Laser Fluorescence Device Does Not Perform Well in Detection of Early Caries Lesions in Primary Teeth: an *In Vitro* Study

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Purpose: To evaluate if using the laser fluorescence (LF) device improves the detection of early or more advanced occlusal caries lesions in primary teeth.

Materials and Methods: A total of 181 occlusal sites in primary teeth were assessed by one examiner using DIAGNOdent. The same examiner and a second examiner evaluated 72 of the sites again to assess intra- and inter-examiner reproducibility. After histological validation, lesions were divided at three thresholds according to the lesion depth: outer half of the enamel (D1), inner half of the enamel (D2) and dentine (D3). The sensitivity, specificity, accuracy and area under ROC curve were calculated for cut-off points for the sample considering the aforementioned thresholds. The parameters were compared using chi-square test and by comparison between unpaired ROC curves.

Results: The overall LF performance was better at dentine threshold than at enamel threshold. The higher specificity was found at D3 and D2, and higher accuracy at D3 ($P < 0.001$). The intra- and inter-examiner agreements were classified as good or excellent for all thresholds.

Conclusions: The LF device performs better at the dentine threshold than at the enamel threshold. This method does not perform well in detecting initial enamel caries lesions.

Key words: dental caries, early detection, laser fluorescence, occlusal surface, primary teeth

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Despite the reduction in the prevalence of dental caries, this disease is far from being eradicated. However, the use of fluoridated products and the improvement of social conditions have contributed to a continued decline in caries extent and to a slower progression of caries (Baelum et al, 2006). Thus, early disease detection and early intervention have become extremely important in the control of dental caries (Baelum et al, 2006).

Diagnostic methods have been developed to detect early caries lesions with good accuracy. For populations with a low incidence of caries lesions, methods with high specificity are preferable (Lussi et al, 1999), in order to reduce the number of false-positive results and thereby avoid unnecessary operative treatment. On the other hand, a diagnostic method should be sensitive enough to detect non-cavitated caries lesions both in the enamel and dentine threshold (Pitts and Stamm, 2004), thus allowing for the use of preventive measures and avoidance of subsequent invasive treat-

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ment (Nyvad, 2004). Therefore, it is essential to consider that the choice for one or another threshold depends on what is expected of the diagnostic method.

Laser fluorescence (LF) is a method that has shown promising results in caries detection, especially regarding its reproducibility (Bader and Shugars, 2004; Aljehani et al, 2006; Lussi and Hellwig, 2006). The LF device consists of a diode laser that emits a light (λ = 655 nm) that is absorbed by dental tissues and is partially re-emitted as a near-infrared fluorescence light. The system collects this fluorescence and provides quantitative measures on a scale from 0 to 99. The higher the number, the deeper the caries lesion (Hibst et al, 2001).

Some authors have reported that LF performs well in detecting early enamel caries lesions (Lussi et al, 1999; Morgan, 2000; Alwas-Danowska et al, 2002). In other studies, LF performed better at detecting dentine caries lesions instead of enamel lesions (Lussi et al, 2001; Bader and Shugars, 2004; Bengtson et al, 2005; Aljehani et al, 2006; Braga et al, 2006; Lussi and Hellwig, 2006; Mendes et al, 2006). Since the LF measures the fluorescence from the organic content of the caries lesions (Hibst et al, 2001), advanced caries lesions would have more bacterial metabolites and therefore the device would theoretically perform better in the detection of this type of lesion. However, to the best of our knowledge, few previous studies have carried out experiments and appropriate statistical analyses to compare the LF performance in detecting early and advanced caries lesions.

Therefore, the aim of the present study was to evaluate the performance of a LF device in detecting occlusal caries lesions in primary teeth considering different thresholds, in order to test whether the LF detects enamel caries lesions better than dentine lesions.

MATERIALS AND METHODS

The protocol of this study was approved by the Ethics Committee of the School of Dentistry, University of São Paulo. Primary teeth (132) from the local Bank of Human Teeth were selected. Samples were stored in saline solution for up to 3 months. Teeth were cleaned with a rotating brush and pumice/water slurry. Photographs of occlusal surfaces were taken for the selection of sites for evaluation. One or two suspected caries sites in each tooth were selected. Thus, 181 sites, presenting no visible cavities, were chosen and independently examined.

A DIAGNOdent instrument (KaVo, Biberach, Germany) was used during the examinations. The device was calibrated against a ceramic standard and then re-calibrated after every tenth tooth. The LF was calibrated on the middle of the buccal surface of every tooth before the examination of each occlusal surface. The tooth was air-dried for 3 seconds (Mendes et al, 2004), and the tip A (for occlusal surfaces) was placed on the previously selected site and rotated around a vertical axis. Three measurements were performed consecutively and the mean value was calculated. Each assessment was conducted following the protocol listed above by a trained examiner.

