PERFORMANCE OF SEVERAL DIAGNOSTIC SYSTEMS ON DETECTION OF OCCLUSAL PRIMARY CARIES IN PERMANENT TEETH

by

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INTRODUCTION

Archeological evidence dates dental caries from more than 100,000 years ago.[1, 2] In the US, dental caries remains the most prevalent chronic disease of children up to 19 years of age. Over 90 percent of dentate adults 20 years of age and older have experienced dental caries at least once.[3] Although dental caries can be observed in all age groups and across gender, it is unequally distributed among the population across racial, educational, socioeconomic, behavioral, psychological, and self-coherent statuses.[3-5] Although a moderate decrease in caries experience has been reported in developed countries, an increase has been observed globally.[6,7]

Dental caries is largely preventable and treatable. The use of dental sealants as a means to prevent dental caries is well established.[8-11] Dental sealants are mostly effective on pits and fissures of newly erupted posterior teeth, but they have also been utilized therapeutically in sealing carious lesions, including frank cavitation.[12,13] In terms of benefit-to-effort ratio, it was observed that one surface is saved from caries or restoration for every two surfaces sealed over a 15-year period,[14] but sealant effectiveness is dependent on its retention. A five-year retention rate ranges from 33 percent to 87 percent.[3,11,15] Dental sealant was strongly endorsed by the Community Preventive Services Task Force, the Centers for Disease Control and Prevention (CDC), and the American Dental Association (ADA).[10,11,16] However, only 27 percent of children and adolescents aged 5 to 19 have received dental sealants.[17]

Since not all caries lesions progress to cavitation, detection of lesions at the earliest stage can potentially improve prognosis by early preventive intervention and avoidance of a repetitive restorative cycle at a high health and monetary cost.[18,19] Longitudinal monitoring is also of utmost importance, especially under sealants.

The International Caries Detection and Assessment System (ICDAS), which is a visual system; two quantitative light-induced fluorescence systems (Inspektor™ Pro) and (QLF-D Biluminator™ 2), and a new approach using photothermal radiometry and luminescence (The Canary System®) are among the most promising systems in caries detection on occlusal surfaces. While the validity of ICDAS and Inspektor Pro is well established, little is published about the Canary System and QLF-D Biluminator 2, and how they compare with ICDAS and Inspektor Pro.

The aim of this two-part *in-vitro* study is to compare the performance of these devices for the detection of caries on the occlusal surfaces of permanent posterior teeth and under sealants. The null hypothesis of this study is that there will be no difference in terms of sensitivity, specificity, or the area under the Receiver Operating Characteristic (ROC) curve for these methods in the detection of caries on occlusal surfaces of posterior permanent human teeth. The alternative hypotheses are that 1) The non-conventional methods Inspektor Pro, QLF-D Biluminator 2 and the Canary System will have higher sensitivity, specificity, and the area under the ROC curve than will ICDAS for detection of caries on occlusal surfaces of posterior permanent human teeth; and that 2) The Canary System will have higher sensitivity, specificity, and the area under the ROC curve than Inspektor Pro and QLF-D Biluminator 2 for the detection of caries under sealants on occlusal surfaces of posterior permanent human teeth.

REVIEW OF LITERATURE

DENTAL CARIES

Archeological findings date dental caries from over 100,000 years ago as observed in early human remains.[1,2] Drilling cavities in human teeth as a form of intervention was evident as early as 12,000 years ago in Pakistan in the Neolithic period for over a period of 1500 years.[20]

Despite a decrease in caries prevalence of 2 DMFT units in many developed and industrialized countries, an increase of 1 DMFT unit has been reported in developing and poor countries between the years 1980 and 2000.[7] Combined collected surveillance data for many countries between 2001 and 2008 shows that dental caries prevalence is increasing globally at an alarming level.[6] Today in the US, dental caries is still considered the most prevalent chronic disease of children up to 19 years of age. Over 75 percent of the dentate population 5 years of age or older have experienced dental caries at least once; 22 percent had untreated dental caries.[3] In 2010 according to the CDC, more than $100 billion were spent on dental treatment in the US alone.[21] Moreover, the disease is unequally distributed among the population across racial, educational, socioeconomic, behavioral, psychological, and self-coherent statuses.[3-5]

Dental caries is preventable and treatable. Fluoride has many applications in preventing tooth caries and plays a major role in decreasing its prevalence; for instance, community water fluoridation in the US has decreased dental caries by 25 percent across a lifespan.[21] The use of dental sealants for preventing dental caries is well established; a

decrease of 60 percent of occlusal caries in posterior teeth has been reported among children aged 6 through 17.[11] Furthermore, dental sealants are found more effective than fluoride varnish in preventing occlusal caries.[22]

DENTAL SEALANT

The use of dental sealants and their effectiveness in preventing dental caries are well established in the literature.[8-11] Dental sealants are mostly effective on pits and fissures of posterior newly erupted teeth but have also been utilized in sealing frank cavitation.[8,10,12,13] In terms of benefit-to-effort ratio, it was observed that one surface is saved from caries or restoration for every two surfaces sealed over a 15-year period.[14] However, sealant effectiveness is dependent on its retention. While retention rates up to 20 years have been observed, a five-year retention rate for sealant varies between 33 percent and 87 percent.[3,11,15]

Several task forces and expert panels have examined many aspects of dental sealants including: the effectiveness of sealants in reducing caries incidence and prevalence; the risk in developing caries in children who are lost to follow-ups; the utility of caries assessment prior to sealant application; and materials and placement techniques.[8,10,11,16] The evidence supports sealing sound and non-cavitated early lesions after visual assessment, even if a follow-up is not possible. Cleaning the surface to be sealed by a toothbrush or a handpiece prophylaxis followed by acid etch is adequate surface treatment. Using a four-handed technique when possible is highly recommended. Material-wise, resin was found to be the material of choice, while the use of bonding agent remains optional.[10,11] Furthermore, a systematic review by Simonsen in 2002[23] and later

updated in 2011[8] recommended that the sealant material be resin, light-cured, non-filled, non-fluoride releasing, and colored.

The advantages of colored resin sealant over clear sealant are recognized.[8] It was found that colored sealants are easier to apply, to monitor and to assess for retention.[14] Subsequently, higher errors in identifying clear sealants on untreated teeth could potentially lead to misdiagnosis of sealed caries lesions.[24] On the other hand, colored sealants hinder continual visual assessment of covered pits and fissures.[8,24]

In regards to dental sealant prevalence in the US, 27 percent of children and adolescents aged 5 to 19 have at least one dental sealant on a permanent tooth.[17] However, there is a disparity among racial and socioeconomic status: 30 percent of white children and adolescents had a dental sealant compared to 23 percent of Mexican Americans and only 17 percent for African Americans. Almost double the number (32 percent) of children and adolescents who live at or above 200 percent of the poverty level have had a dental sealant placed compared to only 17 percent of those who live at or below the poverty level.[17]

Despite dental sealants proven effectiveness, sealants remain underutilized.[8,25] Lack of insurance, third-part billing concerns, and patient acceptance were cited for being a deterrent to sealant placement but not substantiated.[8] On the other hand, Tellez and colleagues[25] suggested that the credit model adopted in dental schools and later reinforced by the compensation model in clinical practice are responsible for lowering dentists' acceptance of dental sealants, especially where clinical practitioners favor surgical treatment over preventive treatment. The underutilization of dental sealants is not limited to the US; similar findings were also reported in Europe and other countries.[26,27] Despite

the controversy about the best approach to increase the prevalence of dental sealants, applying dental sealants through school-based programs is seen as a viable option to increase their prevalence of use in the US as endorsed by the CDC.[11] However, the Pew Center on the States, part of the Pew Charitable Trust, has reported that 40 US states have failed to meet the healthy-people 2010 goal of covering 50 percent of the children.[28]

One concern that has been identified from professional surveys is the fear of sealing over caries and increasing the risk of caries development.[25,26,29] To the contrary of overwhelming evidence that dental sealant does not increase the risk of caries development,[8,10,11,13,27] a study in Germany found school-based sealant programs to benefit only adolescents with low and moderate caries activity, while increasing the risk for caries in those with high caries prevalence at baseline.[30] In Thailand, partial sealant loss was found to increase caries risk for high caries risk children.[31] Nevertheless, simply providing information to dentists does not change the behavioral and long-standing beliefs against dental sealants.[32] Therefore, if the ability to detect caries under the sealant is demonstrated, it may appeal to such concerns and increase the utilization of dental sealants.

Three *in-vitro* studies have evaluated the detection of occlusal caries under sealants.[33-35] In 2006 Deery et al.[33] used DIAGNOdent (KaVo, Biberach, Germany), a laser fluorescence device, and visual examination methods to detect caries under a clear auto-polymerizing resin sealant. The study concluded that placement of clear sealant lowers the sensitivity and increased the specificity of DIAGNOdent and visual examination, which influences the detection process but does not prevent it.[33] Manton and Messer[34] included a clear and an opaque light-curing resin sealant using three detection methods, visual examination, fiber optic transillumination (FOTI), and laser fluorescence

(DIAGNOdent). The study concluded that the clear sealant had little effect on visual and DIAGNOdent methods, while the opaque sealant significantly decreased sensitivity for visual and DIAGNOdent methods; but the opposite was observed with FOTI.[34] Most recently in 2013, Markowitz[35] used the fluorescent camera, Spectra™ (Air Techniques, Melville, NY) along with 2 opaque and 2 clear resin sealants on extracted third molars. The study concluded that Spectra sensitivity slightly decreased for clear sealant and was abolished for opaque sealant.[35]

So far, there are no published studies on the detection of caries under opaque sealant for Inspektor Pro, QLF-D Biluminator 2 or The Canary System. The manufacturer of the Canary System claims it has the ability to detect caries activity under opaque sealants.[36,37]

DETECTION METHODS

The difference between diagnosis and detection is well established.[38] Diagnosis includes both, the detection of the condition and the analysis of the cause and nature of that particular condition. While detection of dental caries can be facilitated through numerous methods, diagnosis is more complex and is influenced by a large number of factors including individual skills and personal biases,[38,39] and the ultimate goal of making a diagnosis is to select the best possible treatment for immediate and long-term health outcomes.[38] Early caries detection potentially allows early diagnosis and consequently yields to early intervention.[18,19,38]

The performance of various detection systems is routinely assessed through comparison against histological validation, the gold standard.[40] Yet, the so-called “Gold

Standard" of histological validation is not perfect and highly debatable.[38,41,42] For instance, measurement parameters, number of stages utilized to divide lesion progression and processing techniques are among of these concerns.[41] Added to that, there are concerns of the effect of dental sealant on the histological validation process, especially for caries free and early lesions.[43, 44] Nevertheless, the depth of the lesion and the use of enhancing dyes are still considered acceptable criteria.[38,41,42]

Performance or the validity of detection methods is routinely reported in terms of sensitivity and specificity. Sensitivity is the ability to correctly detect disease, where specificity is the ability to identify the healthy.[45] Ideally, a detection method should be 100-percent sensitive and 100-percent specific, yet these two characteristics are inversely related; an increase in sensitivity is often associated with a decrease in specificity and vice versa.[45] Sensitivity and specificity are calculated from contingency tables of dichotomous scale, such as sound or carious. Accuracy is expressed in the ability of a device in detecting the true positive and the true negative in a population. Accuracy is reported as "% correct" when representing the correct calls made by a method.

Since sensitivity and specificity are calculated from a dichotomous scale, so that a threshold is chosen to differentiate the state of healthy and diseased.[46] While this might be acceptable at the time of cavitation, a continuous scale with multiple thresholds or cut-off points would certainly be better in describing early caries lesions.[46] Receiver operating characteristics (ROC) analysis is based on the graphic representation of sensitivity and specificity for the entire range of threshold values of a continuous scale in a plot diagram, which makes it suitable for most of the caries detection methods.[46] The ROC plot is also useful in providing visual comparison of multiple methods at the same time, where the

area under the ROC curve (AUC) offers quantitative analysis for the accuracy of methods.[46]

The reliability of detection method is concerned with the repeatability of measurements. Two forms of tests are routinely described for detection methods: inter-examiner agreement and intra-examiner repeatability.[47] Inter-examiner agreement reflects the degree to which different examiners call the same case, while intra-examiner repeatability compares the calls made by the same examiner over several occasions.[46] For dichotomous scale, reliability is routinely reported in kappa or weighted kappa coefficients. However, even though weighted kappa coefficient is still being utilized for a continuous scale, intra-class correlation coefficient (ICC) is considered superior.[47] Furthermore, ICC allows the calculation of agreements for more than two examiners at once, while also accounting for the within-examiner repeatability.[48,49]

The need is constant for improved caries detection aides. Detection aides are sought to be sensitive, accurate, objective, reproducible, and quantifiable.[38,50] Nevertheless, this should be a matter of balance.[38] For instance, a high-sensitivity system at an early lesion stage may yield a positive health outcome for non-operative intervention, while it may yield a negative health outcome if it was used to justify the placement of a restoration.[19]

Zandona and Zero[18] outlined several methods available for detection of early caries lesions. For occlusal caries, visual examination, transillumination, fluorescence, electrical impedance/conductance, ultrasound, optical tomography, luminescence, and photothermal methods have utility. Pretty and Maupomé published a series of articles concerning many of these caries detection aides.[45,46,51,52] Table I lists reported performances of some

common caries detection modules in relation to sensitivity and specificity as reported in the literature.[50,53-62] Table II lists reported pros and cons of such modules as obtained from the literature[18,19,60,63-67].

Most significantly, traditional exams such as visual and radiographic have been found to have low sensitivity for detecting early stages of occlusal caries.[65] This has geared the research efforts to identify a methodology that has higher sensitivity, specificity, and can provide a scale instead of a binary decision. ICDAS, a visual system; two quantitative light-induced fluorescence systems based on auto-fluorescence, the Inspektor Pro and QLF-D Biluminator 2; and The Canary System, based on luminescence and photothermal radiometry, are among promising new systems in caries detection. While ICDAS and Inspektor Pro validity is well-established for the detection of caries on occlusal surfaces, little is published about the Canary System and QLF-D Biluminator 2 and how they compare with ICDAS and Inspektor Pro. This research project, which is limited to these modalities, provides information on how these newer systems compare with each other in detecting caries on occlusal surfaces of permanent posterior teeth. In addition, it will provide information on the validity of Inspektor Pro, QLF-D Biluminator 2, and the Canary System in detecting lesions under opaque resin sealant, which is of great importance to the dental community.

VISUAL EXAMINATION

G.V. Black was the first to explicitly describe methods of visual and tactile detection of dental caries as part of oral examination, which are still in use.[19] With the current emphasis on early intervention, a detection method at the stage of cavitation, as described by G.V. Black and adopted by The World Health Organization (WHO), is no longer adequate.[19]

New methods focusing on the visual examination of the early stages of caries lesion have been developed. Among these are the Nyvad system[68,69] and ICDAS.[70,71] A ball-ended WHO probe is used with both systems under adequate illumination conditions. The Nyvad caries system is a 7-category index that describes lesions as active or inactive and if sound or cavitated. Further differentiation of the state of cavitation is according to cavity size from a micro-cavitation to large cavitation.[69] ICDAS also uses a 7-category scale (Figure 1 and Table III). However, both systems are different; ICDAS uses an ordinal scale to express severity. For instance, score 3 is more severe than score 1 or score 2. On the other hand, Nyvad uses a nominal, non-ordinal, numeral index. For example, a Nyvad score 5 is more severe than score 4 but less severe than scores 2 and 3. However, score 0 denotes a sound state for both systems.

INTERNATIONAL CARIES DETECTION AND ASSESSMENT SYSTEM: (ICDAS)

ICDAS was developed by a group of international cariologists to give detailed descriptions of lesion severity on a 7-category scale (Figure 1 and Table III).[70,71] ICDAS by itself does not describe lesion activity; however, an adjunct system to assess activity

was developed for use with ICDAS.[72] The lesion activity assessment system (LAA) uses visual and tactile cues to describe lesion as active (+) or inactive (-).[72]

For detecting occlusal caries, ICDAS was shown to have a high correlation with histological validation *in vitro*.[56,58,59,73-75] ICDAS was also found to be reliable and reproducible among examiners.[66,76-78] The ICDAS method when combined with other detection aides has demonstrated usefulness in treatment decision-making.[58,66,73,74,76,79] ICDAS has also been found to be reliable for longitudinal monitoring and has the potential of detecting occlusal lesions that are more likely to progress.[70,76,80]

Training and calibration are necessary for ICDAS. [64,66] Several studies by Braga and colleagues[68,81-84] have compared the ICDAS and Nyvad system *in vitro* and *in vivo*, for caries detection on permanent and deciduous teeth. They found that both systems are comparable in regard to accuracy and reliability; however, ICDAS was found to have a slightly higher sensitivity for lesions limited to the outer half of the enamel,[68] which indicates the ability of ICDAS to characterize early lesions more distinctly than Nyvad. Similarly in 2014 Tikhonova et al.[78] conducted an *in-vivo* study comparing the two systems on (N = 140) young adults, ages 18 to 20 years old, and found that while both systems are comparable, ICDAS has slightly higher sensitivity in detecting early lesion activity.[78] Tikhonova and colleagues[78] said using ICDAS could lead to more outcomes using operative treatment than would the diagnoses with the Nyvad system alone.

QUANTITATIVE LIGHT-INDUCED FLUORESCENCE: (QLF)

Inspektor Pro & QLF-D Biluminator 2

Quantitative light-induced fluorescence (QLF) is based upon the phenomenon of tooth auto-fluorescence as first observed by Hartless and Leaver in 1953,[85] in which dentin fluoresces more than enamel, while caries lesions do not fluoresce at all. The source of auto-fluorescence is not fully understood, but it is thought to originate from the dentin in the dentinoenamel junction (DEJ).[86,87] It was shown that fluorescence is lost if the dentin underlying the enamel is removed mechanically, while fluorescence is produced by a thin layer of underlying dentin.[86,87]

The mechanism of action can be explained in the following manner: once a light source of a certain wavelength is shown on a tooth surface, it passes the transparent enamel and excites fluorophores in the DEJ, which reflect light of a less power and higher wavelength. When both wavelengths, shown and reflected, lie within the visible spectrum, they would have two distinct colors.[85,86] In the case of the two QLF systems mentioned earlier, when a blue light of wavelengths in the range of 290 nm to 450 nm is used for excitation, it was observed that, when looking through a blue filter, sound tooth structure fluoresces green.[88,89] If a caries lesion or an internal defect is present, the defect acts as a double barrier against the excitation light reaching the underlying DEJ, and against the fluorophores-emitted light passing back to the outer surface. This obstruction results in a loss of fluorescence and the appearance of a dark (black) area compared with the neighboring sound surface.[86]

The frequent use of QLF in research led to the observation of red fluorescence, in addition to green fluorescence.[87,90] Red fluorescence was first thought to be correlated with higher caries risk[90] and later was correlated with the presence of certain anaerobic bacteria that are responsible for periodontal disease, not caries.[87] In 2013 an *in-vitro* study

correlated the red fluorescence to intrinsic bacterial metabolites (byproducts) due to the presence of specific nutrients in mature biofilm, rather than the presence of specific bacterial species.[91] Also *in vitro,* red fluorescence was correlated to the cariogenicity of the bacterial biofilm.[92] Meanwhile, more research is being done to better understand this phenomenon.

