Validation of quantitative light-induced fluorescence-digital (QLF-D) for the detection of approximal caries *in vitro*

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Directed by Professor Baek-Il Kim

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ABSTRACT

Validation of quantitative light-induced fluorescence-digital (QLF-D) for the detection of approximal caries *in vitro*

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As it was realized that dental caries process is dependent on the balance between pathological factors and protective factors, and that caries progresses when the pathological factors predominate while it can be arrested or reversed when the protective factors predominate, the treatment principles for caries lesions gradually shifted towards preventive treatment of enamel lesions. However, in order to implement preventive therapy, caries lesions need to be detected at their early stage. However, early detection of caries can be difficult, especially approximal caries, due to their anatomical position. Therefore, visual examination alone is likely to underestimate the number of approximal carious lesions. Radiographic examination is another common method for detection of approximal lesions, yet it has been known that radiographic examination often detects

caries lesions at advanced stage, beyond the scope of remineralization interventions. Furthermore, the use of ionizing radiation, which exposes patients to risk, leads into consideration of alternative methods for detection of approximal lesions. These disadvantages of the conventional detection methods have led to a great interest in development of new devices for accurate detection of approximal lesions.

One of the devices is Quantitative Light induced Fluorescence-digital Biluminator™ (QLF-D). This device is able to quantify mineral loss using its excitation light of 405 nm blue light on enamel. Other than the quantitative analysis method, a qualitative analysis method of QLF has been reported as QLF-I. The present study was conducted: firstly, to assess the ability of the QLF-D to distinguish between different stages of approximal caries lesion development by comparing the qualitative analysis method (QLF-DI) and the quantitative analysis method (QLF-DF) with histology examination; secondly, to compare the validity with other various detection methods such as ICDAS II and digital radiography; and finally, to determine the relationships among ICDAS II, QLF-DI, and QLF-DF using these simulation pairs.

A total of 100 permanent molar and premolar teeth were selected from a pool of extracted permanent human teeth from Yonsei University, with ethical approval from the Institutional Review Board for Clinical Research in Yonsei dental hospital (IRB 14-0067). Pairs were formed of the extracted teeth with their marginal ridges in contact to simulate the oral relationship, and the pairs were examined using ICDAS II, digital radiography, and QLF-D. All the examinations were performed by one calibrated dentist, and the recordings for all the examinations were repeated with a 7-day intermission among the different modalities. After completion of all the assessments, all teeth were prepared for histological assessment.

To evaluate reproducibility of all the methods including the novel device, intra-examiner reliability was assessed using Intraclass Correlation Coefficient (ICC). To assess the performance of the QLF-D in comparison with the conventional methods, Sensitivity, specificity, and Area Under the ROC Curve (AUC) were calculated for all the examined methods using ROC, and comparisons were made. Also, the relationships among the QLF-DI, QLF-DF and ICDAS II were assessed using a Spearman's rank correlation.

The intra-examiner reliability analysis showed excellent agreement for ICDAS II, DR, and QLF-DF. The QLF-DI achieved fair-to-good intra-examiner reliability. The sensitivity and specificity values calculated at thresholds D1 and D3 showed that the QLF-DI was the most sensitive method at both enamel (0.95) and dentin thresholds (0.71). When the AUCs were compared, the range of the AUCs was from 0.74 (QLF-DI) to 0.80 (DR and QLF-DF) at enamel threshold, and at dentin threshold, it was from 0.66 (ICDAS II) to 0.76 (ΔF). The highest AUC was obtained for QLF-DF at both enamel (0.80) and dentin thresholds (0.76). In addition, strong correlations were found between the QLF-DI and the ICDAS II with the correlation value of 0.72 ($p<0.01$), and also with the QLF-DF with the correlation value of 0.67 ($p<0.01$).

In conclusion, both the quantitative and qualitative analysis methods for the newly developed QLF-D showed to have relatively high sensitivity and specificity in detecting proximal caries. Also, their performances were similar to those of the traditional methods, from which it can be concluded that the QLF-D is the effective detection method.

Key words: approximal caries, caries detection, quantitative light induced fluorescence-digital (QLF-D), QLF-I

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1. INTRODUCTION

As it was realized that dental caries process is dependent on the balance between pathological factors and protective factors, and that caries progresses when the pathological factors predominate while the caries can be arrested or reversed when the protective factors predominate, the treatment principles for caries lesions gradually shifted towards preventive treatment of enamel lesions where the lesions can have an opportunity to reverse (Featherstone, 2004; Mejare et al., 1985; Mejare and Malmgren, 1986). In order to implement the preventive therapy, caries lesions need to be detected at their early stage. However, early detection of caries can be difficult, especially approximal caries, due to their anatomical position. It was found that 75% of approximal lesions are in the contact area and 25% are beneath the contact area, which makes visual detection complicated (Arnold et al., 1998). Hence, approximal lesions are normally detected when the weakened marginal ridges break down and become cavitated (Rayner and Southam, 1979). Therefore, it is likely to underestimate the number of approximal carious lesions with visual examination only. Radiographic examination is another common method for detection of approximal lesions, yet it has been known that radiographic examination often detects caries lesions at advanced stage, which have already passed the scope of remineralization interventions. Furthermore, the use of ionizing radiation, which exposes patients to risk, leads into consideration of alternative methods for detection of approximal lesions (Claus et al., 2012).

Since the performance of visual examination in detection of early approximal caries lesions is inadequate, enhanced visual scoring systems have been developed. One of them is International Caries Detection and Assessment System (ICDAS), which has been reported by number of studies that it is an accurate and reproducible method to detect early lesions and also to detect longitudinal changes in lesions (Ekstrand et al., 2007; Ferreira Zandona et al., 2010; Pitts and Stamm, 2004). The ICDAS is also theoretically applicable to approximal smooth surfaces (Ekstrand et al., 2011), and the use of ICDAS for approximal caries has been evaluated in a few studies (Neuhaus et al., 2014; Novaes et al., 2009).

