In vitro Detection of Occlusal Caries on Permanent Teeth by a Visual, Light-Induced Fluorescence and Photothermal Radiometry and Modulated Luminescence Methods

Mahmoud Jallad\textsuperscript{a}  Domenick Zero\textsuperscript{b}  George Eckert\textsuperscript{c}  Andrea Ferreira Zandona\textsuperscript{d}

\textsuperscript{a}Community Health and Emergency Services, Cairo, Ill.  \textsuperscript{b}Oral Health Institute, Indiana University School of Dentistry, and \textsuperscript{c}Department of Biostatistics, Indiana University School of Medicine, Indianapolis, Ind., and \textsuperscript{d}Department of Operative Dentistry, University of North Carolina at Chapel Hill School of Dentistry, Chapel Hill, N.C., USA

Key Words
Caries detection · Fluorescence · Human teeth · Inspektor\textsuperscript{TM} · International Caries Detection and Assessment System · In vitro study · Laser · Occlusal caries · Photothermal radiometry and modulated luminescence · Quantitative light-induced fluorescence · QLF-D · The Canary System\textsuperscript{®} · Visual examination

Abstract

Background: The paradigm shift towards the nonsurgical management of dental caries relies on the early detection of the disease. Detection of caries at an early stage is of unequivocal importance for early preventive intervention. Objective: The aim of this in vitro study is to evaluate the performance of a visual examination using the International Caries Detection and Assessment System (ICDAS) criteria, two quantitative light-induced fluorescence (QLF) systems – Inspektor\textsuperscript{TM} Pro and QLF-D Biluminator\textsuperscript{TM} 2 (Inspektor Research Systems B.V., Amsterdam, The Netherlands) – and a photothermal radiometry and modulated luminescence, The Canary System\textsuperscript{®} (Quantum Dental Technologies, Toronto, Ont., Canada) on the detection of primary occlusal caries on permanent teeth. Methods: A total of 60 teeth with occlusal surface sites ranging from sound to noncavitated lesions (ICDAS 0–4) were assessed with each detection method twice in a random order. Histological validation was used to compare methods for sensitivity, specificity, percent correct, and the area under the receiver operating characteristic curve (AUC), at standard and optimum sound thresholds. Interexaminer agreement and intraexaminer repeatability were measured using intraclass correlation coefficients. Results: Interexaminer agreement ranged between 0.48 (The Canary System\textsuperscript{®}) and 0.96 (QLF-D Biluminator\textsuperscript{TM} 2). Intraexaminer repeatability ranges were 0.33–0.63 (The Canary System\textsuperscript{®}) and 0.96–0.99 (QLF-D Biluminator\textsuperscript{TM} 2). The sensitivity range was 0.75–0.96 while that of specificity was 0.43–0.89. The AUC were 0.43 (The Canary System\textsuperscript{®}), 0.87 (ICDAS), 0.90 (Inspektor\textsuperscript{TM} Pro), and 0.94 (QLF-D Biluminator\textsuperscript{TM} 2). Conclusion: ICDAS had the best combination of sensitivity and specificity followed by QLF-D Biluminator\textsuperscript{TM} 2 at optimum threshold.

© 2015 S. Karger AG, Basel
Dental caries remains the most prevalent chronic disease of children in the USA. Despite a moderate decrease in prevalence in developed countries, an increase has been observed globally [Petersen, 2003; Bagramian et al., 2009]. However, dental caries is largely preventable and can be treated by nonsurgical interventions when detected at the earliest stage of the disease [Nyvad, 2004; Zandona and Zero, 2006; Zero et al., 2009]. This represents a paradigm shift aiming to emphasize disease prevention and conservation of tooth structure [Pitts et al., 2013]. This change in paradigm in caries management to a nonsurgical approach has brought into focus the development of new methodologies for early caries detection.

The International Caries Detection and Assessment System (ICDAS) is a visual assessment that provides a detailed description of lesion severity on a 7-category scale (table 1) [Ismail et al., 2007]. For occlusal caries, ICDAS was shown to have high correlation with histological validation in vitro and found to be reproducible and repeatable [Ekstrand et al., 2007; Ismail et al., 2007; Diniz et al., 2009, 2011, 2012; Mitropoulos et al., 2012; Gomez et al., 2013]. ICDAS also demonstrated usefulness in predicting which lesions are more likely to progress and in making treatment decisions when combined with other detection aids [Braga et al., 2010; Diniz et al., 2012; Ferreira Zandona et al., 2012; Jablonski-Momeni et al., 2012; Gomez et al., 2013]. However, training and calibration are necessary [Diniz et al., 2010; Nelson et al., 2011].