The first QLF device tested *in vivo* was in 1995.[88] In 2004 the first commercial QLF device was marketed under the name Inspektor Pro (Inspektor™ Research, Amsterdam, Netherland).[93] In 2012 a newer QLF device was released by the same company under the name QLF-D Biluminator 2 (Inspektor™ Research).[93]

The first QLF equipment, Inspektor Pro (Figure 2), utilizes a light box that contains a blue-green arc-lamp with wavelengths of 290 nm to 450 nm with peak intensity of 370 nm.[94] The excitation light travels to an intraoral wand by fiber optic cable. The wand also contains a charged coupled device (CCD) camera covered with a bandpass blue filter.[88,90] The components are connected to a computer where a two-stage process occurs: 1) Capture of the images using the intraoral camera, and later, 2) Analysis of the images using proprietary software to quantify mineral loss of green fluorescence (Figure 3). Inspektor Pro software (QLF Pro, Inspektor™ Research, Amsterdam, The Netherlands) does not quantify the red fluorescence.

The most recent QLF device, QLF-D Biluminator 2 (Figure 4) is based on the same principle of green and red auto-fluorescence as described earlier. The equipment of QLF-D Biluminator 2 is quite different from that of Inspektor Pro.[94,95] QLF-D Biluminator 2 utilizes an extra-oral digital single lens reflex (DSLR) camera rather than an intraoral camera. The QLF-D Biluminator 2 camera is fitted with a 60-mm macro lens and a

modified filter set.[91,92] The light source is replaced with two sets of blue and white light-emitting diodes (LED) mounted on a ring around the lens surrounded by a metal tube. White LEDs are used for standard white-light images, while the set of narrow-spectrum blue light LEDs of a peak wavelength of 405±20nm provide the excitation fluorescent light.[92,94,95]

In a similar way to Inspektor Pro, QLF-D Biluminator 2 camera is connected to a computer that runs the necessary software for archiving images and analysis. The process of performing analysis is also carried out in two stages similar to Inspektor Pro, but because of the modified set of filters in QLF-D Biluminator 2, captured fluorescent images no longer look green, compared with Inspektor Pro, but have a whitish appearance instead (Figure 5). Acquired images are captured and later analyzed using advanced proprietary software to quantify both green and red fluorescence (Figure 5).[91,92]

The software that performs the analysis for Inspektor Pro reports hard-tissue lesion severity by generating three parameters; average loss of fluorescence in percentage (ΔF [%]); lesion area ($A_{\Delta F}$) and fluorescence radiance loss in percentage (ΔQ [%]).[95] In the case of QLF-D Biluminator 2, the same three parameters are reported in addition to three other parameters; maximum loss of fluorescence in percentage (ΔF_{MAX} [%]); the ratio of red to green fluorescence in percentage (ΔR [%]); and the area of red fluorescence ($A_{\Delta R}$) (Table IV).[95] (ΔF [%]) is computed from the surrounding sound tooth structure, and generally an average loss of fluorescence of more than 5 percent denotes the presence of a lesion. The area is expressed either in square millimeters (mm^2) or in pixels for Inspektor Pro, where it is expressed in pixels only for QLF-D Biluminator 2.

Capturing images requires diminished ambient light settings. In addition, stored images have to meet certain criteria to be analyzed satisfactorily. For instance, a sound tooth surface adjacent to the area in question has to be captured on the same frame for proper analysis. For longitudinal monitoring, ambient conditions need to be alike to avoid false changes in consequent analyses. But most significantly, the analyzing software demands that the operator define the shape of the lesion, identify the sound tooth structure on the frame, and set the desired threshold,[95] which can influence all generated parameters. This makes the analytical process somewhat subjective.[96] Figure 6 shows an example of different analyses of the same lesion that yielded slightly different results.

Green fluorescence QLF, such as Inspektor Pro, has been reported *in vitro* to have a strong correlation with histological validation for occlusal caries of permanent teeth[74,97] and of deciduous teeth as well.[98] Also, it was found to be suitable for detecting caries around restorations that are placed post-extraction[99] and secondary caries around amalgam and tooth-colored restorations.[99-101] QLF also has been correlated with clinicians' treatment decisions for operative intervention.[102] QLF was found to reliable and reproducible among examiners and able to provide longitudinal monitoring.[103,104] Zandona and colleagues, as part of a four-year longitudinal study using ICDAS and Inspektor Pro, demonstrated that Inspektor Pro is able to monitor changes in lesion severity and discriminate among lesions that are rapidly progressing and those that are arrested.[105] However, developmental defects, fluorosis, hypocalcification, and stain may resemble the appearance of caries lesions on fluorescence images.[106] Furthermore, there are no published reports yet on the performance of the new version of QLF, QLF-D Biluminator 2.

THE CANARY SYSTEM

Photothermal Radiometry and Modulated Luminescence: (PTR/LUM)

PTR/LUM is based on the combination of two slightly different responses of the tooth medium from a periodic irradiation with a pulsating laser beam; the first response is the photothermal radiometry (PTR), which signifies the conversion of absorbed optical energy into thermal energy that results in a modulation in the temperature of tooth structure. The second response of the medium is the modulated luminescence (LUM), which signifies the conversion of absorbed optical energy to radiative energy (Figure 7).[107,108]

In other words, when the PTR/LUM laser light is shown on a tooth surface, some photons will get reflected from the surface, others will scatter in the medium and the remainder will get absorbed by the tooth structure. The scattered and absorbed energy yields a change in both optical and thermophysical properties, which is influenced by the change in enamel and dentin structure caused by the caries process.[107,109]

PTR-registered signals are dependent on the penetration depth of thermal waves in two distinct modes: conductively for the first 50 μm to 500 μm and radiatively for as deep as 5 mm beneath the tooth surface. LUM signals register the emitted higher-wavelength light photons produced by the excited-hydroxyapatite absorbed-energy. The relaxation lifetimes of hydroxyapatites differ between sound and caries enamel, which yield different

LUM signals. However, LUM only characterizes the quality of the outer enamel surface.[107] Moreover, LUM can also be influenced by the light reflected and scattered from the surface, which is minimized by the means of adding a special filter.[107,109,110] Heat generated by the PTR/LUM exciting laser inside the tooth is found to be less than 2℃, which makes it safe to use.[108,109,111]

The PTR/LUM system registers four signals: PTR-phase, PTR-amplitude, LUM-phase, and LUM-amplitude. The amplitude signals represent the strength of the signals, while the phase signals represent the delay in the signals. Signals are received via two distinct sensors: an infrared thermal detector for the PTR signals, and a separate photodetector for the LUM signals. These four signals are combined by a proprietary computer software via a specific formula to produce a single numeric number, which is expressed on a scale of 0 to 100 to represent lesion severity.[112,113] The numeric number is referred to as the Canary Number and is the only number available for the end user to see (Figure 8). The Canary Number corresponds to three statuses of tooth conditions: sound (0 to 20); caries (21 to 70), and advanced caries (71 to 100).

PTR/LUM is commercially available as the Canary System (Quantum Dental Technologies; Toronto, Canada). The Canary System (Figure 9) consists of an intra-oral wand (Canary Wand) connected to a control station that also supplies power. The device is connected to a computer loaded with proprietary software to perform necessary analysis and archiving. The wand hosts a semiconductor laser diode, which emits a low-power pulsating laser light of a wavelength of 660 nm at a fixed frequency. Mounted on the tip of the wand are a PTR detector, a LUM detector, an intraoral digital camera, and white LEDs.[113]

The intraoral camera and white LEDs are not part of the PTR/LUM method per se; however, it offers practitioners the convenience of capturing standard intraoral images. The software offers two modes of usage, depending on whether the use of intraoral camera is included: 1) A quick scan mode only performs PTR/LUM technique to produce the Canary Number. The Canary Number obtained by this mode is lost following a few scans and cannot be retrieved; 2) The other scan mode, the detail scan, captures patient information, intraoral images of the tooth, and the Canary Number in an intuitive graphical user interface (GUI) (Figure 10). The use of the device mandates wearing safety eyewear by the patient, practitioner, and any assisting personnel. The device also has to be calibrated at least every 24 hours between uses. The laser beam is very focused with a diameter of 1.5 mm that only covers a very small area of the tooth surface; therefore, the manufacturer recommends multiple readings across each surface of the tooth to register each area of interest, where each reading takes between 5 seconds to 10 seconds to perform. Finally, the device is very sensitive to beam angulation; a small change in the angle corresponds to a unique section of tooth structure, which could yield a different Canary Number, making reliable longitudinal monitoring a real challenge.

In 2004 Jeon et al.[107] reported based on an *in-vitro* study that an experimental PTR/LUM system was found to have higher sensitivity and specificity than visual examination, radiography, and DIAGNOdent for detection of pit and fissure caries. Jeon et al. found that visual examination was conducted according to a custom-made index on a scale of 1 to 10 to indicate the choice of treatment rather than the presence or absence of caries itself. Later in 2007, Jeon et al.[114] reported in an *in-vitro* study that an experimental PTR/LUM system was more reliable than conventional caries detection techniques, such

as visual examination and radiography, in detecting artificially made interproximal demineralized lesions. Moreover, Jeon et al.[109] reported that PTR/LUM can detect early enamel and root caries lesions made via a cyclic model of demineralization/remineralization.

So far, no published studies have used the commercially available Canary System, or any *in-vivo* studies using the early experimental PTR/LUM system.

STUDY OBJECTIVES

This *in-vitro* study is designed to answer two objectives.

Objective I: Evaluate the performance of the visual criteria ICDAS, PTR/LUM method, the Canary System, the quantitative light-induced fluorescence device Inspektor Pro, and the quantitative light-induced fluorescence camera, QLF-D Biluminator 2, for the detection of caries on the occlusal surfaces of permanent teeth.

Objective II: Evaluate the performance of the Canary System, Inspektor Pro, and QLF-D Biluminator 2 for detection of caries under sealants on the occlusal surfaces of permanent teeth.

HYPOTHESIS

Objective I

Null Hypothesis I: There will be no difference in terms of sensitivity, specificity or AUC for ICDAS, the Canary System, Inspektor Pro, and QLF-D Biluminator 2 methods in detection of caries on occlusal surfaces of posterior permanent human teeth.

Alternative Hypothesis I: The non-conventional methods, the Canary System, Inspektor Pro and QLF-D Biluminator 2, will have higher sensitivity, specificity, and

AUC than the ICDAS criteria for detection of caries on occlusal surfaces of posterior permanent human teeth.

Objective II

Null Hypothesis II: There will be no difference in terms of sensitivity, specificity, or AUC for the Canary System, Inspektor Pro and QLF-D Biluminator 2 methods in detection of caries under resin sealants on occlusal surfaces of posterior permanent human teeth.

Alternative Hypothesis II: The Canary System will have higher sensitivity, specificity, and AUC than Inspektor Pro and QLF-D Biluminator 2 for detection of caries under resin sealants on occlusal surfaces of posterior permanent human teeth.

MATERIALS AND METHODS

DESIGN MODEL

This *in-vitro* study was conducted in two parts: The first part assessed the use of ICDAS, the Canary System, Inspektor Pro, and QLF-D Biluminator 2 on the occlusal surfaces of posterior permanent human teeth. In the second part of the study, ICDAS, the Canary System, Inspektor Pro and QLF-D Biluminator 2 were applied on the occlusal surfaces of a second set of posterior human teeth before the teeth were sealed, while only the non-conventional methods, the Canary System, Inspektor Pro and QLF-D Biluminator 2 were applied again after the surfaces were sealed. All teeth were later sectioned and examined under a light stereomicroscope using an enhancing dye for histological validation and data were statistically analyzed. The study was conducted in compliance with Indiana University Institutional Review Board, IU-IRB #1302010696.

PART I: PRIMARY CARIES DETECTION ON OCCLUSAL SURFACES OF PERMANENT TEETH

Specimens Selection and Preparation

Sixty human posterior teeth (N = 60) were chosen from a pool of teeth at the Oral Health Institute of Indiana University School of Dentistry (IUSD). All teeth were received from donor dental offices de-identified and were stored in 0.1-percent thymol solution until selection.[115] Tooth selection criteria included non-restored human posterior teeth with fully formed roots and no lesions above ICDAS score 3 on proximal or smooth surfaces. Teeth, selected by an independent trained examiner, represented ICDAS scores

0-4. Sample distribution included 20 teeth of ICDAS score 0 and 10 teeth for each of ICDAS scores 1 thru 4, where each ICDAS score sample had an equal number of molars and pre-molars. ICDAS scoring was performed according to the criteria set by the International Caries Detection and Assessment System ICDAS Committee (Table III).

Teeth were cleaned with an Abbot-Robinson stiff brown bristle brush (Buffalo Dental Manufacturing Co. Inc.; Syosset, NY). Bristle brushes were mounted on a slow-speed air driven rotary handpiece. Cleaning was performed under a stream of deionized (DI) water in a manner to remove attached soft tissues and debris from tooth crowns. Brushes were replaced as often as needed due to functional wear. Teeth were stored together in a plastic container of 0.1-percent thymol solution at 4℃ until further processing.

DI water rinsing regimen for specimens was performed on all teeth because of the concern that the Canary System manufacturer had with the possible effect of thymol as storage medium on PTR/LUM readings. In the literature, the use of thymol solution as a storage medium is well established.[55,58,61,72-74,76,77, 84,115] Possible effects of thymol on laboratory lesion demineralization and remineralization using artificial saliva were reported.[115] However, there was no precedent protocol on how to rinse thymol completely off teeth or the effect of thymol on any other caries detection devices. Therefore, a rinsing protocol was experimental and was devised after consultation; specimens were thoroughly rinsed with DI water and then moved to fresh containers of DI water. This process was repeated 20 times over a period of 14 days.

A single-blind random sample of teeth (N = 9) were sent to the Canary System manufacturer for measurements to determine if the rinsing protocol described earlier was

adequate for removing the effect of thymol. Of the nine samples, 6 were only stored in 0.1-percent thymol and did not receive any further treatment, where the other 3 teeth were rinsed with DI water according to the devised protocol. Sample teeth were individually wrapped with soaked-wet gauze of the same storing solution, either 0.1-percent thymol or DI water, and placed individually in plastic containers. The Canary System manufacturer provided sample readings, which were statistical analyzed by Indiana University Department of Biostatistics, and showed no significant differences between the two storage solutions.

One occlusal site on each tooth was selected, and the site was marked with black marker (Sharpie Ultra Fine Point Permanent Marker, Newell Rubbermaid Office Products, Oak Brook, IL). The sample was randomly numbered and stored individually in plastic containers filled with DI water at 4℃ until use. During the examination process, the sample was removed from refrigeration and kept at a room temperature until work was concluded, and then the sample was returned to the 4℃ fridge. Teeth were mounted on a block of softened red boxing wax (Patterson Boxing Wax, Patterson Dental; St. Paul, MN) with occlusal surfaces facing up. Two sets of white-light images were taken of each tooth; wet and again after 5 seconds of drying using canned air (Office Max Gas Computer Duster, Office Max; IL). The selected site of each specimen's digital image was masked with an obscuring circle intended not to influence the image-guided examination process (Figure 11). White-light images were taken using a digital stereomicroscope at X1 magnification, (DSM, Nikon-SMZ1500, Nikon Inc.; Japan), a white-light illumination ring that surrounds the lens opening (high intensity illuminator, Nikon NI-150, Nikon Inc.;

Japan), a Nikon digital camera (DXM1200, Nikon Inc.; Japan) and image capturing software (ACT-1 version 2.70, Nikon Inc.; Japan) (Figure 12).

These images were displayed on a 13" laptop liquid crystal display (LCD) of a MacBook Pro (Apple Computers; Cupertino, CA) from a viewing distance to guide examiners during each of the exams.

EXAMINERS

Three examiners (two DDS faculty members and one dental hygienist), all previously calibrated on ICDAS, participated in the study. Prior to the examination, all examiners participated in a discussion, training, and calibration on the different methods of caries detection using a different set of teeth (N = 30) representing ICDAS scores 0 thru 4. Initial training on the Canary System and QLF-D Biluminator 2 was provided on site by each manufacturer, respectively. Inspektor Pro training was provided by one of the faculty examiners experienced on the device. Examiners were considered calibrated on ICDAS and QLF once the intraclass correlation coefficient (ICC) threshold of 0.60 was surpassed for both inter-examiner and intra-examiner reliability.[47] However, inter-examiner agreement for the Canary System was 0.57 and intra-examiner repeatability ranged between 0.46 and 0.58 (Table VI). All assessments for calibration and examination were performed according to manufacturers' instructions and were carried out twice, in a random order, by the three examiners with 7±2 days between the measurements. The random order was designed to be the same for the same device across examiners; for instance, the same exact order was given to all examiners during first assessments of the Canary System, which was different from the order of all other assessments.

EXAMINATION

Visual Examination: ICDAS

Test sites were examined with direct visualization of the wet teeth under headlight LED illumination (Endeavour™ High Resolution Headlight System; Orascoptic, WI) and examined again after 5 seconds drying with canned gas air (Figure 13) using the ICDAS criteria 0 to 6 (Table III and Figure 1).

INSPEKTOR PRO

For Inspektor Pro, each examiner captured the images using QLF Pro™ software version 2.0.0.32 (QLF Pro) in a dark room in a sequential order (Figure 14). Prior to capturing images, each surface was dried for 5 seconds with canned gas air. Examiners were allowed the discretion to retake images ad libitum until an image was deemed acceptable by the examiner.

Images were then analyzed in a devised random order by the same examiner at a separate time independently and guided by the white light images and under the same diminished lighting condition to improve the process as previously described, using QLF software version 2.00h (QLF, Inspektor™ Research Systems). The following parameters were recorded by the software: the average loss of fluorescence in percentage (ΔF [%]); area of the lesion in mm^2; and the relative radiance of the lesion in percentage (ΔQ [%]) (Table IV and Figure 3). The threshold of 5-percent ΔF was set as a cutoff point between sound and caries lesions.

QLF-D BILUMINATOR 2

For the QLF-D camera, the same examiner performed image capturing and analysis separately, similar to Inspektor Pro, in a darkened room. The QLF-D camera was mounted on a generic digital camera tripod (Ambico Camera Tripod Stand) at a close distance and perpendicular to the working bench (Figure 15). For capturing images, a person other than the examiner mounted each tooth on a block of softened red boxing wax with the occlusal surface facing up; the tooth was air dried for 5 seconds with canned gas air and then placed on the bench in the approximate center of the camera's frame. Blue light images were taken using QLF-D Billuminator™ C3 software version 1.23.0.0 (C3, Inspektor™ Research). Examiners were allowed the discretion of using automatic or manual focus and whether to accept or repeat image taking.

Analyses were performed in a random order under diminished lighting conditions. A white-spot lesion analysis of blue light images was performed using a QLF-D QA2 analysis software version 1.23.0.0 (QA2, Inspektor™ Research Systems). The following parameters were recorded by the software: the average loss of fluorescence in percentage (ΔF [%]); the maximum loss of fluorescence in percentage (ΔF_{MAX} [%]); and the area of the lesion in pixels, and the relative volume of the lesion in percentage (ΔQ [%]). The threshold of 5% ΔF was set as a cutoff point between sound and caries lesions (Table IV and Figure 5).

THE CANARY SYSTEM

Teeth were examined after 5 seconds drying with canned gas air. The tip of the 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 (Figure 8). Measurements were taken using the quick scan mode (Figure 10) of the Canary System software version 1.3.8.22 (Quantum Dental Technologies). Examiners wore laser safety goggles during examination (Figure 16). Examiners were allowed the discretion of repeating the measurement, if needed, before it was recorded; however, a maximum of 3 readings per tooth were allowed. The final reading was recorded if more than one reading was taken.

PART II: PRIMARY CARIES DETECTION UNDER SEALANT ON OCCLUSAL SURFACES OF PERMANENT TEETH

Specimens Selection and Preparation

As described earlier in (Part I), 60 human posterior teeth were selected using the same criteria; however, there were slightly more molars (N = 32) than premolars (N = 28). Specimens received the same cleaning and storage protocol with DI water. Site selection and digital images were taken before sealant placement and then again after sealant placement.

