the saliva28 (Figs. 8 to 11).

Production and distribution of this test method was discontinued some time ago.

By further modifying the agar, Laurisch developed a highly selective agar for SM in 1997 by increasing the sugar concentration to 41 %. This agar, together with a Rogosa agar for the detection of LB, was applied to a foil-sealed agar plate using a special procedure.

"Double dip" applied. The film-sealed

"Double dip" goes back to a patent of the Madaus company in Cologne. The film seal extended the

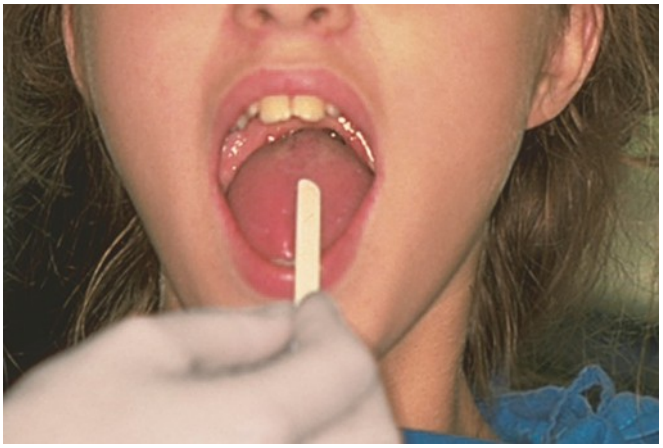


Fig. 8 The plastic spatula is passed over the tongue.

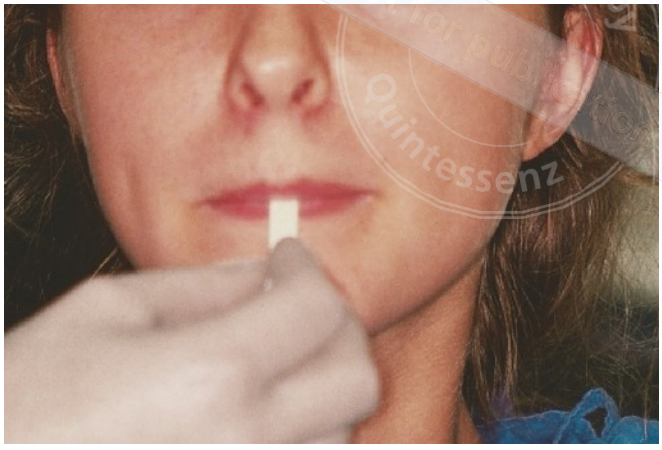


Fig. 9 Through the slightly closed lips, the plastic spatula inoculated with saliva.

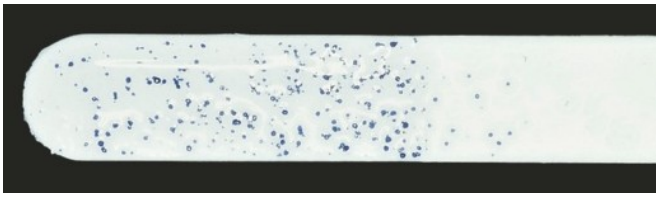


Fig. 10 Little evidence of SM (blue colonies on the spatula).

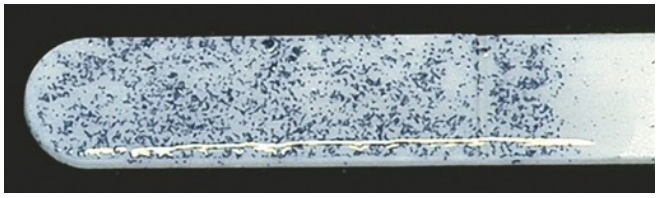


Fig. 11 Much detection of SM on the plastic spatula.

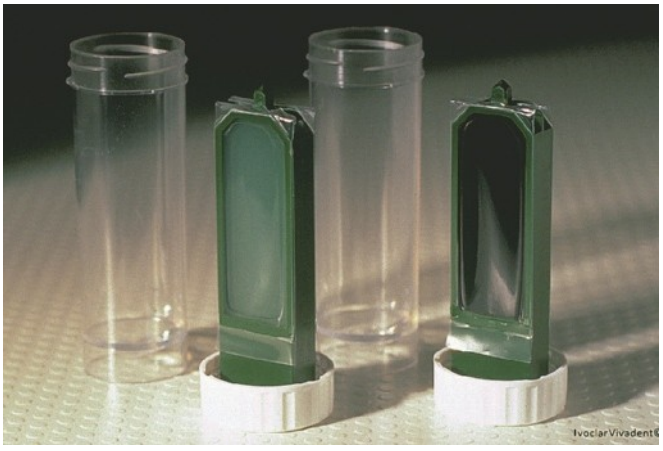


Fig. 12 "Double dip" for the detection of SM and LB, Rogosa agar (light side) and SM-modified MSB agar (dark side, fig. courtesy of Ivoclar Vivadent, Ellwangen).

the shelf life of the MSBAgars to over 12 months, as the bacitracin could not degrade due to the exclusion of air. This detection method was offered by Ivoclar Vivadent (Ellwangen) under the name CRT bacteria in dentistry. After Ivoclar Vivadent had discontinued the production and

Since 2018, this test has been produced by Aurosan (Essen) as a caries screen test on the original Madaus production machine32-34[,51] (Fig. 12). After a saliva sample has been obtained, it is applied with a pipette to the nutrient media of the test device. "double dip". After an incubation period of 48 hours, the germ counts can be read off from a chart diagram (Fig. 13 to 15).

In 2009, GC Germany (Bad Homburg) launched SalivaCheck Mutans for the detection of SM using monoclonal antibodies. The result could be read within 15 minutes. However, the detection limit was 500,000 germs SM/ml saliva. This meant that the field of application was very limited. The test could not be used for prophylaxis in early childhood or for monitoring the progress of patients in recall. The distribution of the test procedure has since been discontinued (Fig. 16 and 17).

Only the SalivaCheck Buffer (GC) is still offered for the determination of functional saliva parameters. These include pH value, secretion

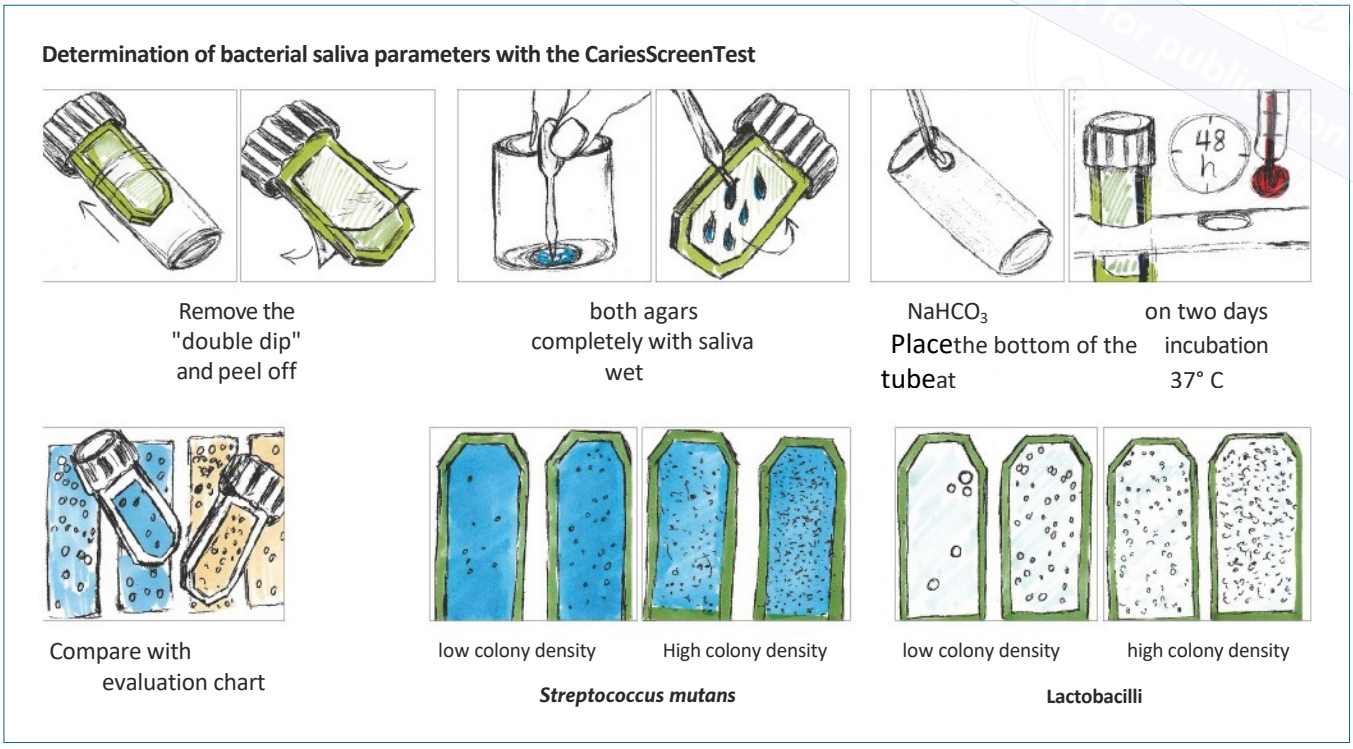


Fig. 13 Evaluation of the saliva sample obtained with the CariesScreenTest (Aurosan, Essen), formerly marketed as CRT bacteria (Ivoclar Vivadent, Ellwangen).

