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# Assessing the use of Quantitative Light-induced Fluorescence-Digital as a clinical plaque assessment

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ABSTRACT

*Background:* The aims of this study were to compare the relationship between red fluorescent plaque (RF plaque) area by Quantitative Light-induced Fluorescence-Digital (QLF-D) and disclosed plaque area by two-tone disclosure, and to assess the bacterial composition of the RF plaque by real time-PCR.
*Methods:* Fifty healthy subjects were included and 600 facial surfaces of their anterior teeth were examined. QLF-D was taken on two separate occasions (before and after disclosing), and the RF plaque area was calculated based on Plaque Percent Index (PPI). After disclosing, the stained plaque area was analyzed to investigate the relationship with the RF plaque area. The relationship was evaluated using Pearson correlation and paired *t*-test. Then, the RF and non-red fluorescent (non-RF) plaque samples were obtained from the same subject for real-time PCR test. Total 10 plaque samples were compared the ratio of the 6 of bacteria using Wilcoxon signed rank test.
*Results:* Regarding the paired *t*-test, the blue-staining plaque area ($9.3 \pm 9.2$) showed significantly similarity with the RF plaque area ($9.1 \pm 14.9$, $p = 0.80$) at $\Delta R20$, however, the red-staining plaque area ($31.6 \pm 20.9$) presented difference from the RF plaque area ($p < 0.0001$). In addition, bacterial composition of *Prevotella intermedia* and *Streptococcus anginosus* was associated with substantially more the RF plaque than the non-RF plaque ($p < 0.05$).
*Conclusions:* The plaque assessment method using QLF-D has potential to detect mature plaque, and the plaque area was associated with the blue-staining area using two-tone disclosure.


## 1. Introduction

Detection of old plaque in oral cavity has distinct advantages to caution against oral disease. Old and mature plaque cause oral disease and it could be a sign to notify risk of oral disease. However, young plaque do not immediately affect patients' oral disease. Two-tone disclosing agent was developed to distinguish old and young plaque due to the result of diffusion phenomenon of active ingredient [1,2]. It has been frequently used in dental clinic for old plaque assessment. However, plaque disclosures have some limitations of which they cannot selectively disclose only plaque, but dye soft debris and pellicle as well [3]. Also, it needs time to remove the plaque at the chair side. Another method of measurement for old plaque is Silness & Löe plaque index [4]. This index has been developed for grading of plaque thickness. However, it is also relatively time consuming and the result may be influenced by the examiner's subjective decision [5].

Quantitative Light-induced Fluorescence-Digital (QLF-D Biluminator™, Inspektor Research Systems BV, Amsterdam, The Netherlands) is a novel dental diagnostic tool which is based on the autofluorescence of teeth. It is the updated version of the first product, the QLF device (Inspektor™ Pro, Inspektor Research Systems BV, Amsterdam, The Netherlands), and it is able to get more clear plaque image in red using improved filter set (D007; Inspektor Research Systems BV, Amsterdam, The Netherlands). When a tooth with plaque is excited by a visible light of 405 nm from the QLF, red fluorescence were shown on the plaque accumulation area [6,7], and the QLF was able to detect and quantify the area. Previous studies have shown that mature plaque may produce red auto-fluorescence and it is associated with products of microbe metabolism which are called porpyrins [5,6]. The porphyrins are known to be produced from late colonizing oral bacteria, such

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as *Porphyromonas gingivalis* and *Prevotella intermedia* which are usually found in heavily accumulated plaque [8]. A recent study reported that the intensity of red fluorescence of plaque which aged in different concentrations of sucrose had a relationship with low pH and cariogenic plaque [7]. Nevertheless, there is still lack of clinical studies on the characteristics of the RF plaque and its potential pathogenicity.

The prevalence of microorganisms can be investigated using the 16s-rRNA-based polymerase chain reaction (PCR) method. The PCR method has been known that it is the most sensitive and rapid method [9], and real-time PCR using the LightCycler™ system is useful to detect and quantify bacteria in clinical samples [10].

To increase the utility of the QLF-D into dental clinic, more studies are required. Therefore, the aims of this study were to evaluate the quantification method of the RF plaque area by QLF-D can replace existing old plaque assessment method by two-tone disclosure, and to compare of bacterial composition ratio between the RF and non-fluorescent plaque (non-RF plaque) by real-time PCR test *in vivo*.

## 2. Materials and methods

### 2.1. Subjects

Ethical approval was obtained from the Yonsei University Dental Hospital (IRB No: 2-2012-0045). This study was performed from December 2012 to June 2013. This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. Total 50 participants through clinical trial recruitment were included, with a mean age of 34.6 years (±11.3). Inclusion criteria were that the participants have sound anterior teeth with good general health. Volunteer who had stained teeth or dental caries region were excluded. Informed consent was given when the participants visited for this study. They were asked to refrain from any oral hygiene behavior and food intake for at least 4 h before visiting.

