Title page

Title

***In vitro* validation of Quantitative Light-induced Fluorescence (QLF) for the diagnosis of enamel fluorosis in permanent teeth**

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Short title

QLF validation for enamel fluorosis in permanent teeth

Keywords

Enamel fluorosis, Quantitative Light Fluorescence, Permanent teeth, Diagnosis.

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This is the author's manuscript of the article published in final edited form as:

Cuevas-Espinosa, D. M., Martinez-Mier, E. A., Ando, M., Castiblanco, G. A., Cortes, F., Rincon-Bermudez, C. M., & Martignon, S. (2017). In vitro Validation of Quantitative Light-Induced Fluorescence for the Diagnosis of Enamel Fluorosis in Permanent Teeth. Caries Research, 51(5), 515–526. https://doi.org/10.1159/000479905

Declaration of interests

All authors declare that they have no conflicts of interest in relation to the subject matter of this paper.

Abstract

This study aimed to validate Quantitative Light-induced Fluorescence (QLF) as a diagnostic tool for mild and moderate enamel fluorosis in permanent teeth, comparing it to visual diagnosis and histological assessment completed using polarized light microscopy (PLM). The buccal surfaces of 139 teeth were visually classified using the Thylstrup and Fejerskov Index (TFI) into sound (TFI 0; n=17), mild (TFI 1-2; n=69) and moderate (TFI 3-4; n=43) fluorosis. Fluorosis was then assessed with QLF (variables ΔF, A and ΔQ at 5-, 15- and 30-radiance thresholds) using as reference areas the entire surface and a region of interest (ROI), identified as the most representative region of a fluorosis lesion. PLM images of longitudinal thin sections including the ROI were assessed for histological changes. Correlations among TFI, PLM, and QLF were determined. A ROC curve was conducted to determine QLF's diagnostic accuracy when compared to the TFI and PLM assessments. This was used to assess the probability that the images were correctly ranked according to severity as determined by PLM and TFI. A positive correlation was found between QLF and PLM, and between QLF and TFI. QLF showed highest sensitivity and specificity for the diagnosis of mild fluorosis. There was also a strong agreement between TFI and PLM. The selection of a ROI resulted in a stronger correlation with TFI and PLM than when the entire surface was used. The study results indicate that defining a ROI for QLF assessments is a valid method for the diagnosis of mild and moderate enamel fluorosis.

Introduction

The use of fluoride to prevent dental caries has been extensively studied [Ast et al., 1950; Dean et al., 1950; Featherstone 1999; Marinho et al., 2003; Marinho VC., 2009]. Community water fluoridation has been used for over 60 years for this purpose. In addition to water, public health programs have used salt and milk as a vehicle for fluoride [WHO, 1984; PAHO, 1986; CDC, 2011]. Despite having clear benefits, detrimental effects have also been associated to fluoride when ingested in excess [Whelton et al., 2006]. Enamel fluorosis is a consequence of chronic intake of high levels of fluoride during enamel development [Fejerskov et al., 1977]. It has raised concerns with respect to increased prevalence of enamel fluorosis, both in non-fluoridated and in fluoridated communities [Clark, 1994; WHO, 2000; Whelton et al., 2004, 2006]. Epidemiological surveillance remains critical in countries where dental fluorosis is endemic in order to monitor public health fluoridation programs. Ideally, these surveillance efforts should be grounded on valid diagnosis methodologies that provide valid data to support appropriate public health decisions.

In 1989, Colombia implemented salt fluoridation as a caries-preventive measure [República de Colombia - Ministerio de Salud, 1996]. Residents of at least 50 municipalities in the country are at high risk of developing enamel fluorosis as a result of their cumulative exposure to multiple sources of fluoride, including water naturally containing fluoride levels above those recommended as optimal (0.7 ppm) [Segura et al., 2001]. The prevalence of enamel fluorosis reported for the country in the last National Oral Health Survey for children age 12 was determined to be 62% using Dean's index [República de Colombia - Ministerio de Salud y Protección Social, 2015], but studies in endemic areas have reported figures as high as 90% [Sanchez et al., 2005; Ramirez et al., 2009; Tellez et al., 2011].