Another potential method for approximal caries detection is a newly developed device called Quantitative Light-induced Fluorescence-digital Biluminator™ (QLF-D). This device is an upgraded version of the first product, the QLF device (Inspektor™ Pro, Inspektor Research Systems BV, Amsterdam, The Netherlands), with a modified filter set (D007; Inspektor Research Systems BV, Amsterdam, The Netherlands), and the principle of this device is based on auto-fluorescence of teeth. When a tooth is excited by a visible light of 405 nm from the QLF, it emits light at higher wavelength, and the emitted light is detected by the device by filtering out the excitation light using the filter (de Josselin de Jong et al., 1995). However, when demineralization occurs, there is loss of fluorescence, and the QLF is able to detect this change and quantify the change of demineralization, compared to the sound tissue by measuring differences in contrast between them using proprietary software. Other than quantitatively detecting mineral loss, the QLF is also able to detect endogenous porphyrins produced by oral bacteria and present as red fluorescence, and the detected red fluorescence is found to be associated with caries risk (Heinrich-Weltzien et al., 2003; Lee et al., 2013). Currently, there is a great deal of interest in the red fluorescence (Pretty, 2006). Regarding detection of enamel lesions, early studies have shown that QLF has high sensitivities and specificities (Angmar-Mansson and ten Bosch, 2001; de Josselin de Jong et al., 1995; Shi et al., 2001). However, the results of the previous studies can only be interpreted in terms of QLF; thus, there is a need to investigate the ability of the QLF-D in detecting caries lesions.

Other than assessing the ability of the QLF in detecting caries lesions using the parameters automatically calculated from the proprietary software, another way of evaluating the performance of QLF was attempted by combining with visual criteria. As ICDAS has been regarded as a validated tool for caries detection, yet within the limitation of visual inspection, a previous study combined ICDAS with a technology-based method such as QLF in order to maximize advantage of each method (Ferreira Zandona et al., 2010). The resulting method was named as QLF-I, in which visual examination was performed using the ICDAS criteria and followed by a QLF examination. The previous study assessed occlusal surfaces and smooth surfaces of 569 children using ICDAS and QLF-I, and reassessed them at 8 months and 12 months (Ferreira Zandona et al., 2010).

Although it was concluded that the combined method has a clinical potential for caries detection, the study was *in vivo*, which needs to be validated *in vitro* (Ferreira Zandona et al., 2010). In addition, the QLF-I analysis method can be considered as a qualitative analysis method of the QLF-D since the method is based on visual classification of caries severity like ICDAS criteria while the quantitative analysis method of the QLF-D using the software provides numerical values. Thus, there is a need to evaluate the QLF-I, qualitative analysis method of the QLF-D against histology, but also related to the quantitative analysis method of the QLF-D, which can be automatically obtained by the QLF software.

In order to assess the performance of detection methods, clinical studies are ideally required. However, it is difficult to conduct such clinical studies because there are many confounding factors to be considered and the extraction of teeth is needed for the gold standard. Therefore, the present study was conducted *in vitro* using simulation pairs of extracted teeth. In order to simulate the anatomy of proximal contacts, simulation pairs were formed of extracted teeth for this study. The aims of the study were: firstly, to assess the ability of the QLF-D in detection of approximal caries at different stages by comparing the two analysis methods QLF-DI, the qualitative analysis method for the QLF-D, and QLF-DF, the quantitative analysis method for the QLF-D, with histology examination; secondly, to compare the validity with other detection methods such as ICDAS and radiography; and finally, to determine the relationships among the QLF-DI, the QLF-DF, and the ICDAS using these simulation pairs.

2. MATERIALS AND METHODS

2.1. Sample selection

A total of 100 permanent molar and premolar teeth were selected from a pool of extracted permanent human teeth from Yonsei University, with ethical approval from the Institutional Review Board for Clinical Research in Yonsei dental hospital (IRB 14-0067). Prior to the extraction, informed and written consent was obtained from all the study participants. After teeth were extracted, they were immediately collected in specimen jars containing distilled water first, and then the teeth were carefully cleaned of soft tissues and calculus, and frozen at -20 ℃ until used. Storing teeth frozen has been proven to not change red fluorescence (Lussi et al., 2006)

Before the teeth were examined using different detection methods, the stored teeth were unfrozen, and selection of teeth was performed. The teeth with enamel hypoplasia or dental fluorosis were excluded. For each selected tooth, a photograph of the lesion on proximal surface was taken to allocate the site to be examined. Each tooth was given an identification number that was maintained throughout the study. The study was conducted following the procedure shown in Figure 1.

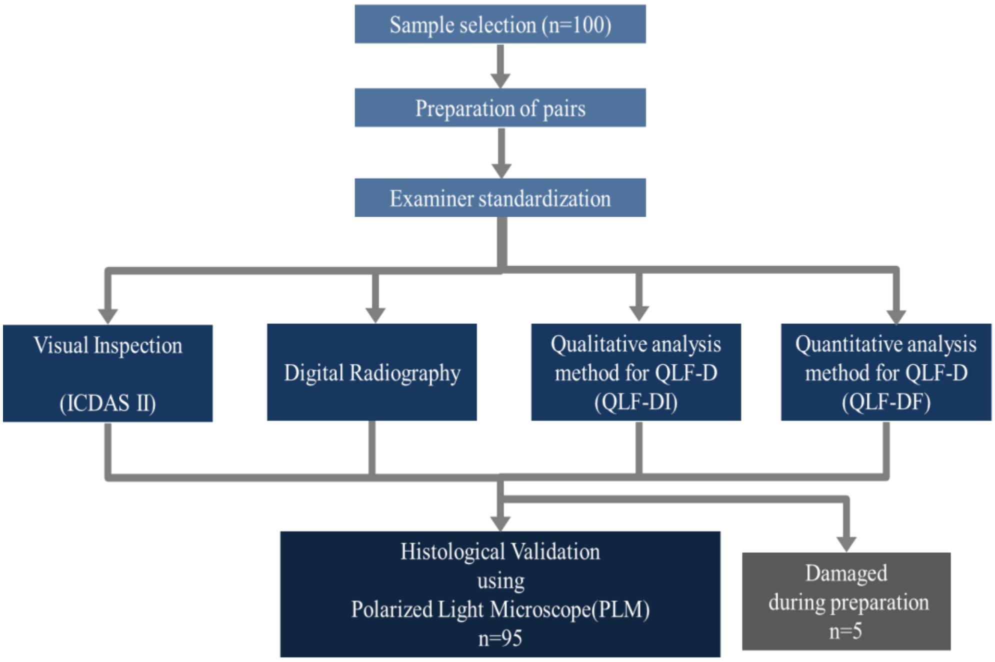

Fig 1. Flow chart of study procedure

2.2. Preparation of simulation pairs

Pairs were formed with marginal ridges in contact to simulate the oral relationship with resin (Ortho-jet, Lang Dental Mfg. Co., Inc., USA) in putty mold (DuoSil Putty set, Bukwang, Busan, Korea), and they were stored individually in containers of distilled water. Each sample was removed from the container, scored and replaced in the container.

