TITLE PAGE

TITLE: In Vitro Detection of Occlusal Caries on Permanent Teeth by a Visual, Light Induced Fluorescence and Photothermal Radiometry and Modulated Luminescence Methods.

AUTHOURS: MAHMOUD JALLAD, Community Health & Emergency Services, Cairo, IL; DOMENICK ZERO, Oral Health Institute, Indiana University School of Dentistry, Indianapolis, IN; GEORGE ECKERT, Department of Biostatistics, Indiana University; and AG FERREIRA. ZANDONA, Department of Operative Dentistry, University of North Carolina at Chapel Hill School of Dentistry, Chapel Hill, NC.

RUNNING HEAD: In vitro Performance of ICDAS, PTR/LUM, QLF and QLF-D.

KEY WORDS: Caries Detection, ICDAS, PTR/LUM, The Canary System, QLF, QLF-D, Inspektor, Occlusal caries, In vitro, Human teeth, Fluorescence, Laser, Visual Examination.

CORRESPONDING AUTHOR:
Andrea Ferreira Zandona, DDS, MSD, PhD
Associate Professor
The University of North Carolina at Chapel Hill - School of Dentistry
Department of Operative Dentistry
436 Brauer Hall, Room 446
Chapel Hill, NC 27599-7450

Jallad, M., Zero, D., Eckert, G., & Ferreira Zandona, A. (2015). In vitro Detection of Occlusal Caries on Permanent Teeth by a Visual, Light-Induced Fluorescence and Photothermal Radiometry and Modulated Luminescence Methods. *Caries Research*, *49*(5), 523–530. http://doi.org/10.1159/000437214

Phone: (919) 537-3978
Fax: (919) 537-3990
azandona@email.unc.edu

DECLARATION OF INTEREST: None of the authors report a conflict of interest. The Study was supported partially by grants from Delta Dental Foundation (Okemos, MI) and a grant from GlaxoSmithKline (GSK, Middlesex, UK).

ABSTRACT:

The paradigm shift towards the non-surgical 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 criteria (ICDAS), two quantitative light-induced fluorescence systems (QLF); Inspektor™ Pro and QLF-D Biluminator™ 2 (Inspektor Research Systems B.V., Amsterdam, The Netherlands) and a Photothermal Radiometry and Modulated Luminescence (PTR/LUM), The Canary System® (Quantum Dental Technologies, Toronto, Canada) on detection of primary occlusal caries on permanent teeth. METHODS: 60 teeth with occlusal surface sites ranging from sound to non-cavitated occlusal 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, % correct and the area under receiver operating characteristic curve (AUC), at standard and optimum sound thresholds. Inter-examiner agreement and intra-examiner repeatability were measured using intraclass correlation coefficient (ICC). RESULTS: Inter-examiner agreement ranged between 0.48 (The Canary System®) and 0.96 (QLF-D Biluminator™2). Intra-examiner repeatability ranged 0.33-0.63 (The Canary System®) and 0.96-0.99 (QLF-D Biluminator™2). Sensitivity ranged 0.75-.096 while specificity ranged 0.43-0.89. AUC was 0.79 (The Canary System®); 0.87 (ICDAS); 0.90 (Inspektor™ Pro); and 0.94 (QLF-D Biluminator™2). CONCLUSION: ICDAS had the best combination of sensitivity and specificity followed by QLF-D Biluminator™ 2 at optimum threshold.

INTRODUCTION:

Dental caries remains the most prevalent chronic disease of children in the US. Despite a moderate decrease in prevalence in developed countries, an increase has been observed globally [Bagramian et al., 2009; Petersen, 2003]. However, dental caries is largely preventable and can be treated by non-surgical 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 non-surgical 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 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 [Diniz et al., 2012; Diniz et al., 2011; Diniz et al., 2009; Ekstrand et al., 2007; Gomez et al., 2013; Ismail et al., 2007; Mitropoulos et al., 2012]. 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; Gomez et al., 2013; Jablonski-Momeni et al., 2012]. 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 [Alfano and Yao, 1981; Bjelkhagen et al., 1982; de Josselin de Jong et al., 1995; Hartles and Leaver, 1953]. The first commercial QLF device was Inspektor™ Pro (Inspektor™ Research, Amsterdam, Netherland). 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 [Gomez et al., 2013; Shi et al., 2001]. It has been correlated with clinicians' treatment decisions for operative intervention [Alammari et al., 2013] and was 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.