The gold standard for enamel fluorosis diagnosis in the laboratory is histology by means of polarized light microscopy (PLM) [Thylstrup & Fejerskov, 1978; Angmar-Månsson et al., 1994]. When it comes to the clinical and epidemiological settings, visual diagnosis using the Thylstrup and Ferjeskov Index (TFI) remains a useful tool [Thylstrup & Fejerskov, 1978, Fejerskov et al., 1988; Nyvad et al., 2009; Pretty et al., 2012]. Nevertheless, the visual diagnosis of mild (TFI 1-2) and moderate (TFI 3-4) forms of enamel fluorosis requires extensive training and has shown significant variation between examiners, posing difficulties for dental professionals and public health authorities [Sabokseir et al., 2016]. In an attempt to overcome the challenges encountered during enamel fluorosis diagnosis, other diagnostic methods have been explored. Quantitative Light-induced Fluorescence (QLF) is a method currently used for the quantitative evaluation of changes in mineral content of dental enamel, mostly in dental caries lesions. It

is based on the three-dimensional reconstruction of the mineralized structure, taking the fluorescence of sound enamel as a starting point [Pretty et al., 2006]. The reconstruction is done using three variables available from the QLF software: *A*, *ΔF* and *ΔQ*. *A* measures the lesion area, *ΔF* represents the depth of the lesion and the fluorescence loss, and *ΔQ* represents the lesion volume as a result of the fluorescence loss multiplied by the lesion area [Pretty et al., 2012]. Since QLF demonstrated measuring early caries lesions [Ten Bosch, 1996; Van der Veen et al., 1996] it has been widely used showing a high correlation between ΔQ and mineral loss [Van der Veen & de Josselin de Jong, 1999; Traneus et al., 2001; Alammari et al., 2012].

The Receiver Operating Characteristics (ROC) analysis has been suggested as an approach to determine the diagnostic accuracy of QLF for enamel fluorosis. The area under the ROC curve represents the probability that an image, in this case, a fluorescence image obtained with QLF, will be correctly ranked according to its severity [Hanley & McNeil, 1982]. Moreover, the representation and interpretation of the area under the ROC curve provides an efficient tool to judge the discrimination ability of test results for predictive purposes [Hanley & McNeil, 1982]. Therefore, the ROC approach can be used to estimate the sensitivity and specificity of a new test (in this case QLF) in relationship to existing and validated techniques, namely visual diagnosis and PLM assessments [Hanley & McNeil, 1982].

Since enamel fluorosis is a condition that comprises a disturbance in enamel mineralization with consequent areas of hypomineralization that result in a lower fluorescence, QLF might prove to be a convenient and objective approach for its diagnosis, both for the clinical and epidemiological settings [McGrady et al., 2012a,b; Pretty et al., 2012]. However, more in vitro evidence of validation, defined as "'the confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled" is needed to support its use [ISO 3534-1:2006]. Therefore, the aim of this study was to validate QLF as a diagnostic tool for mild and moderate fluorosis in permanent teeth, comparing it against visual diagnosis using the TFI and histological assessments using PLM.

Materials and Methods

Sample collection and group assignment

Following IRB approval (UB313-2012, Act 005-2012) permanent teeth were collected from two sites: the surgery clinics of the Dental School at Universidad El Bosque, Bogotá, Colombia and private practices in the municipality of Pitalito, Huila, Colombia. Teeth were extracted for reasons other than inclusion in this study and patients signed a tooth donation consent form. A pool of 300 teeth was collected. Immediately after extraction, soft tissue was removed; teeth were washed with distilled water and stored in a solution of 0.02% thymol diluted with PBS (Phosphate Buffered Saline) at 4°C, until further processing. Teeth with initial non-cavitated to extensive cavitated caries lesions based on the ICDAS classification [ICDAS, 2009], restorations, and with fractures or stains involving the buccal surface, were excluded. Then, differential diagnosis with other developmental enamel defects was conducted by an experienced examiner (SM). First, it was determined whether the defect was diffuse or not. Localized opacities within sound areas were excluded. This led to 139 teeth classified as sound teeth or teeth with enamel fluorosis TFI 1 to TFI 4: 104 molar teeth, 32 premolar teeth and 3 anterior teeth. Together with three other examiners trained by the same examiner, all assessed visually the buccal surface and by consensus teeth were assigned to one of three groups: sound (TFI 0), mild (TFI 1-2), and moderate (TFI 3-4) fluorosis, using the TFI index. Drying was conducted with cotton rolls and visual diagnosis was performed at noon, with a natural source of light.

Visual diagnosis on stereomicroscopic images

Teeth were transported to the Oral Health Research Institute in Indianapolis (IN, USA) and stereomicroscopic images of the buccal surface of each tooth were taken following a standardized procedure (Nikon SMZ 1500; camera Nikon Digital DXM 1200F, software Nikon ACT-1). Images were projected in a dark room with controlled brightness and magnification. A second experienced examiner (EAM) assessed them and classified them into three categories: sound (TFI 0), mild (TFI 1-2) and moderate (TFI 3-4) fluorosis. Disagreements between the visual diagnosis and stereomicroscopic assessment were resolved by consensus among the examiners.