2.3. Examiner standardization

All the examinations were performed by one calibrated dentist. The examiner was experienced in the use of QLF-D for caries detection and quantification. For the ICDAS, the examiner had a 90-min training session through the e-learning program prior to the examination. The course included a theoretical part and evaluation of images of carious teeth. The training for radiographic scoring involved discussion of the radiographic scoring system with a dental professional who was specialized in radiology, and if there was any uncertainty, it was discussed to consensus.

2.4. Detection methods

Four caries detection methods were applied; ICDAS II, Digital radiography, and Quantitative Light-induced Fluorescence-Digital Biluminator ™ (QLF-D), which was analyzed in two different ways, QLF-DI, and QLF-DF.

2.4.1. ICDAS II

The pre-selected site of each simulation pair was examined from buccal, occlusal, and lingual aspects using air syringe and a WHO probe to mimic actual clinical situation, and the lesions were recorded according to the ICDAS II criteria (Committee, 2009):

0 sound

1 first visual change in enamel

2 distinct visual change in enamel when viewed wet

3 localized enamel breakdown due to caries with no visible dentin

4 underlying dark shadow from dentin with or without localized enamel breakdown

5 distinct cavity with visible dentin

6 extensive distinct cavity with visible dentin

2.4.2. Digital Radiography

A pilot study was undertaken in order to ensure the quality of the radiographs. Optimal current, voltage, exposure time, and projection geometry were determined, and a special holder was constructed. The holder arranged the cone to be at a distance of approximately 3 cm from the simulation pairs of teeth and 5 cm from the sensor. Following the pilot study, digital radiography (DR) was performed with the holder using the dental X-ray machine (Kodak 2200 Intraoral X-ray System, Eastman Kodak Co, Rochester, NY, USA) at 60 kV and 7 mA and exposure time of 0.096 seconds.

The radiographs were then viewed by using software (PiViewSTAR; Infinitt, Seoul, Korea) on an 18 in. computer screen under the same lighting conditions at a standard distance. Simulating clinical situations, brightness and contrast could be adjusted. The lesions on radiographs were classified according to the following score (Pitts, 1984):

0 no radiolucency

1 radiolucency restricted to the outer half of the enamel

2 radiolucency involved inner half of enamel, up to (including) DEJ

3 radiolucency confined to outer half of dentine

4 radiolucency involved inner half of dentine with/without apparent pulpal involvement

2.4.3. Qualitative analysis method for Quantitative Light-induced Fluorescence-Digital (QLF-DI)

The pairs were examined and imaged by QLF-D (Figure 2). The images were captured from occlusal, buccal and lingual aspects of the specimens, and the photographing condition is shown in Table 1. The proprietary software (C3 v1.16, Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to capture and store all digital images on a PC automatically. The distance between the specimen and the QLF-D was 10 cm, and the angle between them were maintained to 90 degrees when buccal and lingual sites were captured (Figure 3).

The QLF-D images were then analyzed under darkroom conditions. Since the QLF-DI examination, which was classified as the qualitative analysis method in this study, was meant to be conducted immediately after the ICDAS examination, the images of the lesions were scored according to the QLF-I criteria first (Ferreira Zandona et al., 2010):

0 sound tooth surface

1 slight fluorescence change

2 distinct fluorescence change

3 visible enamel breakdown with a distinct fluorescence change

4 poorly delineated distinct fluorescence change with or without enamel breakdown

5 cavitation visible with distinct fluorescence change (5 and 6)

6 collapsed with 5

2.4.4. Quantitative analysis method for Quantitative Light-induced Fluorescence-Digital (QLF-DF)

Seven days after the QLF-DI examination, the images of the lesions were analyzed using the quantitative method, QLF-DF. A patch was drawn on each lesion and mineral loss was calculated as ΔF (%) at 5% fluorescence loss threshold using QLF-D software (QA2 v1.21, Inspektor Research Systems BV, Amsterdam, The Netherlands). For both

QLF-DI and QLF-DF, the highest value was taken from the three measurements of occlusal, buccal and lingual sites for further analysis.

Before the teeth were sectioned for the histological examination, intra-examiner reliability was assessed for all the methods. The recordings for visual inspection and the acquired images taken under DR and the QLF-D were repeated with a 7-day intermission among the different modalities.

Table 1. Photographing condition of QLF-D in this study

	White light	Blue light
Shutter speed	1/40s	1/20s
Aperture value	13.0	13.0
ISO speed	1600	1600
Pixel size	1296X864	1296X864

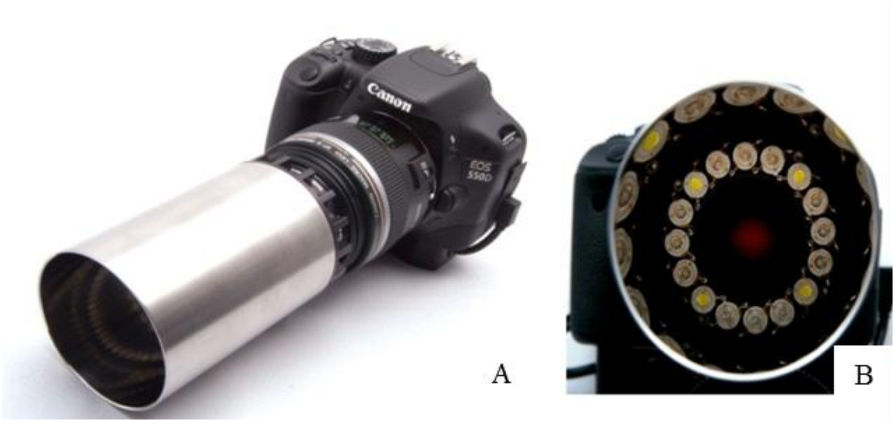

Fig 2. QLF-D device (A: QLF-D from outside, B: white light LED lamps and fluorescence light LED lamps in Biluminator)