Photothermal Radiometry and Modulated Luminescence (PTR/LUM), commercially marketed as The Canary System® (Quantum Dental Technologies, Toronto, Canada), is based on the combination of two slightly different responses of the tooth tissues from a periodic irradiation with a pulsating laser beam; the first response signifies the conversion of absorbed optical energy into thermal energy that results in a modulation in the temperature of tooth structure (PTR). The second response signifies the conversion of absorbed optical energy to radiative energy (LUM) [Hellen et al., 2011; Jeon et al., 2004]. In initial laboratory studies, PTR/LUM is reported to detect lesion as deep as 5 mm and is expressed on a scale of 0-100 to represent lesion severity. PTR/LUM was found to have higher sensitivity and specificity than visual examination, radiography and laser fluorescence [Jeon

et al., 2004]. However, there are no published studies that have used the commercially available The Canary System®.

The aim of this in vitro study is to evaluate the performance of (ICDAS), Inspektor™ Pro, QLF-D Biluminator™ 2 and The Canary System® on detection of primary occlusal caries on permanent teeth.

MATERIALS AND METHODS:

SAMPLE:

Sixty human non-restored 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 of Indiana University School of Dentistry (OHRI-IUSD). Occlusal lesions, selected by an independent trained examiner, represented ICDAS scores 0-4. Teeth initially were stored in 0.1% thymol solution. After cleaning with bristle brush mounted on a slow-speed rotary handpiece, teeth were rinsed with deionized (DI) water twenty times (N=20) over a period of fourteen days, then stored in DI water at 4 °C. One occlusal site on each tooth was selected, marked with black marker (see Figure 1. a) and teeth were photographed using a light stereomicroscope (DSM, Nikon-SMZ1500, Nikon Inc., 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, examiners hand-held the teeth and with direct visualization assessed the teeth first wet then after drying with canned-gas air under headlight LED illumination (Endeavour™ High Resolution Headlight System, Orascoptic, WI, USA) using the full range of ICDAS criteria (0-6).

INSPEKTOR™ PRO:

Each examiner held teeth by hand and captured images, after 5s 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. Average loss of fluorescence in percent (ΔF [%]) was calculated.

QLF-D BILUMINATOR™ 2:

Each examiner captured images at a fixed distance between the mounted QLF-D camera and teeth that were mounted in wax after 5s 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. Average loss of fluorescence in percent (ΔF [%]) was calculated.

THE CANARY SYSTEM®:

Examiners held teeth by hand and then dried the occlusal surface for 5s 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 0-100 (Canary Number) using the quick scan mode.

HISTOLOGICAL VALIDATION:

After all examinations were complete, teeth were embedded in acrylic blocks and 3 sections (1mm thick) were cut at each site using a saw microtome (Leica SP1600, Leica Microsystems, Inc., Buffalo Grove, IL). The sections were bonded to a specimen slide using cyanoacrylate, polished using silicon carbide grinding paper (1000 grit) and photographed using light stereomicroscope. Slides were immersed in 0.1 millimolar (mM) Rhodamine B dye solution for 24 hour, rinsed, dried and re-photographed using light stereomicroscope. Following that, sections were serially ground (200μm) using a precise rotary grinding machine (Exakt 400CS grinder, EXAKT Technologies, Inc., Oklahoma city, OK) and 1000 grit grinding silicon carbide paper. Images were taken following each grind to create a series of 10-15 images of each lesion. Two 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, NC). Intra-examiner repeatability and inter-examiner agreement of all the methods were calculated using intraclass correlation coefficients (ICC). Performance of the methods was calculated using bootstrap analyses for sensitivity, specificity, % correct and the area under the receiver operating characteristic, ROC, curve (AUC). Standard sound threshold was determined at histology score 0; ICDAS score 0; at ≤5% ΔF for QLF methods; and canary number ≤ 20 for The Canary System®. Classification trees using recursive partitioning methods and ROC 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:

Inter-examiner agreement and intra-examiner 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 lesions (5%) had lesions in the inner third of dentin.