QLF image acquisition and analysis

Standardized images of the buccal surface of each tooth were taken with a QLF device (QLF/clin version 3.0.0.35 – Inspektor Research System BV., the Netherlands). QLF settings were as follows: blue-violet light (370 nm wavelength), yellow filter of 540 nm, and a 13 nW/cm^2 exposure with the CCD hand camera [Ando et al., 2001]. The analysis of the images was conducted using two different approaches. 1.) Selecting a Region of Interest (ROI). This region was selected after visual and stereomicroscopic assessment of the surface, by identifying the most representative hypomineralized area on the tooth surface. This area should be over 1mm wide to allow for the posterior acquisition of a longitudinal section

for further exams. The area was marked with a dotted rectangle with a reference sound area immediately adjacent to the ROI (Figure 1). 2.) Using the entire tooth surface (S) and identifying the area of greatest fluorescence to be used as a reference sound area.

Analyses using both reference areas, ROI and S, were performed on each QLF image (Figure 2). The QLF program variables were: average fluorescence loss (ΔF [%]), area (A [mm^2]), and volume of the lesion (ΔQ [$mm^2 \times$ %]). ΔQ is considered as a measure that merges the area and the severity of the lesion. For this study, fluorescence radiance was measured at three different levels: thresholds 5, 15 and 30 (corresponding to the error range for the gray scale three-dimensional reconstruction relative to the fluorescence of the sound area) [de Josselin de Jong et al., 1995]. Lower levels than the determined thresholds were considered hypomineralized enamel.

Polarized light microscopy (PLM) image acquisition and histological assessment

Longitudinal sections of 150 - 200µm including the ROI were obtained with a diamond saw (Series 1000 Deluxe Hard Tissue Microtome- SciFab, Lafayette, Co, USA) and subjected to manual grinding with sandpaper (silicon carbide 2400) until a thickness of 80 -120µm was reached. Sections were imbibed in water and examined under PLM (Zeiss Microscope Axio imager 2, Göttingen, Germany). Standardized digital images were obtained from each section with the aid of a microscope-coupled digital camera (ZEISS Axiocam ERc5s-5X, Göttingen, Germany). Images were coded for blinding purposes. Two trained examiners independently evaluated the images and classified fluorosis according to severity (sound, mild, moderate, and severe). Parameters for the assessment were those previously reported by Thylstrup and Ferjeskov [1978]: positive birefringence, porosity, and presence of a subsurface layer. In case of discrepancies, consensus was achieved among the expert examiners.

Statistical analysis

Normality of numerical data was tested with the Shapiro-Wilk test. Agreement between visual (TFI) and histological (PLM) assessments was evaluated with weighted kappa values. Correlation between visual diagnosis (TFI) and QLF variables was evaluated with Kendall's tau. Interpretation of correlations followed the Range–Relation scale, where values from 0 to 0.25 denote few or no correlation; 0.26 to 0.50 weak correlation; 0.51 to 0.75 moderate to strong correlation, and 0.76 to 1 mean a strong to perfect correlation [Kendall & Gibbons, 1990; Martinez-Ortega et al., 2009]. To establish the specificity and sensitivity of QLF as a tool for the enamel fluorosis assessment, an analysis of the Receiver Operating Characteristics (ROC analysis) was performed. This statistical test assessed QLF's diagnosis accuracy when compared to validated techniques. Additionally, areas under the ROC curve were obtained using

each QLF variable (*A*, Δ*F* and Δ*Q*) in an attempt to identity cut off points that would be useful to separate visual diagnosis scores (TFI 0-1, TFI 1-2, TFI 2-3, and TFI 3-4) and PLM severity scores (sound - mild fluorosis, and mild - moderate fluorosis). Optimal cutoff points were established for two categories (merging sound with mild and mild with moderate). Cut off points with the highest sensitivity were considered more suitable for future use in clinical diagnosis (expressed in mm^2 for A, % for ΔF, and mm^2 ×X % for ΔQ). Analyses were performed on thresholds 5, 15 and 30 and were compared in all cases with both the visual and the histological assessments. Analyses were performed using Stata® (version 11.2 SE; Stata Corporation, College Station Texas, USA).

