Evaluation of a new caries risk test

THE AUTHORS EXAMINE A NEW CARIES RISK TEST THAT CAN BE USED FOR THE DETECTION OF BOTH STREPTOCOCCI MUTANS AND LACTOBACILLI

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.

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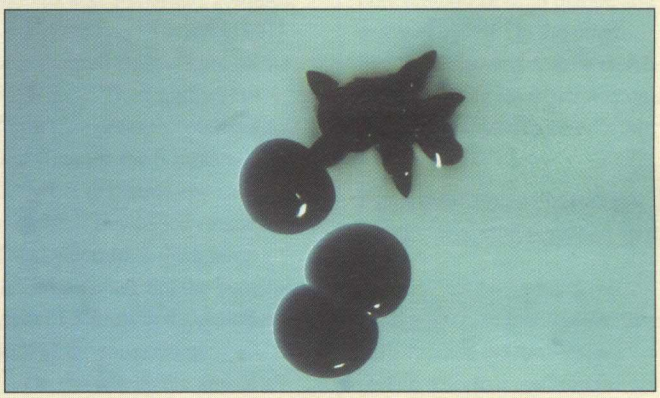


Figure 1: Star-shaped S mutans colony on Mitis-salivarius agar with bacitracin

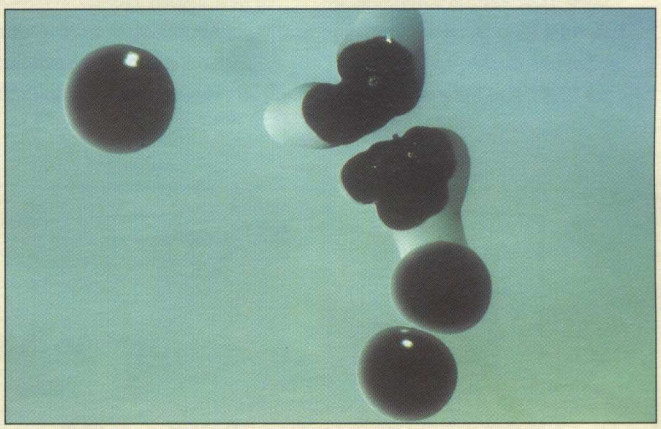


Figure 2: S sobrinus colony with an exudate droplet consisting of extracellular polysaccharides on Mitis-salivarius agar with bacitracin

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 muroloid 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

Oral Streptococci	
Mannitol positive	**Mannitol negative**
Mutans Streptococci	S sanguis S salivarius S gordonii S oralis S milleri
Mutans Streptococci	
S mutans (c, e, f), S sobrinus (d, g) Human	
S ferus (c), S cricetus (a), S rattus (b) (Human), hamster, rat	

al, 1967; Ikeda et al, 1979). Little et al (1977) compared 10 different culture media regarding their bacterial yield of S mutans. They selected reference strains exclusively and were able to prove that TYC-agar is superior to MSB-agar. In the latter, mainly S cricetus of the S mutans group (Table 1) is suppressed by the added bacitracin. However, S cricetus is insignificant as regards its occurrence in the human oral cavity.

Nevertheless, the added bacitracin is significant in so far as it suppresses the concomitant oral flora, particularly S salivarius, and thus facilitates the optical detection of S mutans. Subsequently, Van Palenstein et al (1983) modified the TYC-agar. Analogous to the MSB-agar, they added 20% sucrose and 0.1 units of bacitracin per millilitre and called it trypticase-yeast-extract-cystine-sucrose-bacitracin-agar (TYCSB). S sobrinus colonies again form a cloudy-white halo around the colonies.

On the whole, however, S mutans grow as very distinctive, lens-shaped colonies pressed in the agar. They are difficult to isolate, respectively. For this reason, Schaecken et al (1986) recommended the trypticase-soya-agar and optimised it by adding sucrose and bacitracin (TSY20B) in the same concentrations as in the MSB-agar. TSY20B-agar proved to be of a quality similar to that of TYCSB-agar. However, the former is easier to produce and improves the passability of S mutans and S sobrinus colonies.

