Original Paper

Caries Research

Caries Res 2008;42:297–304
DOI: 10.1159/000148162

Received: November 27, 2007
Accepted after revision: May 28, 2008
Published online: July 29, 2008

Performance of Fluorescence Methods, Radiographic Examination and ICDAS II on Occlusal Surfaces in vitro

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Key Words

Caries detection · DIAGNOdent · DIAGNOdent pen · Fluorescence camera · International Caries Detection and Assessment System · Laser fluorescence · Occlusal caries

Abstract

This study compared the performance of fluorescence-based methods, radiographic examination, and International Caries Detection and Assessment System (ICDAS) II on occlusal surfaces. One hundred and nineteen permanent human molars were assessed twice by 2 experienced dentists using the laser fluorescence (LF and LFpen) and fluorescence camera (FC) devices, ICDAS II and bitewing radiographs (BW). After measuring, the teeth were histologically prepared and assessed for caries extension. The sensitivities for dentine caries detection were 0.86 (FC), 0.78 (LFpen), 0.73 (ICDAS II), 0.51 (LF) and 0.34 (BW). The specificities were 0.97 (BW), 0.89 (LF), 0.65 (ICDAS II), 0.63 (FC) and 0.56 (LFpen). BW presented the highest values of likelihood ratio (LR)+ (12.47) and LR– (0.68). Rank correlations with histology were 0.53 (LF), 0.52 (LFpen), 0.41 (FC), 0.59 (ICDAS II) and 0.57 (BW). The area under the ROC curve varied from 0.72 to 0.83. Inter- and intraexaminer intraclass correlation values were respectively 0.90 and 0.85 (LF), 0.93 and 0.87 (LFpen) and 0.85 and 0.76 (FC). The ICDAS II κ values were 0.51 (interexaminer) and 0.61 (intraexaminer). The BW κ values were 0.50 (interexaminer) and 0.62 (intraexaminer). The Bland and Altman limits of agreement were 46.0 and 38.2 (LF), 55.6 and 40.0 (LFpen) and 1.12 and 0.80 (FC), for intra- and interexaminer reproducibilities. The posttest probability for dentine caries detection was high for BW and LF. In conclusion, LFpen, FC and ICDAS II presented better sensitivity and LF and BW better specificity. ICDAS II combined with BW showed the best performance and is the best combination for detecting caries on occlusal surfaces.

The detection of caries is a key element in the prevention and treatment of lesions, and a difficult task in dentistry [Bader and Shugars, 2004]. Occlusal surfaces are the most caries-affected sites in children and adults because of the special morphology of the pits and fissures and the difficulty of plaque removal. For this reason, the importance of early occlusal caries detection has grown in the last years [Sheehy et al., 2001; Rodrigues et al., in press]. Incipient occlusal lesions have become difficult to detect because of the widespread use of fluorides and their superficial remineralization potential that seems to delay cavitation [Rodrigues et al., in press]. Additionally, the changes in lesion morphology could lead to the presence of occlusal dentine caries under a fissure which seems intact to the naked eye [Lussi et al., 1999]. Visual

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inspection and radiographic examination have been commonly used in clinical practice, but they can detect caries lesions only at an advanced stage [Ricketts et al., 2002].

A new visual method, the International Caries Detection and Assessment System (ICDAS), was devised by an international group of researchers with the goal of designing an internationally accepted caries detection system that would also allow assessment of caries activity [Ekstrand et al., 2007]. In the ICDAS I, devised in 2003, the visual examination was carried out on clean, plaque-free teeth, after careful drying. Later, the criteria were modified and the ICDAS II created. The improvement consisted in an exchange of codes to ensure that the system would reflect increased severity [Ismail et al., 2007; Ekstrand et al., 2007].