Quantitative light-induced fluorescence (QLF) is based on the phenomenon of tooth autofluorescence that dentin fluoresces more than enamel while caries lesions do not fluoresce at all [Hartles and Leaver, 1953; Alfano and Yao, 1981; Bjelkhagen et al., 1982; de Josselin de Jong et al., 1995]. The first commercial QLF device was Inspektor™ Pro (Inspektor™ Research, Amsterdam, The Netherlands). A newer version was introduced in 2012, QLF-D Biluminator™ 2 (Inspektor™ Research) [Heinrich-Weltzien et al., 2003; Lee et al., 2013]. QLF Inspektor™ Pro has been reported to have a strong correlation with histological validation [Shi et al., 2001; Gomez et al., 2013]. It has been correlated with treatment decisions of clinicians for operative intervention [Alammari et al., 2013] and found reproducible among examiners [Tranaeus et al., 2002; Yin et al., 2007]. However, developmental defects, fluorosis, hypocalcification, and stain may resemble the appearance of caries lesions on fluorescence images [Alammari et al., 2013]. Furthermore, there are no published reports yet on the performance of the new version of QLF, the QLF-D Biluminator™ 2.

### Table 1. Scoring criteria for ICDAS and histology (maximum lesion depth)

| Score | Description |
|-------|-------------|
| 0     | No lesions  |
| 1     | Lesion in outer half of enamel |
| 2     | Lesion in inner half of enamel or outer third of dentin |
| 3     | Lesion in middle third of dentin |
| 4     | Lesion in inner third of dentin |

### Materials and Methods

#### Sample

A total of 60 human nonrestored posterior teeth (equal number of molars and premolars) with fully formed roots and no lesions beyond ICDAS score 3 on proximal or smooth surfaces were selected, in compliance with Indiana University Institutional Review Board, from a pool of anonymous donated teeth collected for the
Oral Health Research Institute, Indiana University School of Dentistry (OHRI-IUSD). Occlusal lesions, selected by an independent trained examiner, represented ICDAS scores 0–4. The teeth were initially stored in 0.1% thymol solution. After cleaning with a bristle brush mounted on a slow-speed rotary handpiece, the teeth were rinsed with deionized (DI) water 20 times over a period of 14 days and then stored in DI water at 4 °C. One occlusal site on each tooth was selected and marked with black marker (fig. 1a) and the teeth were photographed using a light stereomicroscope (DSM, Nikon-SMZ1500; Nikon Inc., Tokyo, Japan).

**Examination**

Three examiners, calibrated on a different set of teeth (n = 30), carried out assessments twice (7 ± 2 days apart) in a random order, using ICDAS criteria for visual examinations and manufacturers’ instructions for all other methods.

**ICDAS**

For ICDAS, the examiners hand-held the teeth and with direct visualization assessed the teeth first wet and then after drying with canned-gas air under headlight LED illumination (Endeavour™ High Resolution Headlight System; Orascoptic, Middleton, Wis., USA), using the full range of ICDAS criteria (0–6).

**Inspektor™ Pro**

Each examiner held the teeth by hand and captured images, after 5 s of drying with canned-gas air, in a dark room. Each examiner later performed analyses of the captured images in a random order.
order, under the same diminished lighting condition. The average loss of fluorescence (ΔF) was calculated in percent.

QLF-D Biluminator™ 2

Each examiner captured images at a fixed distance between the mounted QLF-D camera and the teeth were mounted in wax after 5 s of drying with canned-gas air, in a dark room. Each examiner later performed analyses of the captured images in a random order, under the same diminished lighting condition. ΔF was calculated in percent.

The Canary System®

The examiners held the teeth by hand and then dried the occlusal surface for 5 s with canned-gas air. The tip of the Canary wand was positioned perpendicular and as close as possible to the site to be examined and the measurement was recorded on a scale from 0 to 100 (Canary number, CN) using the quick scan mode.