Results

A total of 129 teeth were included in the study as ten teeth were excluded due to damage during processing. Approximately half of the teeth (53.5%) were classified as mild fluorosis (TFI 1 and TFI 2); one third (33.3%) as moderate fluorosis (TFI 3 and TFI 4), and 13.2% as sound (TFI-0) (Table 1).

Visual diagnosis and agreement with histological assessment (PLM)

Table 2 shows the agreement between the visual diagnosis and the histological assessment. The percentage agreement was strong at 94.19% with a weighted kappa of 0.82 ($p<0.001$). The highest disagreement was for the moderate lesions, followed by the mild lesions, while the lesions diagnosed as sound showed almost perfect agreement between visual diagnosis and PLM assessment.

QLF data variables

Table 3 shows the resulting values (mean and SD) of the QLF *A*, *ΔF*, and *ΔQ* variables for TFI sound (TFI 0), mild (TFI 1-2), and moderate (TFI 3-4) enamel fluorosis at QLF radiance thresholds 5, 15, and 30, both for PLM and for visual diagnosis.

QLF correlation with visual diagnosis and histologic assessment

Table 4 shows that statistically significant positive correlations were observed between QLF and visual diagnosis, being higher for the ROI than for the S analysis. All three variables (A, ΔF and ΔQ) showed positive weak to strong correlations in the threshold 15 (0.50; 0.51 and 0.50, respectively $p<0.001$). With respect to specific variables, the highest correlation was found for ΔF at threshold 5 (moderate to strong ktau = 0.53; $p<0.001$), followed by ΔQ at threshold 5 (moderate to strong ktau = 0.51; $p<0.001$). Statistically significant positive correlations were found for all QLF thresholds with histologic assessment

(PLM); they were higher for the ROI than for the S analysis. With respect to the individual ROI analyses variables, the highest correlation was found in ΔF at threshold 5 (moderate to strong, ktau = 0.53; $p<0.001$), followed by ΔQ (ktau = 0.51; $p<0.001$) (Table 4).
When correlating QLF variables with PLM findings, also higher statistical significance was found for the ROI analysis than for the S analysis. Similar to what was observed for QLF and visual diagnosis, the highest correlation for QLF and PLM was observed for the ΔF variable at threshold 5 (ktau = 0.50; $p<0.05$) (Table 4).

Sensitivity and Specificity of dental fluorosis diagnosis with QLF

The specificity and sensitivity of the QLF assessments for the diagnosis between fluorosis and sound teeth were determined using a ROC analysis. The results of the ROC analysis for QLF vs visual diagnosis are shown in Table 5. Results demonstrated that the specificity and sensitivity of QLF for the diagnosis between mild fluorosis (TFI-1) and sound teeth (TFI-0) were high. Figure 3 shows areas under ROC curve (AUC) for each QLF variable when compared to the visual diagnosis of TFI-1 at thresholds 5, 15, and 30. The highest sensitivity and specificity for the QLF variables when compared to the visual scores was found for the ΔQ variable at threshold 5 for TFI-1. In this case, the sensitivity level was 91.89%, the specificity was 88.89% and, the cut-off point was defined in values ≥ 1.4 mm^2%. When the TFI-2 severity was evaluated at threshold 5, the sensitivity of ΔQ was of 72.2% with a specificity of 73% and, the cut-off point was values ≥ 12.3 mm^2 %. The AUC for QLF when compared to the visual diagnosis in assessment of TFI-2 are shown in the Figure 4. For the evaluation of TFI-2 and TFI-3 the variable that showed the highest values was ΔQ at threshold 15. For TFI-4 no statistically significant sensitivity and specificity values were found for any QLF variable at threshold 30.

When comparing the QLF variables to the PLM assessment, we found that the diagnosis of mild fluorosis was more sensitive and more specific. The highest sensitivity and specificity values for the QLF diagnosis of mild fluorosis were found at threshold 5 with the ROI analysis (Table 6). For the assessment of mild fluorosis compared to sound teeth, QLF showed a 90.6% sensitivity and 93.3% specificity. The areas under ROC Curve for QLF, when compared to the PLM assessment of mild fluorosis, are shown in the Figure 6. For the assessment of moderate fluorosis compared with mildly fluorosed teeth, QLF showed a 66% sensitivity and 65.6% specificity for the ΔQ variable at threshold 5 (Table 6).