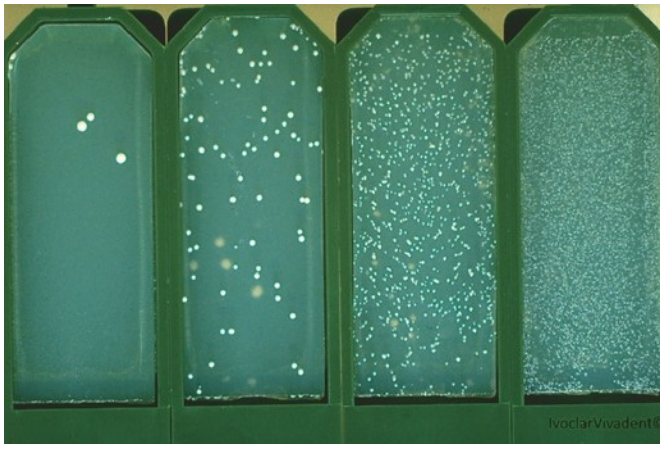


Fig. 14 Evaluation chart for LB (from 1,000 to 1,000,000, from left).

Fig. 15 Evaluation chart for SM (from 1,000 to 1,000,000, from left).

rate and the buffer capacity of the saliva (Fig. 18). Since 2018, the Caries ScreenTest + P (Aurosan) has also been available. In addition to determining the bacterial saliva parameters SM and LB, this also allows the determination of the functional saliva parameters. For the first time, a complete diagnostic tool for the determination of subclinical risk factors is available (Fig. 19 and 20).

Significance of functional saliva parameters

Secretion rate

Determining the salivary flow rate shows whether there is enough saliva. The natural protective function of saliva, the rinsing function, the dilution effect in the case of sugar intake, the removal and the availability of saliva are all important factors.

Fig. 16 Saliva check Mutans (GC, Bad Homburg)

Fig. 17 Evaluation options within 15 min, threshold value 500,000 CFU/ml.

Fig. 18 Buffer capacity test Saliva-Check Buffer (GC).

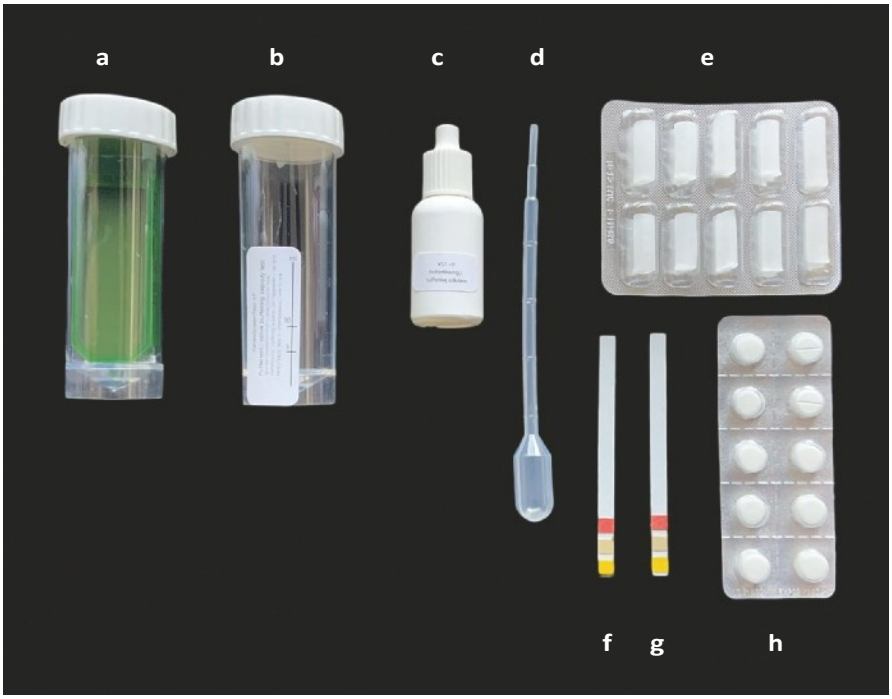


Fig. 19a to h CariesScreenTest + P (Fa. Aurosan) as a complete examination tool for determining bacterial and functional saliva parameters: "Double dips" for detecting SM and LB (a), tubes for collecting the saliva obtained with simultaneous calibration for determining the secretion rate (b), indicator solution for determining the buffer capacity (c), 1-millilitre pipette for collecting the saliva from the saliva sample (for mixing with the test solution (d), paraffin capsules for saliva stimulation (e); pH-value measuring strip for determining the saliva pH (f), pH-value measuring strip for determining the buffer capacity (g), sodium bicarbonate tablet for creating a microaerophilic environment during incubation of the "double dip" (h).

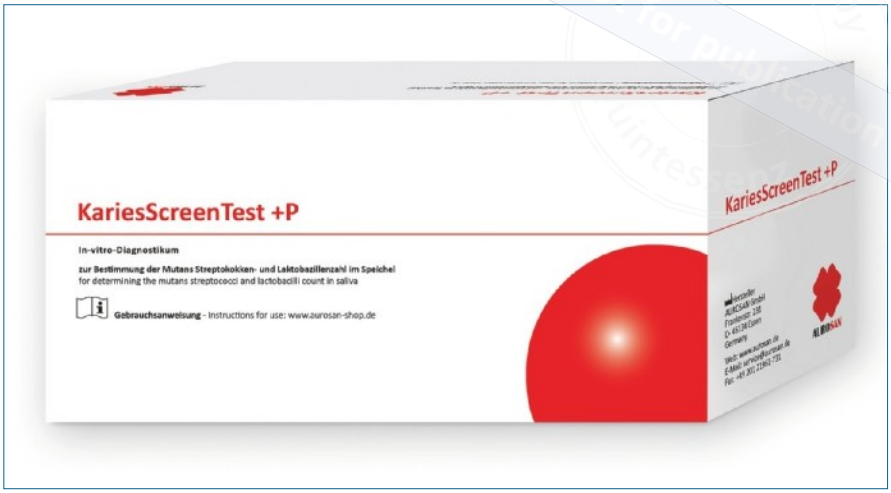


Fig. 20 CariesScreenTest + P (formerly CRT bacteria).

of minerals for remineralisation and the clearance rate depend on the amount of saliva available40,41,44,49,50,52. The secretion rate should be about 1 ml/min. Values below this reduce the clearance rate and the remineralisation potential and are thus favourable to caries.

pH value

The resting pH of the saliva can be determined with indicator test paper (e.g. in SalivaCheck Buffer, CariesScreenTest + P). The resting pH value should be higher than or equal to 7, especially for exposed root surfaces where demineralisation already starts at a pH value of 6.7.

Buffer capacity

Buffer capacity is a key protective mechanism of the oral cavity against food and plaque acids and is related to saliva flow rate. Thus, reduced salivary flow rates show reduced buffer capacities21 in conjunction with corresponding caries findings.

A good buffer capacity is able to neutralise food and plaque acids in the oral cavity. The buffering capacity therefore has an important function for the stability of the pH environment in the oral cavity. Frequent intake of acidic foods leads to a greater pH drop in the oral cavity. The buffering capacity of saliva is no longer sufficient to neutralise this greater amount of acid56,86.

Very good buffer capacities have a pH value of > 6, good ones are between 5 and 6 and poor buffer capacities have a pH value of < 5.

The sodium bicarbonate content of saliva also depends on the secretion rate. High secretion rates always require good buffer capacities21.

