



Occlusal Caries Detection: A Comparison of a Laser Fluorescence System and Conventional Methods

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Abstract

Purpose: The purpose of this study was to compare the effectiveness of a laser fluorescence (LF) device (DIAGNOdent) with a visual and radiographic scoring system for in vitro detection of occlusal caries and to evaluate the effect of 1% NaOCl immersion on the LF readings.

Methods: A total of 54 extracted third molars with macroscopically intact occlusal surfaces were selected. Three examiners assessed 105 sites by visual inspection (VI), bitewing radiography (BWR), and LF. Ten days after the first measurement, all teeth were re-evaluated using the same methods. Then, teeth were immersed in 1% NaOCl solution for 24 hours, and LF readings were taken again. Caries extension was assessed by histology ($\times 40$). The diagnosis methods were compared by means of sensitivity, specificity, intra- and interexaminer reproducibility, and area under receiver operating characteristic (ROC) curve. The mean values of the first, second, and third LF readings from the 3 examiners were compared by one-way analysis of variance and Student-Newman-Keuls test.

Results: No difference was found among the areas under ROC curve. Regarding sensitivity, however, both VI and LF performed similarly ($P > .05$) and superiorly to BWR ($P < .05$). All methods showed a similar performance regarding specificity. The intra- and interexaminer reproducibility was good for all methods. A significant reduction of the LF reading was found after immersion in 1% of NaOCl.

Conclusions: It was concluded that visual inspection is as valid an evaluation method as the laser fluorescence device, which should be considered a better adjunct for occlusal caries diagnosis than BWR. (Pediatr Dent 2005;27:307-312)

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Emphasis has been placed on the diagnosis of occlusal caries in the last few years.¹ The effect of fluoride on superficial occlusal enamel is blamed for late cavitation, allowing extensive dentin demineralization to occur undetected.¹

This different pattern of occlusal caries lesion development has led researchers to correlate macroscopic changes on occlusal surfaces with the histological penetration depth of the lesion. Ekstrand et al^{2,3} successfully demonstrated in a laboratory setting a good correlation between occlusal

signs and the lesion's histological depth. This implies that the results of other workers' visual examinations,⁴ in terms of sensitivity, could be considered a failure in selecting appropriate visual criteria. These visual criteria used the following clinical signs:

1. white or brownish spots visible with and without air-drying;
2. grayish or opaque discoloration;
3. microcavities with progressing stages of demineralization process.

Although promised results have been found, the Ekstrand criteria³ have not been extensively evaluated in other countries where the difference in the caries progress may affect the macroscopic occlusal signs of caries lesions.

Germane to these valuable visual criteria, new noninvasive, instrument-based techniques for detection and quantification

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of demineralization have also been developed and investigated.^{3,5,6,7} One recent introduction is a laser-based fluorescence instrument (LF), KaVo DIAGNOdent (DIAGNOdent KaVo, Biberach, Germany). This system illuminates the tooth surface with pulses of red laser light and analyzes the emitted fluorescence. As the carious lesion progresses, an increase in emitted fluorescent light occurs and a numerical value is assigned to the degree of fluorescence, which may be an indicator of the extent of caries lesions.

The mechanism underlying the enhanced fluorescence in the presence of caries has not been clarified. Hibst et al⁸ proposed that it resulted from the integration of bacterial metabolites and the organic content of lesions rather than crystalline disintegration. This, in part, may explain why the presence of plaque, calculus, and different levels of fissure discoloration on occlusal surfaces may produce fluorescence that, when measured with LF, lead to false-positive answers.^{6,9,10} It is not known whether the removal of exogenous stain by proteolysis might affect the reliability of this new laser-based device.

Although LF's performance has been evaluated in several studies and excellent reproducibility and accuracy for occlusal caries detection has been reported,^{6,7,10,11,12,13} this system should be compared with conventional low-cost methods for caries diagnosis, such as the visual criteria proposed by Ekstrand et al.³ Notwithstanding the promising reports on LF's performance, it is highly important to consider the tradeoffs before investing in such a device.

Therefore, this study's aim was to compare *in vitro* the diagnostic ability of laser fluorescence with Ekstrand's visual and radiographic criteria.³ In addition, this study aimed to evaluate LF's measurements after deproteinization with 1% sodium hypochlorite.