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

Kimmel and Tianoff (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

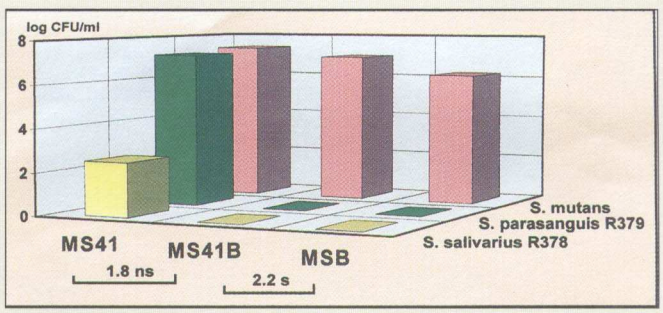


Figure 4: Growth of reference strains on Mitis-salivarius agar with bacitracin and modified sucrose content according to Laurisch (1997) plaque and saliva (log CFU). Bacteria count classes 0 to 3 (from right to left)

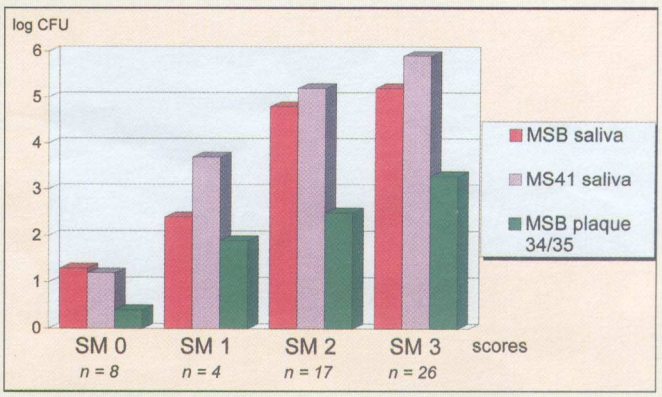


Figure 5: Comparative determination of Lactobacilli using Dentocult LB and CRT

control purposes. Gold et al (1973) examined five plaque samples. Van Palenstein Heldermann et al (1983) used plaque and saliva samples of six patients, while Little et al (1977) only investigated the growth of reference strains.

Only Schaecken et al (1986) were able to express their preference of one agar (TSY20B) over another one (TYC and 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 examination of plaque and saliva samples of 60 children in the Erfurt caries risk study (Kneist et al, 1998c; Kneist et al, 1998a; Kneist et al, 1998b; Stößer et al, 1998). The results were unsatisfactory in so far as not only S sobrinus, but also gram-negative germs in particular (Figure 3), developed glucane halos. Therefore, reliable identification of S sobrinus with TYCSB-agar is not possible. MSB-agar proved to be more reliable.

TABLE 2: FREQUENTLY OCCURRING ORAL LACTOBACILLI

Homofermentative		Heterofermentative
Obligat	**Facultativ**	
L salivarius	L alimentarius	L fermentum
L delbrueckii	L casei	L brevis
ss lactis	L paracasei	L buchneri
ss delbrueckii	ss paracasei	
ss bulgaricus	ss rhamnosus	
L acidophilus	ss tolerans	
L gasseri	L pseudoplantarum	
	L plantarum	
	L rhamnosus	

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 PROGNOSES 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

Caries risk	Bacteria count class
Low	LB 0 and SM 0
Medium	LB > 0 and ≤ 2 and/or SM > 0 and ≤ 1
High	LB ≥ 3 and/or SM ≥ 2; [mixed dentition LB and/or SM ≥ 2 ≥ 10^5/ml saliva

been commercially available. Aaluusua 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, Matsukubuo 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 Bratthall (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 Bratthall (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 (Matsukubuo 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

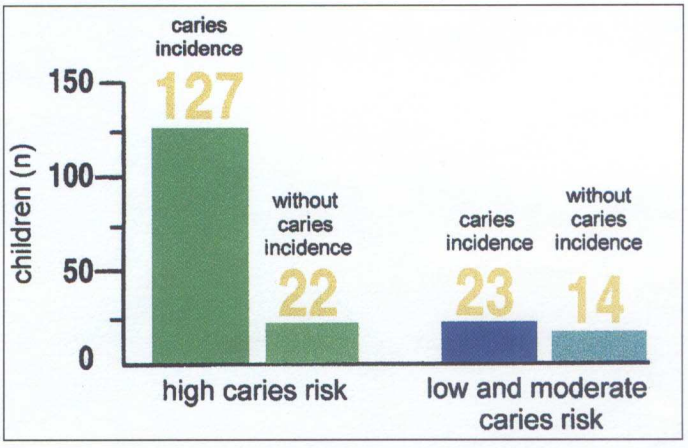
As a counterpart to the S mutans culturing device, Dentocult LB is commercially available for the detection of Lactobacilli (Larmas, 1975). The test consists of a dip-slide covered with Rogosa-agar. In the thirties, Rodriguez (1931) used a selective serum agar for *L acidophilus-odontolyticus*. Until 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 mutans 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). ID