Other new methods have been developed and recommended as diagnostic aids to identify and quantify early caries lesions on smooth and occlusal surfaces [Mendes et al., 2006]. Some of these methods are based on the phenomenon that caries lesions fluoresce more strongly than sound tissues when excited by light at specific wavelengths [Hibst et al., 2001; Bader and Sugars, 2004; Braun et al., 2005; Thoms, 2006]. Both the first laser fluorescence device (LF) and a more recent pen-type LF device (LFpen) function on the same principle: they emit red light at 655 nm and measure fluorescence of bacterial metabolites in infected dentine [Hibst et al., 2001; Lussi and Hellwig, 2006; Lussi et al., 2006]. A recently devised fluorescence camera (FC) device emits blue light at 405 nm, and records fluorescence from the teeth as digital images. However, only limited data are available in the literature and the performance of this device, which is already on the market, has not been evaluated.

The aim of this in vitro study was to compare the performance of different fluorescence-based methods, radiographic examination and ICDAS II on occlusal surfaces.

Materials and Methods

Sample Selection

One hundred and nineteen unstained permanent human molars (35 with microcavities and 84 noncavitated, of which 18 were apparently sound) were selected from a pool of extracted teeth, which were stored frozen at –20°C until use. This storage method does not change the red fluorescence significantly [Francescut et al., 2006]. All teeth had been extracted by dental practitioners in Switzerland (no water fluoridation; 250 ppm F in table salt). Prior to extraction, the patients were informed about the use of their teeth for research purposes and their consent was obtained. The teeth were defrosted for 3 h and calculus and debris were removed using a scaler (Cavitron). They were cleaned for 15 s with water and toothbrush (Trisa ultra super-sensitive; BrushAbo, Switzerland) and for 10 s with a water-powder jet cleaner (PROPHYflex II, KaVo, Biberach, Germany) and sodium hydrogen carbonate powder. To remove powder remnants from the fissures, the teeth were rinsed with the 3-in-1 syringe for 10 s [Lussi and Reich, 2005]. During measurements, teeth were stored in 100% humidity. The occlusal surfaces were photographed at ×6.25 magnification and one spot from each tooth was selected in the fissure surface (test site). All assessments were carried out twice by 2 experienced dentists, with a 1-week interval between measurements.

Assessments with LF Devices

Both the LF system (DIAGNOdent 2095) and the new LFpen (DIAGNOdent 2190) were supplied by KaVo, Biberach, Germany.

The test sites were measured using both LF devices. No calibration training was performed and the examiners were informed about the device functioning. Both devices were first calibrated for every tooth using a ceramic standard, in accordance with the manufacturer's instructions. The fluorescence value of a sound part of the cuspal area on the buccal surface (zero value) was then recorded, to be later subtracted from the peak value. For measurements, tip A (for the LF device) and a cylindrical sapphire fibre tip for occlusal surfaces (for the LFpen device) were used. The device was moved around the test site until the highest value was obtained. The peak values were recorded and the zero value of fluorescence was subtracted. For dentine caries level, the concrete cut-off values were 24 for LF and 17 for LFpen [Lussi and Hellwig, 2006].

Assessments with the FC Device

The FC (VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) is a system that has been modified by exchanging the white LEDs of the camera with six blue GaN-LEDs emitting at 405 nm (optical power 60 mW). An optical long pass filter has been introduced into the beam path in front of the CCD sensor to cut down the excitation light below 495 nm. DDview software (Dürr Dental) was used to digitize the video signal to create the images of 720 × 576 pixels with 3 × 8 bit intensities of RGB channels and resolution of 72 pixels/inch [Thoms, 2006]. These images were analyzed with the software, which quantified the red and green components of fluorescence. This software shows the region of the teeth that emits fluorescence varying from green (approximately 510-nm wavelength) to red (approximately 680-nm wavelength) and an outcome value, ranging from 0 to 3 corresponding to the lesion severity and calculated as the intensity ratio of the red and green fluorescence. Caries lesions were identified when the red/green ratio was higher than that of sound tissue. The fluorescence ratio of caries lesions was taken as the maximum red/green ratio recorded. Images of the teeth were taken using a prototype of the FC system, analyzed by the software, stored in the computer and the values recorded for further analysis. However, no scale for interpretation of these numbers is available in the literature since this method was only recently developed and introduced into the market. For detection at the dentine caries level, the cut-off values were determined by the highest sum of sensitivity and specificity.