Change in interpretation

As caries aetiology concepts evolved, the interpretation of the data obtained by the test procedure also changed. The acceptance of the specific plaque hypothesis led to the transfer of Koch's postulate to caries genesis. Thus, it was believed that the detection of SM alone could provide a reliable indication of the patient's caries risk. However, this opinion was less represented in science than in the concepts for marketing the Dentocult SM Strip Mutans Test. With the introduction of the term "individual caries risk", however, concepts were introduced early on in which the bacterial saliva parameters were presented as only one aspect of a multi-causal process43,44,47.

While the interpretation of the SM numbers found in saliva changed depending on the valid caries genesis, the interpretation of the LB numbers found has remained the same for 80 years27.

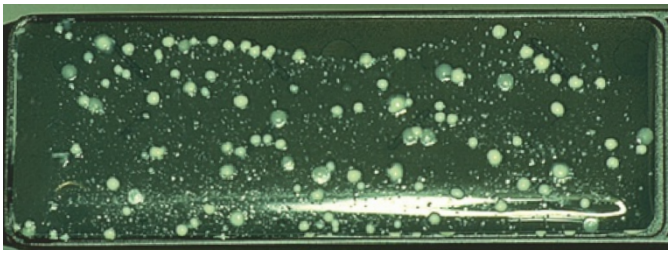


Fig. 21 Dentocult LB (Orion Diagnostica, Espoo, Finland) after incubation.

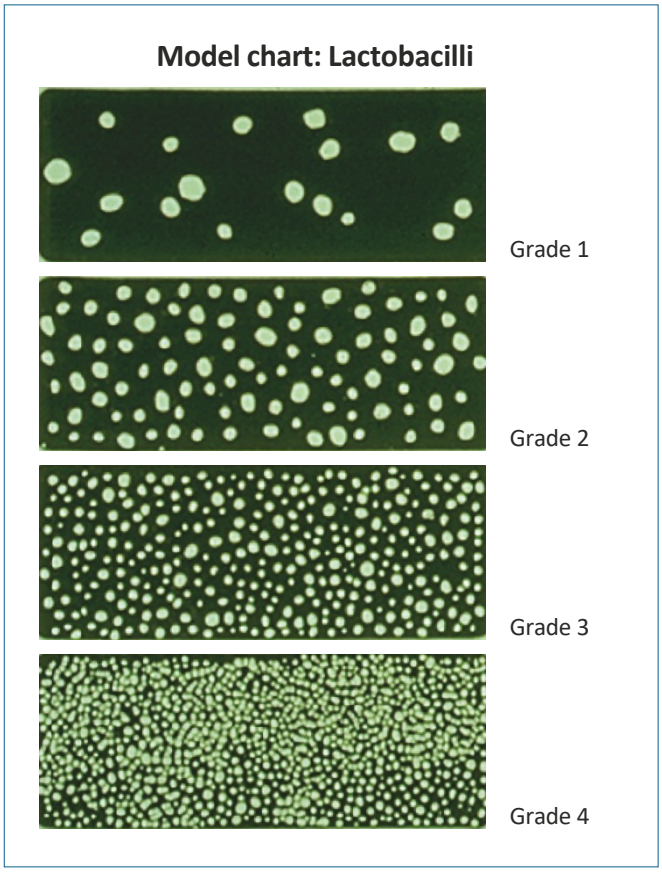


Fig. 22 Evaluation chart of the Dentocult LB.

Jay found in 1947 that sugar reduction led to a reduction in LB numbers, having already pointed out the relationship between diet and caries in [194426]. Kitchin observed the same in a single case study over 3 [years30]. The development of the Rogosa Agar by Rogosa in 1951 simplified the detection of [LB71]. In 1975, Larmas then developed the matching dipslide for further simplified detection. An evaluation [chart45] [was] again used as a reference (Figs. 21 and 22).

Since 1975, dental practice has thus had a suitable, scientifically proven diagnostic method at its disposal to determine the patient's consumption of fermentable carbohydrates and, above all, to check it repeatedly in the context of patient recall care. This makes it possible to determine the patient's compliance during nutritional counselling11[,27,30,64].

Since 1997, the detection of LB on a Rogosa agar has been part of the "double dip" for the detection of SM and LB (Caries Screen Test, formerly CRT bacteria).

On the interpretation of the *Streptococcus mutans* figures

The currently accepted extended ecological plaque hypothesis takes into account the fact that caries can also develop without a relevant amount of SM being detectable. This ecological concept thus contradicts the "infection theory" of the specific plaque hypothesis7[,14,66,75].

Nevertheless, the initial colonisation of the child's oral cavity in the first few years is always of interest.

2 years of life. The initial colonisation with SM depends on the mother's microbial count or the microbiome of the parents8[,9] or caregivers. The threshold value for SM was defined by Berkowitz as 500,000 CFU/ml saliva [determined8].

However, the establishment of SM in the child's oral cavity is only possible with a sufficient nutritional basis in the form of fermentable carbon [hydrates68]. The compliance of the parents is therefore primarily required here9[,12,68].

In 2006, Thenisch was able to show in a systematic review of 981 publications that the detection of SM in saliva at the age of 2 years means a doubling or detection in plaque (plaque smear) means a quadrupling of the caries risk. In children with a positive caries result at 30 months, SM was always detected early9[,67,68,85].

The doubling of the caries risk alone when SM is detected in plaque indicates that it makes a difference whether SM is only found in saliva

is present or already in the plaque. In the spit, SM has no direct influence on caries activity, but it does allow conclusions to be drawn about the SM numbers in the plaque. However, there are often unexpected deviations here. These deviations can also be area-specific on the teeth. This depends on the caries activity at the examined site, whereby the caries-active and the caries-inactive sites can be quite close to each other19,74,76.

Previous studies of the oral microbiota relied heavily on culture-based methods to determine the microorganisms. Thus, it is also possible that refined DNA or RNA examination methods could result in greater deviations in the future. In the present case, however, this does not change the fact that DNA probe methods or methods using the polymerase chain reaction (PCR) technique were able to confirm the transmission of bacteria from mother to child77.

This early detection of SM in the saliva of the child's oral cavity seems to be decisive for the caries prevalence in the following 15 years. This is always higher than when SM is detected later in the oral cavity. Of course, it must also be taken into account that the established diets that are causal for this can only be changed over time20 and sometimes not at all1,3,5,15,20,35,36.

Tenovuo was able to show that the general caries prevalence depends on the time of the first detection of SM in the plaque of the child's oral cavity. The study was carried out by detecting serum antibodies against SM84. Due to an extremely unfavourable nutritional situation and other behaviours that promote colonisation, high bacterial counts of SM establish themselves in the child's oral cavity at an early stage. This, in turn, is always a strong risk indicator for the occurrence of "early childhood". hood caries" (ECC)17,18,63,70 (Figs. 23 to 25).

In cases of high intakes of fermentable carbon hydrates, *Candida albicans* enhances the cariogenic potential of SM in plaque29,72.

Since the agar used in the CariesScreenTest to detect SM is not fungicidal, it can be

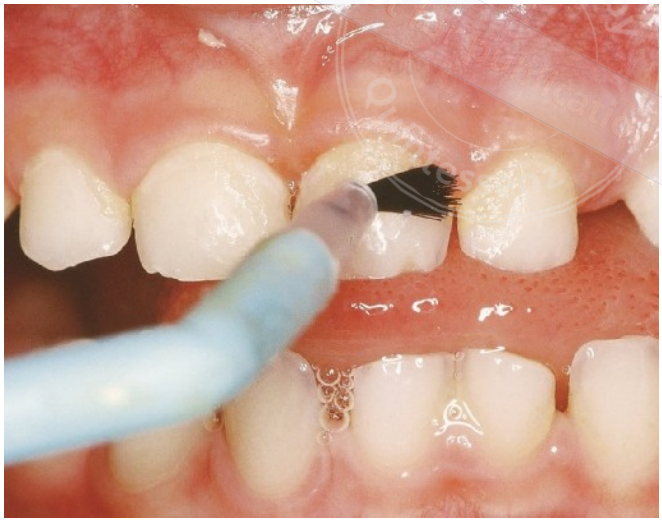


Fig. 23 Plaque smear in children.

Fig. 24 Plaque smear of several teeth after incubation: examination for SM (caries screen test).