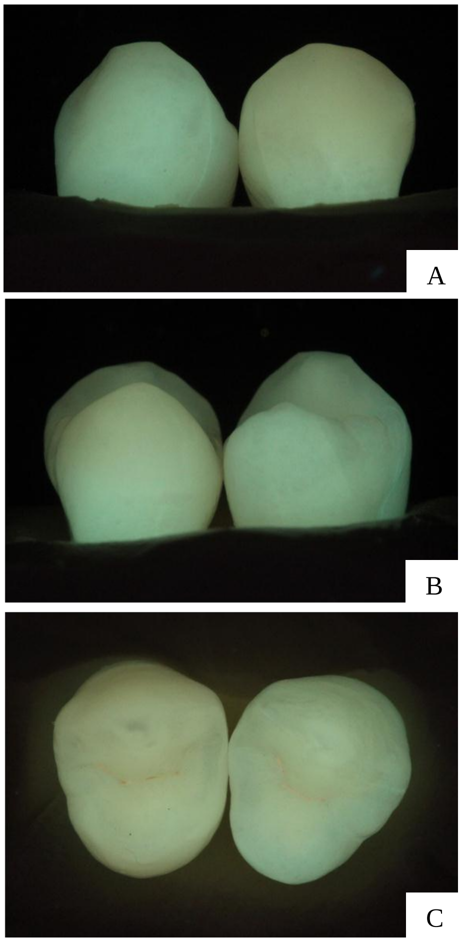

Fig 3. QLF-D images taken in different positions (A: Buccal, B: Lingual, C: Occlusal)

2.5. Histology

After completion of all the assessments, all teeth were prepared for histological assessment. Before sectioning the teeth at the investigation sites, the teeth of more than or equal to ICDAS II code 3, which means cavitated or at least with enamel breakdown, were separately embedded in resin (Ortho-jet, Lang Dental Mfg. Co., Inc., USA) in order to prevent destruction from preparation procedures. The teeth were sectioned buccolingually to a 2 mm thick specimen using a microtome (TechCut 4TM, Allied High Tech Products, Inc., California, USA). The specimens were then ground with silicon carbide paper (800 grit, SiC Sand Paper, R&B Inc., Daejeon, South Korea) to a thickness of 200 ㎛, and photographed at magnifications of 40 and 100 for histological examination with Polarized Light Microscope (PLM, CX31-P, Olympus, Tokyo). The histological score was assigned according to the following classification:

0 no enamel demineralization or a narrow surface zone of opacity

1 enamel demineralization limited to the outer 50% of the enamel layer

2 demineralization involving the inner 50% of the enamel, up to the enamel-dentine junction

3 demineralization involving the outer 50% of the dentine

4 demineralization involving the inner 50% of the dentine

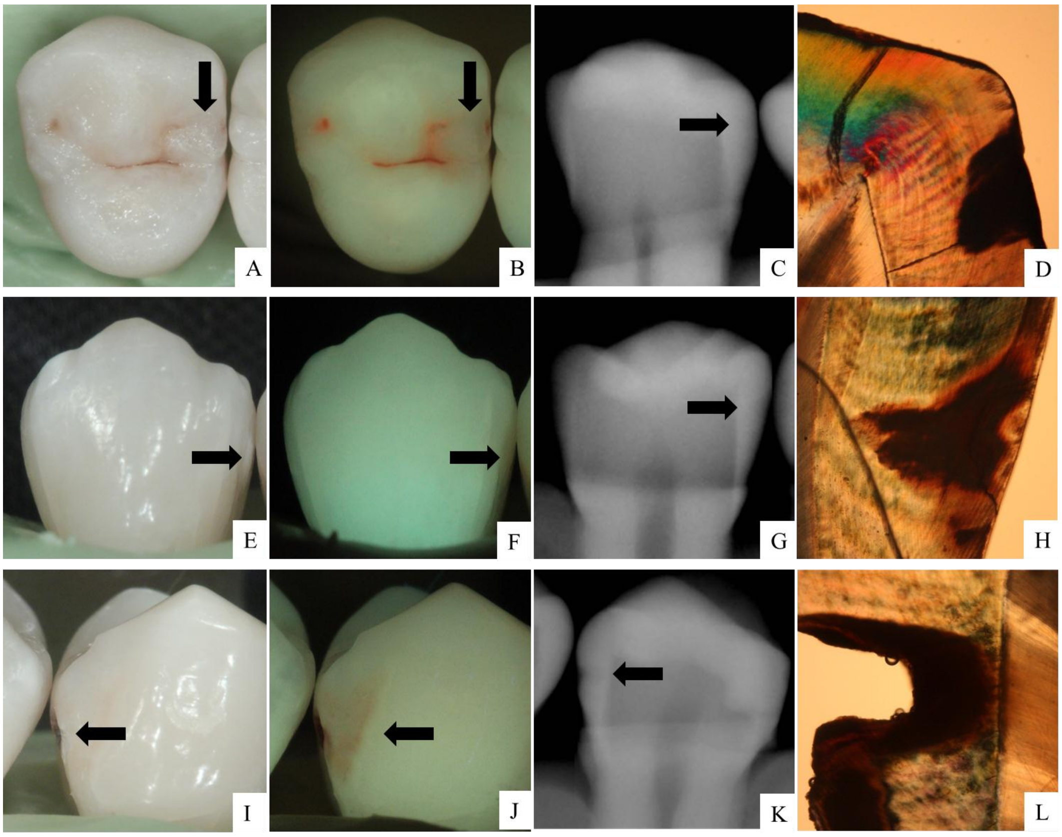

Fig 4. Examples of simulation models under different lights of QLF-D, digital radiography and respective Polarized Light Micrographs (magnified 40 x) (A: simulation model 1 taken from occlusal aspect in white light image, B: simulation model 1 in blue light image, C: radiograph of simulation model 1, D: polarized light micrograph of the simulation model 1 scored as D1, E: simulation model 2 taken from buccal aspect in white light image, F: simulation model 2 in blue light image, G radiograph of simulation model 2, H: polarized light micrograph of the simulation model 2 scored as D2, I: simulation model 3 taken from buccal aspect in white light image, J: simulation model 3 in blue light image, K: radiograph of simulation model 3, L: polarized light micrograph of the simulation model 3 scored as D4. The black arrows indicate demineralized areas.)