Methods

Sample selection and preparation of radiographs

Fifty-four extracted human maxillary and mandibular third molars and premolars, extracted for orthodontic indications and stored for up to 6 months in physiological saline solution, were selected. Inclusion criteria for teeth in this study were:

1. the apparent absence of occlusal restorations and fissure sealants;
2. absence of hypoplastic pits;
3. frank occlusal cavitation.

This study was reviewed and approved by the Ethics Committee of the School of Dentistry, Universidade do Oeste de Santa Catarina (UNOESC), Joaçaba, Brazil.

All teeth were mounted on clear acrylic rods with acrylic resin and properly identified. The occlusal surfaces were cleaned, polished with pumice slurry and toothbrush, and copiously rinsed with water. Then digital photos from the occlusal surfaces were taken, and all sites were identified.

A total of 105 predefined sites in the fissures (generally at the mesial, central, and/or distal fossae) were identified for examination. Bitewing radiographs (BWR) were taken

of each tooth (Ektaspeed, Kodak Ltd, Herts, UK) from the buccal aspect. The teeth were placed perpendicular to the x-ray beam, and the film was parallel to the tooth. The x-ray machine was set at 70 KVP and 7 mA, with a focal point to film distance of 20 cm and exposure time of 0.4 seconds. Radiographs were developed consecutively using an automatic processor. Drawings were made to identify the sites to be analyzed by each observer.

Three examiners participated in this study. One of them trained the other 2 on diagnostic procedures using:

1. 2 representative teeth for each visual scoring system;
2. 2 representative radiographs for each radiographic scoring system, according to Ekstrand's scoring system,³ as described in Table 1.

The examiners were also trained on how to use the LF device, according to the manufacturer's directions.

Examination methods

Teeth were examined by the 3 examiners using visual, radiographic, and LF methods as follows:

1. Visual examination: After removing each tooth one by one from distilled water, the sites were examined under a standard dental operating light at an eye-tooth distance of 20 cm. If no visible signs were seen on the wet occlusal surface, the examiners were allowed to dry the teeth with compressed air. To provide a score for each site (Table 1), the examiners looked for:
 - a. white or brownish discoloration;
 - b. grayish discoloration;
 - c. enamel microcavities in both wet or air-dried surfaces.
2. Radiographic readings: To carry out the radiographic examination, the films were inserted on a viewing box and a black card was placed around each film to cut out extraneous light. An individual drawing was used to locate the precise investigation site on the radiograph in a mesial-distal plane. The evaluation occurred in a darkened room at $\times 2$ magnification.
3. DIAGNOdent readings: The LF device's measurements were made after calibration of the device with the ceramic standard. The assessment of the teeth with the LF's fiber tip was performed according to the manufacturer's instructions. The laser tip (A tip) was positioned on a sound enamel region to provide a baseline measurement before the examination of the target site. The laser tip was positioned on the target site and rotated around its long axis and, to pick up the area where the caries process was most advanced, the highest value was recorded.

Reproducibility

To verify intraexaminer reproducibility, the examiners reperformed all examinations after a period of 7 to 10 days. This time was thought to be adequate to ensure that the observers could not recall specific previous results and findings for any given tooth.

Table 1. Criteria Used for Visual, Radiographic, and Histological Examination³

Score	Visual	Radiographic	Histological
0	No or slight change in enamel after prolonged air-drying (10 s)	No radiolucency visible	No enamel demineralization or a narrow surface zone of opacity (edge phenomenon)
1	Opacity or discoloration hardly visible on the wet enamel, but distinctly visible after air-drying	Radiolucency visible in enamel	Enamel demineralization limited to the outer half of the enamel layer
2	Opacity or discoloration in enamel distinctly visible without air-drying	Radiolucency visible in dentin, but restricted to the outer third of dentin	Demineralization involving between 50% of the enamel and one third of dentin
3	Localized enamel breakdown in opaque or discolored enamel and/or grayish discoloration from the underlying dentin	Radiolucency extending to the middle third of dentin	Demineralization involving the middle third of dentin
4	Cavitation in opaque or discolored enamel exposing dentin	Radiolucency in the pulpal third of dentin	Demineralization involving the inner third of dentin

Second phase of the study

Following this first part of the experiment, all teeth were immersed in a 1% hypochlorite solution in a dark environment (to avoid solution degradation). After 24 hours, the teeth were extensively rinsed with running tap water for 1 hour and the 3 examiners repeated the LF measurements.