Visual Examination – ICDAS II

Visual examination was performed following the ICDAS II [Ismail et al., 2007; Ekstrand et al., 2007], with direct visualization of the teeth under illumination and coded as: (0) sound tooth surface, (1) first visual change in dry enamel, (2) distinct visual change in moist enamel, (3) localized enamel breakdown due to caries with no visible dentine or underlying shadow, (4) underlying dark shadow in dentine with or without localized enamel breakdown, (5) distinct cavity with visible dentine and (6) extensive distinct cavity with visible dentine. Score 3 represented the cut-off for dentine lesions. The teeth were examined in the same room with the aid of a light reflector and a 3-in-1 air syringe.

Bitewing Radiographs

Standardized BW were taken of all the teeth using an X-ray machine (HDX Dental EZ, USA) and double Kodak Insight films (22 × 35 mm, Kodak, Rochester, Minn., USA) at 65 kV, 7 mA and exposure time of 0.09 s. An automatic X-ray film developer XR 24 Pro (Dürr Dental) was used to process the films. The radiographs were then examined independently using an X-ray viewer (Imatec Röntgentechnik, Switzerland) and an X-ray film magnifier (magnification ×2; Svenska Dental Instrument, Sweden) in a dark room to determine whether the occlusal surfaces under study showed: no radiolucency (0), radiolucency in enamel (1), radiolucency in the outer half of dentine (2) and radiolucency in the inner half of dentine (3). Score 2 represented the cut-off for dentine lesions.

Validation

After assessment, the teeth were ground longitudinally on a Knuth-Rotor polishing machine using silicon carbide paper (60-μm grain size) cooled under tap water. Progress of grinding was constantly checked under the microscope (magnification ×6.25) and compared to the initial pictures of the test site. When the periphery of the site was reached, papers of grain size 30, 18, 8 and 5 μm were used. The occlusal cut surfaces were photographed to ensure that the caries lesion was not ground away. The tooth surfaces were then colored with saturated rhodamine B (Fluka, Buchs, Switzerland) dissolved in water. Sites were histologically assessed for caries extension according to the rhodamine B penetration (magnification ×10) as: caries free (D_0), caries extending up to halfway through the enamel (D_1), caries extending into the inner half of enamel (D_2), caries in dentine (D_3) and deep dentine caries (D_4). Subsequently, photographs were taken.

Statistical Analyses

For FC, as no interpretation of the scale was available, the cut-off limits were determined by the highest sum of sensitivity and specificity at each threshold. Sensitivity, specificity, accuracy, area under the ROC curve (A_z) and likelihood ratios (LR+ and LR–) for a positive and negative test were calculated (MedCalc for Windows, version 9.3.0.0, Mariakerke, Belgium) at D_3 threshold for all methods. For the LF devices, the average among the four separate measurements was calculated. The cut-off limits described by Lussi and Hellwig [2006] were used to obtain the sensitivity and specificity. The McNemar test was used to compare the sensitivity, specificity and accuracy among the methods. Cross-tabulation and rank correlation (Spearman's coefficient) with histology were provided.

Using the separate LR+ values for each method, the posttest probability for combinations of the methods was calculated [Lussi et al., 1995] to assess the relative value of using the different methods separately and in combination. At threshold D_3, the pretest odds were 1.02 and the prevalence of disease in the sample was 46%.

A nonparametric statistical test was used to assess the difference among the A_z [Hanley and McNeil, 1983]. The significance level was set at $p < 0.05$.

Intraclass correlation (ICC) and Cohen's unweighted κ values were used to assess inter- and intraexaminer reproducibility [Lin, 1989]. The ICC was used for LF, LFpen and FC since they showed discrete values. The unweighted κ was calculated for all of them, including ICDAS II and BW. For LF, LFpen and FC, the Bland and Altman method was applied to identify systematic differences and the 95% limits of agreement were calculated [Fleiss, 1981; Bland and Altman, 1986].

Results

Histological examination revealed that of the 119 occlusal test sites, 8 were caries free (D_0), 19 had caries extending up to halfway through the enamel (D_1), 37 had caries extending into the inner half of enamel (D_2), 35 had caries in dentine (D_3) and 20 had deep dentine caries (D_4). Cross-tabulation for LF, LFpen, ICDAS II and BW with the corresponding histology were given in the tables 1 and 2.