### 2.2. Quantitative Light-induced Fluorescence-digital examination

Intra-oral photographs with QLF-D were taken on two occasions before and after disclosing procedure with disclosing solution (2-Tone™, Young Dental, Earth City, USA). Facial surface of upper and lower anterior teeth were taken with edge to edge bite. The tooth surfaces were dried before photographing. Two different images, a QLF image and a white light image, were captured at one shooting with a digital SLR camera (model 550D, Canon, Tokyo, Japan) using following condition: shutter speed of 1/30 s (QLF image) and 1/50 s (white light image), aperture value of 5.6 (QLF image) and 8.0 (white light image), focal length of 0.32 mm, and ISO speed of 1600. The camera was vertically placed on the facial surface. The images were automatically stored by default as a bitmap image (BMP). To reduce ambient light, we covered the cone of the QLF-D with a blackout fabric.

### 2.3. Image analysis of the plaque area

Among 600 anterior teeth, 170 teeth (28.3%) showing the stained plaque in blue (blue plaque) with the disclosing agent when an examiner observed with naked eyes, were selected to investigate the relationship of the plaque area between the RF plaque and the disclosed plaque. Plaque area from the plaque images was revealed as Plaque Percent Index (PPI). The index was calculated by the pixel number of tooth and covered plaque area based on planimetric method [11,12]. The QLF image of the RF plaque was analyzed using proprietary software (QA2 v1.21, Inspektor Research Systems BV, Amsterdam, The Netherlands) (Fig. 2(E)). The software provides pixel numbers of whole tooth area and intensities of red fluorescence as the thirteen threshold levels (from $\Delta R0$ to $\Delta R120$). As increasing the threshold level, it means that fluorescence intensity is getting stronger. For example, $\Delta R30$ means that at least 30% of redness difference with respect to that of sound teeth is exist between the plaque and the tooth [13], and $\Delta R120$ is the strongest red intensity of the plaque. And white light image of the disclosed plaque was analyzed using image analysis software (Image-Pro PLUS, Media Cybernetics, MD, USA). An outline was drawn using an irregular AOI options (Fig. 2(A)–(D)). Then the tap called 'count & measure object' and 'select colors' on manual options were used to adjust color-range within histogram base. The images were generated using a function which displays the value of the red, green, and blue channel (RGB). The red and blue values in the histogram were fixed as 255 and the value of green was adjusted to find thresholds of a border line of the red- and blue-staining plaque. When the red-staining plaque area was selected, the blue-staining area was included. Forty teeth were randomly selected and analyzed to decide the optimum thresholds which could be determined as acceptable on visual assessment by a single examiner. The final threshold was decided as 49 for a border line of the stained plaque in red (red plaque) and 29 for that of the blue plaque. Examiner then transferred it to an Excel spreadsheet to calculate the PPI ($PPI_{RF}$, $PPI_{red}$, and $PPI_{blue}$). All analysis was performed by a single examiner.

### 2.4. Real-time PCR test

To investigate the characteristics of the bacterial composition of the RF plaque, real-time PCR test was performed. Among 50 participants, the plaque samples from 10 subjects (20%) were collected. Plaque emitting red fluorescence was collected as the RF plaque sample. And if the plaque was dyed and did not show red fluorescence, it was collected as the non-RF plaque sample to compare of the bacterial composition. The RF and the non-RF plaque samples were obtained from different teeth of the same subject.

The test was performed according to the manufacturer's instructions. Plaque samples were collected from subject's anterior teeth using sterilized dental probe. The samples were put into a 1.5 ml tube containing 1 ml sterilized distilled water then they were stored in a freezer at −70 °C as soon as possible until their use. Whole genomic DNA was extracted using DNeasy Blood & Tissue kit (Quagen, Chatsworth, CA, USA). Then isolated DNA was quantified by Spectrophotometer (Nanodrop ND-1000; NanoDrop Technologies, DE, USA). Real-time PCR amplification reactions were carried out using Master mixture of 1 μl DNA. The Master mixture used in this study was Light Cycler 480 SYBR Green (Roche Diagnostics, Basel, Switzerland) with LC480II(Roche, Basel, Switzerland). The following bacteria were studied: *Streptococcus mutans* [14], *Lactobacillus casei* [15], *Actinomyces israelii* [16], *Streptococcus anginosus* [17], *P. gingivalis* [18], and *P. intermedia* [19].

The condition for initial denaturation of six bacteria was at 95 °C for 10 min. 50 polymerase chain reaction method cycles were as follows; *P. gingivalis*, *S. mutans* : 50 cycles of 95 °C for 20 s, 58 °C for 20 s, and 72 °C for 20 s, and *A. israelii, L. casei, P. intermedia, S. anginosus* and total bacteria: 50 cycles of 95 °C for 20 s, 50 °C for 20 s, and 72 °C for 20 s. After the amplification, melting curve analysis was performed to identify whether the real-time PCR reaction ordinarily was done.

To compare bacterial compositions between the RF and the non-RF plaque, relative quantification was performed. The result expressed as $C_t$ which is the number of cycle passed threshold to detect the mRNA. To normalize the value, we carried out $\Delta C_t$ (target mean $C$—reference mean $C_t$). Higher $\Delta C_t$ means lower expression of mRNA. Each $\Delta C_t$ of the samples was used to compare the