Histological validation

The sites were hemi-sectioned in a buccal to lingual direction using a 0.1-mm-thick diamond saw mounted in a microtome (Labcut 1010, Extec Co, Enfield, Conn). A single, calibrated, blinded examiner evaluated each site's 2 sections under a stereomicroscope with $\times 25$ magnification and reflected light (SZPT Olympus, Tokyo, Japan). The side with more extensive alterations was re-examined under $\times 40$ magnification and classified according to the adopted criteria (Table 1).

The depth of enamel demineralization was assessed on wet sections at the area showing the greatest extension of opacity along the direction of the rods. The depth of dentin demineralization was measured at the area where the color changed from brownish/yellowish to gray discoloration along a line at a right angle to the enamel-dentin junction (EDJ) toward the pulp. The examiner was not aware of previous diagnostic results. As a measure of histological assessment reliability, 25% of the sections were re-examined after 20 days.

Statistical analysis

Reproducibility of the visual and radiographic ranked scoring system was assessed using unweighted kappa statistics. This was performed for repeated readings carried out by each examiner (intraexaminer reproducibility) and for the second series of scores made by pairs of examiners (interexaminer reproducibility). This method was also employed to measure the reliability of histological assessment (intraexaminer reproducibility). Kappa values of 0.4

or less denote marginal reproducibility. Values from 0.4 to 0.75 denote good reproducibility, and values above 0.75 denote excellent agreement.¹⁴ The same procedure was performed for LF values after measurement categorization.

Sensitivity and specificity were calculated using the threshold between 2 and 3 in the visual and radiographic inspection (Table 1). The McNemar test was applied to compare the performance of the diagnostic methods for each examiner. Receiver operating characteristic (ROC) analysis was also performed to compare the diagnostic performance of the 3 methods for occlusal caries diagnosis. In addition, a nonparametric statistical test was applied to estimate the significance of areas under ROC curves.¹⁵

For each examiner, a mean of the LF net values from all sites was made for the first and second measurements (taken before hypochlorite immersion), as well as for the third measurement (taken after NaOCl immersion). These means ($N=3$, for each condition) were compared by one-way analysis of variance (ANOVA) and Student-Newman-Keuls test with a level of confidence set at 95%.

Results

Histological examination showed that: (1) 30 sites=score 0; (2) 16 sites=score 1; (3) 41 sites=score 2; (4) 13 sites=score 3; and (5) 5 sites=score 4. Hence, 18 out of 105 sites were classified as "caries," which represents approximately 17% of the sample.

The continuous scale that correlates the lesion extension and the range of the LF values was obtained by performing 3 ROC analyzes. This analysis was performed after dichotomization of the histological scores into 3 cutoffs: (1) H1 (histological grades=0 and 1); (2) H2 (histological grade=2); and H3 (histological grades 3 and 4). The corresponding cutoff readings observed were: (1) $H1 < 11$; (2) $H2 = 12$ to 16; and (3) $H3 > 16$. The LF threshold was set between H2 and H3.

Table 2 gives unweighted kappa values for intra- and interexaminer reproducibility for each ranked scale. In the 3 methods, all examiners showed good to excellent examiner reproducibility. Regarding the interexaminers' repeatability, kappa statistics showed good reproducibility for all methods. An excellent reliability of the histological assessment was achieved. A kappa value of 0.89 showed excellent agreement between the histological sections' 2 sets or readings.

Sensitivity, specificity, and area under ROC curve (Az) are shown in Table 3. For all examiners, the highest sensitivities were found for visual examination and DIAGNOdent, which were statistically similar ($P>.05$) as detected by the McNemar test 310. The radiographic method was the least reliable in this respect ($P<.05$). The specificity values were similar for all 3 methods ($P>0.05$). Nevertheless, the area under the ROC curves did not show significant differences concerning the diagnostic systems.

Figure 1 shows the means of the 3 LF measurements. One-way ANOVA showed that the factor analyzed was statistically significant ($P=.004$). The Student-Newman-Keuls test, applied for pair-wise comparisons, showed that only the third measurement (after NaOCl immersion) was statistically different ($P<.05$) from the other 2 mean readings.