Standard threshold was (5%) ΔF for both QLF methods and (20) on the canary number for The Canary System®. Optimum threshold was (7%) ΔF for both QLF methods and (25) on the canary number for The Canary System®. For ICDAS, score = 0 was both the standard and the optimum. Table-3 lists sensitivity,

specificity and % correct for detection methods at standard and optimum thresholds along with AUC and correlations with histological scores and depths. AUC was 0.87 (ICDAS), 0.90 (Inspektor™ Pro), 0.94 (QLF-D Biluminator™2) and 0.79 (The Canary System®). Area under the ROC curve (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, $p<.001$) but were slightly lower for histological depth (all ~0.70, $p<.0001$). Correlations of The Canary System® with histological scores and depths were much lower (~0.45, $p>.10$).

DISCUSSION:

Management of dental caries has shifted towards a less interventive 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, 2004b, a; Zandona and Zero, 2006; Zero et al., 2009]. But 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 study's results. For instance, in vitro studies are carried out under ideal laboratory conditions, not representative of practical clinical use. Also, finding sample representative of the whole spectrum of potential measurements and being well-distributed is a big challenge and constitutes an inherently biased

group [Huysmans and Longbottom, 2004]. In this study, sample was selected based on ICDAS criteria, producing bias towards ICDAS method that may have led to over-estimation of ICDAS performance.

Moreover, storage conditions of sample may have an effect on methods performance: 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 [Braga et al., 2010; Cortes et al., 2003; Diniz et al., 2011; Ekstrand et al., 2007; Gomez et al., 2013; Jablonski-Momeni et al., 2012; Mitropoulos et al., 2012; Preston et al., 2007], 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, post-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 [Nyvad, 2004]. The use of light stereomicroscope 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, teeth were cut first into sections and then incrementally ground. This was carried out to minimize the specimen loss associated with the use of microtome saw.

While caries progress on a continuous scale, histological methods predominantly divide lesions progression into about 4-5 stages of relative depths, to normalize the results for various layer

thicknesses of enamel and dentin [Huysmans and Longbottom, 2004]. However, Huysmans and Longbottom [2004] recommend the need for more stages “at least double the number seems desirable”. In this study, five 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 methods’ performance.

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 [Diniz et al., 2011; Pereira et al., 2009]. In this study, manufacturers of QLF and PTR/LUM methods provide standard threshold that separates sound from early enamel lesion ($\Delta F \leq 5\%$ for QLF; $CN \leq 20$ 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 reflects the balance between sensitivity and specificity to boost methods performance, based on results from each individual study. This could explain the variety of thresholds found in the literature. Determining threshold 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 manufacturers’ thresholds ($\Delta F \leq 5\%$ for QLF methods and $CN=20$ for The Canary System) and optimal statistical thresholds ($\Delta F \leq 7\%$ for QLF methods and $CN=25$ for The Canary System) found in this study. Large variation in thresholds is inappropriate to apply in clinical setting when considering treatment decision [Cortes et al.,

2003]. Therefore, it's logical for this study to use the standard threshold as a base of 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 three 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 ICCs for ICDAS are lower than acceptable ICCs for QLF and PTR/LUM, since ICDAS is a subjective measurement, and therefore is 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, 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, % 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 over-treatment. 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 (% 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 over-estimate of sensitivity, whereas under-estimation 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 canary number, 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 over-treatment when using the QLF and PTR/LUM methods. On the other hand and at the statistically optimum threshold of 7% for ΔF for both QLF methods, and 25 for the canary number, specificity is significantly increased for all methods, yielding the highest Youden's 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's possible that the low performance of The Canary System® in the present study may have been influenced by using thymolised saline as the initial storage medium, despite the repeated washing with DI water, a concern later expressed post-sample selection by the device manufacturer, via personal communication. 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 (Figure-1 "c and 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 [Diniz et al., 2011; Jablonski-Momeni et al., 2012; Pereira et al., 2009], 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 [Alammari et al., 2013; Braga et al., 2010; Diniz et al., 2012; Diniz et al., 2011; Gomez et al., 2013; Jablonski-Momeni et al., 2012; Pereira et al., 2009; Zandona and Zero, 2006].