For placing sealant, teeth were mounted on blocks of softened red boxing wax with occlusal surfaces facing up. Occlusal surfaces of teeth were etched for 30 seconds using 35-percent phosphoric acid (Ultra Etch, Ultradent Products, Inc.; South Jordan, UT), and then rinsed for 30 seconds with DI water and dried with canned gas air until a frosted occlusal surface appeared. Opaque resin sealant (Delton® light cure direct delivery system (DDS) – opaque, Dentsply Caulk; York, PA) was placed on the occlusal pits and fissures and spread out carefully with a dental probe to minimize air entrapment. For each tooth, a single dose tube (0.8 ml) was used, while trying to use the least amount needed to cover

and seal all occlusal fissures. The applied sealant was light cured, using Optilux 501 halogen light curing unit, (Optilux 501, Demetron, Kerr Dental; Orange, CA), for 20 seconds keeping the curing tip as near as possible without touching the sealant (Figure 17).

The light-cure unit output was monitored at the beginning, and between every 10 specimens using the built-in Radiometer of Optilux 501 unit and with a second visible light-curing meter (Cure Rite, Dentsply Caulk; Milford, DE). For both meters, light intensity exceeded the minimum threshold, 300 mW/cm^2 per the manufacturer's recommendations. Retention was assessed directly following sealant placement by trying to dislodge the placed sealant with a dental explorer. Specimens were placed back in their individual container immersed in DI water for at least 24 hours before the second set of white-light images were taken using Nikon digital stereomicroscope as described in Part I.

EXAMINERS

Only two examiners, (Ex.1) and (Ex.2), assessed selected lesions for Group 2 prior to and after placement of the sealant following the same protocol as described in Part I.

CARIES DETECTION

Detection methods ICDAS, the Canary System, Inspektor Pro and QLF-D Biluminator 2 were applied as described earlier in Part I prior to the placement of the sealants. A second set of examinations was carried out using the Canary System, Inspektor Pro, and QLF-D Biluminator 2 after the surfaces were sealed. However, a blue color-softened periphery wax was used to mount Group 2 teeth for QLF-D Biluminator 2 rather than red color softened wax as used for Group 1. Different wax color was used to facilitate easier identification of the groups' photos.

HISTOLOGICAL VALIDATION

Histological validation was performed on all teeth. To prepare specimens, teeth were imbedded in acrylic resin blocks. Teeth were placed at the bottom of a 60-ml universal specimen plastic container (Universal Specimen Plastic Container, Medline Industries, Inc.; Mundelein, IL). Containers were then filled with a mixture of a plastic solution of three ingredients:[116] a 94.5-percent of poly methyl methacrylate acrylic resin (PMMA, Pfaltz & Bauer; Waterbury, CT), 5-percent Dibutyl Phthalate Plasticizer (DBP, Sigma Chemicals Co.; St. Louis, MO) and 0.5-percent organic peroxide free radical solution-polymerization initiator (Perkadox-16, Akzo Noble Polymer Chemicals B.V.; Amersfoort, The Netherlands). Containers were capped and left at room temperature for 7 days to polymerize. Once polymerization reached a solid state, blocks were removed from containers, marked, and kept in identifiable containers until cut. Markings were placed on each block to identify the cutting area (Figure 18).

Sectioning specimens was performed using a Leica SP1600 saw microtome (Leica SP1600, Leica Microsystems, Inc.; Buffalo Grove, IL) (Figure 19). Microtome saw blades ran at a constant speed of 600 rpm under constant tap water irrigation. The blade thickness was 280 μm. During cutting, approximately 300 μm of tooth structure was lost with each cut. Between two and four serial longitudinal sections of 1000-μm thick were generated from each specimen. The chosen slices were carefully selected to include the desired lesion in the middle section and to include a section on each of the lesions (Figure 18).

Selected sections of each tooth were bonded with cyanoacrylate to a microscopic specimen slide (Exakt Specimens Slides, Exakt Technologies, Inc.; Oklahoma City, OK). On each slide, a section was marked as most representative of the lesion. Specimens were

polished using silicon carbide grinding paper, size 1000 grit, (Exakt grinding paper K1000, Exakt Technologies, Inc.). Grinding paper was fixed on a rotating grinding table (Exakt 400CS grinder, EXAKT Technologies, Inc.). Slides were polished under of a stream of tap water and then were left to dry in an upright position at a room temperature for at least 24 hours.

Specimens were photographed using a Nikon digital stereomicroscope, SMZ-1500 as described in Part I (Figure 12). Slides were laid flat on a dark black color plate, where a millimeter ruler was taped down in a manner that the edge of the ruler is always visible at the upper side of the image to help in establishing reliable measurements.

Slides were immersed in 0.1 millimolar (mM) Rhodamine B dye solution (Rhodamine B, Fisher Scientific, Inc.; Pittsburg, PA) for 24 hours in an upright position, after which, slides were removed and rinsed under a stream of DI water to remove excess dye. Slides were removed and left to dry at room temperature for 24 hours, then photographed using a digital stereomicroscope under a white light illumination as described earlier.

After that, sections were further grinded serially using a precise rotary grinding machine (Exakt 400CS) with a 1000-grit silicon carbide grinding paper. The machine was set to grind 150±50 μm each time (Figure 19). Following each grind, new sets of images were captured using the light stereomicroscope as described earlier. This grinding process produced up to 15 different histological sections per lesion. This process made it possible to make sure that the best section that represents the maximum depth of the lesion was chosen for analysis (Figure 20).

Five measurements were recorded when applicable for each selected slide: enamel thickness; dentin thickness; lesion depth in enamel; lesion depth in dentin; and maximum sealant thickness (Figure 21). For instance, the first two measurements were recorded for a slide with no lesion and no sealant; and for a slide with a lesion limited to enamel and a sealant; lesion depth in dentin was not recorded. All measurements were carried out using computer software, Image Scope version 11.2.0.780 (Aperio Technologies, Inc.; Vista, CA). Maximum lesion depth and maximum sealant thickness were recorded.

Two examiners, (Ex.3) and (Ex.4) scored 2 sections that were most representative of each lesion independently. Disagreements were resolved by consensus. Lesions were scored histologically according to criteria outlined in Table V.[117]

For illustration, Figure 22 through Figure 26 show readings using all tested methods in addition to histological scorings for sample teeth that represent ICDAS scores 0 thru 4.

STATISTICAL ANALYSIS

Intra-examiner repeatability and inter-examiner agreement of all methods were calculated using intraclass correlation coefficients (ICC). ICCs were used 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. Two-way tables for the categorical assessments and plots for the measurements were used to provide additional information about the repeatability and agreement. ICCs were calculated using mixed-model ANOVAs.

Comparisons between the % correct, sensitivity, specificity, and area under the ROC curve for the ICDAS, the Canary System, Inspektor Pro, and QLF-D Biluminator 2 methods were performed using bootstrap analyses. The correlation of the measurements for each method with the histology scores also was calculated using bootstrap methods. The bootstrap methodology uses resampling techniques to estimate statistics and perform comparisons for values that are not normally distributed. In this case, the bootstrap also provided a way to properly account for the correlations between examiners, between repeats, and between methods. Classification trees using recursive partitioning methods were used to determine new cutoff points for Inspektor Pro and QLF-D Biluminator 2.

SAMPLE SIZE JUSTIFICATION

Data from previous studies indicated a correlation of approximately 0.7 between methods. With a sample size of 20 sound teeth and 10 teeth for each of ICDAS 1 to 4, the study had 80-percent power to detect a difference in the area under the ROC curve of 0.15 (0.75 vs. 0.90), assuming a two-sided test with a 5-percent significance level.

RESULTS

REPEATABILITY AND AGREEMENTS

Group 1

Repeatability for Group 1 using ICC between the two examinations is listed in Table VII. For ICDAS, it was found to range from 0.81 to 0.87. For Inspektor Pro, ΔF repeatability ranged from 0.49 to 0.97; area repeatability ranged from 0.74 to 0.97; and repeatability for ΔQ ranged from 0.87 to 0.97. For QLF-D Biluminator 2, ΔF repeatability ranged from 0.96 to 0.99; repeatability for ΔF_{MAX} ranged from 0.99 to 1.00; area repeatability ranged from 0.94 to 0.98; and ΔQ repeatability ranged from 0.98 to 0.99. And for The Canary System, it was found to range from 0.33 to 0.63 (Table VII).

Agreement between the three examiners, for Group 1 using ICC, was found 0.72 for ICDAS. For Inspektor Pro, agreements among examiners were found 0.73 for ΔF, 0.75 for area and 0.85 for ΔQ. For QLF-D Biluminator 2, agreements among examiners were found 0.96 for ΔF, 0.99 for ΔF_{MAX}, 0.91 for area and 0.96 for ΔQ. For The Canary System, agreement was found to be 0.48 (Table VII).

Group 2: Before Placement of Sealant

Repeatability for Group 2 readings before the placement of the sealant, between the two examinations for examiners (Ex.1) and (Ex.2) using ICC, is listed in Table VIII. For ICDAS, repeatability was 0.87 and 0.90 for (Ex.1) and (Ex.2), respectively. For Inspektor Pro, ΔF repeatability was 0.98 and 0.48, respectively; area repeatability was

0.94 and 0.39, respectively; and repeatability for ΔQ was 0.95 and 0.67, respectively. For QLF-D Biluminator 2, ΔF repeatability was 0.98 for both examiners; repeatability for ΔF_{MAX} was 0.99 for both examiners; area repeatability was 0.93 and 0.91, respectively; and ΔQ repeatability was 0.96 and 0.95 for (Ex.1) and (Ex.2), respectively. And for the Canary System, it was found to be 0.52 and 0.68 for examiners (Ex.1) and (Ex.2), respectively (Table VIII).

Agreement for Group 2 readings before the placement of the sealant, between the two examiners using ICC, was 0.80 for ICDAS. For Inspektor Pro, agreements among examiners were 0.78 for ΔF, 0.59 for area and 0.83 for ΔQ. For QLF-D Biluminator 2, agreements between examiners were 0.96 for ΔF, 0.98 for ΔF_{MAX}, 0.89 for area and 0.95 for ΔQ. For the Canary System, agreement was found to be 0.54. (Table VIII).

Group 2: After Placement of Sealant

Repeatability for Group 2 readings after the placement of the sealant, between the two examinations for examiners (Ex.1) and (Ex.2) using ICC, is listed in Table IX. For Inspektor Pro, ΔF repeatability was 0.24 and 0.37; area repeatability was 0.35 and 0.42; and repeatability for ΔQ was 0.37 and 0.43, respectively. For QLF-D Biluminator 2, ΔF repeatability was 0.80 and 0.84; repeatability for ΔF_{MAX} was 0.57 and 0.84; area repeatability was 0.39 and 0.82; and ΔQ repeatability was 0.53 and 0.85, respectively. For the Canary System, it was found to be 0.47 and 0.22 for examiners (Ex.1) and (Ex.2), respectively (Table IX).

Agreement for Group 2 readings after the placement of the sealant, between the two examiners using ICC, was 0.29 for Inspektor Pro ΔF, 0.43 for area of Inspektor Pro

and 0.45 for ΔQ of Inspektor Pro. For QLF-D Biluminator 2, agreements among examiners were 0.74 for ΔF, 0.69 for ΔF_{MAX}, 0.63 for area, and 0.71 for ΔQ. For the Canary System, agreement was found to be 0.01 (Table IX).

For convenience, repeatability, and agreement values for Group 1 and Group 2 before the sealant placement are combined all together in Table X, where Table XI combined before and after the sealant values for Group 2.

SEALANT THICKNESS

Maximum sealant thickness for Group 2 ranged from 0.35 to 1.65 mm, with mean thickness of 1.08 mm and a standard deviation of 0.26 mm (Table XII). Agreement between devices' readings for Group 2 before and after the sealant ranged from 0.12 to 0.13 for Inspektor Pro, 0.08 to 0.32 for QLF-D Biluminator 2, and 0.05 for the Canary System (Table XIII).

PERFORMANCE OF DETECTION METHODS: SENSITIVITY, SPECIFICITY, % CORRECT AND ROC CURVE

For calculating performance parameters of the detection methods, a cutoff point (threshold) was set to a histological score 0. Histological score 0 was considered sound, where histological scores 1-4 were considered caries. Cutoff points (thresholds) for detection methods were set for ICDAS at score 0, where ICDAS score 0 is considered sound, for both Inspektor Pro and QLF-D Biluminator 2 was set above 5-percent average mineral loss (ΔF), where $\Delta F \leq 5\%$ is considered sound, and 20 (Canary Number) for The Canary System, where 0-20 is considered sound.

GROUP 1

For Group 1, 15 specimens (N = 15) were histologically considered sound (25 percent). Performance parameters for Group 1 are listed in Table XIV in order of sensitivity, specificity, % correct (accuracy), and area under the ROC curve (AUC).

For ICDAS, sensitivity was 0.82, specificity was 0.86, % correct was 0.83 and AUC was 0.87. The ROC curve for ICDAS is presented in Figure 27.

For Inspektor Pro ΔF, sensitivity was 0.89, specificity was 0.60, % correct was 0.82, and AUC was 0.90. The ROC curve for Inspektor Pro ΔF is presented in Figure 28.

For QLF-D Biluminator 2 ΔF, sensitivity was 0.96, specificity was 0.57, % correct was 0.86, and AUC was 0.94. The ROC curve for QLF-D Biluminator 2 ΔF is presented in Figure 29.

For The Canary System, sensitivity was 0.85, specificity was 0.43, % correct was 0.74, and AUC was 0.79. The ROC curve for the Canary System is presented in Figure 30.

Area under the ROC curve was significantly higher for QLF-D Biluminator 2 ΔF than for ICDAS ($p = 0.0023$) and the Canary System ($p = 0.0005$), and higher for Inspektor Pro ΔF than for the Canary System ($p = 0.0214$). Figure 38 shows ROC curves for all detection methods together.

GROUP 2

For Group 2, nine specimens (N = 9) were histologically considered sound (15 percent). Performance parameters for Group 2 before the placement of the sealant are listed in Table XV in order of sensitivity, specificity, % correct (accuracy), and area under the ROC curve (AUC). Table XVI lists performance parameters for Group 2 after the

placement of the sealant. Table XVII provides the combined performance parameters for Group 2 before and after sealant placement.

For ICDAS, sensitivity was 0.73, specificity was 0.92, % correct was 0.76, and AUC was 0.84. The ROC curve for ICDAS is presented in Figure 31.

For Inspektor Pro ΔF for Group 2 before the sealant placement, sensitivity was 0.80, specificity was 0.73, % correct was 0.79, and AUC was 0.87. However, after the placement of the sealant, sensitivity was 0.99, specificity was 0.03, % correct was 0.84, and AUC was 0.67. The ROC curve for Inspektor Pro ΔF is presented in Figure 32 for before the placement of the sealant and in Figure 33 for after the placement of the sealant.

For QLF-D Biluminator 2 ΔF for Group 2 before the sealant placement, sensitivity was 0.76, specificity was 0.69, % correct was 0.75, and AUC was 0.83. However, after the placement of the sealant, sensitivity was 1.00, specificity was 0.00, % correct was 0.85, and AUC was 0.70. The ROC curve for QLF-D Biluminator 2 ΔF is presented in Figure 34 for before the placement of the sealant and in Figure 35 for after the placement of the sealant.

For the Canary System for Group 2 before the sealant placement, sensitivity was 0.92, specificity was 0.19, % correct was 0.81, and AUC was 0.74. However, after the placement of the sealant, sensitivity was 0.54, specificity was 0.50, % correct was 0.53, and AUC was 0.58. The ROC curve for the Canary System is presented in Figure 36 for before the placement of the sealant and in Figure 37 for after the placement of the sealant.

Before placement of the sealant, area under the ROC curve was significantly higher for Inspektor Pro ΔF than for the Canary System ($p = 0.0215$). ICDAS and pre-sealant Inspektor Pro ΔF had significantly higher area under the ROC curve than post-

sealant QLF-D Biluminator 2 ΔF ($p < 0.05$), post-sealant Inspektor Pro ΔF ($p < 0.05$), and post-sealant the Canary System ($p < 0.01$). Pre-sealant QLF-D Biluminator 2 ΔF had significantly higher area under the ROC curve than post-sealant Inspektor Pro ΔF ($p = 0.0447$) and post-sealant the Canary System ($p = 0.0090$). Figure 39 to Figure 41 show visual illustrations of ROC curves before and after sealant placement.

CORRELATION BETWEEN DETECTION METHODS AND HISTOLOGICAL VALIDATION

Correlations between detection methods and histological scores are listed in Table XIX.

For Group 1, ICDAS correlation was 0.81 ($p < 0.001$). For Inspektor Pro, correlations ranged from 0.80-0.81 ($p < 0.001$). For QLF-D Biluminator 2, correlations ranged from 0.79-0.83 ($p < 0.001$). And for The Canary System, correlation was 0.44 ($p > 0.05$) (Table XIX).

For Group 2 before the placement of the sealant, ICDAS correlation was 0.79 ($p < 0.001$). For Inspektor Pro, correlations ranged from 0.73-0.77 ($p < 0.001$). For QLF-D Biluminator 2, correlations ranged from 0.73-0.82 ($p < 0.001$). And for The Canary System, correlation was 0.53 ($p < 0.05$) (Table XIX).

For Group 2 after the placement of the sealant, Inspektor Pro correlations ranged from 0.20-0.26 ($p > 0.05$). For QLF-D Biluminator 2, correlations were 0.47 and 0.50 for ΔF and $\Delta F_{MAX,}$ respectively, at significance level ($p < 0.05$), and 0.19 and 0.35 for the area and ΔQ, respectively, at significance level ($p > 0.05$). And for The Canary System, correlation was 0.08 ($p > 0.05$) (Table XIX).

Cross tabulations between ICDAS and histological validation measurements for each exam are presented in Table XX thru Table XXII for Group 1 and in Table XXIII and Table XXIV for Group 2. Over-scoring, under-scoring, and agreement between ICDAS and histological scores, as described by the ICDAS coordinating committee,[70] were highlighted in different colors.

Cross-tabulations between histological scores and detection methods' ratings are listed for Group 1 in Table XXV, for Group 2 before sealant in Table XXVI, and for Group 2 after the placement of the sealant in Table XXVII.

Agreement between the two histological methods, with and without the use of Rhodamine B as an enhancing dye, was calculated using Cohen's kappa and weighted kappa. For Group 1, agreement was 0.86 and 0.90 for Cohen's kappa and weighted kappa, respectively. For Group 2, agreement was 0.89 and 0.93 for Cohen's kappa and weighted kappa, respectively (Table XXVIII).

FIGURES AND TABLES

FIGURE 1. Clinical characteristics of International Caries Detection System (ICDAS).[70]

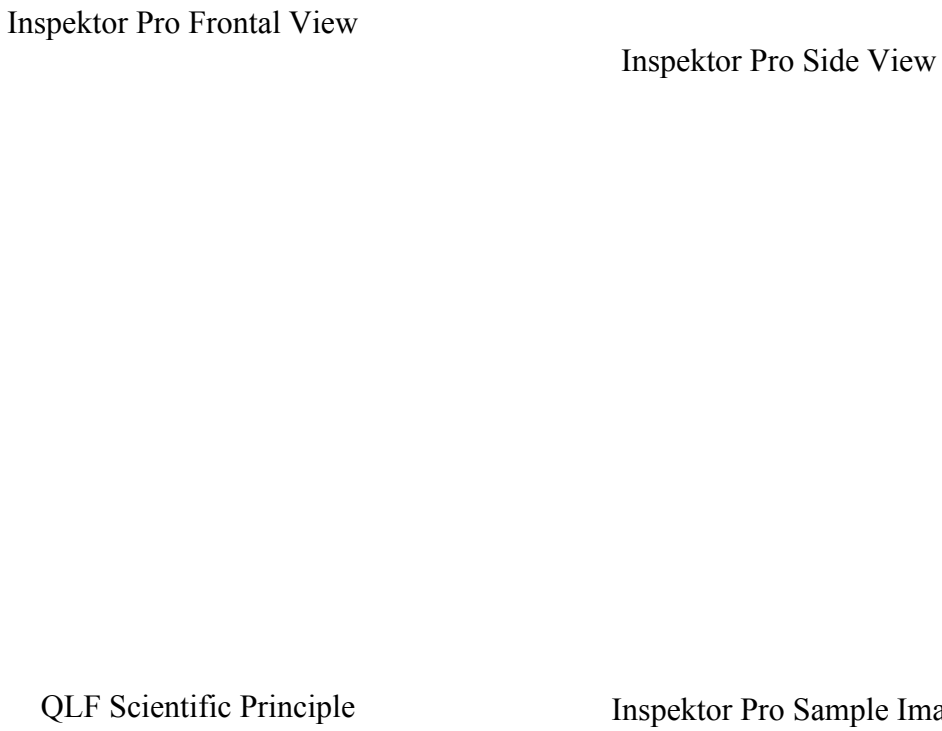


FIGURE 2. Inspektor Pro, equipment images on top. Bottom left shows the scientific principle for QLF. Bottom right image shows a sample tooth without and with QLF.[94, 95]

FIGURE 3. Inspektor Pro lesion analysis. Top: Sample tooth. Bottom: Sample analysis. Bottom images clockwise: QLF image; lesion patch design; computed parameters; lesion fluorescence rendering.