2.6. Statistical analysis

To evaluate reproducibility of all the methods including the novel device, intra-examiner reliability was assessed using Intraclass Correlation Coefficient (ICC). The sensitivity, the specificity were calculated for each diagnostic method at enamel threshold (D1) and dentin threshold (D3). At the enamel threshold, all enamel and dentin lesions were regarded as disease positive, and at the dentin threshold, sound surfaces and enamel lesions were classified as disease negative while dentine lesions were regarded as caries. For the ICDAS II, DR, and the QLF-DI, the cutoff point of (D1) was between 0 and 1, and the cutoff point of (D3) was between 2 and 3. For ΔF values of the QLF-DF, as there was no scale available for detecting proximal lesions, the cut-off limits were determined by the maximum combination of sensitivity and specificity (Med Calc 12.7.0.0.,Mariarke, Belgium). Receiver Operating Characteristics (ROC) statistics were performed to evaluate the degree of agreement between the detection methods and the histology, and the Area Under the Curve (AUC) values were calculated. Also, the relationships among the QLF-DI, QLF-DF and ICDAS were assessed using a Spearman's rank correlation. The significance level for all the statistical tests was set at a = 0.05 (PASW statistics ver.18.0, SPSS, Chicago, IL, USA).

3. RESULTS

95 surfaces were analyzed as 5 teeth were damaged during preparation for histology examination.

3.1. Intra-examiner reliability

The intra-examiner reliability analysis showed that the ICDAS II, the DR, and the QLF-DF had excellent intra-examiner reliability whereas the QLF-DI showed to have fair-to-good reliability (Table 2).

Table 2. Intra-examiner agreement obtained for the methods in detecting proximal caries

	ICDAS II	Digital Radiography	QLF-DI	QLF-DF
ICC	0.96*	0.88*	0.74*	0.78*
95% CI	0.91-0.98	0.76-0.94	0.53-0.87	0.59-0.89

ICC, intraclass correlation coefficient.*p<0.0001

3.2. Determination of cut-off values for QLF-DF

Before sensitivity and specificity were calculated for the detection methods, the ΔF values of the QLF-DF were box-plotted related to histological scoring to observe the distribution (Figure 5), and ROC curve analysis was performed to find the optimum QLF-DF threshold values for enamel and dentine lesions (Figure 6 and 7). The optimal cut-off limits for QLF-DF are shown in table 3.

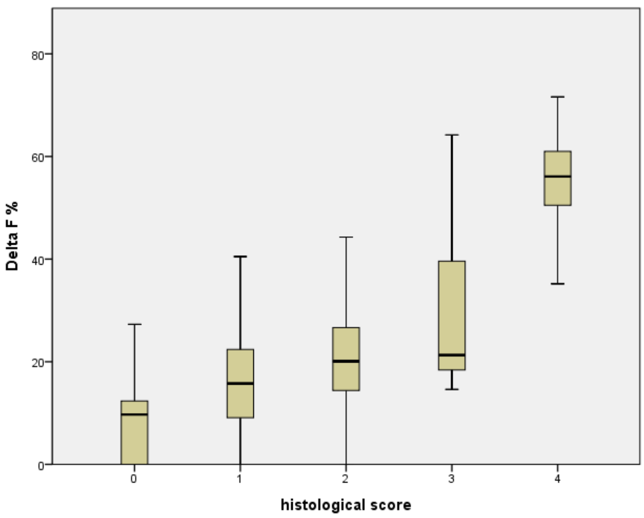

Fig 5. Box plot showing ΔF values related to the histological scoring. Box plots show median, 1st, and 3rd quartiles, minimum and maximum values

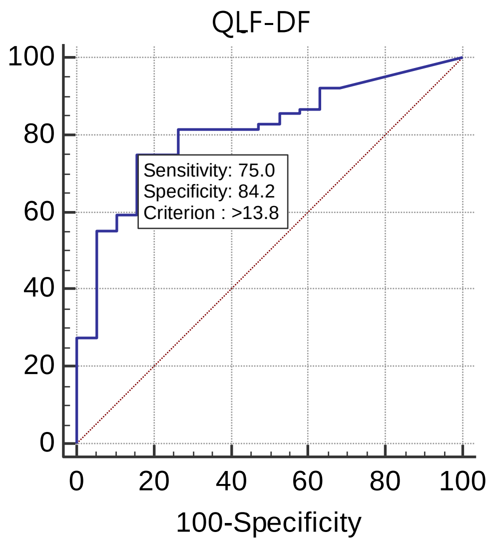

Fig 6. Selected ROC curve of the QLF-DF at enamel threshold

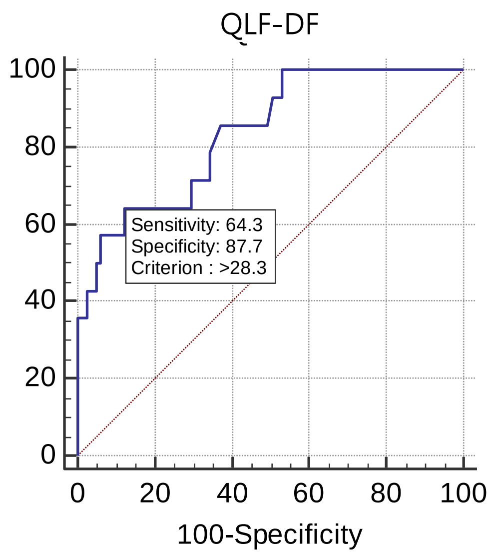

Fig 7. Selected ROC curve of the QLF-DF at dentin threshold

Table 3. Optimal cut-off values of the QLF-DF for detection of proximal caries

Histology	QLF-DF
Sound	≤ 13.8
Enamel lesion	$13.8 < \Delta F \leq 28.3$
Dentin lesion	>28.3

3.3. Comparison of detection methods with histology

Table 4 presents the cross-tabulation for ICDAS II, DR, QLF-DI and QLF-DF with the corresponding histological score. Table 5 shows the agreement between the detection methods and the histological classification, and histological examination of the 95 surfaces revealed that 19 surfaces were sound, 62 surfaces had caries within enamel (D1, D2), while 14 surfaces had caries in dentine (D3, D4).