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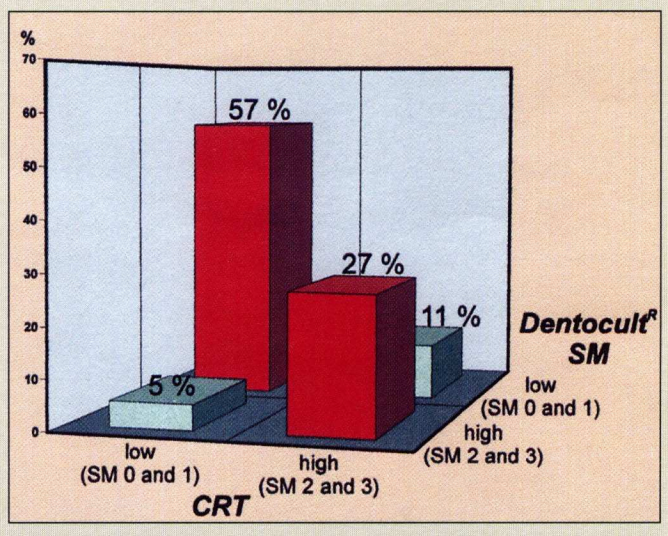

Figure 9: Comparative determination of S mutans using Dentocult SM Strip Mutans and CRT

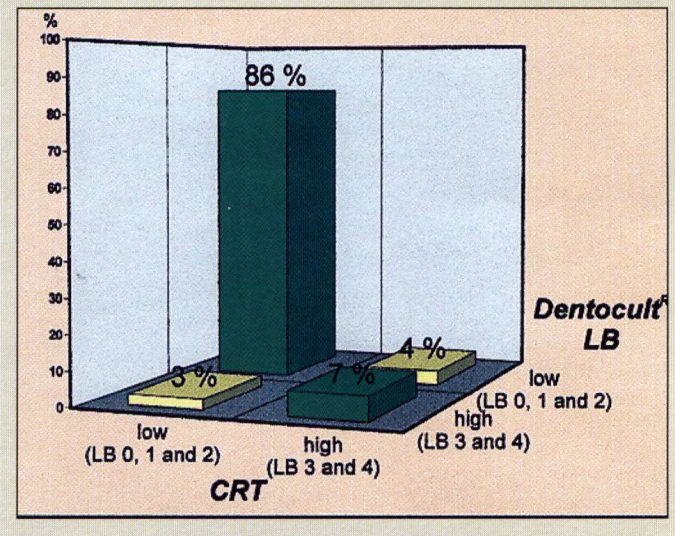

Figure 10: Detection of S mutans by means of Dentocult SM Strip Mutans and CRT. Bacteria count classes according to the bacteria counts in

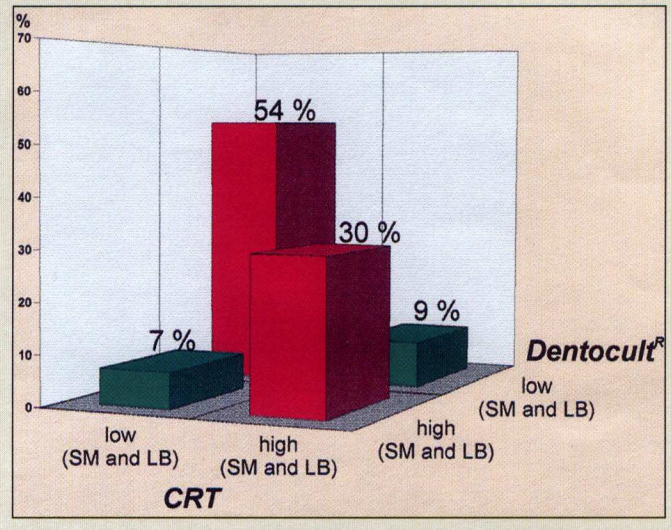

Figure 11: Comparative determination of the caries risk using Dentocult SM Strip Mutans and LB, as well as CRT

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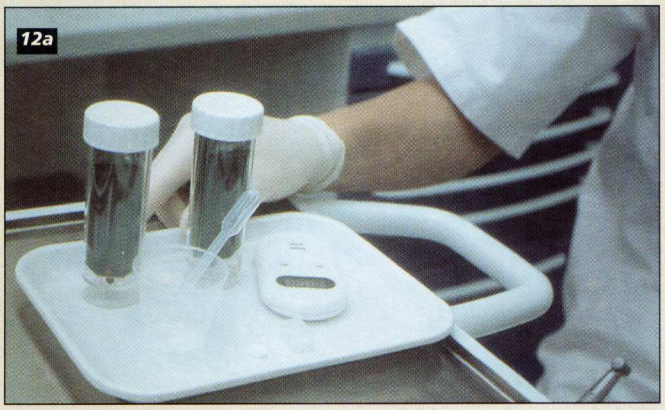