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 investigations 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:

Supplementary data associated with this article can be found, in the online version. 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, IN) for serving as examiners. We also thank the staff of Oral Health Research Institute of Indiana University for their help. This study was partially supported by a grant from Delta Dental Foundation (Okemos, MI) and a grant from GlaxoSmithKline (GSK, Middlesex, UK). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Conceived and designed the experiment: MJ, FZ. Performed examination: MJ, FZ. Performed the experiment: MJ. Analyzed the data: GE. Wrote the paper: MJ, DZ, GE, FZ.

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. Journal of dentistry 2013;41:127-132.

Alfano RR, Yao SS: Human teeth with and without dental caries studied by visible luminescent spectroscopy. Journal of dental research 1981;60:120-122.

Bagramian RA, Garcia-Godoy F, Volpe AR: The global increase in dental caries. A pending public health crisis. American journal of dentistry 2009;22:3-8.

Banting DW, Amaechi BT, Bader JD, Blanchard P, Gilbert GH, Gullion CM, Holland JC, Makhija SK, Papas A, Ritter AV, Singh ML, Vollmer WM: Examiner training and reliability in two randomized clinical trials of adult dental caries. Journal of public health dentistry 2011;71:335-344.

Bjelkhagen H, Sundstrom F, Angmar-Mansson B, Ryden H: Early detection of enamel caries by the luminescence excited by visible laser light. Swedish dental journal 1982;6:1-7.

Braga MM, Mendes FM, Ekstrand KR: Detection activity assessment and diagnosis of dental caries lesions. Dental clinics of North America 2010;54:479-493.

Cortes DF, Ellwood RP, Ekstrand KR: An in vitro comparison of a combined foti/visual examination of occlusal caries with other caries diagnostic methods and the effect of stain on their diagnostic performance. Caries research 2003;37:8-16.

de Josselin 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 research 1995;29:2-7.

Diniz MB, Boldieri 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. Operative dentistry 2011;36:133-142.

530 Diniz MB, Lima LM, Santos-Pinto L, Eckert GJ, Zandona AG, de
531 Cassia Loiola Cordeiro R: Influence of the icdas e-
532 learning program for occlusal caries detection on dental
533 students. Journal of dental education 2010;74:862-868.
534 Diniz MB, Rodrigues JA, Hug I, Cordeiro Rde C, Lussi A:
535 Reproducibility and accuracy of the icdas-ii for occlusal
536 caries detection. Community dentistry and oral
537 epidemiology 2009;37:399-404.
538 Ekstrand KR, Martignon S, Ricketts DJ, Qvist V: Detection and
539 activity assessment of primary coronal caries lesions: A
540 methodologic study. Operative dentistry 2007;32:225-
541 235.
542 Ekstrand KR, Ricketts DN, Kidd EA: Reproducibility and
543 accuracy of three methods for assessment of
544 demineralization depth of the occlusal surface: An in
545 vitro examination. Caries research 1997;31:224-231.
546 Ferreira Zandona A, Santiago E, Eckert G, Fontana M, Ando M,
547 Zero DT: Use of icdas combined with quantitative light-
548 induced fluorescence as a caries detection method.
549 Caries research 2010;44:317-322.
550 Ferreira Zandona A, Santiago E, Eckert GJ, Katz BP, Pereira de
551 Oliveira S, Capin OR, Mau M, Zero DT: The natural
552 history of dental caries lesions: A 4-year observational
553 study. Journal of dental research 2012;91:841-846.
554 Fleiss JL: Statistical methods for rates and proportions, ed 2d.
555 New York, Wiley, 1981.
556 Francescut P, Zimmerli B, Lussi A: Influence of different
557 storage methods on laser fluorescence values: A two-
558 year study. Caries research 2006;40:181-185.
559 Gomez J, Zakian C, Salsone S, Pinto SC, Taylor A, Pretty IA,
560 Ellwood R: In vitro performance of different methods in
561 detecting occlusal caries lesions. Journal of dentistry
562 2013;41:180-186.
563 Hartles RL, Leaver AG: The fluorescence of teeth under
564 ultraviolet irradiation. The Biochemical journal
565 1953;54:632-638.
566 Heinrich-Weltzien R, Kuhnisch J, van der Veen M, de Josselin de
567 Jong E, Stosser L: Quantitative light-induced
568 fluorescence (qlf)--a potential method for the dental
569 practitioner. Quintessence international 2003;34:181-
570 188.
571 Hellen A, Mandelis A, Finer Y, Amaechi BT: Quantitative
572 evaluation of the kinetics of human enamel simulated
573 caries using photothermal radiometry and modulated
574 luminescence. J Biomed Opt 2011;16:071406.