The measurements were repeated twice for approximately 50% of the sample (72 sites). One of these assessments was performed by the same examiner as for the first assessment, but 1 month later. The other was completed by a different examiner, who was blinded to the previous readings. This strategy permitted calculation of the intra- and inter-examiner reproducibility of the method.

After the examinations, non-demineralised sections approximately 250 µm thick were prepared using a 0.3-mm thick diamond saw mounted in a microtome (Labcut 1010, Extec, CT, USA). The tooth slices were manually polished with silicon carbide paper (400, 600, 1000 and 1200 grit in sequence). The examination of each section was performed separately by two examiners using a stereomicroscope at 16 to 40 times magnification and reflected light (SZPT Olympus, Tokyo, Japan). In case of any discrepancy, the examinations were repeated until agreement could be reached.

The sites were classified according to a five-point scale:

- D0, no caries
- D1, caries lesion limited to the outer half of the enamel
- D2, caries extending into the inner half of the enamel but not to the dentine-enamel junction
- D3, caries limited to the outer half of the dentine
- D4, caries involving the inner half of the dentine.

Receiver operating characteristics (ROC) analysis was performed for D1, D2 and D3 thresholds considering the readings obtained. Best cut-off points were obtained specifically for this sample at each one of the thresholds by a combination of sum of sensitivity and specificity found with ROC analysis. The sensitivity, specificity, and accuracy were also calculated based on these cut-off points. The areas under ROC curves for different thresholds were submitted to comparison

Table 1 Inter- and intra-agreement (Kappa values) in detecting occlusal caries lesions in primary teeth

Threshold	Intra-examiner agreement	Inter-examiner agreement	
		1st examination	2nd examination
D1	0.69	0.81	0.67
D2	0.50	0.66	0.66
D3	0.52	0.65	0.84

Table 2 Area under the curve (A_z) and the best cut-off point obtained from ROC analysis, and specificity, sensitivity and accuracy at D1, D2 and D3 thresholds

Threshold	Best cut-off point	A_z	Sensitivity	Specificity	Accuracy
D1	6	0.680[b]	0.64[a]	0.66[b]	0.65[b]
D2	11	0.726[b]	0.57[a]	0.79[a,b]	0.70[b]
D3	16	0.884[a]	0.76[a]	0.88[a]	0.86[a]

Different letters express statistically significant difference within the same column ($P < 0.001$)

of unpaired ROC curves (Hanley and McNeil, 1983) and other parameters were compared using Chi-square test.

The intra- and inter-examiner reproducibility was assessed by Kappa test, also considering the different thresholds adopted.

RESULTS

The tested LF device presented the best intra-examiner reproducibility at D1 and the best inter-examiner reproducibility at D3. In general, the inter- and intra-examiner agreement varied from 0.50 to 0.84 (Table 1). These values can be classified as good (0.75–0.40) or excellent (> 0.75) (Fleiss, 1988).

The best cut-off points selected by ROC analysis are shown in Table 2. The areas under ROC curves (A_z) showed better global performance of this LF device for detecting dentine caries lesions (D3 threshold) than enamel caries lesions, in both D1 and D2 thresholds. Concerning the depth of enamel lesions, the method produced a similar A_z for initial or advanced lesions (Table 2).

At all tested thresholds, the LF device had similar sensitivity ($P > 0.05$). Regarding the specificity, it was higher for D3 than at D1 ($P < 0.001$) and similar between D3 and D2 ($P > 0.05$). Moreover, this LF device was more accurate at D3 than at other thresholds ($P < 0.001$) (Table 2).

DISCUSSION

While some researchers have stated that the LF is able to detect early caries lesions (Lussi et al, 1999; Morgan, 2000; Alwas-Danowska et al, 2002), other studies do not support this assertion, and report that the LF detects dentine caries lesions better (Shi et al, 2000; Lussi et al, 2001; Bader and Shugars, 2004; Bengtson et al, 2005; Braga et al, 2006; Lussi and Hellwig, 2006; Mendes et al, 2006). Therefore, the present study aimed to compare the performance of the DIAGNOdent device among different thresholds, including D1, D2 and D3 thresholds.

The diagnostic thresholds determine the limits between what is sound and what is diseased. Concerning dental caries, depending on the evaluated population, varying diagnostic thresholds can affect data that are obtained in a study of caries prevalence (Pitts and Fyffe, 1988; Assaf et al, 2006).

Reliability is a great advantage of an objective method, such as this LF device, because it does not depend on individual judgment of the examiner. In the

present study, both the intra- and inter-examiner agreement were classified as good or excellent (Fleiss, 1988). This was also observed in previous studies performed in primary teeth (Attrill and Ashley, 2001; Lussi and Francescut, 2003; Aljehani et al, 2006; Mendes et al, 2006). For visual inspection, the inclusion of initial lesions implies more differences in the examiner's clinical opinion and experience (Pitts and Fyffe, 1988; Assaf et al, 2006). In contrast, the LF method presented similar agreement rates at all thresholds evaluated, which is also an advantage of a quantitative diagnostic method. Nevertheless, the LF was not useful in detecting early enamel caries lesions, owing to its lower validity.