Histological Validation

After all examinations were complete, the teeth were embedded in acrylic blocks and 3 sections (1 mm thick) were cut at each site using a saw microtome (Leica SP1600; Leica Microsystems, Inc., Buffalo Grove, Ill., USA). The sections were bonded to a specimen slide using cyanoacrylate, polished using silicon carbide grinding paper (1,000 grit), and photographed using a light stereomicroscope. The slides were immersed in 0.1 mM of rhodamine B dye solution for 24 h, rinsed, dried and rephotographed using a light stereomicroscope. Following that, the sections were serially ground (200 μm) using a precise rotary grinding machine (EXAKT 400CS grinder; EXAKT Technologies, Inc., Oklahoma City, Okla., USA) and 1,000-grit grinding silicon carbide paper. Images were taken following each grind to create a series of 10–15 images of each lesion; 2 sections were selected to represent the lesion at its maximum depth and later scored by 2 examiners independently. Disagreements were resolved by consensus after examining the sections together. Lesion depth histological score classification is presented in table 1 [Ekstrand et al., 1997].

Statistical Analysis

Analysis was performed using SAS software version 9.3 (SAS Institute Inc., Cary, N.C., USA). Intraexaminer repeatability and interexaminer agreement of all the methods were calculated using intraclass correlation coefficients (ICC). Performance of the methods was calculated using bootstrap analyses for sensitivity, specificity, percent correct (the sum of true positive and true negative values in a dichotomous table of a diagnostic method) and the area under the receiver operating characteristic curve (AUC). Standard sound threshold was determined at histology score 0, ICDAS score 0, ≤5% ΔF for QLF methods, and CN ≤20 for The Canary System®. Classification trees using recursive partitioning methods and receiver operating characteristic curves were used to determine the optimum cutoff points (thresholds) for the detection methods. The correlation of the measurements for each method with the histology scores and histology lesion depths were calculated. Data from previous studies indicated correlation of approximately 0.7 between methods. With a sample size of 20 sound teeth and 10 teeth for each of ICDAS 1–4, the study was a priori determined to have 80% power to detect a difference in AUC of 0.15 (0.75 vs. 0.90), assuming a two-sided test with 5% significance level.

Results

Figure 1 shows an example of readings by all methods for the same sample along with histological sections.

Examiners Repeatability and Agreement

Interexaminer agreement and intraexaminer repeatability values, using ICC, are presented in table 2. Agreement ranged from 0.48 (The Canary System®) to 0.96 (QLF-D Biluminator™ 2 ΔF). Repeatability ranged from 0.33 to 0.63 for The Canary System® and from 0.96 to 0.99 for QLF-D Biluminator™ 2 ΔF.

Performance

Out of the 60 sites, 15 (25%) were sound, 10 (17%) had lesions limited to the outer half of enamel, 27 (45%) had lesions extending to the inner half of enamel or to the outer third of dentin, 5 (8%) had lesions in the middle third of dentin, and 3 (5%) had lesions in the inner third of dentin.

The standard threshold was ΔF 5% for both QLF methods and 20 on the CN for The Canary System®. The optimum threshold was ΔF 7% for both QLF methods and 25 on the CN for The Canary System®. For ICDAS, score = 0 was both the standard and the optimum. Table 3 lists sensitivity, specificity and percent correct for detection methods at standard and optimum thresholds along
with AUC and correlations with histological scores and depths. The AUC was 0.87 (ICDAS), 0.90 (Inspektor™ Pro), 0.94 (QLF-D Biluminator™ 2), and 0.79 (The Canary System®). The AUC was significantly higher for QLF-D Biluminator™ 2 than for ICDAS (p = 0.0023) and The Canary System® (p = 0.0005) and higher for Inspektor™ Pro than for The Canary System® (p = 0.0214). Correlations of ICDAS, Inspektor™ Pro and QLF-D Biluminator™ 2 with histological score were strong (all ∼0.80, p < 0.001) but were slightly lower for histological depth (all ∼0.70, p < 0.0001). Correlations of The Canary System® with histological scores and depths were much lower (∼0.45, p > 0.10).

## Discussion

Management of dental caries has shifted towards a less interventional approach, with emphasis on preventive interventions to induce lesion remineralization at early disease stages. This trend requires early caries detection devices that are accurate and valid [Pretty and Maupome, 2004a, b; Zandona and Zero, 2006; Zero et al., 2009]. However, for successful longitudinal monitoring, which is vital for assessing the success of preventive intervention, reliability becomes as important as accuracy itself.