Huysmans MC, Longbottom C: The challenges of validating diagnostic methods and selecting appropriate gold standards. Journal of dental research 2004;83 Spec No C:C48-52.

Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, Pitts NB: The international caries detection and assessment system (icdas): An integrated system for measuring dental caries. Community dentistry and oral epidemiology 2007;35:170-178.

Jablonski-Momeni A, Stucke J, Steinberg T, Heinzel-Gutenbrunner M: Use of icdas-ii, fluorescence-based methods, and radiography in detection and treatment decision of occlusal caries lesions: An in vitro study. International journal of dentistry 2012;2012:371595.

Jeon RJ, Han C, Mandelis A, Sanchez V, Abrams SH: Diagnosis of pit and fissure caries using frequency-domain infrared photothermal radiometry and modulated laser luminescence. Caries research 2004;38:497-513.

Lee ES, Kang SM, Ko HY, Kwon HK, Kim BI: Association between the cariogenicity of a dental microcosm biofilm and its red fluorescence detected by quantitative light-induced fluorescence-digital (qlf-d). Journal of dentistry 2013;41:1264-1270.

Mitropoulos P, Rahiotis C, Kakaboura A, Vougiouklakis G: The impact of magnification on occlusal caries diagnosis with implementation of the icdas ii criteria. Caries research 2012;46:82-86.

Nelson S, Eggertsson H, Powell B, Mandelaris J, Ntragatakis M, Richardson T, Ferretti G: Dental examiners consistency in applying the icdas criteria for a caries prevention community trial. Community dental health 2011;28:238-242.

Nyvad B: Diagnosis versus detection of caries. Caries research 2004;38:192-198.

Pereira AC, Eggertsson H, Martinez-Mier EA, Mialhe FL, Eckert GJ, Zero DT: Validity of caries detection on occlusal surfaces and treatment decisions based on results from multiple caries-detection methods. European journal of oral sciences 2009;117:51-57.

Petersen PE: The world oral health report 2003: Continuous improvement of oral health in the 21st century--the approach of the who global oral health programme. Community dentistry and oral epidemiology 2003;31 Suppl 1:3-23.

Pitts NB, Ekstrand KR, Foundation I: International caries detection and assessment system (icdas) and its

international caries classification and management system (iccms) - methods for staging of the caries process and enabling dentists to manage caries. Community dentistry and oral epidemiology 2013;41:e41-52.

Preston KP, Higham SM, Smith PW: The efficacy of techniques for the disinfection of artificial sub-surface dentinal caries lesions and their effect on demineralization and remineralization in vitro. Journal of dentistry 2007;35:490-495.

Pretty IA, Maupome G: A closer look at diagnosis in clinical dental practice: Part 1. Reliability, validity, specificity and sensitivity of diagnostic procedures. Journal 2004a;70:251-255.

Pretty IA, Maupome G: A closer look at diagnosis in clinical dental practice: Part 2. Using predictive values and receiver operating characteristics in assessing diagnostic accuracy. Journal 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. Microscopy research and technique 2012;75:605-608.

Shi XQ, Tranaeus S, Angmar-Mansson B: Comparison of qlf and diagnodent for quantification of smooth surface caries. Caries research 2001;35:21-26.

Tranaeus S, Shi XQ, Lindgren LE, Trollsas K, Angmar-Mansson B: In vivo repeatability and reproducibility of the quantitative light-induced fluorescence method. Caries research 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 research 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.

Zero DT, Fontana M, Martinez-Mier EA, Ferreira-Zandona A, Ando M, Gonzalez-Cabezas 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 1:25S-34S.

LEGENDS:

Table 1. Scoring Criteria for ICDAS and Histology (Maximum Lesion Depth).