Considering the general decrease in the prevalence of dental caries throughout the world, the employment of diagnostic thresholds that permit the early detection of pathological alteration in dental mineralised tissue has become desirable. More sensitive diagnostic methods and criteria, including the recording of non-cavitated lesions, are necessary (Pitts and Stamm, 2004; Warren et al, 2006). If an initial lesion is detected before the cavitation stage, it can be arrested easily (Kidd, 1984) and probably will prevent future invasive treatment and more serious damage of dental tissues (Kidd, 1984; Angmar-Mansson et al, 1998; Stookey et al, 1999; Nyvad, 2004). Based on such criteria, some authors have defended the investigation of methods for early detection of caries (Stookey et al, 1999).

Concerning the LF, previous studies have demonstrated that the device is able to detect early caries lesions (Lussi et al, 1999; Morgan, 2000; Alwas-Danowska et al, 2002). A previous *in vitro* study showed better results regarding the performance of the LF in detecting occlusal caries lesions in advanced enamel caries lesions (D2 threshold) (Alwas-Danowska et al, 2002). On the other hand, the majority of the studies have demonstrated higher values related to the performance in detecting caries lesions at the dentine threshold than at the enamel threshold (Shi et al, 2000; Lussi et al, 2001; Bader and Shugars, 2004; Mendes et al, 2004; Bengtson et al, 2005; Braga et al, 2006; Lussi and Hellwig, 2006; Mendes et al, 2006). Nevertheless, these previous studies did not perform adequate statistical analyses. Furthermore, most of these studies were performed with permanent teeth. A systematic review reported that there is a lack of evidence related to caries detection in primary teeth (Bader et al, 2002). In the present study, we carried out comparisons among the LF performance in different thresholds using primary teeth, and we observed that the LF presented better performance when used at the D3 threshold.

The analysis of the values of the accuracy and the areas under the ROC curves confirmed that the LF device performed better in detecting dentine caries lesions than enamel caries lesions. This finding is in agreement with previous studies (Mendes et al, 2004; Braga et al, 2006; Mendes et al, 2006). Dentinal caries are more advanced lesions and often harbour more bacterial metabolites. As the LF device measures the fluorescence from the organic content of the caries lesions (Hibst et al, 2001), it is expected that the device performs better at the dentine threshold than at the enamel threshold.

Moreover, detection at the D2 threshold was better than detection of early enamel caries lesions (D1 threshold). The only previously published study that had used D1 threshold found values of specificity higher than sensitivity, showing the poor performance of the LF device in detecting initial enamel lesions (Mendes et al, 2006).

In contrast to observations made on permanent teeth, the LF device has shown a specificity similar or slightly higher than the sensitivity for primary teeth (Bader and Shugars, 2004). This trend was also observed for our sample for all thresholds investigated in the present study.

Recently, a new LF device designed for occlusal and approximal caries detection, named DIAGNOdent pen (Kavo, Biberach, Germany), was introduced (Lussi and Hellwig, 2006; Lussi et al, 2006). In approximal caries lesions, the device presented a higher likelihood ratio in detecting caries lesions at D1 threshold than at D2 and D3 thresholds in permanent teeth (Lussi et al, 2006). Nevertheless, in occlusal caries lesions, the new DIAGNOdent pen produced better results at D2 and D3 thresholds than D1 (Lussi and Hellwig, 2006). Further studies using the new device must be performed in permanent and primary teeth.

One could argue that the results of the present study might be influenced by the storage of the samples. A previous study showed that the best way to store the samples is frozen at -20°C (Francescut et al, 2006). However, in this study, the storage in saline solution was not evaluated (Francescut et al, 2006). We do not believe that the storage of the samples in the present study will affect fluorescence measurement. Furthermore, if the saline solution induced a decrease in the fluorescence readings, this reduction would be more pronounced in dentine caries lesions, reducing the performance of caries detection at this threshold, which is contradicted by the findings of the present study.

Some dentine lesions may progress slowly without a clinically visible crack at the enamel surface, i.e. hid-

den caries (Weerheijm, 1997), and visual caries detection becomes difficult. Therefore, a diagnostic method performing well at the dentine threshold and able to identify this kind of lesion could be a good adjunct to visual inspection and radiographic examination. The LF device satisfies all of these conditions. Moreover, the LF device has been reported to give better accuracy and reproducibility compared with radiographic examination (Shi et al, 2000; Attrill and Ashley, 2001; Lussi and Francescut, 2003; Mendes et al, 2006).

In conclusion, the LF device performs better at the dentine threshold. This method does not show good performance in detecting initial enamel caries lesions in occlusal surfaces of primary teeth.

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