This in vitro study has several limitations that impact its clinical implications and, therefore, contemplation should be exercised in extrapolating the results of the study. For instance, in vitro studies are carried out under ideal laboratory conditions and are not representative of practical clinical use. Also, finding a sample representative of the whole spectrum of potential measurements and being well distributed is a big challenge, and the use of extracted teeth constitutes an inherently biased group [Huysmans and Longbottom, 2004]. In this study, the sample was selected based on ICDAS criteria, producing bias towards the ICDAS method that may have led to overestimation of ICDAS performance.

Moreover, the storage conditions of the sample may have an effect on the performance of methods – the effect of storage temperature (frozen vs. refrigerated) on fluorescence readings has been reported [Francescut et al., 2006] and the use of thymol solution as disinfectant had an effect on laboratory lesion demineralization and remineralization [Preston et al., 2007]. However, the use of thymol solution as a storage medium remains a common practice for extracted teeth [Cortes et al., 2003; Ekstrand et al., 2007; Preston et al., 2007; Braga et al., 2010; Diniz et al., 2011; Jablonski-Momeni et al., 2012; Mitropoulos et al., 2012; Gomez et al., 2013] and repeated washing with DI water was carried out in order to eliminate any effect of thymol on the device readings – a concern later expressed, after the sample selection, by the manufacturers of the Canary System®, via personal communication.

The methodology of histological validation shows large variations in the literature. Ideally, it should relate to the parameters that the detection method is evaluating [Nyhad, 2004]. The use of light stereomicroscopy of tooth sections with enhancing dye such as rhodamine B has been reported [Huysmans and Longbottom, 2004; Rodrigues et al., 2012], which makes it standard for comparison, despite the presence of more accurate methods. In this study, the teeth were cut first into sections and then incrementally ground. This was carried out to minimize any effect of thymol on the device readings – a concern later expressed, after the sample selection, by the manufacturers of the Canary System®, via personal communication.

The methodology of histological validation shows large variations in the literature. Ideally, it should relate to the parameters that the detection method is evaluating [Nyhad, 2004]. The use of light stereomicroscopy of tooth sections with enhancing dye such as rhodamine B has been reported [Huysmans and Longbottom, 2004; Rodrigues et al., 2012], which makes it standard for comparison, despite the presence of more accurate methods. In this study, the teeth were cut first into sections and then incrementally ground. This was carried out to minimize any effect of thymol on the device readings – a concern later expressed, after the sample selection, by the manufacturers of the Canary System®, via personal communication.

The methodology of histological validation shows large variations in the literature. Ideally, it should relate to the parameters that the detection method is evaluating [Nyhad, 2004]. The use of light stereomicroscopy of tooth sections with enhancing dye such as rhodamine B has been reported [Huysmans and Longbottom, 2004; Rodrigues et al., 2012], which makes it standard for comparison, despite the presence of more accurate methods. In this study, the teeth were cut first into sections and then incrementally ground. This was carried out to minimize any effect of thymol on the device readings – a concern later expressed, after the sample selection, by the manufacturers of the Canary System®, via personal communication.

The methodology of histological validation shows large variations in the literature. Ideally, it should relate to the parameters that the detection method is evaluating [Nyhad, 2004]. The use of light stereomicroscopy of tooth sections with enhancing dye such as rhodamine B has been reported [Huysmans and Longbottom, 2004; Rodrigues et al., 2012], which makes it standard for comparison, despite the presence of more accurate methods. In this study, the teeth were cut first into sections and then incrementally ground. This was carried out to minimize any effect of thymol on the device readings – a concern later expressed, after the sample selection, by the manufacturers of the Canary System®, via personal communication.
more stages: ‘at least double the number seems desirable’. In this study, 5 stages of depth progression were used, as utilized by Ekstrand et al. [2007]. The histological classification system used here lacks the distinction between inner enamel and outer dentin lesions but because of the threshold used here no effect was expected on calculating the performance of methods.

The selection of cutoff threshold remains debatable and difficult to defend. For instance, an early threshold between sound and earliest stage of enamel caries signifies where preventive treatment could start, while placing a threshold at the middle of dentin could be used to justify a restorative approach [Pereira et al., 2009; Diniz et al., 2011]. In this study, manufacturers of QLF and PTR/LUM methods provide standard threshold that separates sound from early enamel lesion (ΔF ≤5% for QLF; CN ≤20 for PTR/LUM) but there is no suggested threshold by device manufacturer to signify the transition among histological depths.