Fig. 25 Plaque smear of several teeth after incubation: examination for LB (CariesScreenTest).

yeast fungi also grow on it. Differentially, these can be detected by a positive catalase reaction (Fig. 26).

Since colonisation of the child's oral cavity with SM is only possible with a sufficient supply of substrate, the importance of advising the pregnant woman or the new mother on correct nutritional behaviour as well as adequate age-appropriate dental care and a fluoridation concept coordinated with this becomes apparent. At the same time, however, this is also the starting point for the timely

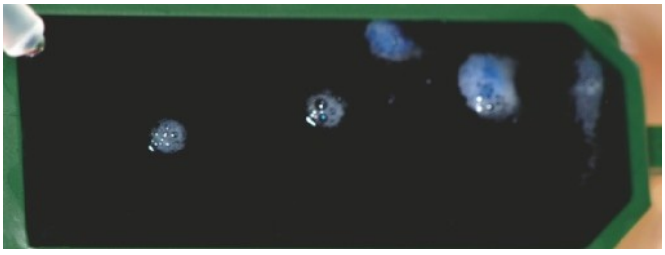


Fig. 26 Foaming of the yeast colonies with the addition of $_{H2O2}$.

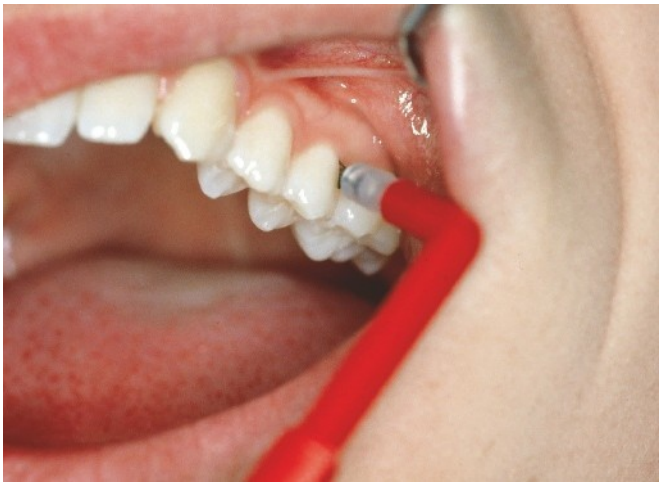


Fig. 27 Plaque smear from the approximal space 26m.

Fig. 28 A high LB count in the approximal space indicates an increased caries risk (caries screen test).

the biome of the mother or parents by a "saliva test" and thus define the risk of transmission. Preventive therapies by parents lead to a reduction of caries-relevant germs - in this case especially SM - and thus reduce the risk of transmission[38].

As a rule, SM can be detected in higher proportions in caries patients than in caries-free patients. Since high bacterial counts favour further caries prevalence, it is clear that, in addition to conservative measures, preventive measures must be taken.

The use of the "no-go" approach should be an indispensable part of treatment37[57,69].

A high proportion of LB in the plaque always indicates a possible caries progression. An analysis of the plaque situation in an approximal space is possible with the help of a smear technique (Figs. 27 and 28).

These scientific findings, gained over decades, also partly form the basis for the extended ecological plaque hypothesis (Fig. 29).

The homeostatic situation of the healthy microbiome is characterised by a balance of potentially pathogenic and apathogenic germs - with a pronounced diversification. The continued consumption of fermentable carbohydrates leads to a proliferation of acidogenic and acid-forming germs, which increasingly displace the apathogenic flora. Clinically visible (highlighted in light grey in Fig. 29) is the constant increase in plaque and insufficient oral hygiene. Clinically invisible (highlighted in yellow in Fig. 29), acid-forming and acid-tolerant germs proliferate in the plaque as the pH value decreases.

Acid tolerance and subsequent selection of low pH NonSM seem to play a crucial role in destabilising plaque homeostasis.

According to the pH value reduction, an acidogenic phase (pH value < 6.5 and destabilisation of homeostasis), an acidic phase, in which a further selection of acidophilic germs occurs, and the dysbiotic phase (pH value < 5.5)[80] are distinguished.

In this acidic environment, SM and other acid-producing bacteria, especially LB, can promote lesion development through excessive growth by maintaining an environment characterised by a persistently low pH. LB and SM thus displace the low-acid producing NonSM that initiated the initial pH reduction. The plaque is now dominated by strong acid producers as diversification decreases[79]. Therefore, high proportions of SM and LB can be seen as biomarkers for sites with particularly rapid caries progression.

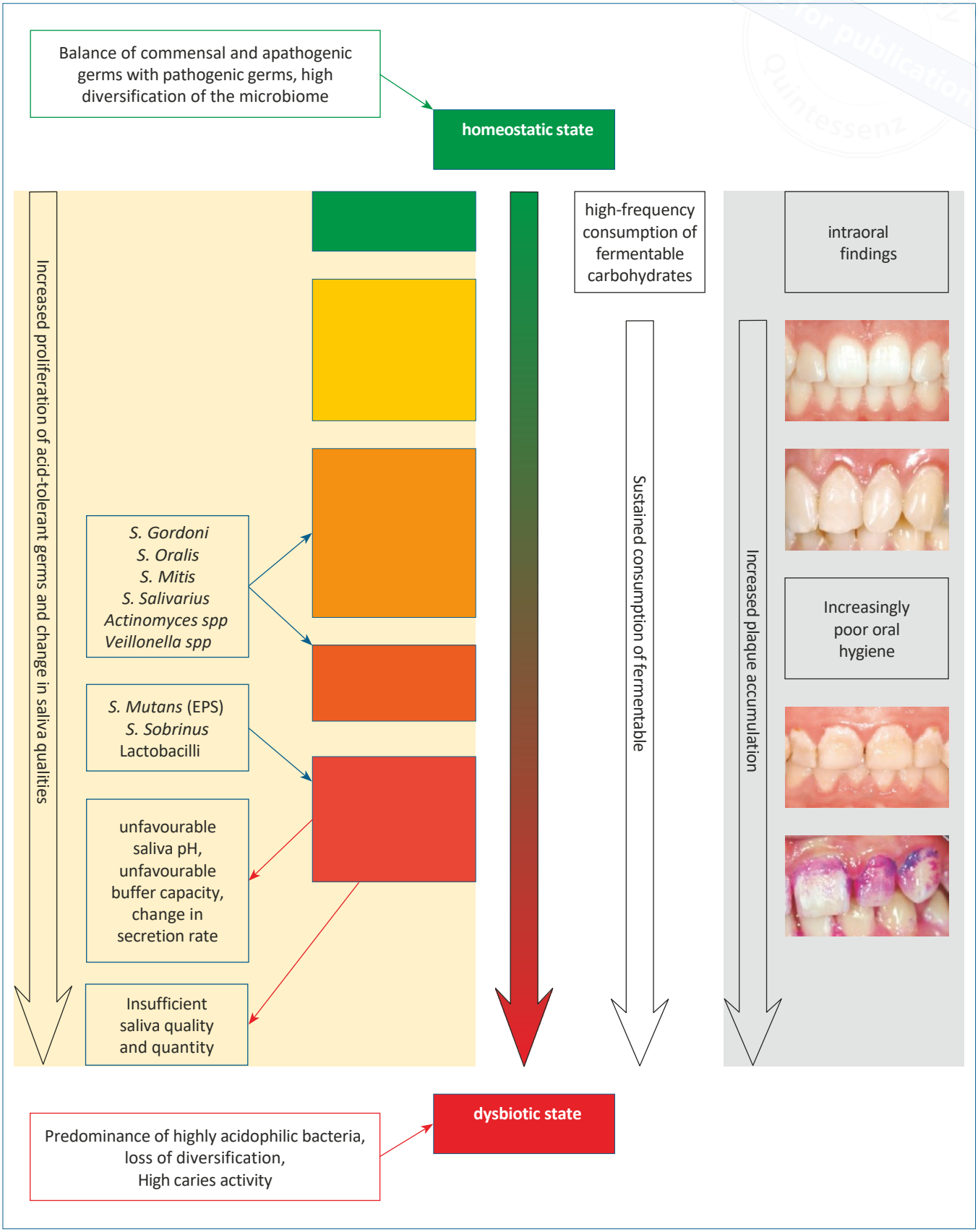


Fig. 29 The extended ecological plaque hypothesis according to Laurisch, modified after [Conrads14], [Marsh58] and [Zijnge89].

Ultimately, a dysbiotic state develops, which is characterised by the fact that the diversification of the microbiome has been lost and acidogenic and aciduric germs dominate in the plaque.