The optimal cut-off limits for FC device determined by the point at which the sum of sensitivity and specificity was maximal are shown in table 1. Specificity, sensitivity, accuracy, A_z, LR+ and LR– are shown in table 3 (threshold D_3). The highest sensitivities were observed for FC (0.86), LFpen (0.78) and ICDAS II (0.73), with no statistically significant difference among them. However, BW and LF showed highest specificities (0.97 and 0.89, respectively). Rank correlations (Spearman's coefficient) with histology were 0.53 (LF), 0.52 (LFpen), 0.41 (FC), 0.59 (ICDAS II) and 0.57 (BW).

Table 4 gives an overview of the probabilities of correct detection of dentine caries when the methods are used independently or in combination. When ICDAS II was combined with BW at threshold D_3 the posttest probability was 95.7%. The combination of ICDAS II, BW and any third method did not increase the posttest probability significantly.

Reproducibilities are represented in table 5. The mean differences as well as the limits of agreement (mean ± 1.96 SD) for both inter- and intraexaminer reproducibility for the LF, LFpen and FC can be observed in the Bland-Altman plots (fig. 1). The range between the upper and the lower limits of agreement was 46.0 and 38.2 for LF, 55.6 and 40.0 for LFpen and 1.12 and 0.80 for FC, for both intra- and interexaminer reproducibilities.

Table 1. Cross-tabulation for LF, LFpen and FC devices with the corresponding histology

Histological score	LF cut-off values				LFpen cut-off values				FC cut-off values				Total
	0–7	7.1–14	14.1–24	>24[1]	0–6	6.1–13	13.1–17	>17[1]	0–1.262	1.263–1.299	1.300–1.319	>1.319[1]	
0	8				7	1			7	1			8
1	7	5	3	4	5	4	1	9	11	1		7	19
2	13	11	10	3	6	10	2	19	17	1	2	17	37
3	4	8	10	13	1	6	3	25	5			30	35
4	1	1	3	15		1	1	18	2		1	17	20
Total	33	25	26	35	19	22	7	71	42	3	3	71	119

[1] Reference for the calculation of sensitivity, specificity, accuracy and LR+ at D_3 threshold.

Table 2. Cross-tabulation for ICDAS II system and BW with the corresponding histology

Histological score	ICDAS II score							BW score				Total
	0	1	2	3[1]	4	5	6	0	1	2[1]	3	
0	8							8				8
1	3	6	7	3				19				19
2	1	2	13	21				35	2			37
3	2		6	24		3		27	3	4	1	35
4		5		9	3	3		5	1	11	3	20
Total	14	13	26	57	3	6		94	6	15	4	119

[1] Reference for the calculation of sensitivity, specificity, accuracy and LR+ at D_3 threshold.

Table 3. Specificity, sensitivity, accuracy, area under the ROC curve (A_z), LR+ and LR– of different methods at D_3 threshold

	Specificity	Sensitivity	Accuracy	A_z	LR^+	LR^-
Method	D_3	D_3	D_3	D_3	D_3	D_3
LF	0.89[a]	0.51[a]	0.74[a]	0.809[a]	4.65	0.55
LFpen	0.56[b]	0.78[b]	0.64[b]	0.794[a, b]	1.79	0.39
FC	0.63[b]	0.86[b]	0.72[a]	0.752[a, b]	2.28	0.23
ICDAS II	0.65[b]	0.73[b]	0.68[a]	0.753[a, b]	2.11	0.41
BW	0.97[c]	0.34[c]	0.63[b]	0.715[b]	12.47	0.68

D_3: D_0–D_2 = sound; $D_{3,4}$ = decayed. Within columns, significant differences are represented by different superscript letters (McNemar test, $\alpha = 0.05$).