Discussion

The aim of this study was to evaluate the validity of QLF as a diagnostic tool for the diagnosis of mild and moderate enamel fluorosis in comparison to visual diagnosis using TFI and histological assessments by PLM. We employed two different approaches to achieve our aim and found that one of those approaches provided better results. The correlations found in our study are significantly greater when employing a ROI than when analyzing the entire surface (S). This may be explained by the fact that the ROI was chosen to represent the area that most closely resembled the severity for that tooth, identified through visual assessment by the operator. The correlation values found in this study suggest that the ROI analysis is useful for the diagnosis of enamel fluorosis. However, a limitation for this approach is that it depends on the operator´s ability to choose a sound area before performing the QLF analysis. In general, we found higher correlations in our study, than those previously reported for other studies [McGrady et al., 2012]. The approach used by McGrady et al. [2012a,b] involved a different methodology. In our study we had availability to a visual examination of the teeth and this allowed for the exclusion of teeth with localized enamel defects and caries lesions. For the ROI approach we selected the most marked fluorotic area within the buccal surface and used the surrounding enamel as reference, and for the S approach we used the entire tooth surface and identified the area of greatest fluorescence to be used as a reference sound area. The QLF analyses were conducted at thresholds 5, 10 and 15. We combined anterior and posterior teeth with different degrees of fluorosis severity from TFI-1 to TFI-4. Correlations among TFI, PLM, and QLF were determined and QLF's diagnostic accuracy was compared to the TFI and PLM assessments through a ROC curve using the PLM as gold standard. On the other hand McGrady et al. [2012a,b] had availability to pictures of teeth and QLF images. Included teeth were upper incisor teeth. They used a software "mask" manually drawn around the whole buccal surface and then applied the automated algorithm to conduct the analysis using a convex hull approach, so that extrinsic staining and enamel fractures could be discarded and then the areas of enamel hypomineralization would be compared against the thresholded. They set the threshold at a level of 5.

The sample of this study consisted of a greater number of molar and premolar teeth compared to anterior teeth. Other studies on fluorosis have also used posterior teeth [Ando et al., 2001; Alamari et al., 2013]. More recent studies using QLF consider only maxillary anterior teeth due to the feasibility of the acquisition of the images [Pretty et al., 2006, 2012; McGrady et al., 2012a, 2012b]. Taking into account the fact fluorosis corresponds to a developmental defect of the enamel with a diffuse presentation pattern, enamel fluorosis findings are similar on anterior versus posterior teeth with variations with respect to the fluorosis severity, which can be different between group of teeth [Thylstrup & Fejerskov, 1978]. The methodology used in the current study with all groups of teeth was the same and the results showed no

differences between groups; therefore, the results of this *in vitro* study could be strained to future clinical investigations.

In this study, the correlations between QLF and visual diagnosis or PLM showed weak to strong positive values. Our results add to the body of evidence that supports the use of QLF for the diagnosis of enamel fluorosis. QLF offers multiple advantages, which make its use desirable, including the fact that images can be quantitatively analyzed. Other advantages include that images can be analyzed remotely and at a later time; use analysis approaches as the ROI, and the possibility of storing them for longitudinal analyses.

The analysis results of this study allowed us the establishment of appropriate references to evaluate QLF and validate it the use of QLF for the assessment of mild and moderate fluorosis against PLM. Our results described a moderate positive correlation for the three QLF variables (A, ΔF and ΔQ) in the ROI analysis and the PLM. The moderate strength of the correlation could be explained by the fact that PLM measures three severity scores (sound, mild and moderate) with an ordinal scale, while QLF variables outcomes are continuous, resulting in discrepancies that make correlations difficult to establish. This difficulty has been described in previous studies where the lack of an appropriate gold standard is emphasized [Pretty et al., 2006; McGrady et al., 2012b].

In addition, a challenge faced by prior studies has been the identification and delimitation of the affected area [Pretty et al., 2006; McGrady et al., 2012a,b]. Such challenge can be explained by the fact that enamel fluorosis includes diffuse, confluent areas of hypomineralization, that may difficult the selection of the reference (sound) area required to perform the QLF three-dimensional reconstruction. Also, the naturally curved tooth surface can lead to in some inaccuracies [Pretty et al., 2006]. In order to overcome the major challenges faced by others, we approached the QLF analysis: through an analysis of the surface or S analysis (as enamel fluorosis has been conventionally studied) and through a novel methodology that evaluates a region of interest, or ROI analysis, which had not been used until now.

In the present study, QLF showed a high sensitivity and specificity for the assessment of mild fluorosis. This finding is of clinical relevance, since mild fluorosis is the most difficult to assess. In contrast, sensitivity and specificity were lower for assessment of moderate fluorosis. This finding may be explained by the smaller sample size for moderate fluorosis in relation to the sample size for mild fluorosis teeth in our study. Another reason for this finding is that when the severity is higher, the lesion distributes over the entire surface, making the selection of the reference area more difficulty. In contrast,

McGrady's studies [2012a,b] used the QLF methodology including the entire surface; they reported 80% sensitivity and 85% specificity with an area under the curve of 0.91 and a high precision for the assessment of TFI-3.