The extended ecological plaque hypothesis repeatedly underlines the well-known fact that a proliferation of caries-relevant germs occurs with the regular consumption of fermentable carbohydrates23,62.

The role of SM is not limited to acid formation alone: it is the main producer in the formation of extracellular polysaccharides and thus the guarantor of an undisturbed "quorum sen sing" in the plaque, as the matrix it forms protects the tooth-bearing biotope from the natural defence functions of the oral cavity - secretion rate and buffer capacity42,83.

This means that SM still holds a key position for caries risk and caries activity. It also maintains the acidic environment in which LB can overgrow the system. In fact, LB are able to produce acid up to a pH of 3, while SM stop producing acid at a pH between 4 and 5. LB - not actively involved in plaque formation themselves - thus use the pH environment created by SM to drive caries progression. Therefore, they are also present in active carious lesions54,59,78,87.

It can be seen that the extended ecological plaque hypothesis contains elements of the non-specific and specific plaque theories, but brings both more in line with clinical course and preventive understanding. It is important for understanding not only to know what germs are present, but also to be aware of what these germs do31,79.

However, since the number of SM and LB is not the only predictor of caries, it is important in risk diagnostics to determine other factors53. Various concepts therefore supplemented bacterial saliva parameters with clinical parameters at an early stage43,50,44. In 1990 Axelsson summarised the state of knowledge at that time in a risk concept, which is shown in Table 4.

It can be seen that the risk criteria include both clinical and subclinical risk parameters. Additional factors are in particular the functional saliva parameters. Findings between high caries and no caries risk differ in the number and severity of the individual risk parameters. The intermediate findings according to Axels son are shown in Table 5.

König defined the individual risk areas by establishing individual risk ratings for 4 parameters - namely tooth status, salivary secretion, nutritional situation and microorganisms41 (Figs. 30 to 33).

The Cariogram10,73 supplements the previously explained risk parameters with further risk parameters and the existing fluoridation concept. It is a method that was already introduced by Bratthall and others at the end of the 1990s and enables an interpretation and weighted analysis of the various factors involved in the development of caries10. The cariogram offers the ideal prerequisites for a systematic approach: It graphically illustrates the caries risk or the chance to avoid new caries in the future. The Cariogram can be downloaded from the internet, but it is only available in the English 64-bit version. The mobile version is also available as an app.

Figure 34 explains all the risk parameters highlighted in the cariogram. After weighting, the green area indicates the probability that no new caries is to be expected. In the final, digitally generated risk assessment, individual risk factors are summarised in complexes (Tab. 6).

The dark blue areas clearly show the importance of bacterial saliva parameters for risk diagnostics. It is therefore obvious that the predictive value of the results of the Cariogram software is higher if saliva parameters and the number of SM are determined and included in the evaluation65.

All the concepts presented determine a static situation of the oral environment at the time of the examination. The dynamic component of the changes in the biotope oral cavity is thereby

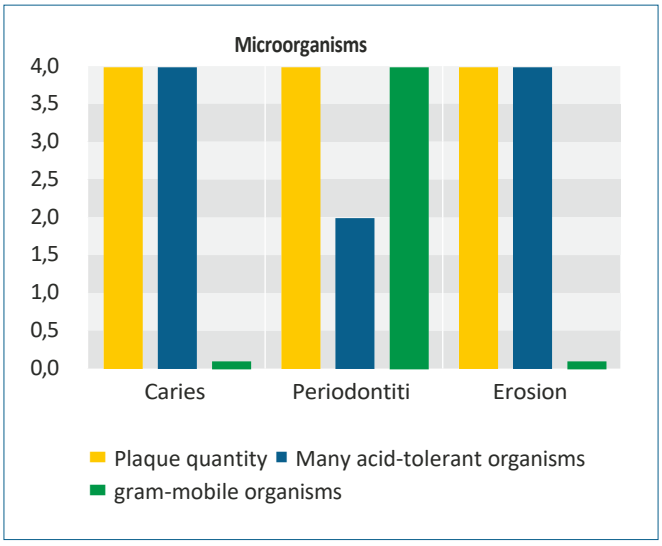


Fig. 30 Importance of microorganisms.

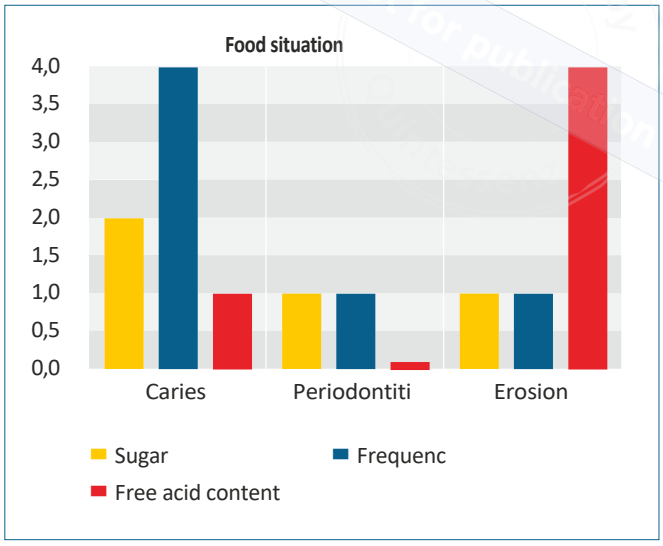


Fig. 31 Importance of the nutritional situation.

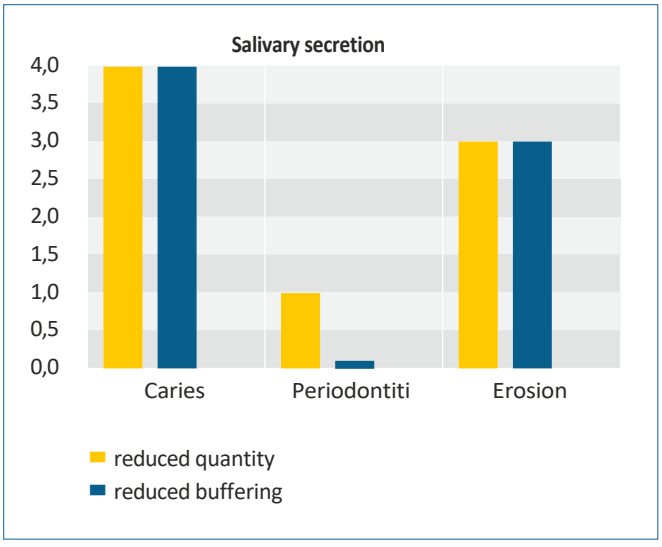


Fig. 32 Significance of salivary secretion.

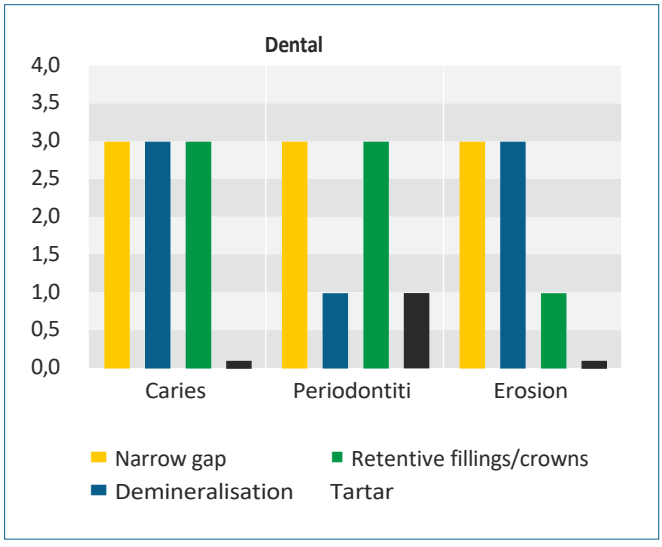


Fig. 33 Significance of dental status.

Tab. 4 Risk concept according to [Axelsson6]. The subclinical risk parameters are marked in yellow.