Table 4. Cross-tabulation for the caries scores detection methods with the corresponding histology scores

Histological score	ICDAS II						Digital Radiography					QLF-DI						QLF-DF			Total
	0	1	2	3	4	5,6	0	1	2	3	4	0	1	2	3	4	5	0	1	2	
S	13	4	1	0	1	0	17	1	1	0	0	10	5	1	2	1	0	16	3	0	19
D1	10	3	9	7	5	0	16	7	9	2	0	1	15	13	3	2	0	13	17	4	34
D2	4	4	7	7	6	0	5	11	9	3	0	3	2	10	9	4	0	6	16	6	28
D3	1	1	3	2	0	2	1	1	4	2	1	0	1	3	2	1	2	0	5	4	9
D4	0	0	0	0	1	4	0	1	0	0	4	0	0	0	1	0	4	0	0	5	5
Total	28	12	20	16	13	6	39	21	23	7	5	14	23	27	17	8	6	35	41	19	95

S, sound; D1, demineralization in outer enamel; D2, demineralization in inner enamel; D3, demineralization in outer dentin; D4, demineralization in inner dentin.

QLF-DF cutoffs: 0, sound; 1, enamel lesion; 2, dentin lesion.

Table 5. Agreement between the detection methods and gold standard histology

Histology	Number of surfaces	Number of surfaces (%) showing agreement with histological classification			
		ICDAS II	Digital Radiography	QLF-DI	QLF-DF
S	19	13(68)	17(90)	10(53)	16(84)
E	62	23(37)	36(58)	40(65)	33(53)
D	14	9(64)	7(50)	10(71)	9(64)

S, sound; E, enamel lesion; D, dentin lesion

3.4. AUC analysis for all detection methods

The sensitivity and specificity values calculated at thresholds D1 and D3 are presented in Table 6 and 7. The QLF-DI was the most sensitive method at both enamel (0.95) and dentin thresholds (0.71), yet it showed moderate specificity at enamel threshold (0.53). The DR was the most specific method at both enamel (0.89) and dentin thresholds (0.94), while it showed moderate sensitivity at dentin threshold (0.50). The AUCs were also calculated, and the range of the AUC was from 0.74 (QLF-DI) to 0.80 (DR and QLF-DF) at enamel threshold, and at dentin threshold, from 0.66 (ICDAS II) to 0.76 (QLF-DF). The highest AUC was obtained for QLF-DF at both enamel and dentin thresholds. (Table 6 and 7, and Figure 8 and 9).

Table 6. Sensitivity, specificity, area under receiver operating characteristics (AUROC) curve of caries detection methods at enamel histological thresholds

	Cut-off points	Sensitivity	Specificity	AUROC (SE)
ICDAS II	0/1	0.80	0.68	0.74 (0.07)
Digital Radiography	0/1	0.71	0.89	0.80 (0.05)
QLF-DI	0/1	0.95	0.53	0.74 (0.08)
QLF-DF	ΔF>13.8	0.75	0.84	0.80 (0.06)

SE, standard error.

Table 7. Sensitivity, specificity, area under receiver operating characteristics (AUROC) curve of caries detection methods at dentinal histological thresholds

	Cut-off points	Sensitivity	Specificity	AUROC (SE)
ICDAS II	2/3	0.64	0.68	0.66 (0.08)
Digital Radiography	2/3	0.50	0.94	0.72 (0.09)
QLF-DI	2/3	0.71	0.74	0.73 (0.08)
QLF-DF	ΔF>28.3	0.64	0.88	0.76 (0.08)

SE, standard error.

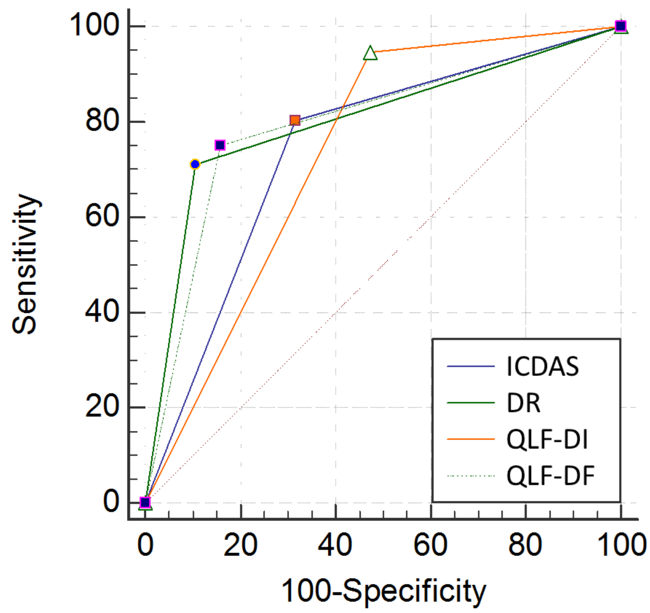

Fig 8. ROC curves of the detection methods at enamel threshold

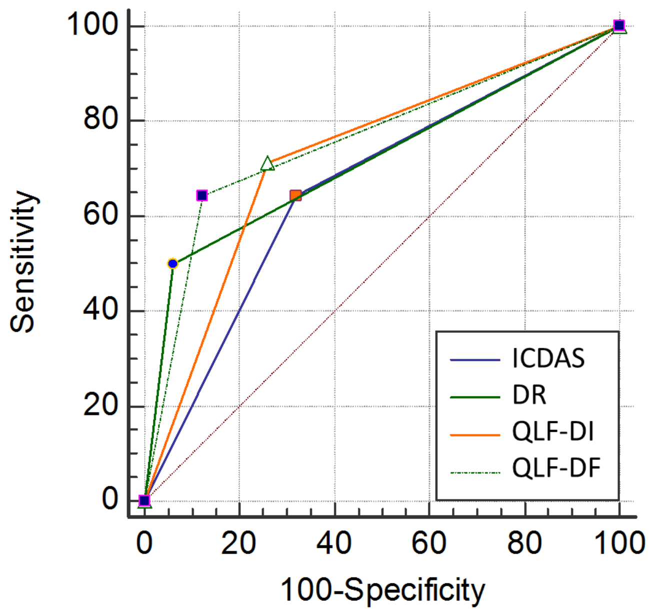

Fig 9. ROC curves of the detection methods at dentin threshold

3.5. Correlations of QLF-DI with QLF-DF and with ICDAS II

The correlations between the QLF-DI and the QLF-DF and between the QLF-DI and the ICDAS II were assessed using Spearman's correlation analysis. Strong correlations were found between the QLF-DI and the ICDAS II with the correlation value of 0.72 ($p<0.01$), and also with the QLF-DF with the correlation value of 0.67 ($p<0.01$).