In the current study, in addition to establishing values of sensitivity and specificity, we also attempted to establish cutoff points for each fluorosis score and the QLF variables A, ΔF and ΔQ. We found that the thresholds where the sensitivity and specificity had highest values for fluorosis diagnosis were 5 and 15, confirming the findings of a similar study in dentinal caries lesions [Kühnisch et al., 2006]. Our results indicate that the sensitivity and specificity of the QLF technique decreases with increasing the threshold. This may be due to the fact that threshold 5 includes some noise within the ROI; for this reason, the sensitivity is higher, while in the 30-threshold the analysis may lose sensitivity because this method would exclude any gray scales above the radiance level of 70% of reconstructed sound fluorescence radiance as hypomineralized enamel. The QLF variables ΔQ and ΔF were the most consistent and the ones better defining the lesions possibly because as the severity of the lesions increases, so does fluorescence loss. Our results are in agreement with a previous study that reported that ΔQ correlates stronger with the visual assessment of TFI than the area or severity alone [Pretty et al., 2006]. In the current study strong correlations were also found with PLM assessments.

Based on our results, we conclude that QLF using a ROI is a valid *in vitro* diagnostic method for mild enamel fluorosis and promising for moderate enamel fluorosis. Therefore, QLF could be used as a tool for the assessment and monitoring of mild and moderate severities of enamel fluorosis, which are an indicator of fluoride over-exposure, in order to provide elements to make appropriate public health decisions.

Acknowledgments

This study was conducted with the financial support of COLCIENCIAS, Colombia Contract 442-2012 and U.S. National Institute of Health (NIH) grant R21 DE16034. We would like to thank to UNICA, Universidad El Bosque, and Frank Lippert and Anderson Hara from the Oral Health Research Institute, Indiana University, for their valuable scientific and technical assistance. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The roles of all authors were: Conceived and designed the experiments: SM, EAMM, DMCE, and MA. Performed the clinical examination: SM, EAMM, GAC, DMCE and CMRB. Performed statistical analyses: FC, DMCE and CMRB. Wrote the paper: DMCE, SM, EAMM, MA, GAC and CMRB.

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Legends

Table headings:

Table 1. Sample distribution according to the TFI scores and the severity of enamel fluorosis.

Table 2. Agreement between the visual and histological diagnosis for enamel fluorosis.

Table 3. Mean and standard deviation (SD) of QLF variables (*A*, ΔF, and ΔQ) at thresholds 5, 15, and 30 for TFI sound, mild and moderate enamel fluorosis by PLM and visual diagnosis (n=129).

Table 4. Enamel fluorosis QLF correlation (at thresholds 5, 15, and 30) with PLM and visual diagnosis.

Table 5. Receiver Operating Characteristics of QLF according to visual diagnosis with Thylstrup & Fejerskov Index.

Table 6. Receiver Operating Characteristics of QLF according to fluorosis severity with PLM.

Figure headings and legends:

Figure 1. Selection of Region of Interest (ROI).

Solid lines mark longitudinal section. Rounded rectangle with dot line indicates area for QLF and PLM analyses.

Figure 2. QLF analysis schematization.

a). Selection of the entire surface (S) for QLF analysis. The continuous line marks the selected area with a dotted line placed in the brightest fluorescent area, which is the benchmark for reconstruction in grayscale. c). Selection of ROI for QLF analysis. The dotted line is located in the area with greatest fluorescence. b.) and d.) Software's patch reconstruction in grayscale.

Figure 3. Areas under ROC curve for QLF when compared to the visual diagnosis in assessment of TFI-1.

Figure 4. Areas under ROC curve for QLF when compared to the visual diagnosis in assessment of TFI-2.

Figure 5. Areas under ROC curve for QLF when compared to the PLM assessment of mild fluorosis.

Table 1. Sample distribution according to the TFI scores and the severity of enamel fluorosis.

	TFI fluorosis severity					
	Sound	Mild fluorosis		Moderate fluorosis		
Distribution of teeth	TFI-0 n (%)	TFI-1 n (%)	TFI-2 n (%)	TFI-3 n (%)	TFI-4 n (%)	**Total (n)**
	17 (13.2)	36 (27.9)	33 (25.5)	31 (24.0)	12 (9.3)	
Total	17 (13.2)	69 (53.5)		43 (33.3)		**129**

Table 2. Agreement between the visual and histological diagnosis for enamel fluorosis.