No caries risk	high caries risk
SM negative	SM values > 500,000 ml/min.
excellent oral hygiene habits	Very poor oral hygiene habits
Low LB values	LB values > 100,000 ml/min.
Very low DMF or DMFT index	Very high DMFT value with buccal/lingual DFS
No active initial caries	Very much initial caries
adequate salivary secretion	Saliva secretion rate < 0.7 ml/min.
low consumption of sticky, sugary products	Heavy consumption of sticky, sugary products
Buffer capacity with a pH value > 5.5	Buffer capacity with a pH value < 4.5

Tab. 5 Intermediate findings of the risk concept according to Axelsson[6]. The subclinical risk parameters are also marked in yellow.

low caries risk	Caries risk
SM positive	SM positive
good oral hygiene habits	poor oral hygiene habits
low LB values	high LB values
low DMF or DMF-T index, respectively	High proximal DMF
little initial caries	much initial caries
Salivary secretion rate > 1 ml/min.	Saliva secretion rate 1 ml/min.
low consumption of sticky, sugary products	Heavy consumption of sticky, sugary products
Buffer capacity with a pH value < 5.5	Buffer capacity with a pH value < 5

Tab. 6 Risk determination based on the individual risk factors nutrition, bacteria, susceptibility and circumstances.

Nutrition	Bacteria	Receptivity	Accompanying circumstances
Frequency of food intake and composition of food	Plaque quantity and qualitative composition of plaque (number of SM/LB)	Resistance of the tooth substance (fluoridation) and quality of the saliva (secretion rate, buffer capacity, saliva pH value)	Carious tooth damage in the past and general state of health
The area is shown in dark blue in the cariogram.	The area is shown in red in the cariogram.	The area is shown in light blue in the cariogram.	The area is shown in yellow in the cariogram (Figs. 35 to 37).

not recorded. However, a repeated risk analysis in the sense of monitoring after the implementation of preventive measures enables a more dynamic interpretation61,81.

Conclusions

The significance and interpretation of saliva tests and the determination of bacterial and functional risk parameters have changed over time depending on the current caries aetiology. The extended ecological plaque hypothesis contains elements of the non-specific and specific plaque theory, but brings the clinical course and our preventive understanding into better harmony. For this understanding, it is important not only to know what germs are there, but also to be aware of what these germs do. Plaque consists of potentially pathogenic and apathogenic germs.

germs with a large diversification. For the development of a pathogenic plaque, it is primarily not the genotype of the bacteria that is decisive, but the phenotype. This develops from the individual conditions that can turn a potentially cariogenic germ into a cariogenic germ. This process is important for the preventive concepts of the dental practice, because this process is reversible if the acidogenic or aciduric conditions in the plaque can be changed. Unfortunately, the key to successful therapy lies in an area that is often the most difficult to change: the nutritional situation20.

SM is a crucial component of plaque due to its distinct ability to form extracellular polysaccharides, which also creates the acidic pH environment for LB. The detection of these germs in the plaque is associated with a high caries activity at this site. A detection

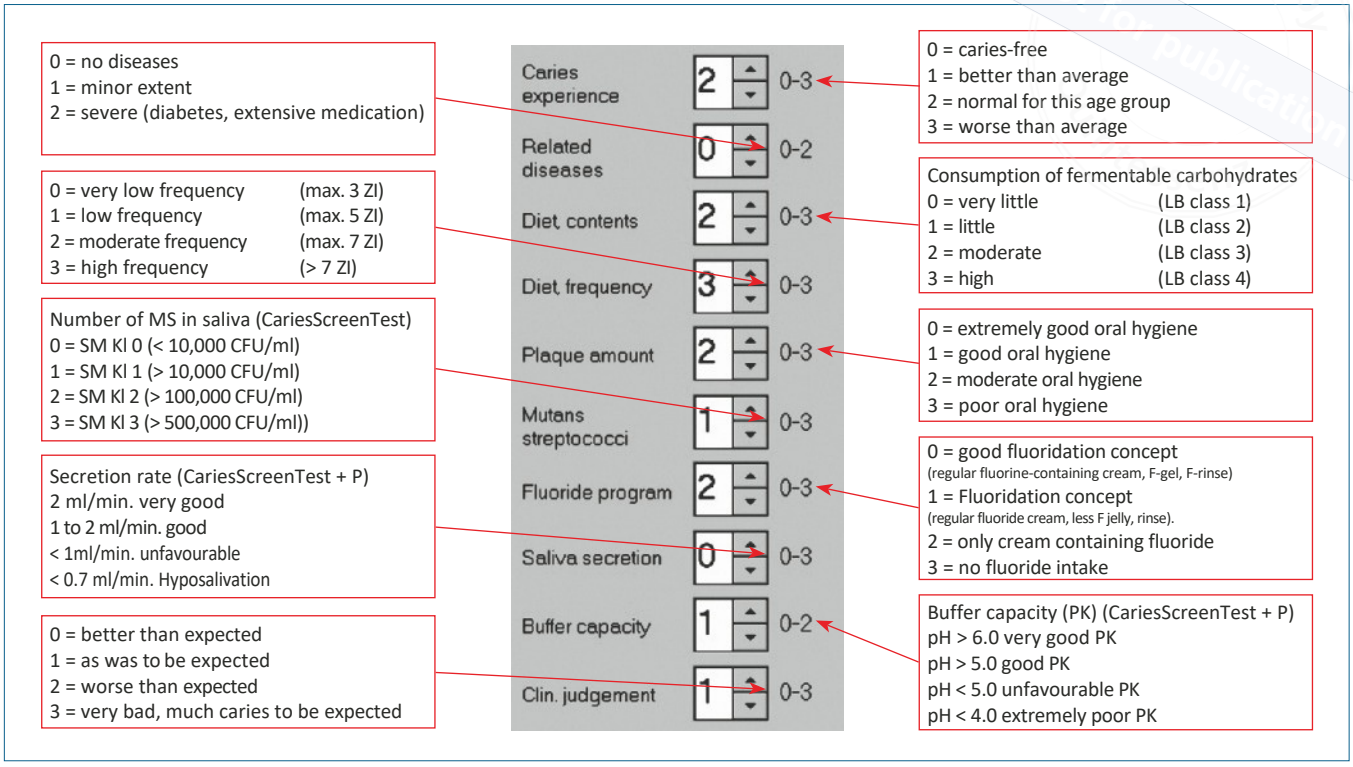


Fig. 34 Risk parameters determined in the cariogram.

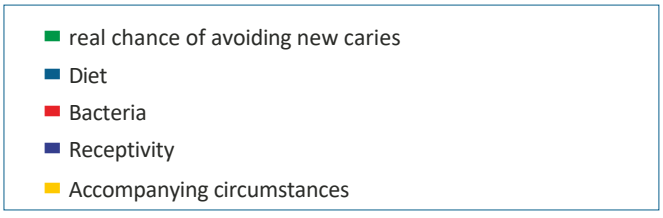


Fig. 35 Labelling of the summarised risk factors with corresponding colours.

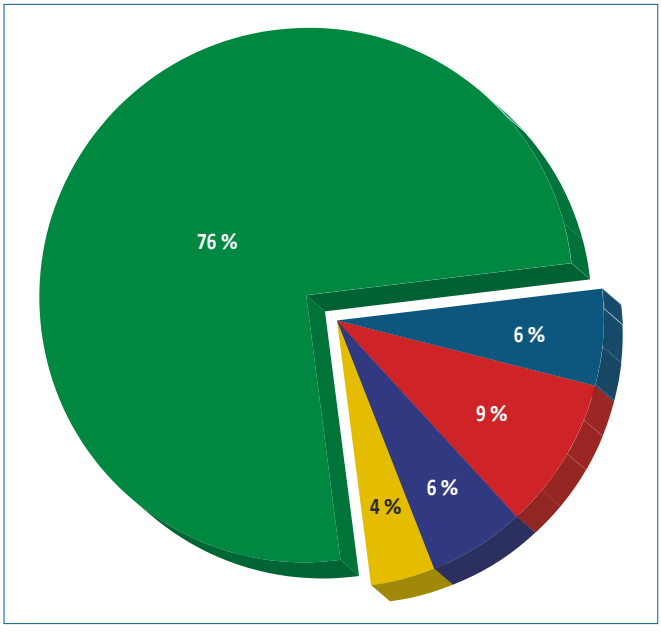


Fig. 36 Low caries risk: The probability of no caries is 76 % (green area).