Discussion

Based on the sensitivity and specificity values, the present study confirmed the reliability of Ekstrand's visual ranked scoring system, designed for clinical use, during occlusal caries assessment.³ Although the sensitivity and specificity were not as high in a Brazilian sample as those published by Ekstrand et al,^{2,3} they were within the range of the highest published values for visual inspection.⁴ This is in agreement with other studies that have evaluated this vi-

sual scoring system.¹⁶ As long as an appropriate criterion is selected for occlusal caries diagnosis, the visual inspection performance can be similar to that provided by the LF device, with the advantage of the visual inspection being inexpensive for dental practitioners.

It is worth mentioning, however, that some studies^{13, 16, 17} that have evaluated Ekstrand's visual system³ did not reach similar findings. Although no scientific evidence was found to explain this apparent controversy, one could speculate that lack of experience in using this visual scoring system could be the reason.

Higher values of specificity than the ones reported in this investigation were expected to be obtained with the visual inspection. Therefore, all histological sections of false-negative results were re-examined in the present investigation. Interestingly, the great majority was visually classified as score 2 (sound). In 60% to 71% of the cases (depending on the examiner), however, these lesions were not confined to enamel, but extended into the outer third of the dentin. Unfortunately, no distinguishable visual difference on the occlusal surface was detected between the specimens, with histological demineralization restricted to enamel and those lesions that extended into the outer third of the dentin. Had the authors subclassified score 2 into 2a (lesion restricted to enamel) and 2b (lesion into the outer third of dentin) and set the threshold between 2a and 2b, the specificities would be higher (92.4%, 82%, and 94% for examiners 1, 2, and 3, respectively), with little reduction on the sensitivities values (69.8%, 82.1%, 68.1%, respectively for each examiner). This aspect, however, deserves further investigation.

The LF device performed well on the occlusal sites of teeth with macroscopically intact surfaces. This finding, however, is not universal. Although some researchers have

Table 2. Unweighted Kappa Values for Intra- and Interexaminer Reproducibility for Ranked Scoring Systems

Diagnostic methods	Intraexaminer reproducibility			Interexaminer reproducibility		
	Examiner 1	Examiner 2	Examiner 3	Examiners 1-2	Examiners 1-3	Examiners 2-3
Visual	0.77	0.79	0.67	0.74	0.54	0.55
Radiographic	0.75	0.95	0.85	0.74	0.43	0.41
DIAGNOdent	0.63	0.58	0.68	0.66	0.62	0.55

Table 3. Performance of the Diagnostic Methods in Diagnosing Occlusal Caries Lesions Using Threshold Between 2 and 3 in Each Scoring System: Sensitivity, Specificity, and Area Under Receiver Operating Characteristic Curve (Az)*

Examiner	Sensitivity (%)			Specificity (%)			Az		
	1	2	3	1	2	3	1	2	3
Visual examination	77a	83.3c	72.2e	80.5g	76.7h	81.6i	84j	78l	82m
Radiographic examination	27.8b	27.8d	27.8f	94.3g	95.4h	93.1i	76j	75l	75m
DIAGNOdent	72.2a	77.8c	77.8e	67.1g	70.9h	71.3i	71j	67l	72m

reported good to excellent performance of the LF device,^{6,18,19,20} others have not observed this trend.^{13,16} Several factors may account for this difference. There is a great variation among the recommended cutoffs for interpretation of the LF measurements.

Dental practitioners who use the LF device in their offices are presented with different clinical guidelines from the manufacturer (KaVo Clinical Guidelines) and from in vitro and in vivo studies,^{6,7,16,18} which confuses dentists in daily practice. Recently, an in vitro study demonstrated that the LF performance seemed to be influenced by the cutoff limits chosen to detect dentin caries.²¹ This means that a rather small difference on the LF scale can be a shift from nonoperative to operative intervention. This is an even worse situation, considering that—regardless of LF's recommended cutoff limits—in any scale, LF's results are strongly skewed towards the lower end of the scale. The difference between 2 readings is less pronounced in shallow lesions than in deeper lesions.