		Visual diagnosis			
		Sound (n)	Mild fluorosis (n)	Moderate fluorosis (n)	**Total (n)**
PLM	Sound	15	0	0	15
	Mild fluorosis	2	59	3	64
	Moderate fluorosis	0	10	40	50
	Total	17	69	43	**129**

Table 3. Mean and standard deviation (SD) of QLF variables (*A*, *ΔF*, and *ΔQ*) at thresholds 5, 15, and 30 for TFI sound, mild and moderate enamel fluorosis by PLM and visual diagnosis (n=129).

Diagnostic method	Severity of fluorosis	QLF analysis approach		QLF variable								
				A	***ΔF***	***ΔQ***	***A***	***ΔF***	***ΔQ***	***A***	***ΔF***	***ΔQ***
				Threshold 5			**Threshold 15**			**Threshold 30**		
PLM assessment	Sound (n=15)	S	Mean	32.4	32.0	1101.4	27.3	36.4	1052.3	18.5	43.9	855.1
			±SD	11.9	8.0	593.1	11.3	6.9	595.4	11.3	5.3	599.5
		ROI	Mean	0.0	6.4	0.5	0.0	3.1	0.0	0.0	0.0	0.0
			±SD	0.0	1.3	0.7	0.0	6.5	0.0	0.0	0.0	0.0
	Mild (n=64)	S	Mean	38.7	35.0	1388.9	33.8	39.0	1342.7	23.6	46.4	1111.9
			±SD	9.4	5.7	426.5	9.5	4.5	433.4	7.8	3.6	407.1
		ROI	Mean	0.7	15.3	13.1	0.4	17.5	10.1	0.0	14.4	2.4
			±SD	0.5	6.4	11.9	0.4	9.0	11.2	0.1	16.0	5.1
	Moderate (n=50)	S	Mean	39.4	38.0	1514.4	35.7	41.1	1478.3	26.5	47.2	1270.0
			±SD	7.8	5.7	385.6	7.6	5.3	389.3	7.3	3.9	411.8
		ROI	Mean	1.0	23.3	25.1	0.8	25.8	22.9	0.3	23.8	12.4
			±SD	0.5	8.3	17.4	0.4	7.3	17.9	0.4	17.0	18.0
Visual diagnosis	Sound (n=17)	S	Mean	32.3	32.0	1083.2	27.0	36.2	1032.8	18.1	43.8	833.5
			±SD	11.1	7.6	560.2	10.7	6.6	563.2	10.7	5.0	568.2
		ROI	Mean	0.0	6.3	0.5	0.0	2.7	0.0	0.0	0.0	0.0
			±SD	0.0	1.3	0.7	0.0	6.2	0.0	0.0	0.0	0.0
	Mild (n=69)	S	Mean	38.9	36.0	1405.6	34.1	39.4	1360.0	23.9	46.6	1131.0
			±SD	8.3	5.2	385.7	8.4	4.2	392.8	7.1	3.4	377.4
		ROI	Mean	0.7	16.1	14.1	0.4	18.6	11.0	0.0	15.4	3.1
			±SD	0.5	6.8	12.5	0.4	8.6	12.0	0.1	16.3	7.2
	Moderate (n= 43)	S	Mean	39.5	38.0	1528.7	36.0	41.0	1493.0	26.8	47.1	1285.6
			±SD	9.3	6.5	439.5	9.1	5.9	441.9	8.2	4.3	450.7
		ROI	Mean	1.0	23.7	26.1	0.8	26.2	24.0	0.3	24.5	13.0
			±SD	0.5	8.1	17.5	0.4	7.3	17.8	0.4	16.8	18.3

Note: S indicates analysis done by entire surface and ROI indicates analysis done by selected region of interest.

Table 4. Enamel fluorosis QLF correlation (at thresholds 5, 15, and 30) with PLM and visual diagnosis.