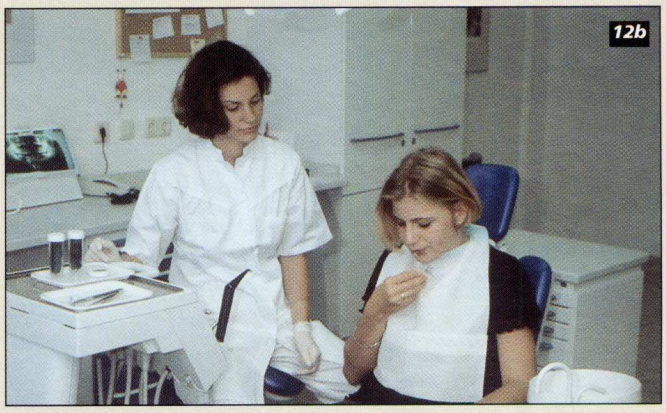

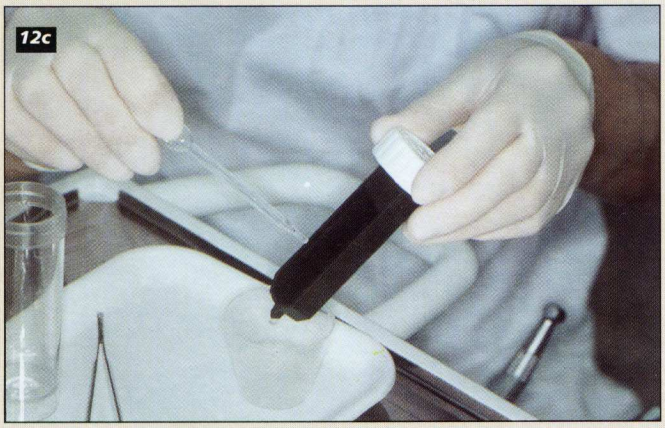

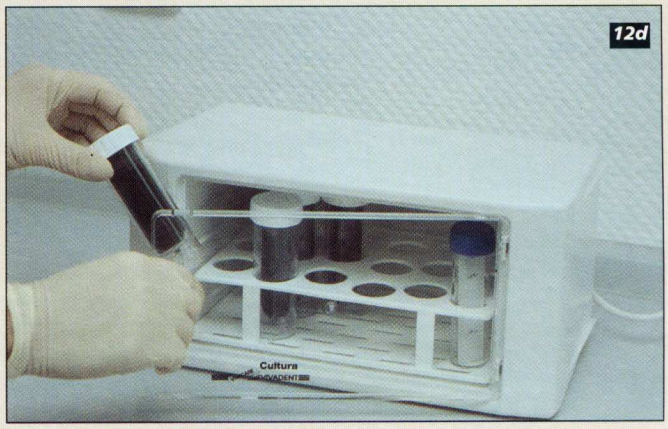

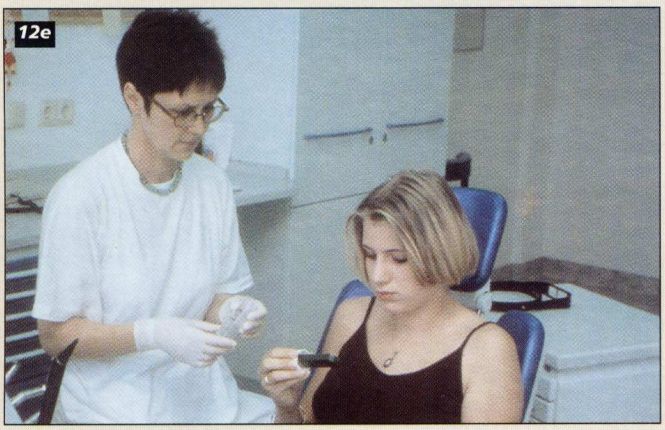

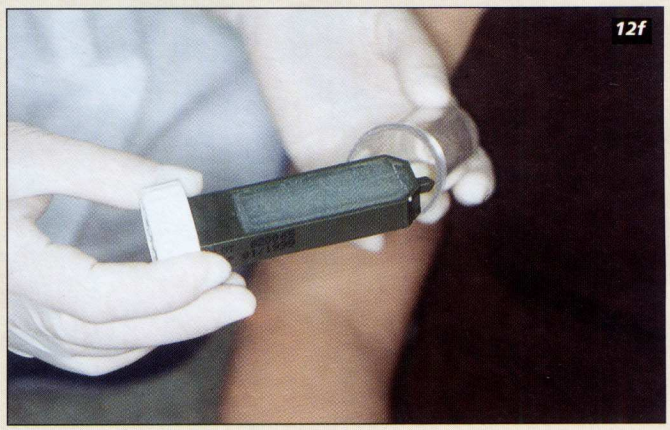

Figure 12a-f: Application of the CRT in the dental practice. From saliva stimulation to evaluation

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