Thresholds generated by analytical software are usually different than those of manufacturers [Diniz et al., 2012]. The former reflect the balance between sensitivity and specificity to boost method performance based on results from each individual study. This could explain the variety of thresholds found in the literature. Determining thresholds is very complex, which may be influenced by many factors including the expected difference between in vitro and in vivo settings. This may explain the difference between the thresholds of manufacturers (ΔF ≤5% for QLF methods; CN = 20 for The Canary System®) and optimal statistical thresholds (ΔF ≤7% for QLF methods; CN = 25 for The Canary System®) found in this study. Large variation in thresholds is inappropriate to apply in the clinical setting when considering treatment decision [Cortes et al., 2003]. Therefore, it is logical for this study to use the standard threshold as a base for comparisons between methods.

While ICDAS agreement is commonly reported by the means of kappa, ICC is considered superior to kappa in multilevel measures [Banting et al., 2011]. ICC was used in the current study rather than kappa statistics to allow estimation of the repeatability across all 3 examiners at once, rather than by each examiner, and to allow estimation of the agreement across all examiners rather than separately for each pair of examiners, while also accounting for the within-examiner repeatability [Fleiss, 1981]. The interpretation of the ICC depends on the measurement that is being made. Acceptable ICC for ICDAS are lower than acceptable ICC for QLF and PTR/LUM, since ICDAS is a subjective measurement and is therefore inherently harder to repeat. All detection methods in this study had acceptable agreement except for The Canary System® (table 2). Despite the training and calibration done prior to starting the study, the examiners found The Canary System® to be more sensitive to angulation. Reproducibility of QLF-D Biluminator™ 2 was significantly higher than all other methods but this may have been influenced by having the teeth mounted in wax at a fixed distance from the QLF-D camera, whereas teeth in all other methods were hand-held. For ICDAS, similar agreement was reported using ICC by Diniz et al. [2011]. For Inspektor™ Pro, this study reported findings lower than those reported by Yin et al. [2007]. However, repeatability variation among examiners may have been affected by the fact that each examiner analyzed their own set of different images, adding a layer of variation. If the analyses of the Inspektor™ Pro images had been made by a single trained analyst, the variation could have potentially been smaller [Yin et al., 2007]. Nevertheless, more studies are needed to assess the reliability of QLF-D Biluminator™ 2 and The Canary System®.

For assessing methods performance, no single parameter can be used in lieu of all others. Methods that maintain a balance in sensitivity, specificity, percent correct and AUC would be preferred [Pretty and Maupome, 2004a]. A method with comparatively high sensitivity and low specificity can affect treatment decision, which may increase the potential for overtreatment. Disease distribution within a sample is usually specified in order to represent the whole spectrum of potential measurements of the detection methods being evaluated. However, in a dichotomous histological scale, with a threshold between scores 0 and 1, a sample can become readily skewed in its distribution, which may yield to unrealistic performance. In this study, the caries to sound lesion ratio was 3:1, giving higher weight to sensitivity than specificity in calculating accuracy (percent correct). In addition, sensitivity and AUC can be affected by the distribution of the extents of the lesion in the sample. Increasing numbers of deeper (large) lesions, which are easier to detect, will lead to an overestimate of sensitivity, whereas underestimation will occur if there is a relative overabundance of small white spot lesions [Huysmans and Longbottom, 2004].

At the standard thresholds of 5% for ΔF for both QLF methods and 20 for the CN, using Youden's index (sum of sensitivity and specificity minus 1) [Youden, 1950], ICDAS had an acceptable performance and was highest (0.68) among all methods studied. For the QLF methods, AUC values were the highest (0.94), although specificity was significantly lower than for ICDAS (0.60 for Inspektor™ Pro and 0.57 for QLF-D). Specificity was lowest...
(0.43) for The Canary System®. This implies the possibility of considerable overtreatment when using the QLF and PTR/LUM methods. On the other hand, at the statistically optimum threshold of 7% for ΔF for both QLF methods and 25 for the CN, specificity is significantly increased for all methods, yielding the highest Youden index for QLF-D Biluminator™ 2 (0.73). Of course, changing the thresholds for the methods requires more investigation to determine whether these new thresholds are limited to conditions similar to this in vitro study or can be generalized. Gomez et al. [2013] have used 8% for Inspektor™ Pro ΔF as a threshold and found similar findings to the current study for sound surfaces in vitro. Sample selection criteria in Gomez et al. [2013] were very similar to this study.