QLF	Variable / QLF analysis	*A*			*ΔF*			*ΔQ*		
		Threshold			Threshold			Threshold		
		5	15	30	5	15	30	5	15	30
PLM	S	0.13[a]*	0.18[a]*	0.20[a]*	0.23[a]*	0.19[a]*	0.15[a]*	0.20[a]*	0.20[a]*	0.20[a]*
	ROI	0.42[b]*	0.48[b]*	0.38[b]*	0.51[c]*	0.48[b]*	0.38[b]*	0.48[b]*	0.48[b]*	0.36[b]*
VISUAL	S	0.15[a]*	0.21[a]*	0.24[a]*	0.25[a]*	0.21[a]*	0.18[a]*	0.23[a]*	0.24[a]*	0.24[a]*
	ROI	0.45[b]*	0.50[c]*	0.38[a]*	0.53[c]*	0.51[c]*	0.40[b]*	0.51[c]*	0.50[c]*	0.39[a]*

Kendall´s tau correlation. *Statistically significant p value (<0.05). [a]Poor positive correlation. [b]Weak positive correlation. [c]Moderate to strong positive correlation.

Table 5. Receiver Operating Characteristics of QLF according to visual diagnosis with Thylstrup & Fejerskov Index.

QLF Variable / Visual	ROC	*A* Threshold 5	*A* Threshold 15	*A* Threshold 30	*ΔF* Threshold 5	*ΔF* Threshold 15	*ΔF* Threshold 30	*ΔQ* Threshold 5	*ΔQ* Threshold 15	*ΔQ* Threshold 30
TFI 0-1	**CP (≥)**	0.30	0.10	0.10	7.3	15.00	30.30	1.40	0.10	0.10
	Se (%)	81.08	62.16	10.81	81.08	75.68	29.73	91.89	70.72	24.32
	Sp (%)	94.44	100.00	100.00	77.78	77.78	100.00	88.89	83.33	100.00
	AUC (%)	0.94	0.81	0.55	0.90	0.83	0.64	0.95	0.81	0.62
TFI 1-2	**CP (≥)**	0.60	0.40	0.10	15.70	19.50	30.30	12.30	7.60	0.10
	Se (%)	72.22	72.22	52.78	69.44	72.22	61.11	72.22	75.00	63.89
	Sp (%)	72.97	70.27	89.19	70.27	76.57	70.27	72.97	75.68	75.68
	AUC (%)	0.76	0.77	0.70	0.78	0.78	0.72	0.80	0.78	0.73
TFI 2-3	**CP (≥)**	1.10	0.70	0.10	20.90	23.90	31.73	17.90	15.50	2.40
	Se (%)	55.88	61.76	58.82	52.94	52.94	52.94	55.88	61.76	52.94
	Sp (%)	55.56	58.33	47.22	52.78	52.78	55.56	55.56	61.11	52.78
	AUC (%)	0.55	0.61	0.56	0.58	0.56	0.55	0.58	0.60	0.56
TFI 3-4	**CP (≥)**	0.90	0.70	0.10	20.20	23.60	31.50	17.70	15.70	1.70
	Se (%)	41.67	33.33	41.67	41.67	41.67	41.67	41.67	41.67	41.67
	Sp (%)	38.24	38.24	41.18	41.18	44.12	47.06	41.18	41.18	41.18
	AUC (%)	0.36	0.39	0.46	0.54	0.54	0.52	0.42	0.44	0.46

Abbreviations: CP: Cut-off point; Se: Sensitivity; Sp: Specificity; AUC: Area under the ROC curve.

Table 6. Receiver Operating Characteristics of QLF according to fluorosis severity with PLM.

QLF Variable / PLM	ROC	*A* Threshold 5	*A* Threshold 15	*A* Threshold 30	*ΔF* Threshold 5	*ΔF* Threshold 15	*ΔF* Threshold 30	*ΔQ* Threshold 5	*ΔQ* Threshold 15	*ΔQ* Threshold 30
PLM (Sound – Mild)	**CP (≥)**	0.30	0.10	0.10	7.90	15.30	30.00	1.80	0.10	0.10
	Se (%)	85.94	75.00	28.13	84.38	79.69	45.31	90.63	79.69	42.19
	Sp (%)	93.33	100.00	100.00	86.67	80.00	100.00	93.33	80.00	100.00
	AUC (%)	0.94	0.87	0.64	0.92	0.88	0.72	0.95	0.87	0.71
PLM (Mild – Moderate)	**CP (≥)**	0.80	0.50	0.10	18.50	21.60	30.80	14.20	11.50	0.20
	Se (%)	64.00	68.00	58.00	68.00	66.00	64.00	66.00	62.00	62.00
	Sp (%)	64.06	64.06	71.88	67.19	65.63	64.06	65.63	62.50	62.50
	AUC (%)	0.65	0.72	0.67	0.76	0.74	0.68	0.72	0.74	0.67

Abbreviations: CP: Cut-off point; Se: Sensitivity; Sp: Specificity; AUC: Area under the ROC curve.