QLF-D Biluminator™ 2 Frontal View

QLF-D Biluminator™ 2 Side View

QLF-D Biluminator™ 2
Scientific Principles

QLF-D Biluminator™ 2 Sample Image

FIGURE 4. QLF-D Biluminator 2, equipment images on top. Bottom left: the scientific principles for QLF-D Biluminator 2. Bottom right: sample image without and with QLF-D Biluminator 2.[94, 95]

FIGURE 5. QLF-D Biluminator 2 Lesion Analysis. Top: Sample tooth. Bottom: Sample analysis. Bottom images left to right: QLF-D image with sound margins drawn; lesion fluorescence rending; computer parameters.

FIGURE 6. QLF analyses Inspektor Pro. Same specimen was analyzed differently each time yielding different results. Operator design and choices influence the results. From the top image down: Recommended design; two examples of incorrect choices of sound margins; incorrect capturing of lesion borders.

FIGURE 7. Scientific principles for photothermal radiometry (PTR) module.

FIGURE 8. The Canary System scale and zones.[112]

FIGURE 9. The Canary System Equipment[112] (top); Canary wand in use (bottom). Device is held perpendicular to the occlusal surface.

FIGURE 10. The Canary System Scan Modes: Quick on top. Detail on Bottom. Quick Scan Mode: View only the current reading and up to 5 previous readings. Detail Scan Mode: Archives patient's information, images and all previous readings.

FIGURE 11. White light image of a specimen (#10). Wet and dry images on top. Image of the same specimen after lesion is obscured on bottom.

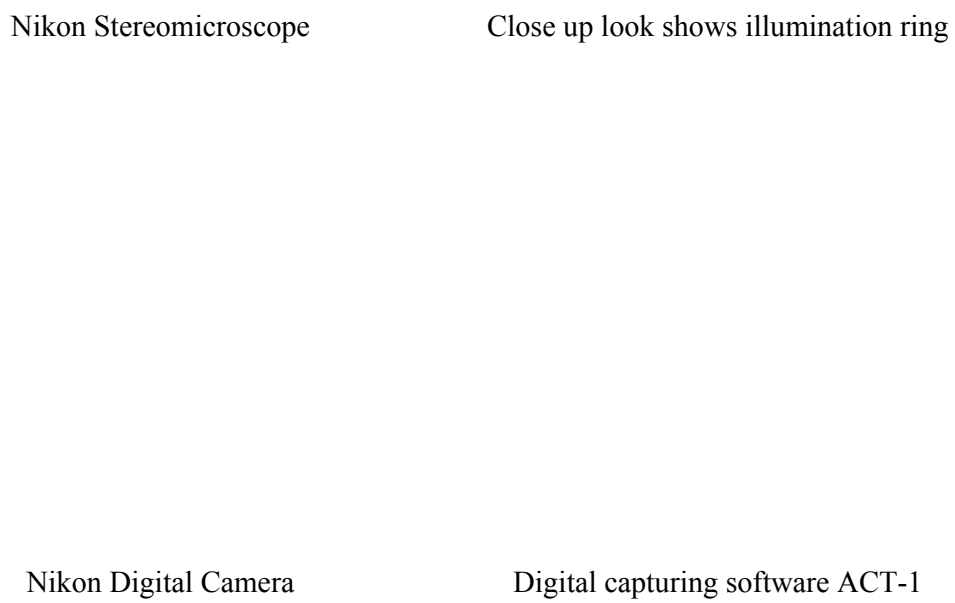


FIGURE 12. Nikon stereomicroscope equipment.

FIGURE 13. ICDAS examination setup. (Top) Examiner glancing at guiding image before proceeding with examination. (Bottom) Examiner using canned air to dry examined tooth while wearing headlight illumination.

FIGURE 14. Inspektor Pro setup. (Top) Examiner glancing at guiding image before proceeding with examination. For illustration, setup photo is taken in a bright ambient condition. (Bottom) Inspektor Pro image capturing was taken in dark ambient conditions.

FIGURE 15. QLF-D Biluminator 2 setup. For illustration, setup photo is taken in a bright ambient condition. (Bottom) Image capturing was taken in dark ambient conditions.

FIGURE 16. The Canary System setup. Examiner wearing laser safety goggles while performing the test.

Acid etching mounted specimens.

Post etchant frosty appearance.

Applying single dose sealant.

Spreading sealant with explorer.

Light curing sealant.

Post sealant photo of specimen.

FIGURE 17. Sealant placement process in summary.

Specimens placed in plastic containers

Container filled with PMMA solution.

Specimens embedded in PMMA block.

Cut area was marked.

Serial cuts with microtome between lines

Sections identified.

Specimen sections fixed on a slide (Left) without dye. (Right) with dye.

FIGURE 18. Specimens' preparation for histological validation in summary.

FIGURE 19. Specimens preparing equipment for histological validation. (Top) Diamond microtome: specimen cutting saw. (Bottom) Precise automated grinding table.

FIGURE 20. Specimen histological sections; occlusal view, and key ,(top row); multiple sections were processed per tooth as illustrated.

Specimen white-light image with measurements.

Specimen white-light image with measurements after dye enhancement.

FIGURE 21. Histological validation measurements. Lesion depth was measured for each section before and after the use of the enhancing dye.

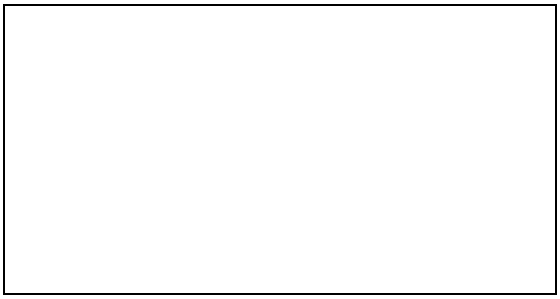
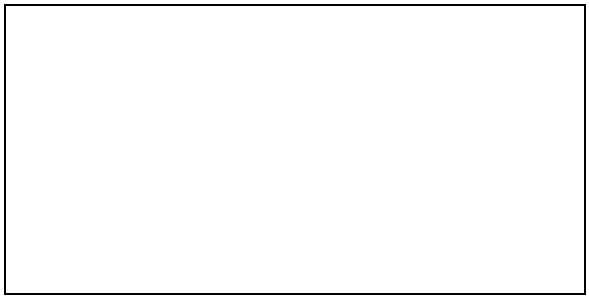
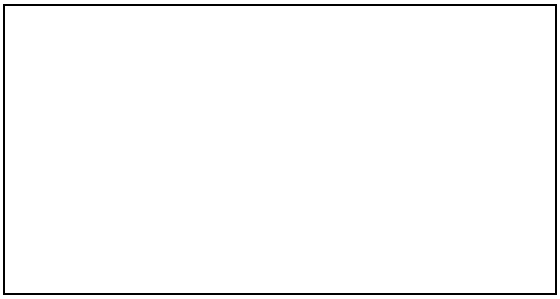
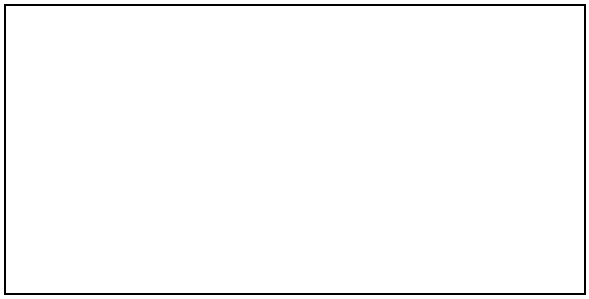
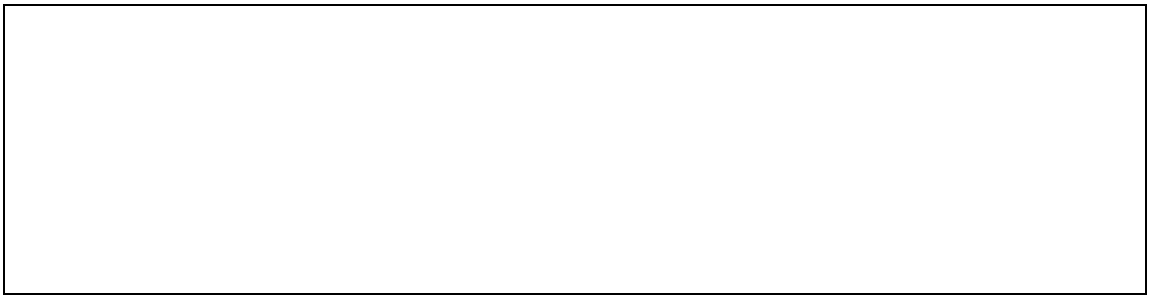
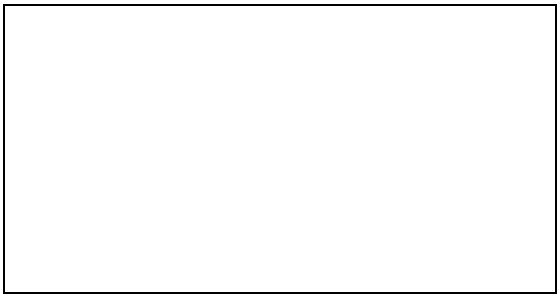
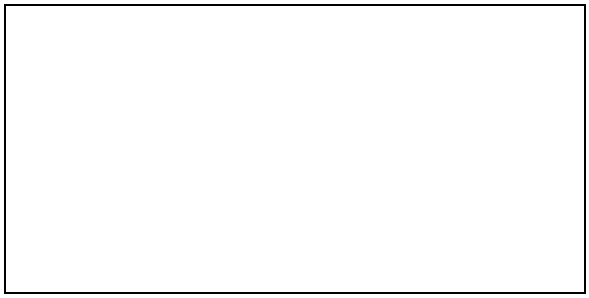
	
Sample Wet Image ICDAS=0	Sample Dry Image ICDAS=0
	
Sample Inspektor Pro Analysis	Sample QLF-D Biluminator 2 Analysis
	
Sample Canary Number	
	
Sample Histology Score without Dye	Sample Histology Score with Rhodamine B

FIGURE 22. Sample specimen represents ICDAS score 0 with reading of same specimens using all devices and histological score.

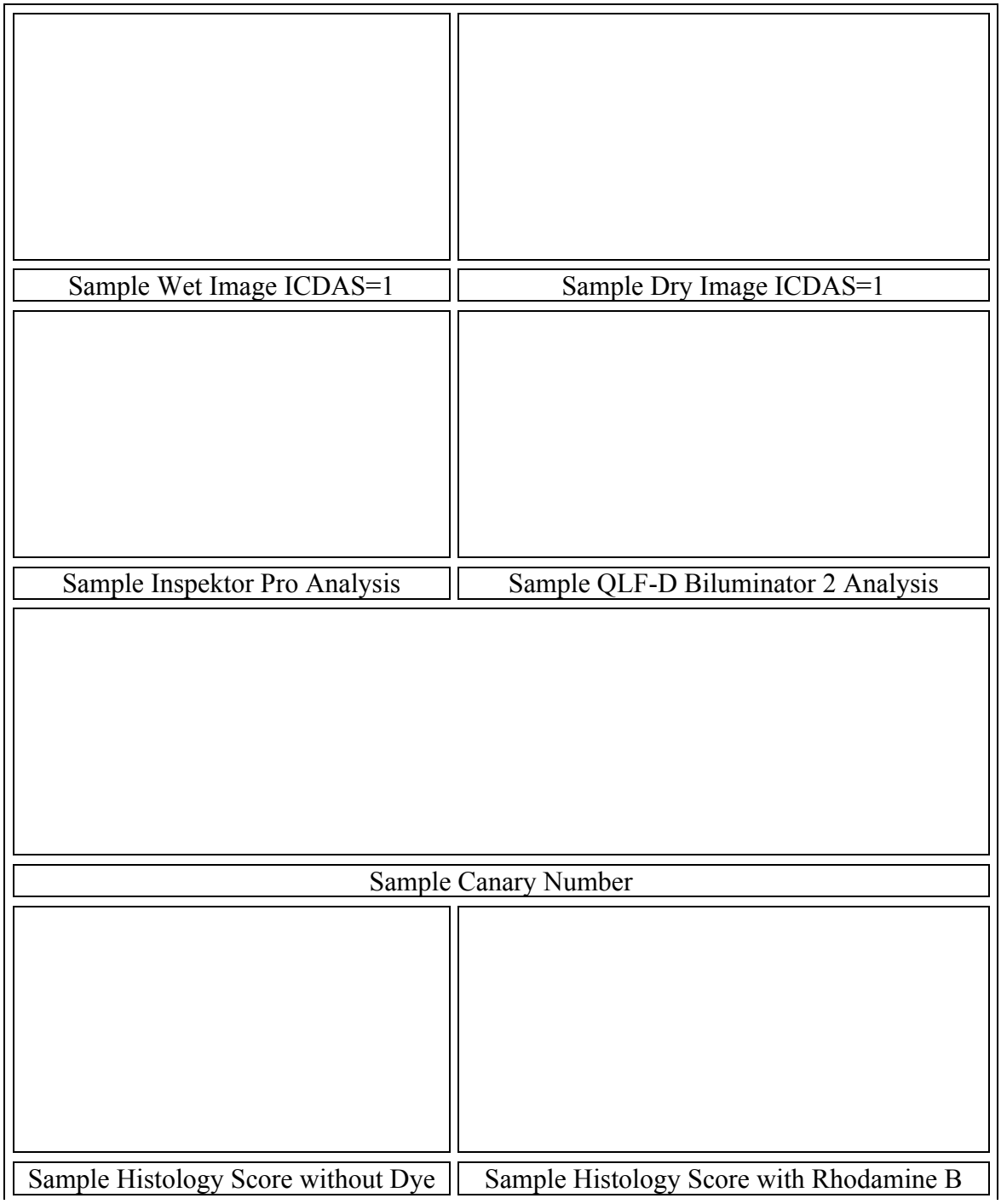


FIGURE 23. Sample specimen represents ICDAS score 1 with reading of same specimens using all devices and histological score.

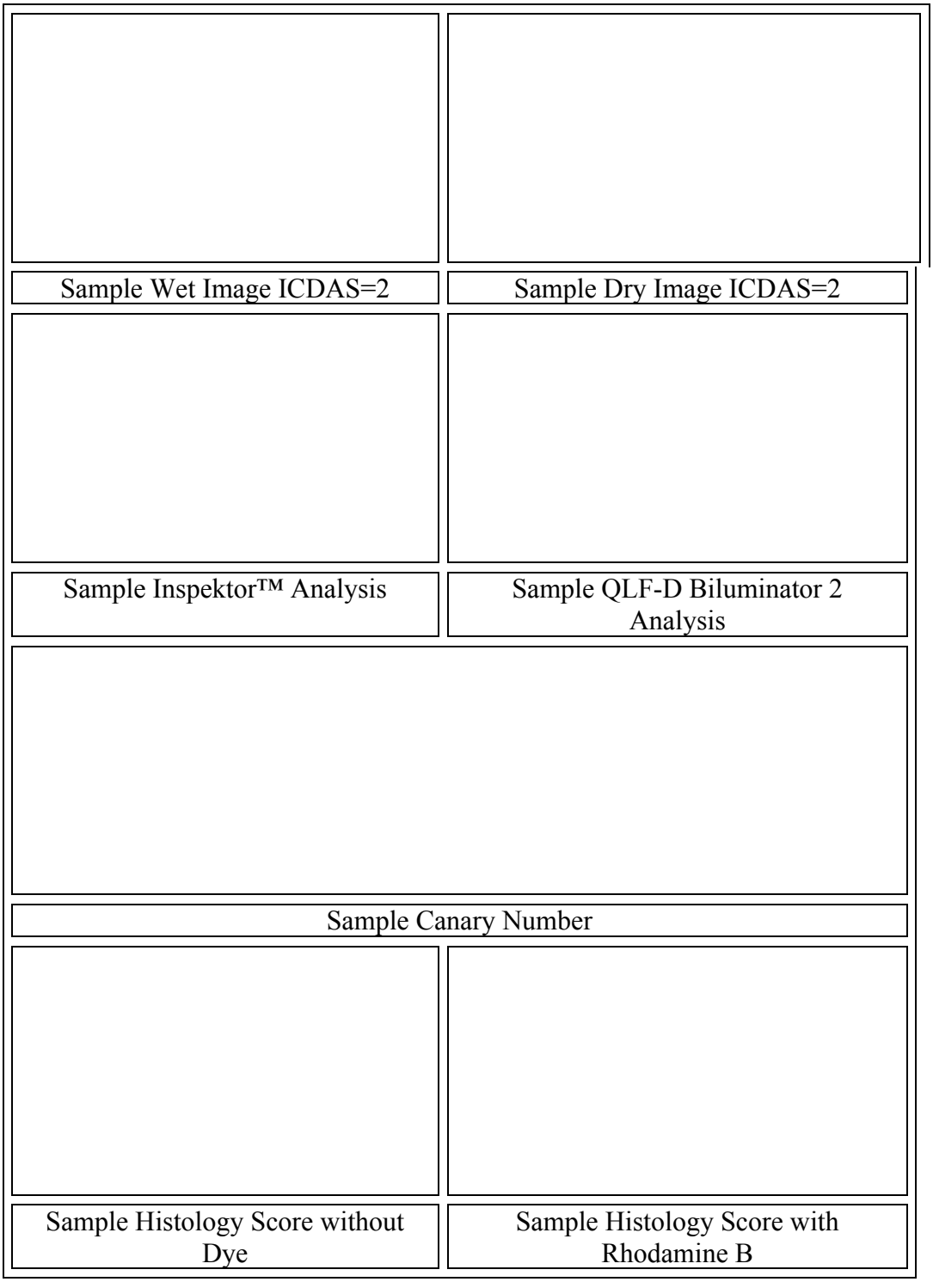


FIGURE 24. Sample specimen represents ICDAS score 2 with reading of same specimens using all devices and histological score.

Sample Wet Image ICDAS=3	Sample Dry Image ICDAS=3
Sample Inspektor Pro Analysis	Sample QLF-D Biluminator 2 Analysis
Sample Canary Number	
Sample Histology Score without Dye	Sample Histology Score with Rhodamine B

FIGURE 25. Sample specimen represents ICDAS score 3 with reading of same specimens using all devices and histological score.