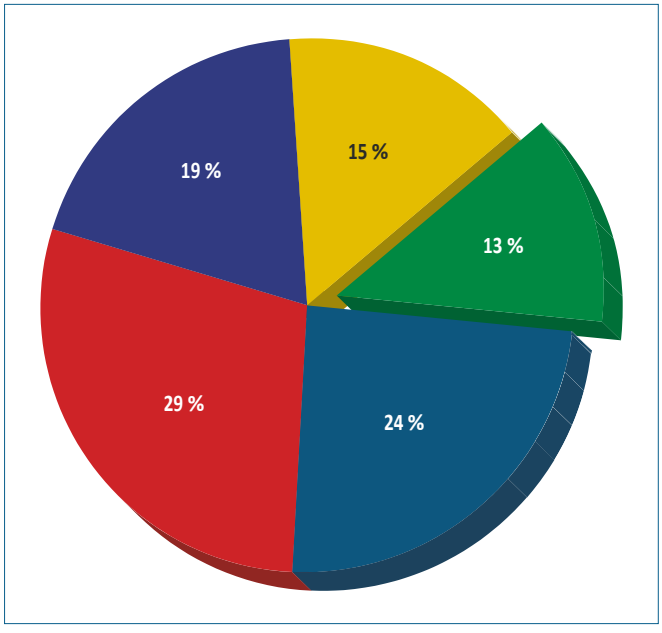


Fig. 37 High caries risk: The probability of no caries is only 13 % (green area).

of germs in saliva in high numbers does not necessarily correlate with caries activity on certain tooth surfaces, but gives clear indications that the oral cavity biotope is not in a homeostatic state. The smear technique, however, gives us more precise information about the suspected caries activity on the corresponding tooth surface.

Good salivary pH, secretion rate and buffering capacity are also important factors in a homeostatic situation. Therefore, bacterial and functional salivary parameters can be an important part of caries risk diagnostics. They are also an integral part of all risk concepts cited here. Their importance does not only result from their significance in diagnostics. In addition, repeated examinations of subclinical risk parameters in the recall also give us objectifiable indications of changing risk parameters and the patient's compliance at an early stage.

Due to the multi-causality of the disease, it is indispensable to assess the relevant risk parameters. determine. But that alone is not enough. The much more crucial question is why. Only by answering this question can adequate preventive therapies be developed that reflect the current state of scientific knowledge.

Risk diagnostics including clinical and subclinical risk parameters enables diagnosis-based prevention. This in turn is not only a medical necessity, but also positively influences the success of our preventive efforts22[,46,90].

The presence of plaque - key evidence of the non-specific plaque theory - should not be the sole basis of diagnostics on which we base our preventive service concepts.

Note

The author is the developer of the CRT bacte- ria, which was produced and distributed by Ivoclar Vivadent until 2018. In cooperation with Aurosan, he has developed the successor product KariesScreenTest and KariesScreenTest + P.

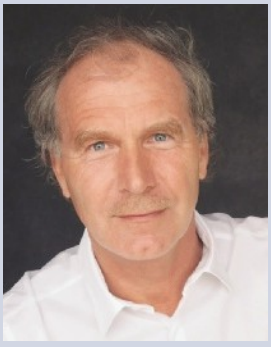
Literature

1. Aas JA, Griffen AL, Dardis SR et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008;46(4): 1407-1417.
2. Alaluusua S, Savolainen J, Tuompo H, Grönroos L. Slide-scoring method for estimation of Streptococcus mutans levels in saliva. Eur J Oral Sci 1984;92(2): 127-133.
3. Alaluusua S. Longitudinal study of salivary IgA in children from 1 to 4 years old with reference todental caries. Scand J Dent Res 1983;91(3):163-168.
4. Anderson AC, Rothballer M, Altenburger MJ et al. In vivo shift of the microbiota in oral biofim in response to frequent sucrose consumption. Sci Rep 2018; 8142020.
5. Anderson MH, Shi W. A probiotic approach to caries management. Pediatr Dent 2006;28(2):151-153.
6. Axelsson P. Method for determining caries risk. Phillip J 1990;7;181-187.
7. Banas JA, Drake DR. Are the mutans streptococci still considered relevant to understanding the microbial etiology of ental caries? BMC Oral Health 2018;18:129.
8. Berkowitz RJ, Turner J, Green P. Maternal salivary levels of Strepto-coccus mutans and primary oral infection of infants. Arch Oral Biol 1981;26(2):147-149.
9. Berkowitz RJ. Acquisition and transmission of mutans streptococci. J Calif Dent Assoc 2003;31(2):135-138.
10. Bratthall D. Dental caries: Intervened - interrupted - interpreted. Concluding remarks and cariography. Eur J Oral Sci 1996;104:486-491.
11. Burt BA, Pai S. Sugar consumption and caries risk: A systematic review. J Dent Educ 2001;65(10):1017-1023.
12. Caufield PW, Griffen AL. Dental caries. An infectious and trans-missible disease. Pediatr Clin North Am 2000;47(5):1001-1019.
13. Clarke JK. On the bacterial factor in the Ætiology of dental caries. Br J Exp Pathol 1924;5(3): 141-147.
14. Conrads G. The oral microbiome and its cariogenic species. ZM 2020;23/24:2294-2296.
15. Corby PM, Lyons-Weiler J, Bretz WA et al. Microbial risk indicators of early childhood caries. J ClinMicrobiol 2005;43 (11):5753-5759.

16. DAJ [Deutsche Arbeitsgemeinschaft für Jugendzahnpflege e. V.]. Principles for measures to promote oral health within the framework of group prophylaxis according to § 21 SGB V of 15.06.1993, amended on 24.06.1998 and 20.06.2000. Internet: https://www.daj.de/Grundsaetze.49.0.html. Accessed: 15.12.2020.
17. de Carvalho FG, Silva DS, Hebling J et al. Presence of mutans streptococci and Candida spp. in dental plaque/dentine of carious teeth and early childhood caries. Arch Oral Biol 2006;51: 1024-1028.
18. Divaris K: Predicting dental caries outcomes in children: A "risky" concept. J Dent Res 2016;95(3): 248-254.
19. Duchin S, van Houte J. Relationship of streptococcus mutans and lactobacilli to incipient smooth surface dental caries in man. Arch Oral Biol 1978;23:779-786.
20. Ellrott T. How children learn to eat. Nutrition - Science and Practice 2007;1:167-173.
21. Ericsson Y. Clinical investigation of the salivary buffering action. Acta Odontol Scand 1959;17:131.
22. Featherstone JDB, White JM, Hoover CI et al. A randomized clinical trial of anticaries therapies targeted according to risk assessment (caries management by risk assessment). Caries Res 2012;46:118-129.
23. Fitzgerald RJ, Jordan HV, Stanley HR. Experimental caries and gingival pathologic changes in the gnotobiotic rat. J Dent Res 1960;39:923-935.
24. G-BA [Federal Joint Committee]. Guideline of the Federal Joint Committee on screening examinations for dental, oral and maxillofacial diseases in the version of 17 January 2019. Internet: www.g-ba.de/richtlinien/29. Access: 15.12.2020.
25. Gold OG, Jordan HV, van Houte J. A selective medium for streptococcus mutans. Arch Oral Biol 1973;18:1357-1364.
26. Jay P. Nutrition and dental caries. J New Jersey Dent Soc 1944;15:20.
27. Jay P. The reduction of oral lactobacillus acidophilus counts by the periodic restriction of carbohydrate. Am J Orthod 1947;33:162.
28. Jensen B; Bratthall, D: A new method for the estimation of mutans streptococci in human saliva. J Dent Res 1989;68(3): 468-471.
29. Kim D, Sengupta A, Niepa THR et al. Candida albicans stimulates Streptococcus mutans microcolony development via cross-kingdom biofilm-derived metabolites. Sci Rep 2017;7: 41332.
30. Kitchin PS, Permar D. Results of 3 year study of the effectiveness of carbohydrate restriction in reducing salivary lactobacillus counts. J Dent Res 1948;27(3): 316-319.
31. Kneist S, Callaway A. From generalists to specialists: the role of bacteria in the extended ecological plaque hypothesis. ZMK 2013;29 (9):548-559.
32. Kneist S, Laurisch L, Heinrich-Weltzien R, Stösser L. A modified mitis salivarius medium for caries diagnostic test. J Dent Res 1998;77: Abstr. 2712.
33. Kneist S, Laurisch L, Heinrich-Weltzien R. The new CRT - microbiological background and efficiency evaluation. Oralprophylaxe 1999;21:180–185.
34. Kneist S. Heinrich-Weltzien R, Laurisch L. Evaluation of a new caries risk test. Independ Dent 1999;7/8:76-84.
35. Köhler B, Andreen I, Jonsson B. The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. Oral Microbiol Immunol 1988;3(1):14-17.
36. Köhler B, Andréen I. Mutans streptococci and caries prevalence in children after early maternal caries prevention: A follow-up at eleven and fifteen years of age. Caries Res 2010; 44:452-458.
37. Köhler B, Bjarnason S. Mutans streptococci, lactobacilli and caries prevalence in 15 to 16-year olds in Goteborg. Part II. Swed Dent J 1992;16(6):253-259.
38. Köhler B, Bratthall D, Krasse B. Preventive measures in mothers influence the establishment of the bacterium streptococcus mutans in their infants. Arch Oral Biol 1983;28(3):225-231.
39. Köhler B, Bratthall D. Practical method to facilitate estimation of streptococcus mutans levels in saliva. J Clin Microbiol 1979;9(5): 584-588.
40. König K, Goepel C. The importance of saliva for oral and dental health. Zahnärztl Mitt 1992;4:42-46.
41. König K. Karies und Parodontopathien. Stuttgart: Thieme, 1987.
42. Koo H, Xiao J, Klein MI. Extracellular polysaccharides matrix - An often forgotten virulence factor in oral biofilm research. Int J Oral Sci 2009;1: 229-223.
43. Krasse B. Biological factors as indicators of future caries. Ind Dent J 1988;38:225.
44. Crass B. The quintessence of caries risk. Berlin: Quintessenz, 1986.
45. Larmas M. A new dip slide method for the counting of salivary lactobacilli. Proc Finn Dent Soc 1975;71:31-35.
46. Laurisch L. Determining caries risk: why, how and what are the therapeutic consequences? BZB 2013;1/2:48-53.