It is possible that the low performance of The Canary System® in the present study may have been influenced by using a thymol solution as the initial storage medium, despite the repeated washing with DI water. Any such effect could not be identified or quantified with certainty in this study. The Canary System® is still considered relatively new and further investigation into its performance is needed.

Within the constraints of the in vitro conditions of this study, QLF-D Biluminator™ 2 agreement and performance were comparable to, indeed slightly better than, those of Inspektor™ Pro. These findings support the ability of QLF-D Biluminator™ 2 to replace Inspektor™ Pro for quantifying green fluorescence. The analysis process was simpler and since the captured images have a whitish tint instead of green, they are more clinically acceptable, as expressed by the examiners (fig. 1c, d). Nevertheless, further investigations are needed to assess the performance of the QLF-D Biluminator™ 2.

The most important value a detection method can offer is to help in forming a diagnosis that facilitates a treatment decision or to provide a means of reliable longitudinal monitoring of lesion progression or regression. While most treatment decisions are made during the visual examination [Pereira et al., 2009; Diniz et al., 2011; Jablonski-Momeni et al., 2012], Ferreira Zandona et al. [2010] described the potential of using ICDAS combined with Inspektor™ Pro in predicting lesions that are more likely to progress. On the other hand, Pereira et al. [2009] reported a substantial increase in invasive treatment when multiple detection methods are combined. Numerous studies advocate the use of other detection methods as an adjunct to visual examination and not as a replacement [Zandona and Zero, 2006; Pereira et al., 2009; Braga et al., 2010; Diniz et al., 2011, 2012; Jablonski-Momeni et al., 2012; Alammari et al., 2013; Gomez et al., 2013].

Within the constraints of the in vitro conditions used, ICDAS remains acceptable for caries detection, as demonstrated by its ability to detect early caries lesions and high correlation with histological lesion depth. Further investigation into both QLF-D Biluminator™ 2 and The Canary System® is required, especially in the area of identifying appropriate measurement thresholds in relation to treatment decisions.

Appendix

Online supplementary data associated with this article can be found in the online version (for all online suppl. material, see www.karger.com/doi/10.1159/000437214). Additional high-resolution images of sample can be found online at http://www.mrjallad.com.

Acknowledgment

This study was conducted in Indiana University School of Dentistry in fulfillment of a Master Degree. We thank Dr. Gossweiler and Mrs. Patel, of Preventive and Community Dentistry (Indiana University School of Dentistry, Indianapolis, Ind., USA), for serving as examiners. We also thank the staff of the Oral Health Research Institute of Indiana University for their help. This study was partially supported by a grant from Delta Dental Foundation (Okemos, Mich., USA) and a grant from GlaxoSmithKline (GSK, Brentford, UK). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

M.J. and A.F.Z. conceived and designed the experiment and performed the examination. The experiment was performed by M.J. and data were analyzed by G.E. The paper was written by M.J., D.Z., G.E., and A.F.Z.

Disclosure Statement

None of the authors report a conflict of interest.

References

Alammari MR, Smith PW, de Josselin de Jong E, Higham SM: Quantitative light-induced fluorescence (QLF): A tool for early occlusal dental caries detection and supporting decision making in vivo. J Dent 2013;41:127–132.

Alfano RR, Yao SS: Human teeth with and without dental caries studied by visible luminescence spectroscopy. J Dent Res 1981;60:120–122.
Ekstrand KR, Martignon S, Ricketts DJ, Qvist V, de Josselin de Jong E, Sundstrom F, Westerling H, Diniz MB, Boldieri T, Rodrigues JA, Santos-Pinto Braga MM, Mendes FM, Ekstrand KR: Detection and activity assessment of primary changes in initial enamel caries with laser fluorescence. J Dent 2013;41:180–186.

Hartles RL, Leaver AG: The fluorescence of teeth under ultraviolet irradiation. Biochem J 1953;54:632–638.

Heinrich-Weltzien R, Kuhnisch J, van der Veen M, de Josselin de Jong E, Stosser L: Quantitative light-induced fluorescence (QLF) – a potential method for the dental practitioner. Quintessence Int 2003;34:181–188.