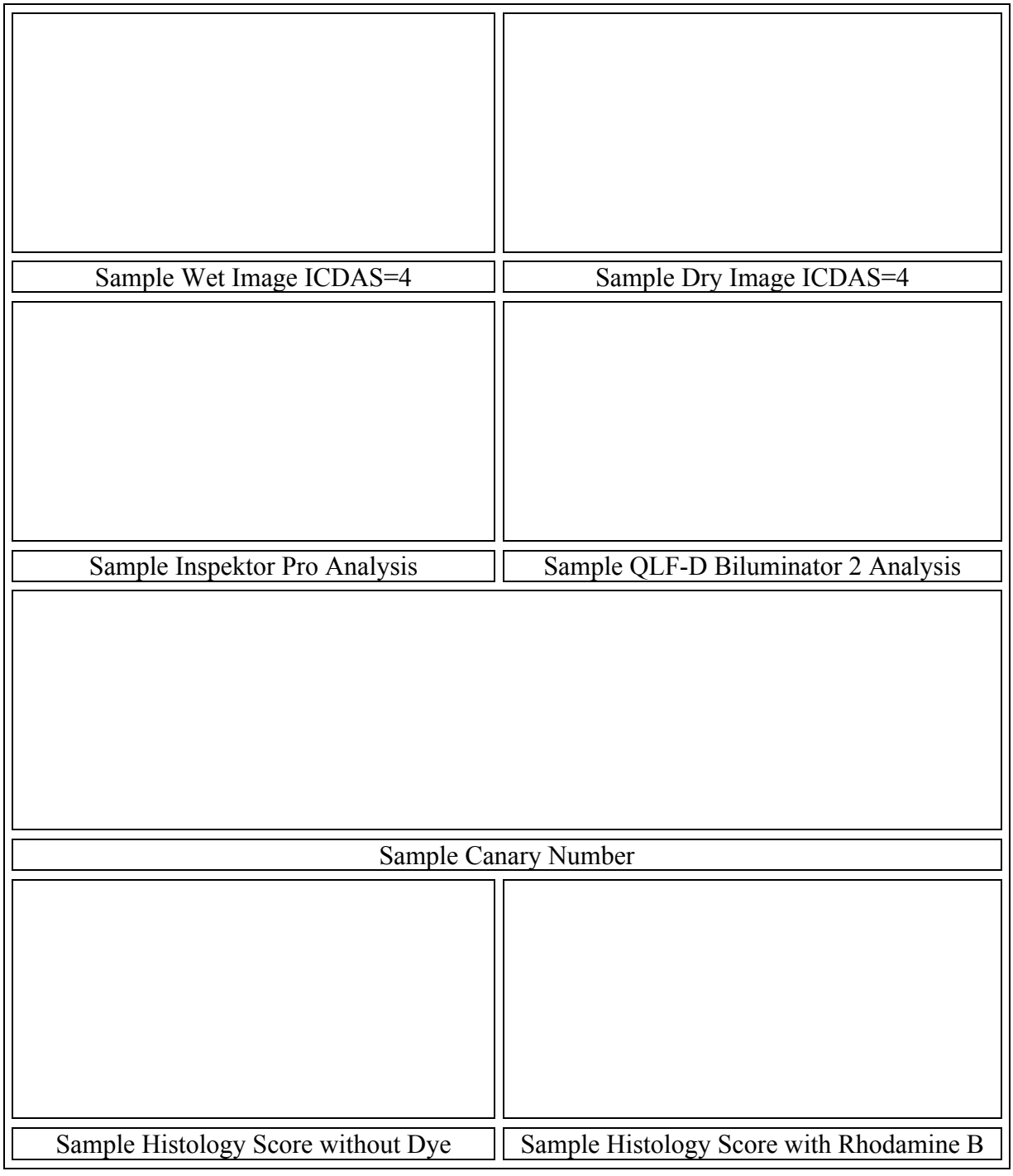


FIGURE 26. Sample specimen represents ICDAS score 4 with reading of same specimens using all devices and histological score.

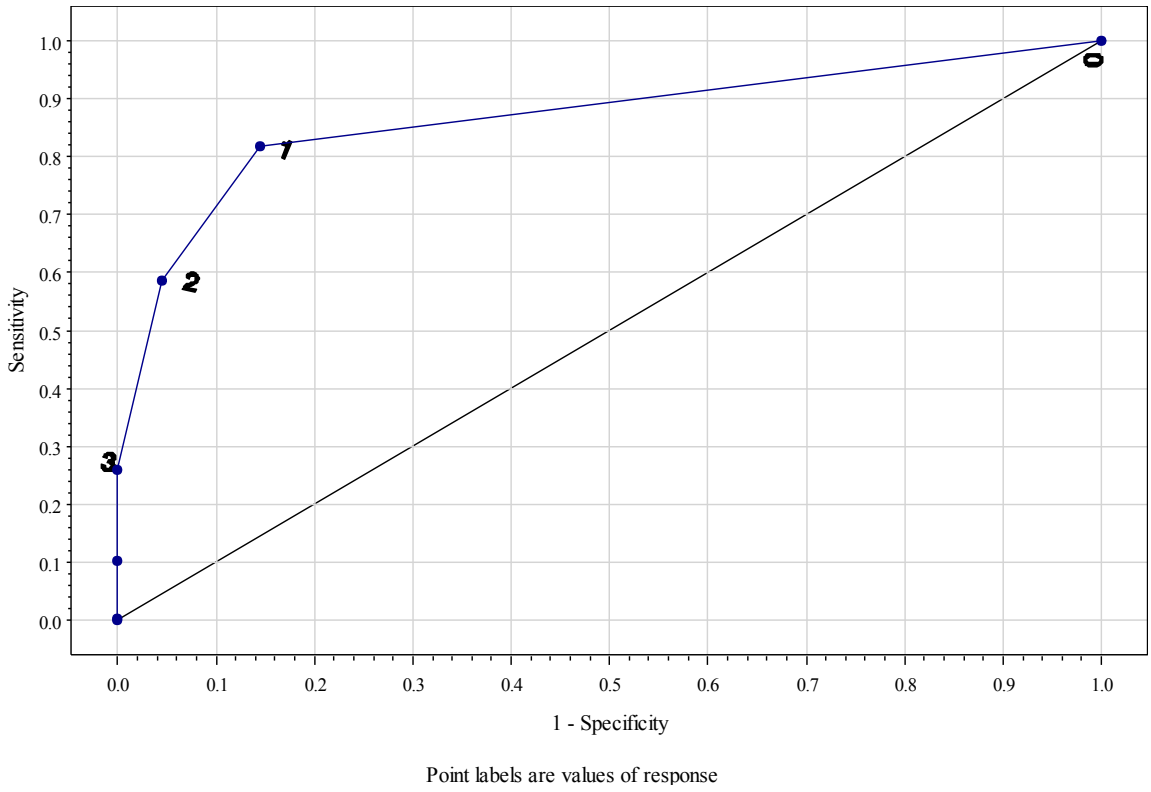


FIGURE 27. ROC plot for group 1 and ICDAS measurement. Area under curve = 0.87.

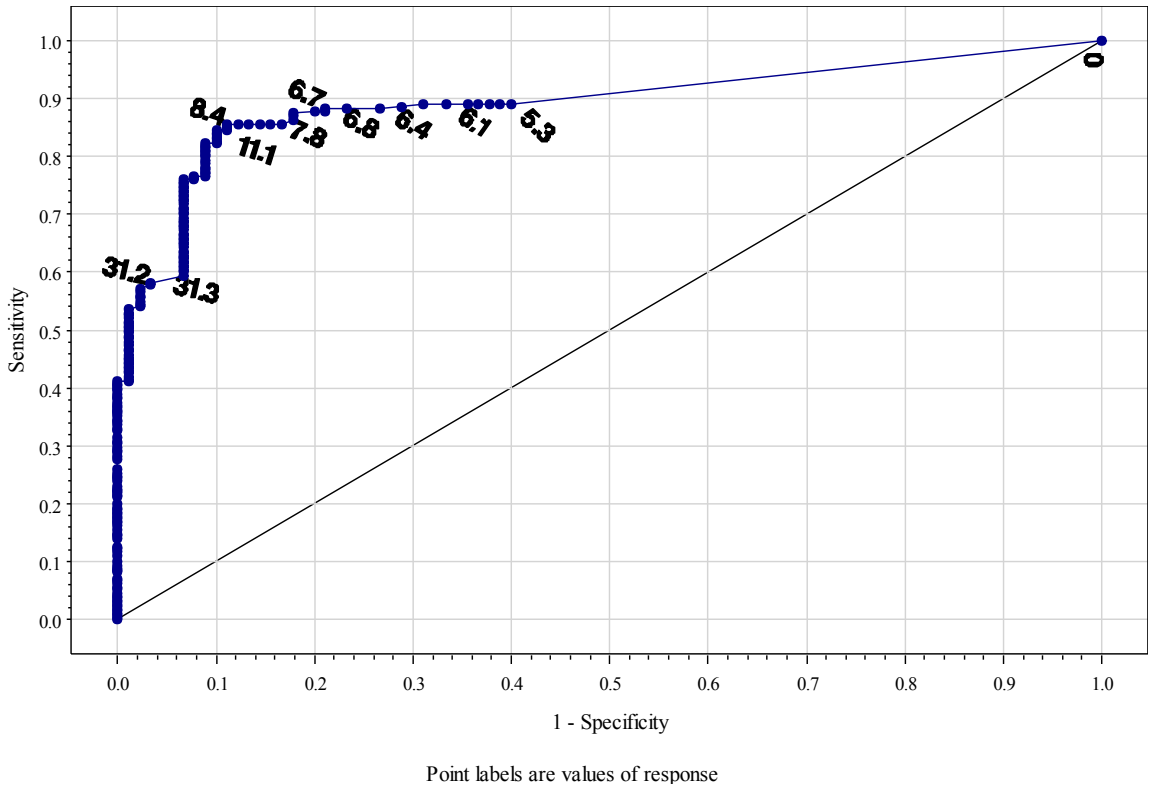


FIGURE 28. ROC plot for group 1 and Inspektor Pro ΔF [%] measurement. Area under curve = 0.90.

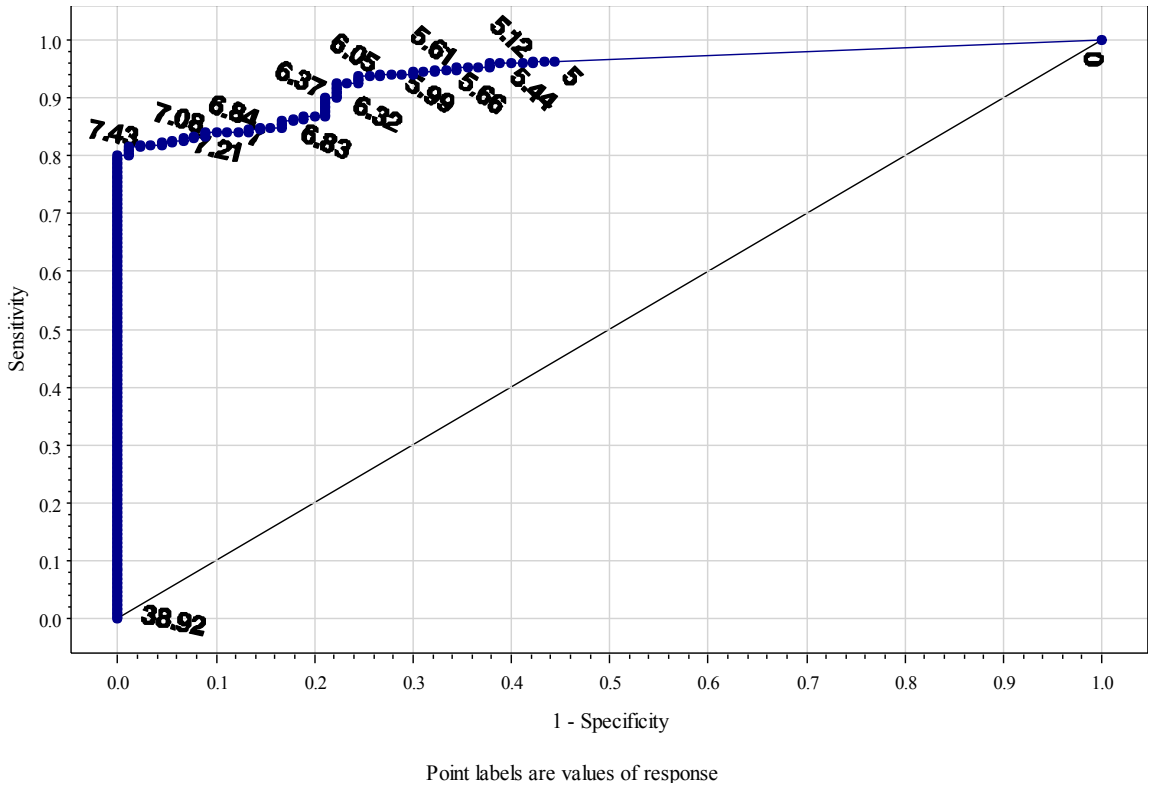


FIGURE 29. ROC plot for group 1 and QLF-D Biluminator 2 ΔF [%] measurement. Area under curve = 0.94.

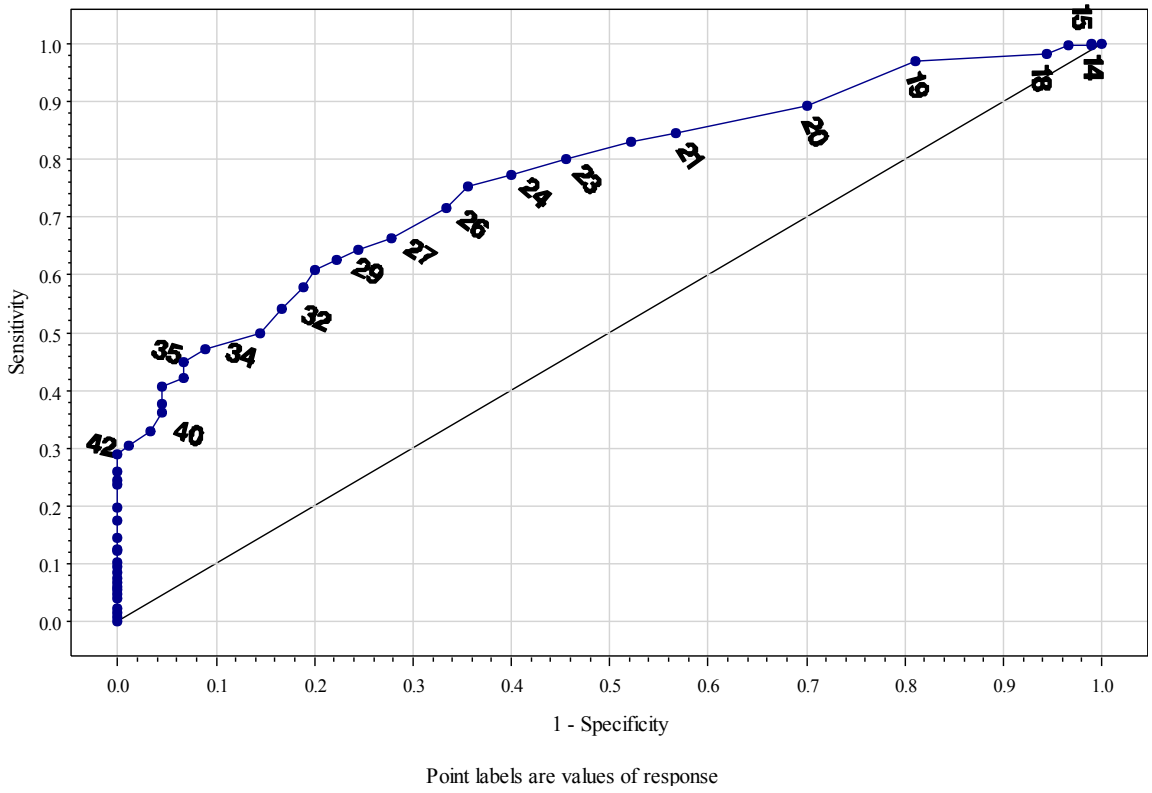


FIGURE 30. ROC plot for group 1 and Canary Number measurement. Area under curve = 0.79.

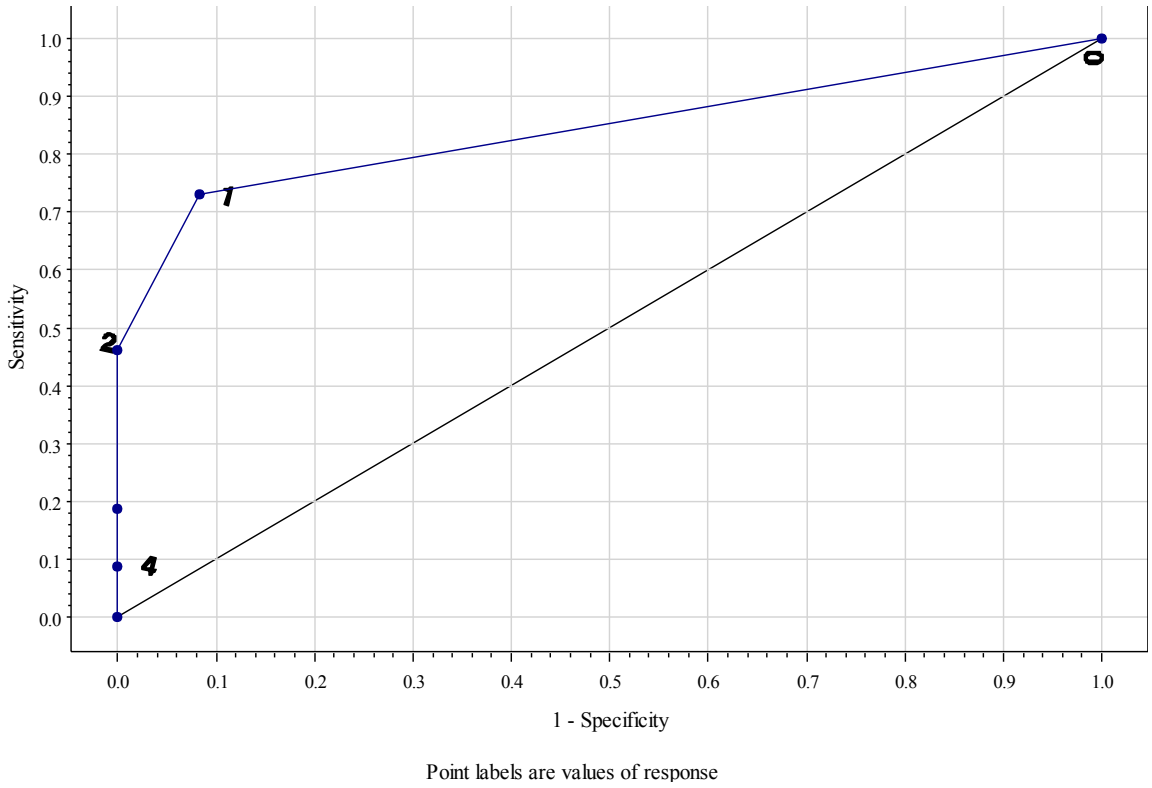


FIGURE 31. ROC plot for group 2 and ICDAS measurement. Area under curve = 0.84.