47. Laurisch L. Determining the individual caries risk - a prerequisite for tailor-made prophylaxis. Oral Prophylaxis 1988;10:126.
48. Laurisch L. A microbiological detection method for the determination of caries-relevant germs. ZWR 1989;98:872.
49. Laurisch L. Individual prophylaxis. Cologne: DÄV-Hanser, 1994.
50. Laurisch L. Caries risk determination: Why and how? Zahnärzteblatt Sachsen 2003;10: 27-32.
51. Laurisch L. New selective culture medium for the detection of Streptococcus mutans. Patent specification no. 197 24 970.1. German Patent Office Munich 1997.
52. Lehmann RR. Ecology of the oral cavity. Stuttgart: Thieme, 1991.
53. Lencová E, Broukal Z, Spížk J. Point-of-care salivary microbial tests for detection of cariogenic species-clinical relevance thereof-review. Folia Microbiol 2010;55(6):559-568.
54. Loesche WJ, Syed SA. The predominant cultivable flora of carious plaque and carious dentine. Caries Res 1973;7(3): 201-216.
55. Loesche WJ. Role of streptococcus mutans in human dental decay. Microbiol Rev 1986;50:353-380.
56. Lussi A, von Salis-Marincek M, Ganss C et al. Clincal study monitoring the pH on tooth surfaces in patients with and without erosion. Caries Res 2012;46(6):507-512.
57. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiol 2003; 149(2):279-294.
58. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994;8:263-271.
59. Matsui R, Cvitkovitch D. Acid tolerance mechanisms utilised by streptococcus mutans. Future Microbiol 2010 Mar;5(3):403-417.
60. Miller WD: Die Mikroorganismen der Mundhöhle. Leipzig: Thieme, 2nd ed. 1892.
61. Nyvad B, Takahashi N. Integrated hypothesis of dental caries and periodontal diseases. J Oral Miciobiol 2020;12(1):1710953.
62. Orland FJ, Blayney JR Harrison RW et al. Experimental caries in rats inoculated with enterococci. J Am Dent Assoc 1955;50(3):259-272.
63. Parisotto TM, Steiner-Oliveira C Silva CM et al. Early childhood caries and mutans streptococci: A systematic review. Oral Health Prev Dent 2010;8(1):59-70.
64. Peres MA, Sheiham A, Liu P et al. Sugar consumption and changes in dental caries from childhood to adolescence. J Dent Res 2016;95(4):388-394.
65. Petersson GH, Isberg PE, Twetman S. Caries risk assessment in school children using a reduced Cariogram model without saliva tests. BMC Oral Health 2010;10:5.
66. Philip N, Suneja B, Walsh L. Beyond streptococcus mutans: clinical implications of the evolving dental caries aetiological paradigms and its associated micro biome. Br Dent J; 2018; 224(4):219-225.
67. Plonka KA, Pukallus ML, Barnett AG et al. A longitudinal case-control study of caries development from birth to 36 months. Caries Res 2013;47: 117-127.
68. Plonka KA, Pukallus ML, Barnett AG et al. Mutans streptococci and lactobacilli colonization in predentate children from the neonatal period to seven months of age. Caries Res 2012;46:213-220.
69. Powell V, Leroux BG, Martin JA, White BA. Identification of adult populations at high risk for dental caries using a computerized database and patient records: A pilot project. J Public Health Dent 2000;60(2):82-84.
70. Raja M, Hannan A, Ali K. Association of oral candidal carriage with dental caries in children. Caries Res 2010;44: 272-276.
71. Rogosa M, Mitchell JA, Wiseman R. A selective medium for the isolation and enumeration of oral lactobacilli. J Dent Res 1951;30:682-689.
72. Sampaio AA, Souza SE, Ricomini-Filho AP et al. Candida albicans increases dentine demineralization provoked by streptococcus mutans biofilm. Caries Res 2019;53(3):322-331.
73. Schilke R. Caries risk diagnosis in the light of the extended ecological plaque hypothesis. Quintessenz 2021;12 [in preparation].
74. Simón-Soro A, Belda-Ferre P, Cabrera-Rubio R, Alcaraz LD, Mira A. A tissue-dependent hypothesis of dental caries. Caries Res 2013;47:591-600.
75. Simón-Soro A, Mira A. Solving the etiology of dental caries Trends Microbiol 2015;23(2): 76-82.
76. Simón-Soro Á, Tomás I, Cabrera-Rubio R et al. Microbial geography of the oral cavity. J Dent Res 2013;92:616-621.
77. Subramaniam P, Suresh R. Streptococcus mutans strains in mother-child pairs of children with early childhood caries. J Clin Pediatr Dent 2019;43(4): 252-256.
78. Svensäter G, Larsson UB, Greif EC, Cvitkovitch DG, Hamilton IR. Acid tolerance-Response and survival by oral bacteria. Oral Microbiol Immunol 1997;12(5):266-273.
79. Takahashi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Res 2008;42:409-418.

80. Takahashi N, Nyvad B. The role of bacteria in the caries process: Ecological perspectives. J Dent Res 2011;90:294-303.
81. Tanner ACR, Kressirer CA, Rothmiller S, Johansson I, Chalmers NI. The caries microbiome: Implications for reversing dysbiosis advances in dental research 2018;29(1):78-85.
82. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001;65 (10):1028-1037.
83. Tanzer JM. Dental caries is a transmissible infectious disease: The Keyes and Fitzgerald revolution. J Dental Res 1995; 74:1536-1542.
84. Tenovuo J, Lehtonen OP, Aaltonen AS. Caries developoment in children in relation to the presence of mutans streptococci in dental plaque an of serum antibodies against whoe cells and protein antigen I/II of streptococcus mutans. Caries Res 1990;24(1): 59-64.
85. Thenisch NL, Bachmann LM, Imfeld T et al. Are mutans streptococci detected in preschool children a reliable predictive factor for dental caries risk? A systematic review. Caries Res 2006;40:366-374.
86. Twetman S, Fontana M. Patient caries risk assessment. Monogr Oral Sci 2009;21:91-101.
87. van Houte J, Gibbons RJ, Pulkkinen AJ. Ecology of human oral lactobacilli. Infect Immun 1972;6(5):723-729.
88. Young DA, Nový BB, Zeller GG et al. The American Dental Association Caries Classification System for Clinical Practice: A report of the American Dental Association Council on Scientific Affairs. J Am Dent Assoc 2015;146(2): 79-86.
89. Zijnge V, van Leeuwen MB, Degener JE et al. Oral biofilm architecture on natural teeth. PLoS One 2010; 5e9321.
90. Zimmer S. Therapy without a clean diagnosis cannot lead to a cure. DZW 2019. Internet: https://www.dzw.de/dentistry/prophylaxis?page=1. retrieval: 15.12.2020.



Lutz Laurisch

Dr. med. dent.

E-mail: Lutz@Dr-Laurisch.de

Practice Dres. Laurisch
Arndtstraße 25
41352 Korschenbroich