Table 4. Probability of different methods and their combination for the detection of caries at D_3 threshold

Method	D_3	
	Posttest odds	Posttest probability, %
LF	3.95	79.8
LFpen	1.52	60.3
FC	1.93	65.9
ICDAS II	1.79	64.1
BW	10.60	91.4
ICDAS II +		
LF	8.34	89.3
LFpen	3.21	76.2
FC	4.09	80.4
BW	22.36	95.7
ICDAS II + BW +		
LF	103.97	99.0
LFpen	40.02	97.5
FC	50.98	99.0

Table 5. Unweighted κ values and ICC for inter- and intraexaminer reproducibility of different methods

Method	Interexaminer		Intraexaminer	
	κ	ICC	κ	ICC
LF	0.58	0.90	0.60	0.85
LFpen	0.55	0.93	0.54	0.87
FC	0.58	0.85	0.61	0.76
ICDAS II	0.51	–	0.61	–
BW	0.50	–	0.62	–

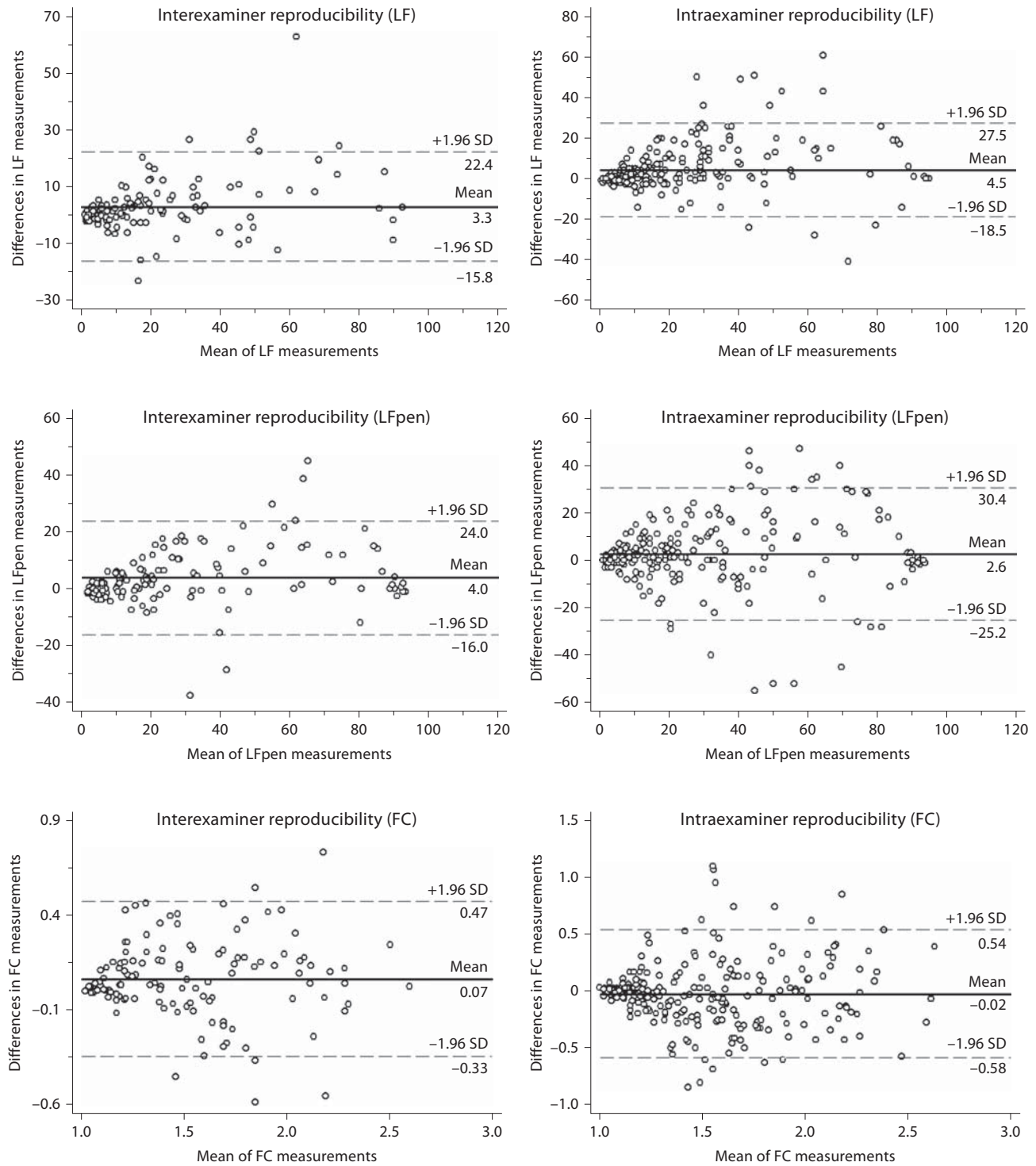

Fig. 1. Bland-Altman plots for intra- and interexaminer reproducibility of LF, LFpen and FC.