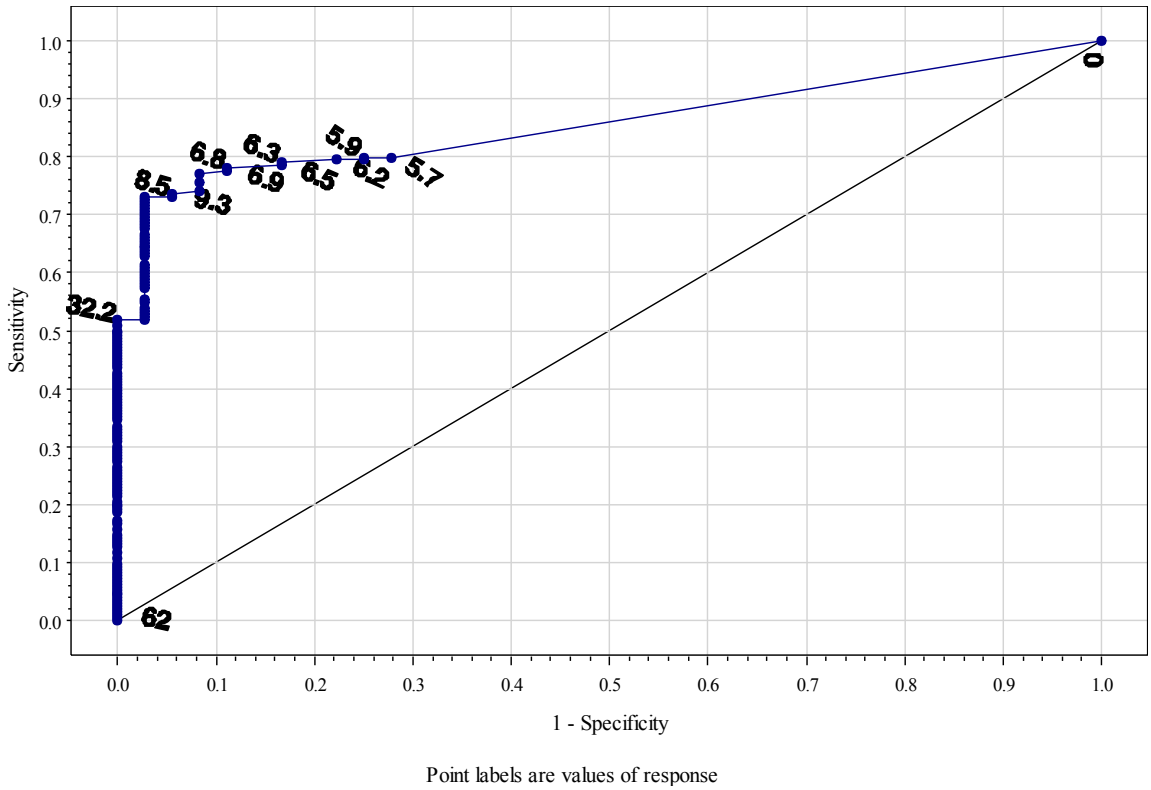


FIGURE 32. ROC plot for group 2 and Inspektor Pro ΔF [%] measurement. Area under curve = 0.87.

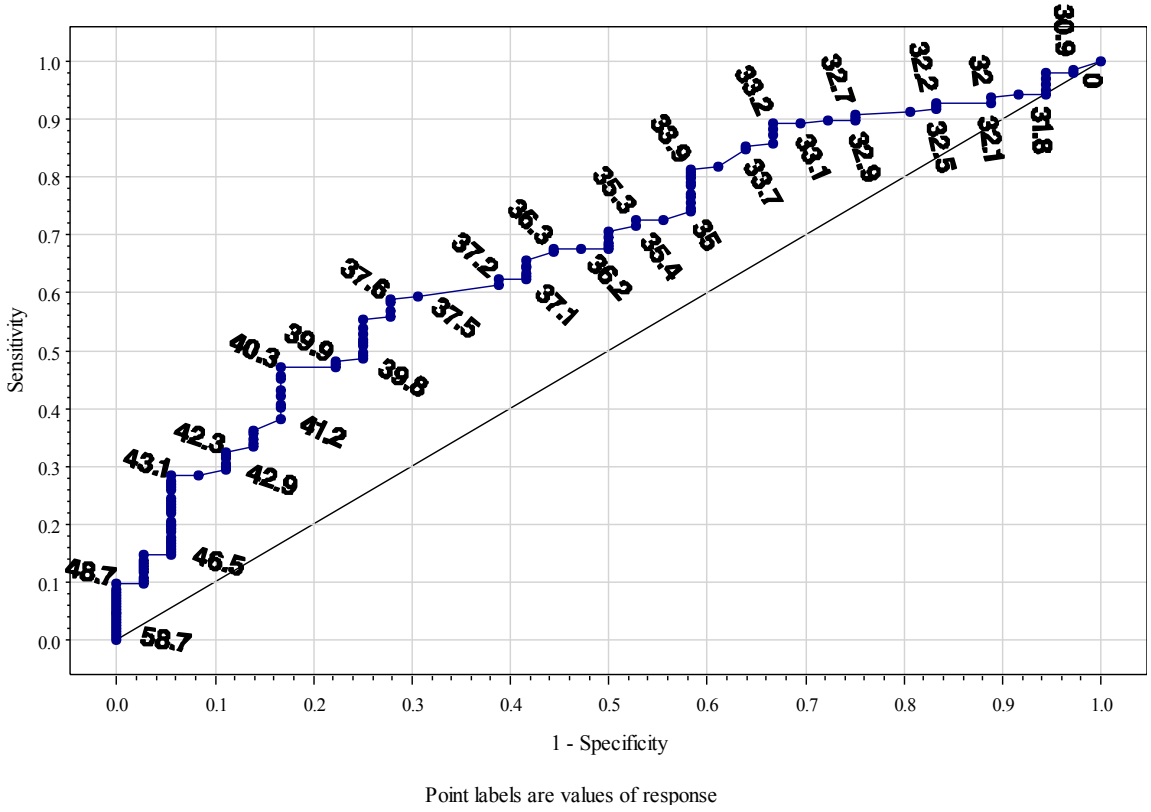


FIGURE 33. ROC plot for group 2-sealed and Inspektor Pro ΔF [%] measurement. Area under curve = 0.67.

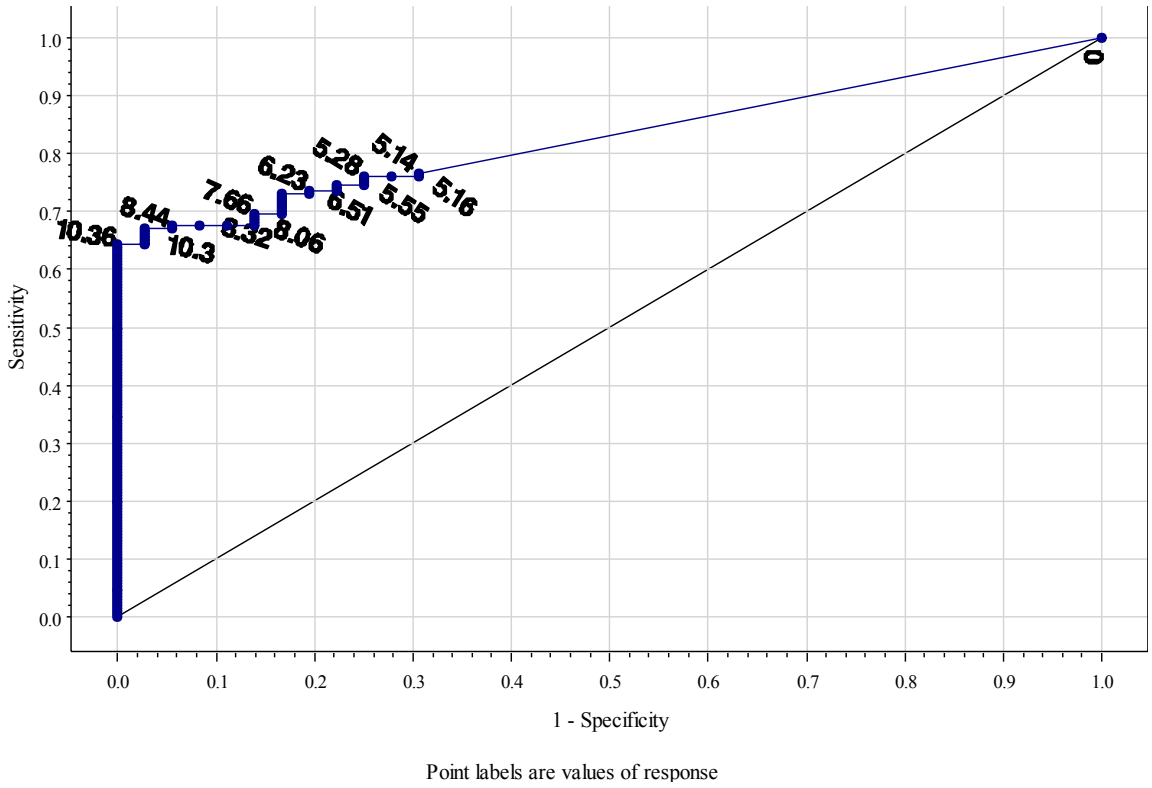


FIGURE 34. ROC plot for group 2 and QLF-D Biluminator 2 ΔF [%] measurement. Area under curve = 0.83.

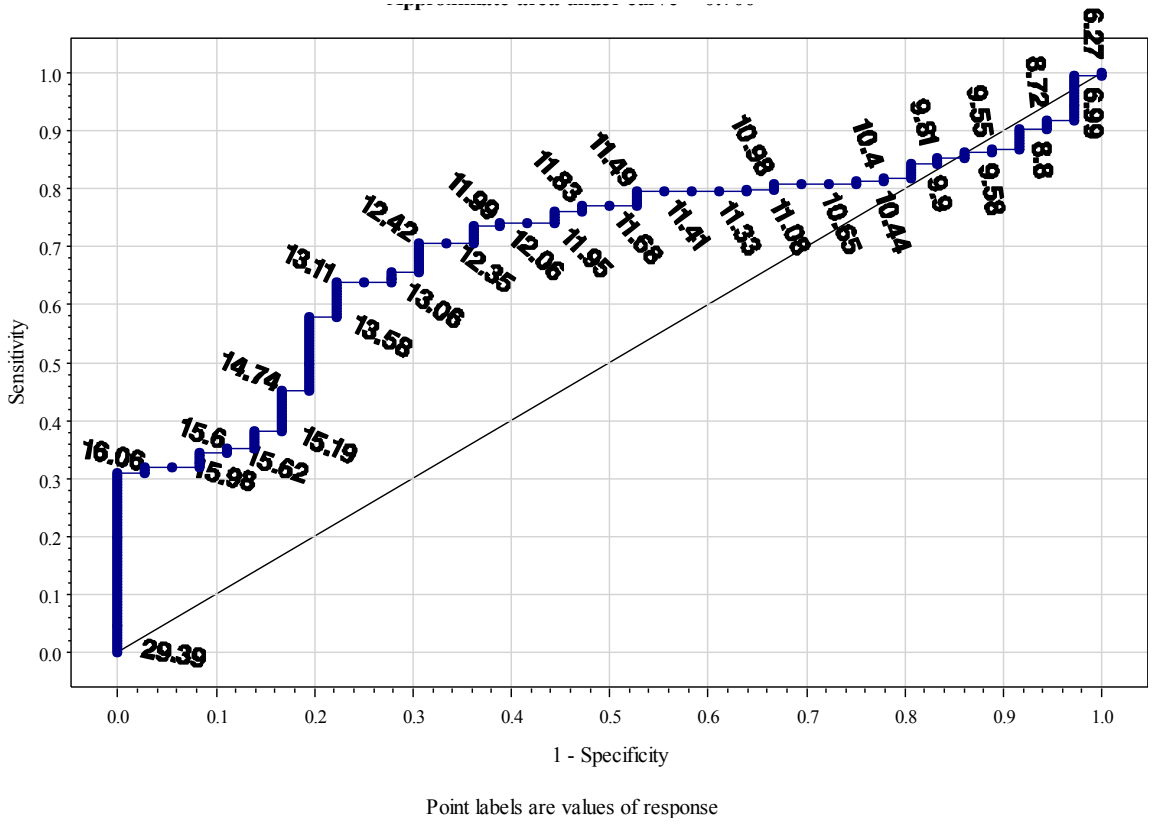


FIGURE 35. ROC plot for group 2-sealed and QLF-D Biluminator ™ 2 ΔF [%] measurement. Area under curve = 0.70.

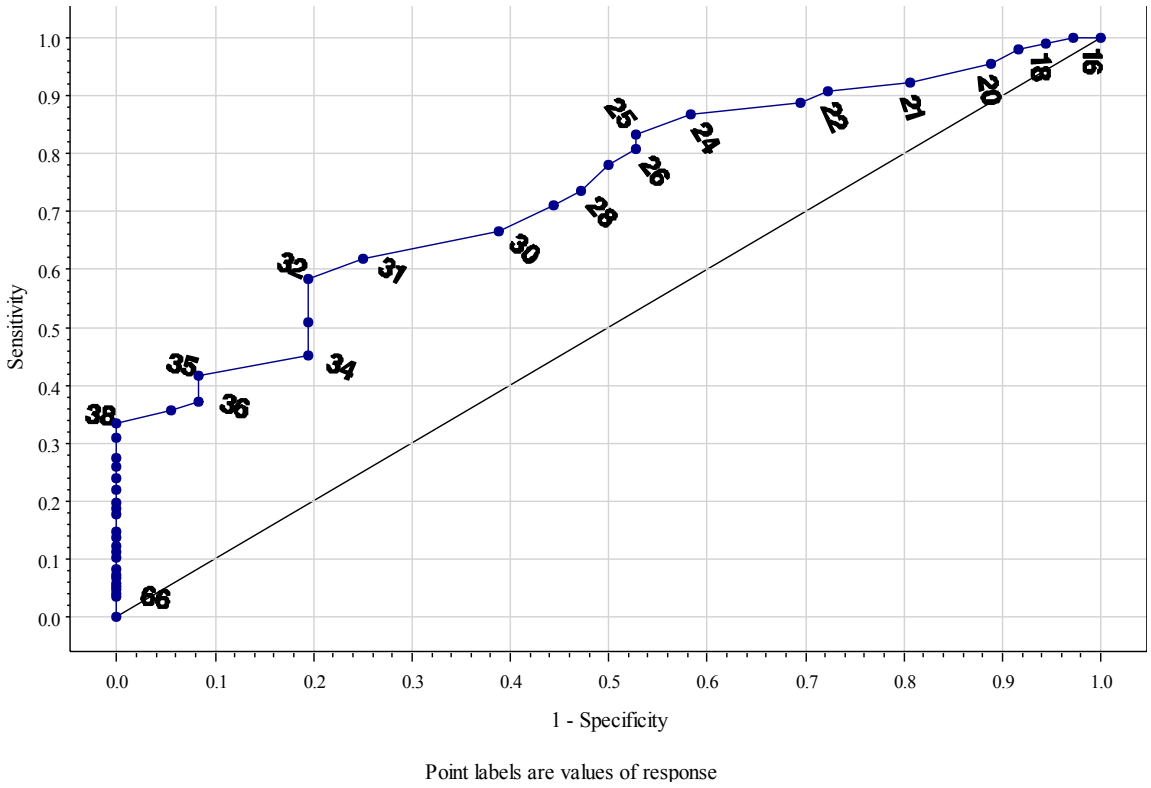


FIGURE 36. ROC plot for group 2 and Canary Number measurement. Area under curve = 0.74.

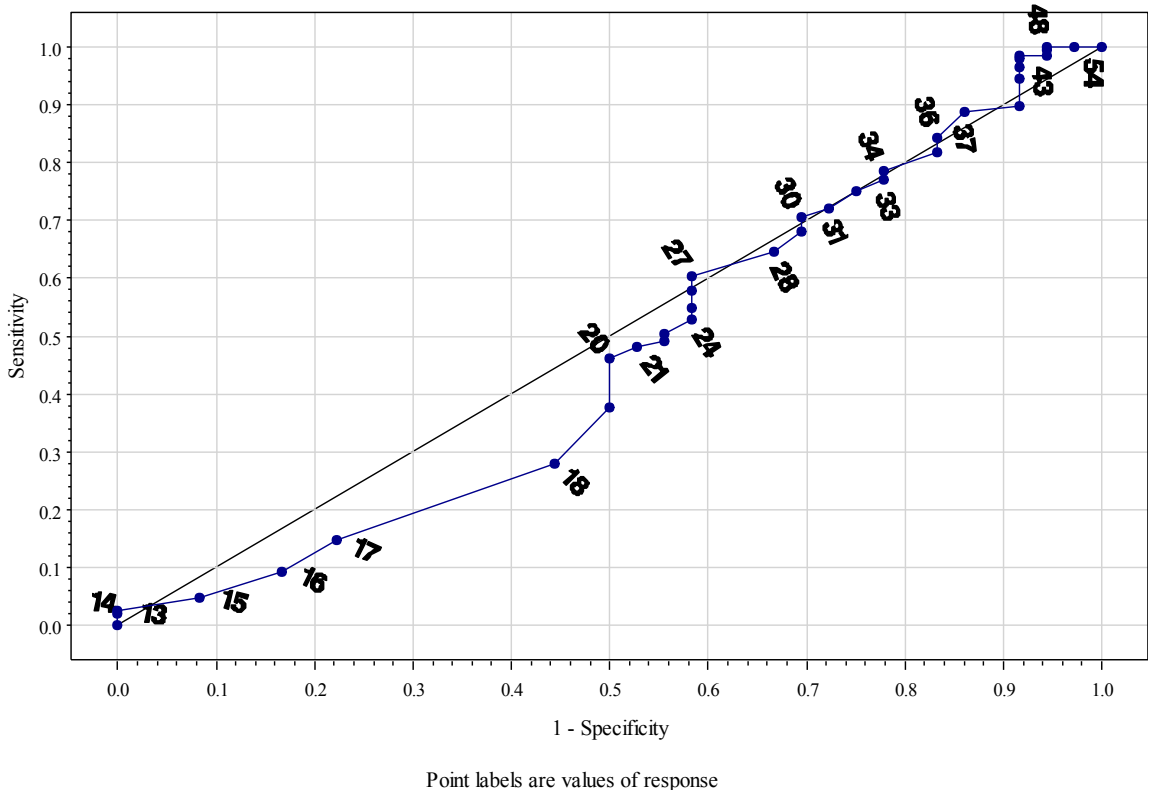


FIGURE 37. ROC plot for group 2-sealed and Canary Number measurement. Area under curve = 0.58.

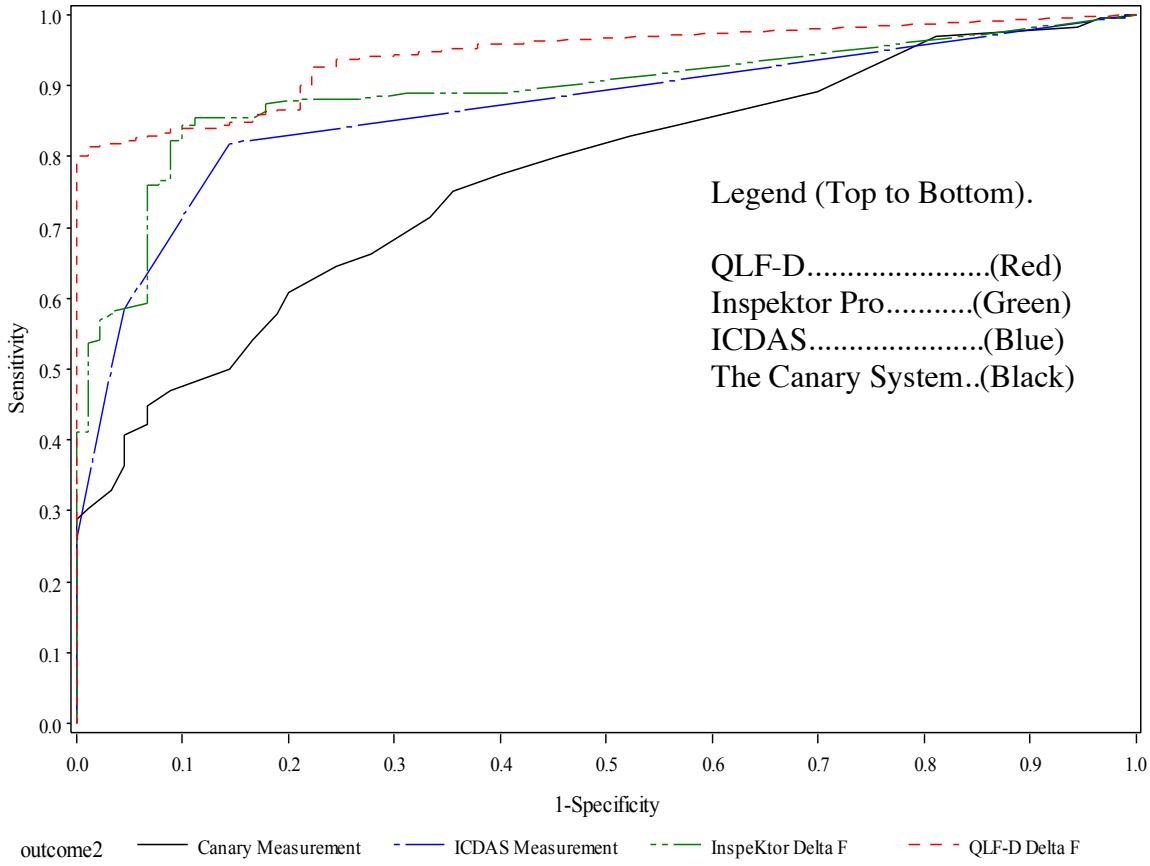


FIGURE 38. Comparison of ROC plots for all detection methods (Group 1).