Table 2. Inter- and intra-examiner agreements using Intraclass Correlation Coefficient ICC (95% CI).

Table 3. Sensitivity, specificity, % correct, Youden's Index, area under receiving operating characteristic curve (AUC) and correlations with histology scores and depths.

Figure 1. Example of readings by all method for the same sample along with histological sections.

Figure-1 (a) photo of occlusal surface of lower molar with ICDAS (3) lesion identified between black markings;

Figure 1 (b) The Canary System showing canary number (55);

Figure-1 (c) analysis of Inspektor Pro image with ΔF value (44%);

Figure-1 (d) analysis of QLF-D Biluminator 2 image with ΔF value (16.7%);

Figure-1 (e) light stereomicroscope images of histological section without enhancing dye with histological score (3);

Figure-1 (f) light stereomicroscope images of histological section with (Rhodamine B) with histological score (4).

ICDAS: International caries detection and assessment system.

ICC: Intraclass Correlation Coefficient. (Statistical term)

CI: Confidence Interval. (Statistical term)

QLF: Quantified Light-Induced Fluorescence.

ΔF: Average loss of fluorescence.

PTR/LUM: Photothermal Radiometry and Modulated Luminescence.

CN: Canary Number on a scale (0~100).

AUC: area under the receiving operating characteristic (ROC) curve.

p : p-value (statistical term).

% correct: percent correct (the sum of true positive and true negative values in a dichotomous table of a diagnostic method).

OHRI-IUSD: Oral Health Research Institute of Indiana University School of Dentistry.

DI: Deionized.

704 TABLES:

Table 1. Scoring Criteria for ICDAS and Histology.

ICDAS		***Histology (Maximum Lesion Depth)***	
Score	***Description***	***Score***	***Description***
0	*Sound tooth surface*	0	*No lesions*
1	*First visual change in enamel*	1	*Lesion in outer ½ of enamel*
2	*Distinct visual change in enamel/dentin*	2	*Lesion in inner ½ of enamel or outer ⅓ of dentin*
3	*Enamel breakdown*	3	*Lesion in middle ⅓ of dentin*
4	*Underlying dark shadow from dentin with or without enamel breakdown*	4	*Lesion in inner ⅓ of dentin*
5	*Distinct cavity with visible dentin*		
6	*Extensive distinct cavity with visible dentin*		

705

706

Table 2. Inter- and intra-examiner agreement using Intraclass Correlation Coefficient ICC (95% CI).

	Agreement			
Detection Method	*Inter-examiner*	*Intra-examiner*		
		Ex. 1	*Ex. 2*	*Ex. 3*
ICDAS	0.72	0.87	0.81	0.85
(QLF) InspeKtor™ Pro ΔF	0.73	0.97	0.51	0.49
QLF-D Biluminator™ 2 ΔF	0.96	0.98	0.96	0.99
(PTR/LUM) The Canary System® CN	0.48	0.33	0.63	0.58

707

708 *Table 3. Sensitivity, specificity, % correct, Youden's Index (J), area under receiving operating characteristic curve (AUC)*
709 *and correlations with histology scores and depths.*

Detection Method	*Threshold*	*Sensitivity*	*Specificity*	*% Correct*	*J*	*AUC*	*Correlation with Histology Score*	*Correlation with Histology Depth*
ICDAS	Sound	0.82	0.86	0.83	0.68	0.87	0.81	0.72
(QLF)	ΔF (5%)	0.89	0.60	0.82	0.49	0.90		
Inspektor™ Pro	ΔF (7%)	*0.87*	*0.82*	*0.86*	0.69		0.80	0.69
QLF-D	ΔF (5%)	0.96	0.57	0.86	0.53	0.94	0.79	0.67
Biluminator™ 2	ΔF (7%)	0.84	0.89	0.85	0.73			
(PTR/LUM)	CN (20)	0.85	0.43	0.74	0.28	0.79	0.44	0.45
The Canary System®	CN (25)	*0.75*	*0.64*	*0.73*	0.39			