Discussion

Within the limitations of an in vitro study, each method showed different sensitivities and specificities. In agreement with our study, Attrill and Ashley [2001] observed lower sensitivity of radiographic examination for both enamel and dentine and found it a worse method for occlusal caries detection than LF or visual examination. Burin et al. [2005] reported that visual inspection is as valid an evaluation method as LF, which should be considered a better adjunct for occlusal caries detection than radiographic examination. Only BW showed a significantly lower value of accuracy than the other methods, which is in agreement with Ricketts et al. [2002].

Of the laser fluorescence devices, LF showed higher specificity and lower sensitivity and LFpen lower specificity and practically the same sensitivity as was found by Lussi and Hellwig [2006]. In a recent in vivo comparison of LF, visual and radiographic examination, it was concluded that LF may be a useful supplement to visual examination, and its diagnostic performance seems to be good for occlusal caries detection [Toraman Alkurt et al., 2008]. Other in vivo investigations demonstrated that LF provided good ability to detect dentine caries lesions [Diniz et al., in press]. The difference between the sensitivity and specificity of LF and LFpen could be due to the difference between both cut-off limits, since the fluorescence values obtained using both devices are also not similar.

FC showed a good performance for detecting dentine caries in terms of sensitivity. This method could represent a useful tool to aid the diagnostic process like the other methods tested. Its performance has been assessed and the fluorescence spectrum at the white spot lesions has shown two dominant emission peaks at 640 and 700 nm. Therefore, the role of fluorophores compatible with porphyrins on the fluorescence spectrum can be assumed [Thoms, 2006]. Owing to the lack of a scale for the interpretation of FC values, we calculated optimal cut-off limits for the various thresholds from the maximum sum of sensitivity and specificity as a function of FC values. The prototype used showed cut-off values so close to each other that its use in the clinical practice would be difficult, if not impossible. Caution must be used when these in vitro cut-offs are used for clinical assessment, although in this study no change in the fluorescence values due to storage should be expected [Francescut et al., 2006]. In spite of the high sensitivity obtained for the FC device, the Spearman's coefficient showed a weak correlation with the histology, which could be also observed in the cross-tabulation. The difficulty of this device in detecting enamel caries lesions could be observed. The FC device also presented the lowest value of LR–, which shows how much the odds of the disease decrease when a test is negative, at the D_3 threshold. It is hoped that the FC device could be improved in order to provide more widely spaced values, since this device should not be a dichotomous instrument.

A similar device – quantitative laser-induced fluorescence (QLF) – almost equal in design has the same excitation wavelength (peak at 405 nm) and a closely similar cut-off filter (520 nm). The red fluorescence is detected and stored in the same way for both devices. The QLF device has been extensively tested and is reported to be a valuable tool for early detection, quantification and monitoring of noncavitated caries lesions by measuring the reduction in autofluorescence associated with mineral loss [Kühnisch and Heinrich-Weltzien, 2004]; these studies did not include the red fluorescence methodology of QLF.

The same difficulty in detecting enamel lesions was observed when the BW was assessed. However, the ICDAS II showed the best correlation with the histology, and combining the cross-tabulation results this method could be suggested as the best to detect changes in enamel.

The A_z confirmed the good performance of the methods in detecting either the presence or the absence of occlusal caries lesions. The advantages of the ROC curve are: (a) it includes several cut-off points; (b) it shows the relationship between the sensitivity and specificity, and (c) it is not affected by the prevalence of disease [Obuchowski, 2003]. Burin et al. [2005] did not find statistical difference in A_z between LF, visual and radiographic examination. In our study, the LF presented the highest A_z value when compared to the other methods. However, this value was statistically significant different only from the BW A_z value.