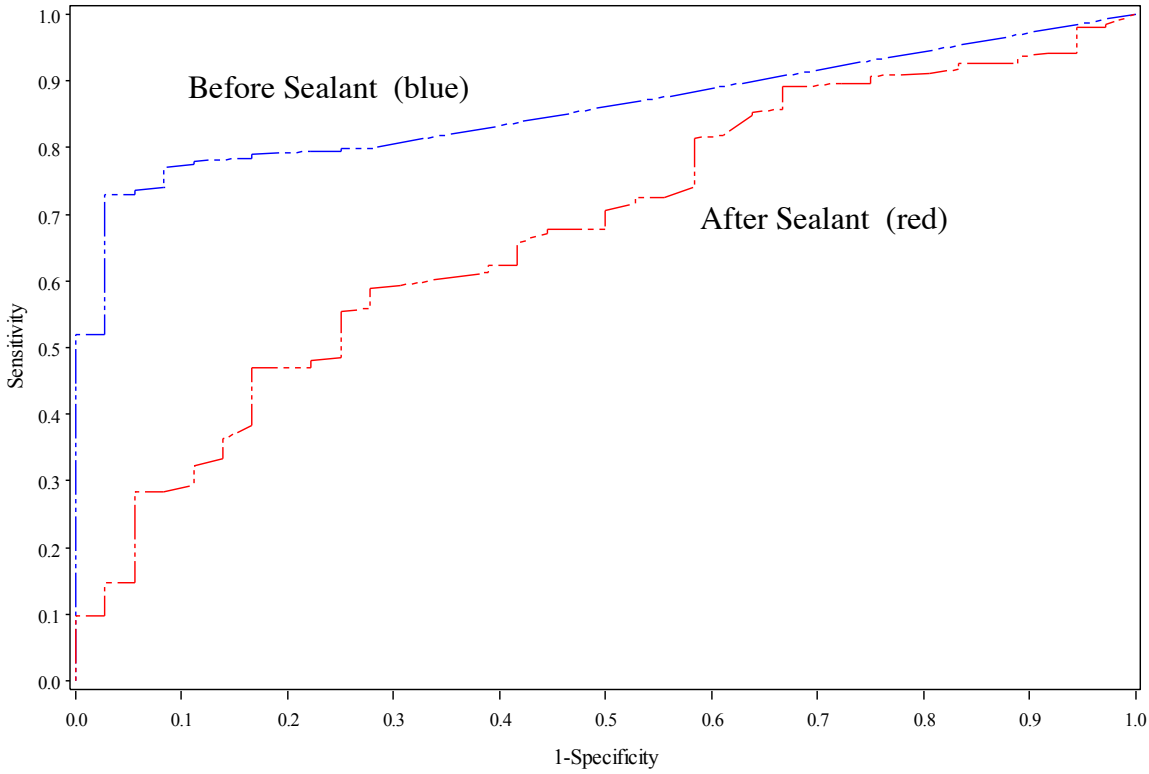


FIGURE 39. Comparison of ROC plots for Inspektor Pro ΔF. Group 2 (before and after sealant).

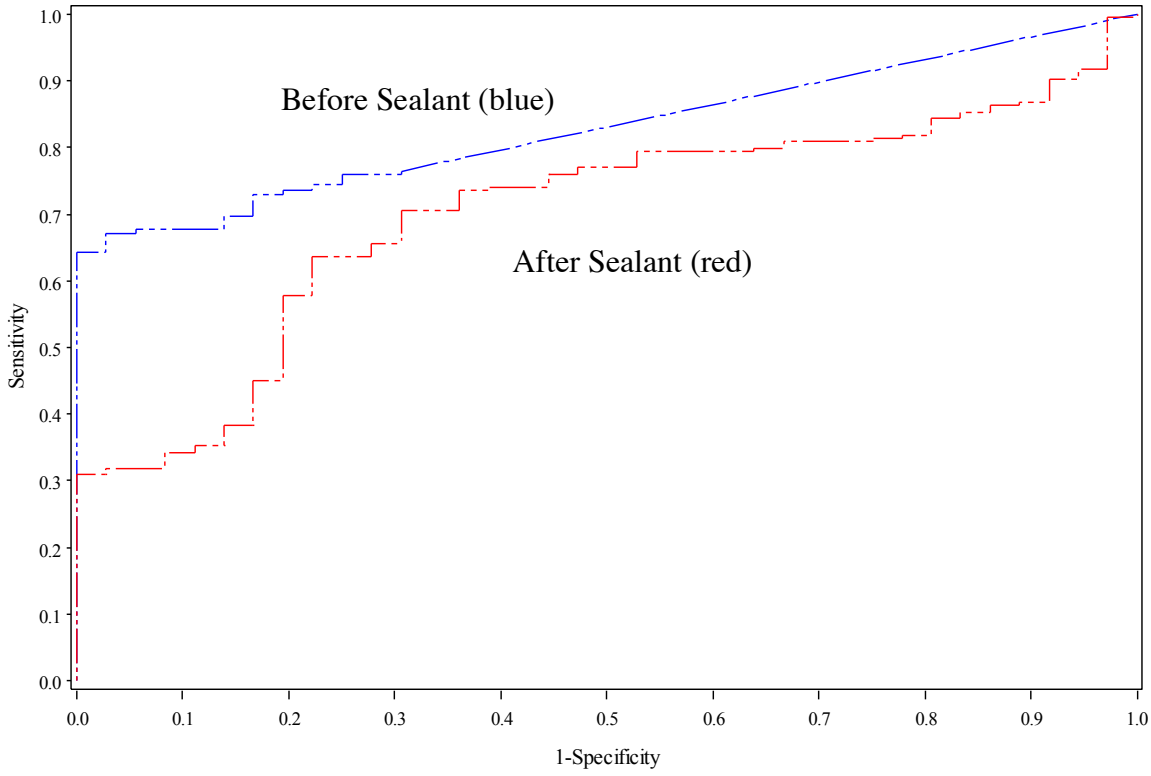


FIGURE 40. Comparison of ROC plots QLF-D Biluminator 2 ΔF. Group 2 (before and after sealant).

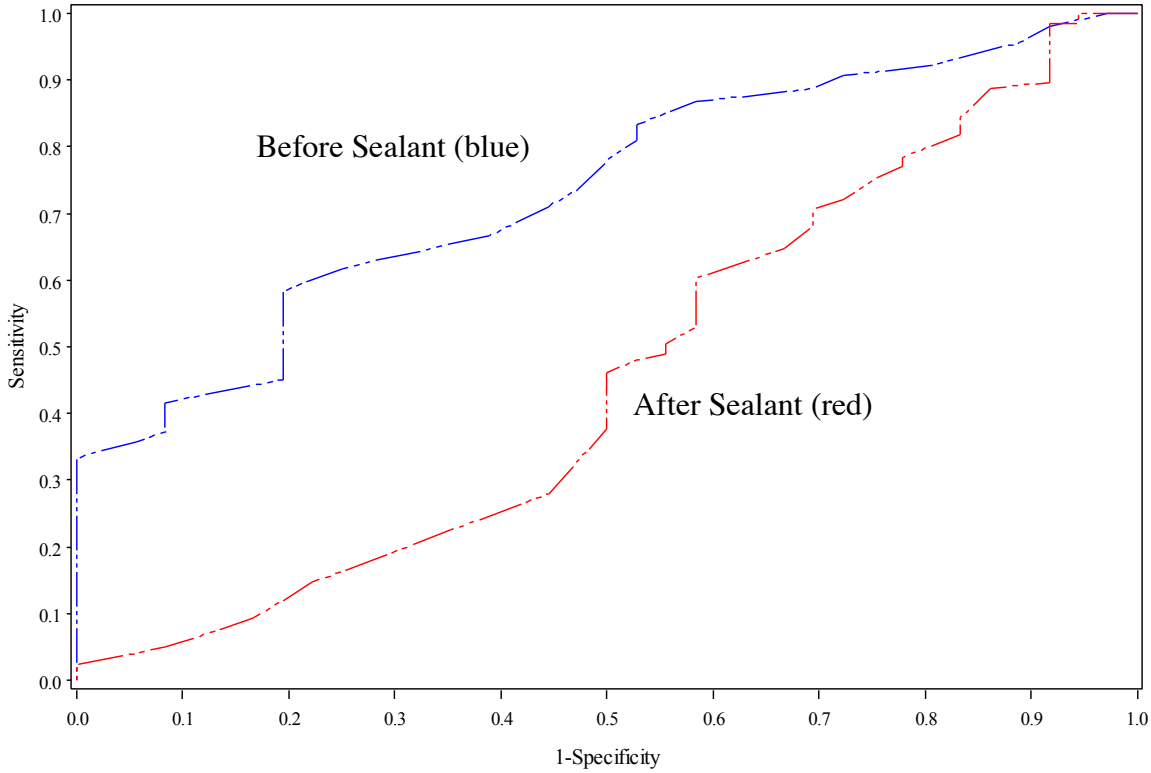


FIGURE 41. Comparison of ROC plots The Canary System. Group 2 (before and after sealant).

TABLE I

Performance of various caries detection aides: reported sensitivity, specificity and other remarks.

Detection Method		Threshold	Sensitivity Mean	Specificity Mean	Remarks
Visual	Visual				Higher performance are obtainable via combination aids; Visual-Tactile[50] & Magnification[58]
		Occlusal	66[50]	89[50]	
		Proximal	94[50]	92[50]	
	ICDAS				
		Enamel	93[56]	60[56]	
		Dentin	52[56]	77[56]	
X-ray	Conventional				Others show: *No difference[53] *Slight increase[54] between digital and conventional.
		Enamel	48[59]	97[59]	
		Dentin	61[59]	95[59]	
	Digital				
		Enamel	48[59]	97[59]	
		Dentin	51[59]	84[59]	
Ultrasound	Ultrasound				Limited number of studies available
		Cavitated	82[57]	75[57]	
		Non-Cavitated	49[57]	N/A[57]	
Fiber Trans Illumination	FOTI				Digital FOTI has same performance.
		Enamel	98[55]	50[55]	
		Dentin	66[55]	96[55]	
OCT	SS-OCT				Limited number of studies available
		Enamel	98[62]	75[62]	
		Dentin	60[62]	98[62]	
Electrical	ECM				
		Enamel	80[55]	71[55]	
		Dentin	68[55]	90[55]	
	ACIST	Unavailable			
Fluorescence	DIAGNOdent				Intraoral fluorescence camera (VistaProof) has same performance as Diagnodent[61]
		Occlusal	80[50]	86[50]	
	QLF				
	Inspektor™	Enamel	74[56]	80[56]	
	Pro	Dentin	85[56]	49[56]	
LUM	Luminescence	Unavailable			
PTR/LUM	The Canary System	Unavailable			

TABLE II
Comparisons between caries detection aides

Detection Method		Pros.	Cons.
Visual	Visual	∗ Most used.[18] ∗ Quick and easy.[65] ∗ All surfaces.[65] ∗ Not invasive.[65]	∗ Severity not reported.[18] ∗ Subjective.[65]
	ICDAS	∗ All surfaces.[19] ∗ Early detection.[19] ∗ High agreement inter- & intra-examiner[19].	∗ Training & Calibration is necessary.[64, 66]
X-ray	Conventional	∗ Superior in approximal.[65] ∗ Secondary caries[65].	∗ No early detection.[65] ∗ Activity not reported.[65] ∗ Low dose radiation.[65]
	Digital	∗ Image enhancement[60] ∗ Digital subtraction[60] ∗ Longitudinal monitoring[60] ∗ Decrease exposure[60]	∗ Lower performance[60] ∗ Low dose radiation.[65] ∗ Expensive.[65]
Fiber Trans Illumination	FOTI	∗ All surfaces[60] ∗ Detects fracture and cracks[63] ∗ High specificity[67]	∗ Subjective[60] ∗ Poor longitudinal monitoring[60]low sensitivity.[67] ∗ Similar performance as visual and radiography.[67]
OCT Optical Coherence Tomography	OCT	∗ Longitudinal monitoring.[63] ∗ Insensitive to saliva, stain, ambient light or plaque.[63]	∗ No data yet.
Electrical	ECM Electronic Caries Monitor	∗ Longitudinal monitoring.[60] ∗ Site or surface specific[60] ∗ Root caries detection	∗ Affected by temperature, thickness and hydration[60] ∗ Time consuming[63]
	ACIST AC Impedance Spectroscopy Technique	∗ Insensitive to stain.[63] ∗ Quantitative.[63] ∗ Longitudinal monitoring.[63]	∗ Occlusal and smooth surfaces only.[63] ∗ Cannot check for secondary caries.[63]
Fluorescence	DIAGNOdent	∗ Quantifiable[60] ∗ Occlusal, approximal and smooth surfaces.[65] ∗ Early detection for hidden dentin caries[67]	∗ Sensitive to stain, calculus and plaque[60] ∗ High false positive.[67] ∗ Only primary lesions[63] ∗ Not for early enamel lesion.[67]
	QLF Inspektor ™ Pro	∗ Quantifiable[60] ∗ Longitudinal monitoring[60]	∗ Sensitive to stain, calculus and plaque[60] ∗ Sensitive to fluorosis[63]
PTR/LUM	The Canary System®	∗ Depth profiling.[63] ∗ Early detection.[63] ∗ Insensitive to stain.[63] ∗ Check for secondary caries[63]	∗ Limited data available.

TABLE III

ICDAS assessment system.[71]

Score	Description
Code 0:	Sound tooth surface
Code 1:	First visual change in enamel
Code 2:	Distinct visual change in enamel/dentin
Code 3:	Enamel breakdown
Code 4:	Underlying dark shadow from dentin with or without enamel breakdown
Code 5:	Distinct cavity with visible dentin
Code 6:	Extensive distinct cavity with visible dentin

TABLE IV

Quantitative parameters obtained by QLF

Name (Symbol)	Unit	Description
Delta F (ΔF)	%	Average loss in green fluorescence in percent.
Lesion Area ($A_{\Delta F}$)	mm^2 or pixels	Lesion area; given in square millimetre or pixels for Inspektor Pro and in pixels for QLF-D Biluminator 2.
Delta Q (ΔQ)	$\%.mm^2$ or %.pixel	Green fluorescence radiance loss. The mathematical result of multiplying Delta F and Lesion Area ($\Delta F \times A_{\Delta F}$).
Delta F Max (ΔF_{MAX})	%	Maximum loss of green fluorescence in percent.
Delta R (ΔR)	%	Percentage of increase of the ratio of the red and green fluorescence with respect to the ratio of sound tissue.
Red Fluorescence Area ($A_{\Delta R}$)	Pixels	Area of red fluorescence given in pixels.

TABLE V

Histological findings assessment system.[117]

Classification	Measurements
0	No lesions
1	Lesion in outer ½ of enamel
2	Lesion in inner ½ of enamel or outer 1/3 of dentin
3	Lesion in middle 1/3 of dentin
4	Lesion in inner 1/3 of dentin

TABLE VI

Calibration readings reliability; intra-class correlation coefficients ICC

Method	**Device**	**Variable**	**Inter-Examiner ICC**	**Intra-Examiner ICC EX. (Ex.1)**	**EX. (Ex.2)**	**EX. (Ex.3)**
ICDAS			0.74*	0.80	0.77	0.78
QLF	Inspektor Pro	Δ F %	0.68*	0.95	0.90	0.84
		Area mm^2	0.67*	0.98	0.77	0.81
		Δ Q %. mm^2	0.81*	0.97	0.75	0.81
	QLF-D Biluminator 2	Δ F %	0.95†	0.96	0.94	N/A
		Δ F Max %	0.96†	0.99	0.96	N/A
		Area pixels	0.77†	0.76	0.86	N/A
		Δ Q %.pixels	0.76†	0.75	0.84	N/A
PTR/LUM	The Canary System	Canary #	0.57*	0.46	0.58	0.56

*** Represents inter-examiner agreement between 3 examiners: (Ex.1), (Ex.2) and (Ex.3).**
† Represents inter-examiner agreement between 2 examiners: (Ex.1) and (Ex.2).

TABLE VII

Group 1 readings reliability; intra-class correlation coefficients ICC

Method	**Device**	**Variable**	**Inter-Examiner ICC**	**Intra-Examiner ICC**		
				EX. (Ex.1)	**EX. (Ex.2)**	**EX. (Ex.3)**
ICDAS			0.72	0.87	0.81	0.85
QLF	Inspektor Pro	Δ F %	0.73	0.97	0.51	0.49
		Area mm^2	0.75	0.97	0.80	0.74
		Δ Q %. mm^2	0.85	0.97	0.89	0.87
	QLF-D Biluminator 2	Δ F %	0.96	0.98	0.96	0.99
		Δ F Max %	0.99	0.99	0.99	1.00
		Area pixels	0.91	0.98	0.94	0.97
		Δ Q %.pixels	0.96	0.99	0.98	0.99
PTR/LUM	The Canary System	Canary #	0.48	0.33	0.63	0.58

TABLE VIII

Group 2 readings reliability before sealant placement.
intra-class correlation coefficients ICC

Method	Device	Variable	Inter-Examiner ICC	Intra-Examiner ICC	
				EX. (Ex.1)	EX. (Ex.2)
ICDAS			0.80	0.87	0.90
QLF	Inspektor Pro	ΔF %	0.78	0.98	0.48
		Area mm^2	0.59	0.94	0.39
		ΔQ %. mm^2	0.83	0.95	0.67
	QLF-D Biluminator 2	ΔF %	0.96	0.98	0.98
		ΔF Max %	0.98	0.99	0.99
		Area pixels	0.89	0.93	0.91
		ΔQ %.pixels	0.95	0.96	0.95
PTR/LUM	The Canary System	Canary #	0.54	0.52	0.68

TABLE IX

Group 2 readings reliability after placement
of sealant. Intra-class correlation coefficients ICC

Method	Device	Variable	Inter-Examiner ICC	Intra-Examiner EX. (Ex.1)	EX. (Ex.2)
QLF	Inspektor Pro	Δ F %	0.29	0.24	0.37
		Area mm^2	0.43	0.35	0.42
		Δ Q %. mm^2	0.45	0.37	0.43
	QLF-D Biluminator 2	Δ F %	0.74	0.80	0.84
		Δ F Max %	0.69	0.57	0.84
		Area pixels	0.63	0.39	0.82
		Δ Q %.pixels	0.71	0.53	0.85
PTR/LUM	The Canary System	Canary #	0.01	0.47	0.22

TABLE X

Reliability for Group 1 and Group 2 before sealant placement (ICC)

Method	Device	Variable	Group #	Inter-Examiner	Intra-Examiner ICC		
				ICC	EX. (Ex.1)	EX. (Ex.2)	EX. (Ex.3)
ICDAS			Group 1	0.72*	0.87	0.81	0.78
			Group 2	0.80†	0.87	0.90	N/A
QLF	Inspektor Pro	Δ F %	Group 1	0.73*	0.97	0.51	0.84
			Group 2	0.78†	0.98	0.48	N/A
		Area mm^2	Group 1	0.75*	0.97	0.80	0.81
			Group 2	0.59†	0.94	0.39	N/A
		Δ Q %. mm^2	Group 1	0.85*	0.97	0.89	0.81
			Group 2	0.83†	0.95	0.67	N/A
	QLF-D Biluminator 2	Δ F %	Group 1	0.96*	0.98	0.96	N/A
			Group 2	0.96†	0.98	0.98	N/A
		Δ F Max %	Group 1	0.99*	0.99	0.99	N/A
			Group 2	0.98†	0.99	0.99	N/A
		Area pixels	Group 1	0.91*	0.98	0.94	N/A
			Group 2	0.89†	0.93	0.91	N/A
		Δ Q %.pixels	Group 1	0.96*	0.99	0.98	N/A
			Group 2	0.95†	0.96	0.95	N/A
PTR/LUM	The Canary System	Canary #	Group 1	0.48*	0.33	0.63	0.56
			Group 2	0.54†	0.52	0.68	N/A

* Represents inter-examiner agreement between 3 examiners: (Ex.1), (Ex.2) and (Ex.3).
† Represents inter-examiner agreement between 2 examiners: (Ex.1) and (Ex.2).