This study attempted to calculate post hoc diagnostic thresholds. This approach maximizes LF's performance measures. It gives further evidence, however, on the best thresholds that must be used with this device. A recent systematic review of this LF device's ability to detect caries has shown a great variation among the thresholds used for detection of occlusal dentinal caries in permanent and primary teeth.²² Among 16 in vitro studies, only 6 reported dentinal caries with values over 12 in the LF scale. The other studies reported dentinal caries with values above 15 to 17, demonstrating that the present study is in agreement with the great majority of LF studies.²²

Another source of variation among LF in vitro studies is the storage medium of the sample, which can influence LF readings. In one study, an increase in LF readings was observed after storage of the teeth in 10% neutral-buffered formalin.²³ A significant decrease in LF readings was observed after chemical irrigation with NaOCl and H₂O₂.²⁴ Confirming the aforementioned findings, the present investigation, as well as another in vitro study, detected a reduction of approximately 53% of the mean LF measurements after immersion in NaOCl.²⁵

In addition to the aforementioned findings, further evidence that LF conceivably reflects changes in mineral content and does not adequately measure small changes in the mineral content^{8,26} includes:

1. the lack of alteration of LF measurements after acid etching²⁴;
2. the device's inability to detect in vitro remineralization²⁶;
3. the poor correlation of LF readings and mineral loss.²³

It was demonstrated that bacterial colonies grown from caries swabs, and their metabolites showed fluorescence when excited by the diode laser.⁸ The molecules responsible for the caries fluorescence increase seem to be porphyrins, which are synthesized by several microorganisms in caries lesions. One could say that as soon as the teeth are extracted and removed from the oral cavity, the organic

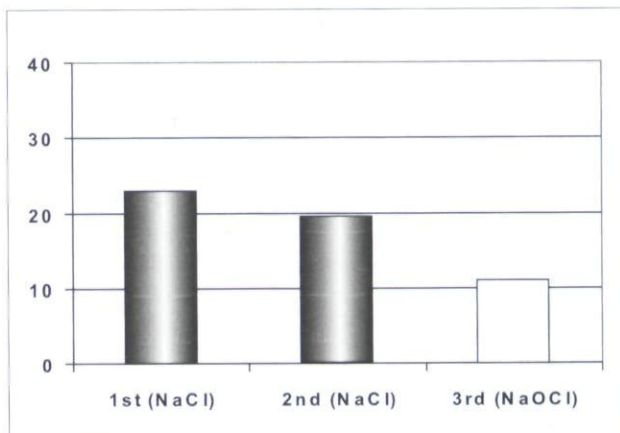


Figure 1. Mean laser fluorescence readings of occlusal caries lesions from the first and second sets of measurements (before sodium hypochlorite immersion) and the third measurement (taken after sodium hypochlorite immersion). Different column colors indicate statistically significant differences among groups ($P=.004$). The standard deviation is 1.6, 3.3, and 2.6, respectively.

components at its surface (ie, biofilm, calculus, etc), as well as the organic components of the tooth itself (ie, collagen), will start presenting significant compositional and structural changes.

Of course, these changes will be exacerbated by the use of chemicals for decontamination and/or fixation of the specimens for storage until the LF readings can be performed. It could be that the marked reduction of the LF measurements, followed by sodium hypochlorite immersion, is due to the fact that the NaOCl, which is a nonspecific proteolytic agent, denatures organic components, causing a marked reduction on the LF readings.

These findings may also explain why the presence of discoloration, exogenous stain, plaque, and calculus may produce fluorescence that, when measured with LF, lead to false-positive answers.^{19,27}

The lowest sensitivity values were observed for the radiographic inspection. If the cutoff limit were set between 0 and 1, highest sensitivity and specificity values would be observed. This means that the radiographic examination underestimates the actual lesion depth and, therefore, can only be used as an adjunct for caries diagnosis. Similar findings were observed in 3 comparisons of BWR with LF readings.^{11,19,28} Sensitivity was lower for radiographs in all 3 studies, while specificity was higher for radiographs in 2 studies.^{11,19}

Conclusions

Based on this study's results, the following conclusions can be drawn:

1. Laser fluorescence and visual inspection showed similar performance in terms of sensitivity and specificity.
2. The performances of laser fluorescence, visual inspection, and radiographic inspection were similar in terms of specificity.
3. Laser fluorescence and visual inspection were superior to radiographic inspection in terms of sensitivity.

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