4. DISCUSSION

As there was a paradigm shift in caries management from a surgical model to a preventive and management model, more attention has been paid to development of simple, quantifiable, and reliable caries detection methods. However, when a new detection device is developed, it also requires accurate examination of the device compared to the established methods. For proximal caries detection, visual examination and radiography have been the conventional diagnostic methods. When the visual plus tactile methods were used only for caries detection, the sensitivity and specificity were reported to be 0.29 and 0.89, which increased to 0.36 and 0.98 when aided with radiography (Mejare et al., 1985). However, these values still indicate that the combination of visual examination and radiography is deficient for detecting early proximal caries lesions.

The QLF has been shown to be a sensitive, valid, and reproducible detection method, and it has been reported that the device enables not only the detection of early caries but also longitudinally monitoring the progression or regression of the lesions (Stookey, 2004). These abilities of the device are based on auto-fluorescence of tooth. When there is a lesion, less fluorescence is emitted from tooth because the lesion actually blocks the excitation light of the device and also the back-scattered fluorescence from dentin, which is necessary for producing fluorescence (van der Veen and de Josselin de Jong, 2000). This phenomenon results in a reduction of fluorescence, and this device is able to detect and quantify the contrast in fluorescence between the lesion and the sound area (van der Veen and de Josselin de Jong, 2000). There have been several previous studies that evaluated the QLF on proximal caries, and one of the earliest study found strong correlation between the QLF and the histological gold standard with the coefficient value of 0.85 for approximal lesion (Shi et al., 2001). Another *in vitro* previous study reported that the QLF could be used to detect interproximal caries lesions at the D2 and D3 level when applied from both buccal and lingual directions (Buchalla et al., 2002). Although the potential of the QLF for the proximal caries detection has been observed *in vitro*, there is no clinical study to validate the performance of QLF. The QLF-D, the newly developed device used in the present study, is based on the same principle but with an enhanced light source and filter system. The present study was performed in order to evaluate the performance of the QLF-D in detecting approximal caries. Thus, simulation pairs were formed of extracted teeth, and 95 surfaces in total were examined using the QLF-D with two different analysis methods, the QLF-DI, the qualitative analysis method, and the QLF-DF, the quantitative analysis method that provides mineral loss in %, and the two analysis methods were validated against histological gold standard, and compared with ICDAS II and digital radiography.

High sensitivity and specificity at both enamel and dentin thresholds were observed for the both analysis methods of the QLF-D, except for the moderate specificity at enamel threshold found for the QLF-DI (Table 6 and 7). This finding suggests that the QLF-D system was able to detect demineralization on proximal surfaces using both analysis methods. Moreover, the QLF-DI, the qualitative analysis method of QLF-D, showed to be highly correlated with the ICDAS II with the spearman's coefficient value of 0.72 ($p<0.01$), and this result corroborates with the finding of the previous study where the correlations between ICDAS and QLF-I examination were 0.79, 0.74 and 0.77 for occlusal/ smooth surfaces/ and combined surfaces (Ferreira Zandona et al., 2010). Based on the results of this study, the present study could be considered as the *in vitro* study that validates the use of qualitative analysis method of the QLF-D as an adjunct to visual techniques in proximal caries detection. In particular, this adjunctive detection method would be appropriate for patients with high prevalence due to its high sensitivity and low specificity. In addition, strong correlation between the QLF-DI and the QLF-DF not only supports the validation, but also suggests that the QLF-DI method may be able to substitute the QLF-DF under certain circumstances. Since the QLF-DI is less time consuming procedure than the QLF-DF, it may be advantageous for epidemiological studies. However, since the QLF-DI is based on subjective visual assessment rather than the use of an automated algorithm, it should be taken account that the accuracy of the QLF-DI can be limited.

The AUROC curves were calculated for all the methods. The highest AUC values at both enamel and dentin thresholds obtained for the QLF-DF confirm that the QLF-D is one of the most accurate caries detection method that enables quantification of caries lesions. In this study, only ΔF (%) was selected from the parameters of the QLF-D such as lesion area and ΔQ, which represents lesion "volume". This was due to the possibility of effects of angle variation on the ΔQ. It was reported that significant changes in ΔQ were observed when the angle of image acquisition was varied buccolingually and cervicocoronally, whereas there was no significant effect of angle variation on the ΔF (Ando et al., 2004).

Although the validity of the QLF-D was confirmed from the present study, it should always be considered that there are factors affecting the validity of the QLF-D including presence of plaque and stain (Amaechi and Higham, 2002). Hence thorough cleaning is necessary for accurate examination using the QLF-D like it was done in the present study.

Diagnostic performance of the traditional detection methods, i.e. visual examination and DR, were evaluated. Visual inspection using ICDAS II was more sensitive in detecting enamel and dentinal carious lesions; however, DR was more specific in enamel and dentinal lesions. Regarding the visual inspection using ICDAS II, the sensitivities at enamel and dentin level were lower than those obtained in a previous study (Mitropoulos et al., 2010), yet considering the previous study was performed on free proximal surfaces, the results of the present study reflect the actual clinical situation where there is limited visibility to proximal surfaces. This study evaluated the DR only, excluding the conventional film radiography, and from the results of the present study, the performance of DR in detecting approximal caries was found to be fair. Although DR has been recently introduced, DR has advantages over conventional radiography including reduced radiation exposure, elimination of dark room processing, ability to manipulate images, and ready storage and communication (Christensen, 2004). However, the problem with use of ionizing radiation, which exposes patients to risk, cannot be resolved, leading into a need of searching other detection methods (Claus et al., 2012).