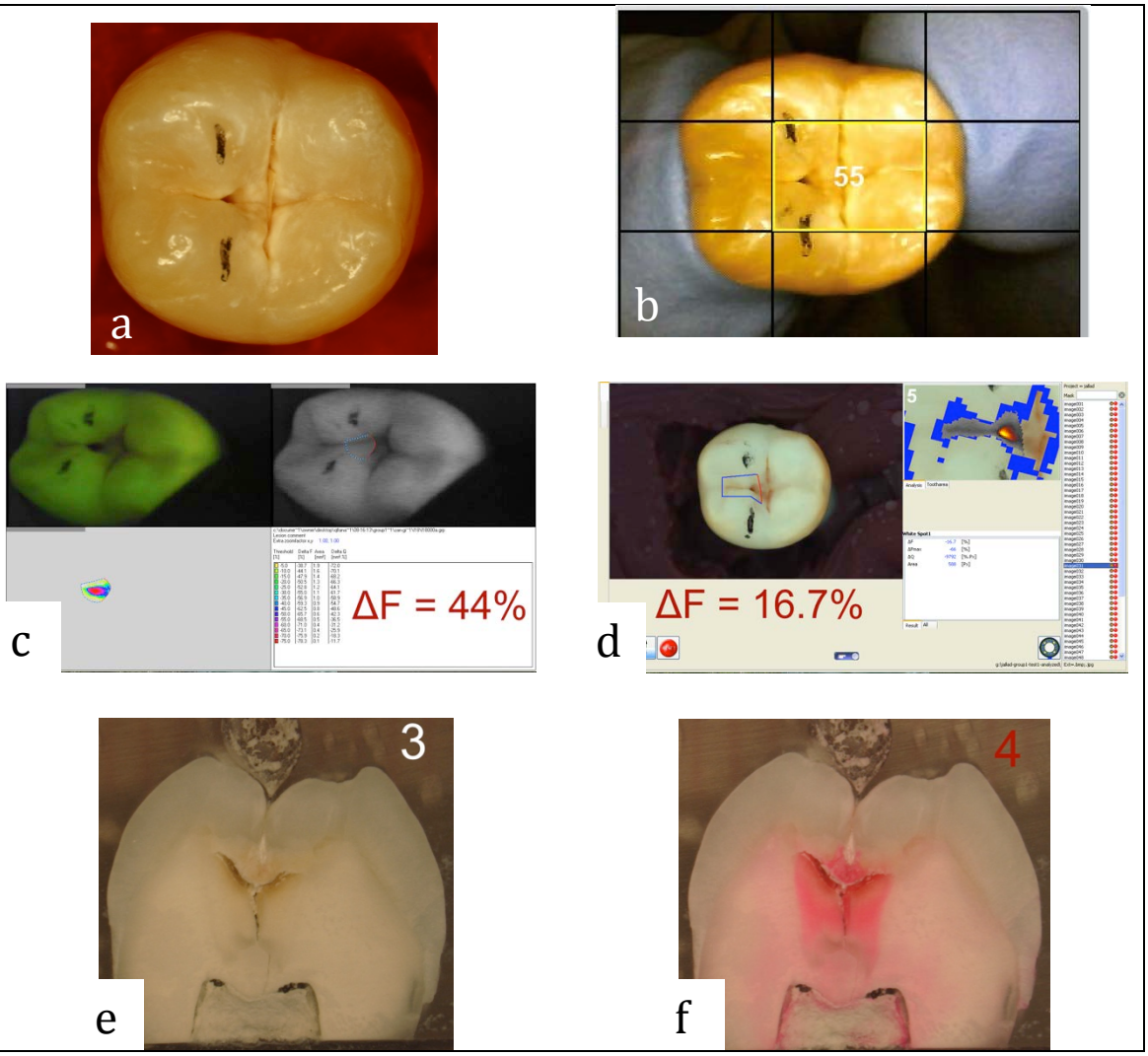

Figure 1. Example of readings by all method for the same sample along with histological sections. (a) photo of occlusal surface of lower molar with ICDAS (3) lesion identified between black markings; (b) The Canary System showing canary number 55; (c) analysis of Inspektor Pro image with ΔF value; (d) analysis of QLF-D Biluminator 2 image with ΔF value; and (e and f) light stereomicroscope images of histological section before and after enhancing dye (Rhodamine B) with histological score on top right corner.