The ICC values obtained for both intra- and interexaminer reproducibilities agreed with those found by Kühnisch et al. [2007a], who observed high values for both LF and LFpen. Lussi and Hellwig [2006] also observed values of ICC (>0.98) for both LF devices and κ varying from 0.83 (LF) to 0.89 (LFpen) for intraexaminer reproducibility, which is in agreement with this study. High ICC was also observed by Alwas-Danowska et al. [2002] and Rodrigues et al. [in press] for LF. The good reproducibility means that both devices can be used for monitoring the caries process [Lussi and Hellwig, 2006].

The Cohen's κ values for ICDAS II found in this study were lower than those found by Ekstrand et al. [2007] for

both intra- and interexaminer reproducibility. It should be considered that these results involve subjective aspects such as background knowledge and individual clinical experience of the examiners [Fung et al., 2004], which could explain the difference between the studies.

In Bland and Altman plots, there should ideally be no systematic deviation (mean difference = 0) and only a small range between the upper and the lower limits of agreement. The line shown in the plot for LF and LFpen (with deviation from 2.6 to 4.5 from the zero line) indicates the mean of the differences between two measurements. These values would only be zero in an ideal situation where no differences between the measurements were observed. Kühnisch et al. [2007a] suggested that the range should not exceed ±20 LF units. Although the present study showed ranges of 38.2 for LF and 40.0 for LFpen for interexaminer reproducibility, we could not say that these limits of agreement could be considered good and the reproducibility of LFpen seems to be lower. In the Bland-Altman plot for LFpen intraexaminer agreement, a diamond-shaped array of points was observed. This phenomenon reflects a device output limitation effect [Huysmans et al., 2005]. The shape is defined by concentrations of points close to 99 and 0 representing perfect agreement of extreme measurements, and clusters of points at the top and bottom corners of the diamond representing disagreement of extreme measurements [Huysmans et al., 2005]. This result implies that the operation of the LFpen warrants further investigation. The ideal situation would be for all the paired values to be close to each other and at the same time close to the zero line. It could also be observed for both LF devices that the pairwise differences were smallest for the lowest values of fluorescence. For FC, considering that the highest mean value obtained using this device was 2.6, the interval in which the measurements were more reliable was between 1 and 1.5.

As the main principle of the FC device is based on an intraoral camera function, the possibility of capturing images of the fluorescing carious teeth or with plaque and showing them to the patient make the device useful in the clinical practice. Additionally, the facility of picture storage is another advantage of this method, due to the possibility to follow the caries lesion's progress or arrest.

It is important to point out that both LF devices present some limitations. A high value of fluorescence may result from other sources than caries, such as the presence of stains, disturbed tooth development or mineralization [Sheehy et al., 2001; Souza-Zaroni et al., 2006]. Such alterations could lead to some bias, increasing the sensitivity as a false-positive result. Nevertheless, in the present study, teeth with such alterations were not included in the sample. At the same time, the exclusion of stained teeth could have made the performance of the fluorescence-based methods appear better than it would if a random sample had been used.

As described earlier, the LR+ can be used to calculate the posttest odds of a test, which then become the pretest odds for a second independent test with a known LR+, resulting in the posttest probability of their combination [Lussi et al., 1995]. The tests of combinations of two or three methods using LR+ confirmed the important role of conventional methods, such as visual and radiographic examination, when combined with adjunct methods, for detection of dentine caries. Such combinations seem to improve the process of pit-and-fissure caries detection.

Each method has different characteristics and specific modes of functioning, and thus varies in sensitivity and specificity. In the present study, some methods presented better sensitivity (LFpen, FC and ICDAS II) and others better specificity (LF and BW). However, the posttest probability for dentine caries detection was high for LF and BW. Therefore, a combination of methods would be the best choice in order to detect caries on occlusal surfaces, as also suggested by some authors [Shi et al., 2000; Ricketts et al., 2002; Souza-Zaroni et al., 2006; Rodrigues et al., in press]. The ICDAS II combined with BW showed the best posttest probability and appears to be the best combination.

Acknowledgment

The authors wish to thank the Federal Commission for Scholarships for Foreign Students in Switzerland for financial support.

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