Since the study was performed by a single trained examiner, the intra-examiner reliability was checked throughout the study. The ICC values showed excellent reproducibility for ICDAS II, DR, and QLF-DF and fair-to-good reproducibility for QLF-DI (Table 2). Reproducibility of ICDAS II and the DR could only be compared to the finding of the previous study (Novaes et al., 2010), and they reported of 0.864 for the reproducibility of the ICDAS II, which was lower than that reported by the present study, and 0.889 for that of the radiography, which was in accordance with that of the present study. The lower value may be due to the fact that the previous study focused on comparing performances of different detection methods in children. Regarding the QLF-D, it also showed high intra-examiner reliability for both qualitative and quantitative analysis methods, which increases the validity of this newly developed device (0.74 and 0.78, respectively; see Table 2). However, as this study relies in only one examiner for all methods, it may be difficult to interpret the results in generalization. Therefore, further study with various examiners is necessary. In addition, although the present study was *in vitro* study which validated the detection methods versus the histological gold standard, there can be a limitation in translating the results into the clinical situation. Further research will be required to assess the novel technology *in vivo*.

5. CONCLUSION

This study assessed the performance of the novel technology based method QLF-D in detecting proximal caries with two different qualitative and quantitative analysis methods, and compared it to those of the traditional methods. Relatively high sensitivity and specificity were achieved for the two analysis methods of the QLF-D, and their performances were similar to those of the traditional methods, indicating the potential for the QLF-D as an effective detection method. The QLF-D would help not only managing caries from early stage but also avoiding unwanted operative treatment. When this device complements the traditional methods, it will help dental professionals to determine accurate clinical diagnosis and make appropriate treatment plan.

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국문초록

Quantitative light induced fluorescence-digital을 이용한 인접면 우식 탐지

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고혜연

치아우식증이 예방 치료를 통해 재광화가 이루어질 수 있다는 사실이 알려지면서 치료에 대한 패러다임이 침습적 치료에서 예방 치료 쪽으로 변화되었고 이러한 변화는 우식병소의 조기 탐지에 대한 중요성을 부각시키는 계기가 되었다. 그러나 인접면 우식병소의 경우에는 해부학적 구조로 인하여 시진만으로 치아우식을 탐지하는데 한계가 있고 방사선 사진 또한 병소 깊이를 과소평가하게 되는 경향이 있어 법랑질에 국한된 우식병소를 확인하기에는 판독의 정확성이 떨어진다. 최근 소개된 Quantitative Light induced Fluorescence-Digital (QLF-D)는 초기우식병소의 탐지에 널리 사용되던 QLF에 새로운 필터를 장착한 장비로서 405 nm의 파장의 빛을

이용하여 우식병소와 건전치면의 형광 발현의 차이를 탐지하고 수치화할 수 있는 장점이 있으나 치아의 평활면과 교합면에 발생한 우식병소에 국한되어 활용되고 있다. 이에 본 연구의 목적은 QLF-D를 이용한 인접면 우식 탐지법의 신뢰도와 타당도를 *in vitro* 상에서 평가하고 기존의 우식 탐지법인 시진 및 방사선 사진의 타당도와 비교하고자 하였다. 또한 QLF-D의 정성적인 분석방법(QLF-DI)과 정량적인 분석방법(QLF-DF) 및 시진과의 관련성을 알아보고자 하였다.

본 연구는 연세대학교 치과대학병원 연구심의위원회(IRB No: 14-0067)의 승인을 받아 진행되었다. 발치된 치아 중 우식의 심도(severity)가 다양한 소구치와 대구치 100개를 선별하여 치아 2 개씩 짝을 지어 변연융선의 높이가 같도록 매몰한 후 ICDAS II 기준에 따른 시진과 방사선 사진을 이용하여 매몰된 치아의 인접면 우식의 심도를 평가하였다. QLF-D로 촬영한 이미지는 QLF-I 기준(QLF+ICDAS)을 이용한 정성적인 방법(QLF-DI)과 전용 분석 프로그램을 이용하여 ΔF 값을 산출하는 정량적인 방법(QLF-DF)을 통해 인접면 우식을 평가하였다. 모든 평가를 마친 뒤, 편광현미경을 이용하여 병소 깊이를 조직학적으로 평가하였다. D1(법랑질에 국한된 우식 병소)과 D3(상아질까지 침범된 우식병소)병소를 탐지하는 모든 검사법의 타당도를 평가하고 비교하기 위해 우식 심도의 조직학적 구분에 비교하여 시진, 방사선 사진, QLF-DI 및 QLF-DF 탐지법의 민감도, 특이도 및 Area under the ROC curve(AUC)를 분석하였다. 모든 검사법의 신뢰도는 검사자내 일치도로 평가하였다. QLF-D의 정성적인 분석방법인 QLF-DI과 정량적인 분석방법인,

QLF-DF 및 ICDAS II과의 관련성은 Spearman's rank correlation으로 검정하였다.

타당도 평가 결과, 정성적인 방법인 QLF-DI는 D1에서 0.74(민감도: 0.95, 특이도: 0.53), 정량적인 방법인 QLF-DF는 0.80(민감도:0.75, 특이도:0.84)의, D3에서 QLF-DI는 0.73(민감도: 0.71, 특이도: 0.74), 정량적인 방법인 QLF-DF는 0.76(민감도:0.64, 특이도:0.88)의 타당도를 보였다. D1에서 ICDAS II의 AUC는 0.74, 방사선 사진의 AUC는 0.80이였고 D3에서 ICDAS II의 AUC는 0.66, 방사선 사진의 AUC는 0.72 로 기존의 탐지 방법인 시진 및 방사선 사진과 비교하였을 때 QLF-DF가 D1과 D3 병소를 탐지하는데 가장 높은 타당도를 보이는 것으로 평가되었다. 검사자내 일치도 평가 결과, ICDAS II, DR, QLF-DF 검사법이 "excellent"범위의 신뢰도를 보였고 QLF-DI는 "fair-to-good"범위의 신뢰도를 보였다. QLF-DI는 ICDAS II와 0.72($p<0.01$)의 QLF-DF와는 0.67($p<0.01$)의 높은 상관성을 보였다.

결론적으로, QLF-D의 두 가지 분석방법을 이용한 인접면 우식 탐지법은 높은 신뢰도와 타당도를 보였고 기존의 검사방법과 비교하였을 때 유사하였다. 따라서 QLF-D는 인접면 우식을 평가하는데 효과적인 장비로 사료된다.

핵심되는 말: 인접면 우식증, 우식 탐지, quantitative light induced fluorescence-digital (QLF-D), QLF-I