Hellen A, Mandelis A, Finer Y, Amaechi BT: Comparision of a combined FOTI/visual examination of occlusal caries with other caries diagnostic methods and the effect of stain on their diagnostic performance. Caries Res 2003;37:8–16.

de Jesselin de Jong E, Sundstrom F, Westerling H, Tranaeus S, ten Bosch JJ, Angmar-Mansson B: A new method for in vivo quantification of changes in initial enamel caries with laser fluorescence. Caries Res 1995;29:2–7.

Diniz MB, Bokdiiri T, Rodrigues JA, Santos-Pinto L, Lussi A, Cordeiro RC: The performance of conventional and fluorescence-based methods for occlusal caries detection: an in vivo study with histologic validation. J Am Dent Assoc 2012;143:339–350.

Diniz MB, Lima LM, Eckert G, Zandona AG, Cordeiro RC, Pinto LS: In vitro evaluation of ICDAS and radiographic examination of occlusal surfaces and their association with treatment decisions. Oper Dent 2011;36:133–142.

Diniz MB, Lima LM, Santos-Pinto L, Eckert GJ, Zandona AG, de Cassia Loiola Cordeiro R: Influence of the ICDAS e-learning program for occlusal caries detection on dental students. J Dent Educ 2010;74:862–868.

Diniz MB, Rodrigues JA, Hug I, de Cassia Loiola Cordeiro R, Lussi A: Reproducibility and accuracy of the ICDAS-II for occlusal caries detection. Community Dent Oral Epidemiol 2009;37:399–404.

Ekstrand KR, Martignon S, Ricketts DJ, Vquist V: Detection and activity assessment of primary coronal caries lesions: a methodologic study. Oper Dent 2007;32:225–235.

Ekstrand KR, Ricketts DJ, Kidd EA: Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: an in vitro examination. Caries Res 1997;31:224–231.

Ferreira Zandona A, Santiago E, Eckert GJ, Katz BP, Pereira de Oliveira S, Capin OR, Mau M, Zero DT: The natural history of dental caries lesions: a 4-year observational study. J Dent Res 2012;91:841–846.

Fleiss JL: Statistical Methods for Rates and Proportions, ed 2. New York, Wiley, 1981.

Francescut P, Zimmerli B, Lussi A: Influence of different storage methods on laser fluorescence values: a two-year study. Caries Res 2006;40:181–185.

Gomez J, Zakian C, Salsone S, Pinto SC, Taylor A, Pretty IA, Ellwood R: In vitro performance of different methods in detecting occlusal caries lesions. J Dent 2013;41:180–186.

Pretty IA, Maupome G: A closer look at diagnosis in clinical dental practice. Part 2. Using predictive values and receiver operating characteristic in assessing diagnostic accuracy. J Can Dent Assoc 2004b;70:313–316.

Rodrigues JA, Neuhaus KW, Diniz MB, Hug I, Stich H, Karlsson L, Lussi A: Comparison among gold standard techniques used for the validation of methods for occlusal caries detection. Micros Res Tech 2012;75:605–608.

Shi XQ, Tranaeus S, Angmar-Mansson B: Comparison of QLF and DIAGNOdent for quantification of smooth surface caries. Caries Res 2001;35:21–26.

Tranaeus S, Shi XQ, Lindgren LE, Tollas K, Attadeh-Moghadam K, Ricketts DJ: In vivo repeatability and reproducibility of the quantitative light-induced fluorescence method. Caries Res 2002;36:3–9.

Yin W, Feng Y, Hu D, Ellwood RP, Pretty IA: Reliability of quantitative laser fluorescence analysis of smooth surface lesions adjacent to the gingival tissues. Caries Res 2007;41:186–189.

Youden WJ: Index for rating diagnostic tests. Cancer 1950;3:32–35.

Zandona AF, Zero DT: Diagnostic tools for early caries detection. J Am Dent Assoc 2006;137:1675–1684; quiz 1730.

Zandona AF, Fontana M, Martinez-Mier EA, Ferreira-Zandona A, Ando M, Gonzalez-Cabaza C, Bayne S: The biology, prevention, diagnosis and treatment of dental caries: scientific advances in the United States. J Am Dent Assoc 2009;140(suppl 1):255–345.