TABLE XI:

Reliability for Group 2 before and after the placement of sealant (ICC)

Method	Device	Variable	Sealant	Inter-Examiner ICC	Intra-Examiner EX. (Ex.1)	EX. (Ex.2)
QLF	Inspektor Pro	Δ F %	Before	0.78	0.98	0.48
			After	0.29	0.24	0.37
		Area mm^2	Before	0.59	0.94	0.39
			After	0.43	0.35	0.42
		Δ Q %. mm^2	Before	0.83	0.95	0.67
			After	0.45	0.37	0.43
	QLF-D Biluminator 2	Δ F %	Before	0.96	0.98	0.98
			After	0.74	0.80	0.84
		Δ F Max %	Before	0.98	0.99	0.99
			After	0.69	0.57	0.84
		Area pixels	Before	0.89	0.93	0.91
			After	0.63	0.39	0.82
		Δ Q %.pixels	Before	0.95	0.96	0.95
			After	0.71	0.53	0.85
PTR/LUM	The Canary System	Canary #	Before	0.54	0.52	0.68
			After	0.01	0.47	0.22

TABLE XII

Descriptive analysis of dental sealant for Group 2
1 millimeter = 1000 μm

Maximum Thickness Parameter	**Value in millimeters.**
Mean	1.08
Median	1.06
Standard Deviation	0.26
Standard Error	0.03
Minimum	0.35
Maximum	1.65

TABLE XIII

: Agreement between devices' readings with and without sealant (ICC)

Method	Device	Variable (Unit)	Intra-class correlation coefficient ICC
QLF	Inspektor Pro	Δ F (%)	0.14
		Area (mm^2)	0.12
		Δ Q (%. mm^2)	0.13
	QLF-D Biluminator 2	Δ F (%)	0.32
		Δ F Max (%)	0.21
		Area (pixels)	0.08
		Δ Q (%.pixels)	0.21
PTR/LUM	The Canary System	Canary #	0.05

TABLE XIV

Group 1 performance parameters; sensitivity, specificity, percentage correct and are under ROC curve AUC

Method	Device	Variable	Sensitivity	Specificity	% Correct	AUC
ICDAS			0.82	0.86	0.83	0.87
QLF	Inspektor™ Pro	Δ F %	0.89	0.60	0.82	0.90
	QLF-D Biluminator™ 2	Δ F %	0.96	0.57	0.86	0.94
PTR/LUM	The Canary System®	Canary #	0.85	0.43	0.74	0.79

TABLE XV

Group 2 before sealant performance parameters. sensitivity, specificity, percentage correct and area under ROC curve AUC

Method	Device	Variable	Sensitivity	Specificity	% Correct	AUC
ICDAS			0.73	0.92	0.76	0.84
QLF	Inspektor™ Pro	Δ F %	0.80	0.73	0.79	0.87
	QLF-D Biluminator™ 2	Δ F %	0.76	0.69	0.75	0.83
PTR/LUM	The Canary System®	Canary #	0.92	0.19	0.81	0.74

TABLE XVI

Group 2-sealed performance parameters: sensitivity, specificity, percentage correct and are under ROC curve AUC

Method	Device	Variable	Sensitivity	Specificity	% Correct	AUC
QLF	Inspektor™ Pro	Δ F %	0.99	0.03	0.84	0.67
	QLF-D Biluminator™ 2	Δ F %	1.00	0.00	0.85	0.70
PTR/LUM	The Canary System®	Canary #	0.54	0.50	0.53	0.58

TABLE XVII

Performance parameters for group 1, group 2 before and after the sealant;. sensitivity, specificity, percentage correct and are under ROC curve AUC

Method	Device	Variable	Group	Sensitivity	Specificity	% Correct	AUC
ICDAS			Group 1	0.82	0.86	0.83	0.87
			Group 2 Presealed	0.73	0.92	0.76	0.84
QLF	Inspektor ™ Pro	Δ F %	Group 1	0.89	0.60	0.82	0.90
			Group 2 Presealed	0.80	0.73	0.79	0.87
			Group 2 Sealed	0.99	0.03	0.84	0.67
	QLF-D Biluminator ™ 2	Δ F %	Group 1	0.96	0.57	0.86	0.94
			Group 2 Presealed	0.76	0.69	0.75	0.83
			Group 2 Sealed	1.00	0.00	0.85	0.70
PTR/ LUM	The Canary System®	Canary #	Group 1	0.85	0.43	0.74	0.79
			Group 2 Presealed	0.92	0.19	0.81	0.74
			Group 2 Sealed	0.54	0.50	0.53	0.58

TABLE XVIII

Performance parameters for recommended and experimental cutoff points (thresholds)*

Method	Group	Variable (Threshold)	Sensitivity	Specificity	% Correct	AUC
Inspektor™ Pro	Group 1	Δ F (≤ 5%)	0.89	0.60	0.82	0.90
		Δ F (≤ 7%)	0.87	0.82	0.86	
	Group 2 Presealed	Δ F (≤ 5%)	0.80	0.73	0.79	0.87
		Δ F (≤ 7%)	0.77	0.92	0.79	
QLF-D Biluminator ™ 2	Group 1	Δ F (≤ 5%)	0.96	0.57	0.86	0.94
		Δ F (≤ 7%)	0.84	0.89	0.85	
	Group 2 Presealed	Δ F (≤ 5%)	0.76	0.69	0.75	0.83
		Δ F (≤ 7%)	0.71	0.83	0.73	
	Group 2 Sealed	Δ F (≤ 5%)	1.00	0.00	0.85	0.70
		Δ F (≤ 12.5%)	0.69	0.70	0.69	
The Canary System®	Group 1	CN (≤ 20)	0.85	0.43	0.74	0.79
		CN (≤ 25)	0.75	0.64	0.73	
	Group 2 Presealed	CN (≤ 20)	0.92	0.19	0.81	0.74
		CN (≤ 25)	0.83	0.47	0.78	

*Sensitivity, specificity, percentage correct and are under ROC curve AUC, Group 1 (white background), group 2 before sealant (reverse contrast) and group 2 after sealant (blue background)..

TABLE XIX

Correlation of detection methods and histology scores
Group 1 (white background), group 2 before sealant (reverse contrast) and group 2 after sealant (blue background)

Method	Device	Variable	Correlation		
			Group 1	Group 2 Before Sealant	Group 2 After Sealant
ICDAS			0.81*	0.79*	N/A
QLF	Inspektor Pro	Δ F %	0.80*	0.77*	0.26$
		Area mm^2	0.81*	0.73*	0.20$
		Δ Q %. mm^2	0.81*	0.76*	0.21$
	QLF-D Biluminator 2	Δ F %	0.79*	0.73*	0.47†
		Δ F Max %	0.79*	0.77*	0.50†
		Area pixels	0.81*	0.82*	0.19$
		Δ Q %.pixels	0.83*	0.82*	0.35$
PTR/ LUM	The Canary System	Canary #	0.44$	0.53†	0.08$

*** Represents significance level p<0.001**
† Represents significance level p<0.05
$ Represents NO significance.

TABLE XX

Cross-tabulation between histological validation
and ICDAS for examiner (Ex.1) and Group 1

	ICDAS Scores (Ex.1) (Group 1)	Histological Score					Total ICDAS
		0	1	2	3	4	
Test 1	0	15	6	3			24
	1		3	7			10
	2		1	12	3		16
	3			4	1		5
	4			1	1	2	4
	5					1	1
	Total Histology	15	10	27	5	3	60

	ICDAS Scores (Ex.1) (Group 1)	Histological Score					Total ICDAS
		0	1	2	3	4	
Test 2	0	15	5	3			23
	1		2	2	1		5
	2		3	17	2		22
	3			4		1	5
	4			1	2	2	5
	5						
	Total ICDAS	15	10	27	5	3	60

LEGEND	Agreement	Under-scored	Over-scored

TABLE XXI

Cross-tabulation between histological validation and ICDAS for examiner (Ex.2) and Group 1

	ICDAS Score (Ex.2) (Group 1)	Histological Score					Total Histology
		0	1	2	3	4	
Test 1	0	11	5	4	1		21
	1	3	4	12	1		20
	2	1	1	8	2		12
	3			3	1	1	5
	4					2	2
	5						
	Total ICDAS	15	10	27	5	3	60

	ICDAS Score (Ex.2) (Group 1)	Histological Score					Total Histology
		0	1	2	3	4	
Test 2	0	12	6	3			21
	1	3	4	10	2		19
	2			10	2		12
	3			3	1	1	5
	4			1		2	3
	5						
	Total ICDAS	15	10	27	5	3	60

LEGEND	Agreement	Under-scored	Over-scored

TABLE XXII

Cross-tabulation between histological validation and ICDAS for examiner (Ex.3) and Group 1

	ICDAS score (Ex.3) (Group 1)	Histological Score					Total Histology
		0	1	2	3	4	
Test 1	0	12	5	2			19
	1	1	3	4			8
	2	2	2	10	2		16
	3			8	3	1	12
	4			3		2	5
	5						
	Total ICDAS	15	10	27	5	3	60

	ICDAS score (Ex.3) (Group 1)	Histological Score					Total Histology
		0	1	2	3	4	
Test 2	0	11	5	2			18
	1	2	2	6			10
	2	2	3	8	1		14
	3			8	1	1	10
	4			3	3	2	8
	5						
	Total ICDAS	15	10	27	5	3	60

LEGEND	Agreement	Under-scored	Over-scored

TABLE XXIII

Cross tabulation between histological validation and ICDAS for examiner (Ex.1) and Group 2

	ICDAS Score (Ex.1) (Group 2)	Histological Score					Total Histology
		0	1	2	3	4	
Test 1	0	9	11	2			22
	1		5	8		1	14
	2		1	7	3	2	13
	3			4	2		6
	4				2	3	5
	5						
	Total ICDAS	9	17	21	7	6	60

	ICDAS Score (Ex.1) (Group 2)	Histological Score					Total Histology
		0	1	2	3	4	
Test 2	0	8	10	2			20
	1	1	2	6			9
	2		5	11	3	3	22
	3			1	2		3
	4			1	2	3	6
	5						
	Total ICDAS	9	17	21	7	6	60

LEGEND	Agreement	Under-scored	Over-scored

TABLE XXIV

Cross tabulation between histological validation and ICDAS for examiner (Ex.2) and Group 2

	ICDAS Score (Ex.2) (Group 2)	Histological Score					Total Histology
		0	1	2	3	4	
Test 1	0	9	11	5			25
	1		6	7	1	1	15
	2			7	2	1	10
	3			2	3	2	7
	4				1	2	3
	5						
	Total ICDAS	9	17	21	7	6	60

	ICDAS Score (Ex.2) (Group 2)	Histological Score					Total Histology
		0	1	2	3	4	
Test 2	0	7	10	3	1		21
	1	2	6	10	1	1	20
	2		1	6	3	1	11
	3			2	1	1	4
	4				1	3	4
	5						
	Total ICDAS	9	17	21	7	6	60

LEGEND	Agreement	Under-scored	Over-scored

TABLE XXV

Cross tabulation between histological validation and number of observations of each detection method for group 1

ICDAS Score	Histological Score					Total (%) Methods
	0	1	2	3	4	
0	77 (21%)	33 (9%)	15 (4%)	1 (0%)		126 (35%)
1	9 (3%)	18 (5%)	41 (11%)	4 (1%)		72 (20%)
2	4 (1%)	9 (3%)	65 (18%)	14 (4%)		92 (26%)
3			31 (9%)	6 (2%)	5 (1%)	42 (12%)
4			10 (3%)	5 (1%)	12 (3%)	27 (8%)
5					1 (0%)	1 (0%)
Total (%) Histology	90 (25%)	60 (17%)	162 (45%)	30 (8%)	18 (5%)	360 (100%)
Inspektor™ ΔF	0	1	2	3	4	Total (%) Methods
ΔF ≤ 5% (Sound)	54 (15%)	25 (7%)	5 (1%)			84 (23%)
ΔF > 5% (Caries)	36 (10%)	35 (10%)	157 (44%)	30 (8%)	18 (5%)	276 (77%)
Total (%) Histology	90 (25%)	60 (17%)	162 (45%)	30 (8%)	18 (5%)	360 (100%)
QLF-D ΔF	0	1	2	3	4	Total (%) Methods
ΔF ≤ 5% (Sound)	51 (14%)	10 (3%)				61 (17%)
ΔF > 5% (Caries)	39 (11%)	50 (14%)	162 (45%)	30 (8%)	18 (5%)	299 (83%)
Total (%) Histology	90 (25%)	60 (17%)	162 (45%)	30 (8%)	18 (5%)	360 (100%)
Canary Number (CN)	0	1	2	3	4	Total (%) Methods
CN ≤ 20 (Sound)	39 (11%)	16 (4%)	23 (6%)	2 (1%)	1 (0%)	81 (23%)
20 < CN ≤ 70 (Caries)	51 (14%)	44 (12%)	134 (37%)	28 (8%)	16 (4%)	273 (76%)
CN ≥ 71 (Advanced Caries)			5 (1%)		1 (0%)	6 (2%)
Total (%) Histology	90 (25%)	60 (17%)	162 (45%)	30 (8%)	18 (5%)	360 (100%)
LEGEND	Agreement		Under-scored		Over-scored	

TABLE XXVI

Correlation of agreement between histological validation and number of observations of each detection method for group 2 before sealant placement.

CDAS Score	Histological Score					Total (%) Methods
	0	1	2	3	4	
0	33 (14%)	42 (18%)	12 (5%)	1 (0%)		88 (37%)
1	3 (1%)	19 (8%)	31 (13%)	2 (1%)	3 (1%)	58 (24%)
2		7 (3%)	31 (13%)	11 (5%)	7 (3%)	56 (23%)
3			9 (4%)	8 (3%)	3 (1%)	20 (8%)
4			1 (0%)	6 (3%)	11 (5%)	18 (8%)
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
Inspektor™ ΔF	0	1	2	3	4	Total (%) Methods
ΔF ≤ 5% (Sound)	26 (11%)	39 (16%)	2 (1%)			67 (28%)
ΔF > 5% (Caries)	10 (4%)	29 (12%)	82 (34%)	28 (12%)	24 (10%)	173 (72%)
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
QLF-D ΔF	0	1	2	3	4	Total (%) Methods
ΔF ≤ 5% (Sound)	25 (10%)	48 (20%)				73 (30%)
ΔF > 5% (Caries)	11 (5%)	20 (8%)	84 (35%)	28 (12%)	24 (10%)	167 (70%)
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
Canary Number (CN)	0	1	2	3	4	Total (%) Methods
CN ≤ 20 (Sound)	7 (3%)	14 (6%)	2 (1%)			23 (10%)
20 < CN ≤ 70 (Caries)	29 (12%)	54 (23%)	82 (34%)	28 (12%)	24 (10%)	217 (90%)
CN ≥ 71 (Advanced Caries)						
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
LEGEND	Agreement		Under-scored		Over-scored	

TABLE XXVII

Correlation of agreement between histological validation and number of observations of each detection method for group 2-sealed

Inspektor™ ΔF	Histological Score					TOTAL (%) Methods
	0	1	2	3	4	
ΔF ≤ 5% (Sound)	1 (0%)	2 (1%)		1 (0%)		4 (2%)
ΔF > 5% (Caries)	35 (15%)	66 (28%)	84 (35%)	27 (11%)	24 (10%)	236 (98%)
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
QLF-D ΔF	0	1	2	3	4	Total (%) Methods
ΔF ≤ 5% (Sound)						
ΔF > 5% (Caries)	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
Canary Number (CN)	0	1	2	3	4	Total (%) Methods
CN ≤ 20 (Sound)	18 (8%)	33 (14%)	41 (17%)	9 (4%)	11 (5%)	112 (47%)
20 < CN ≤ 70 (Caries)	18 (8%)	35 (15%)	43 (18%)	19 (8%)	13 (5%)	128 (53%)
CN ≥ 71 (Advanced Caries)						
Total (%) Histology	36 (15%)	68 (28%)	84 (35%)	28 (12%)	24 (10%)	240 (100%)
LEGEND	Agreement		Under-scored		Over-scored	

TABLE XXVIII

Agreement between histological validation methods;
without the use of enhancing dye and with Rhodamine B as dye

Group 1		Histological Score with Rhodamine B					Total (%)
		0	1	2	3	4	
Histological Score without Dye.	0	15	3	1			19 (32%)
	1		6				6 (10%)
	2		1	26			27 (45%)
	3				5	1	6 (10%)
	4					2	2 (3%)
Total w/ Dye (%)		15 (25%)	10 (17%)	27 (45%)	5 (8%)	3 (5%)	60 (100%)

	Kappa	Weighted Kappa
Group 1 Agreement	0.86	0.90

Group 2		Histological Score with Rhodamine B					Total (%)
		0	1	2	3	4	
Histological Score without Dye.	0	9	1				10 (17%)
	1		15	2			17 (28%)
	2		1	19			20 (33%)
	3			1	6		7 (12%)
	4					6	6 (10%)
Total w/ Dye (%)		9 (15%)	17 (28%)	22 (37%)	6 (10%)	6 (10%)	60 (100%)

	Kappa	Weighted Kappa
Group 2 Agreement	0.89	0.93

LEGEND	Agreement	Under-scored	Over-scored

TABLE XXIX

Comparison between ICDAS and non-conventional methods in terms of sensitivity, specificity and AUC for Group 1; significance level key at the bottom

Method	**Device**	**Variable**	**Comparison ICDAS vs.**		
			Sensitivity	Specificity	AUC
QLF	Inspektor Pro	Δ F %	No Significance	† ICDAS > Inspektor	No Significance
	QLF-D Biluminator 2	Δ F %	*ICDAS < QLF-D	† ICDAS > QLF-D	†ICDAS < QLF-D
PTR/ LUM	The Canary System	Canary #	No Significance	*ICDAS > Canary	No Significance

*** Represents significance level p < 0.001**

† Represents significance level p< 0.05

DISCUSSION

DESIGN RATIONALE

The rationale behind keeping these two groups separated came after considering three possible effects that could have altered the histological examination process, especially for caries free and early lesions. These three factors were: 1) The effect of acid etch treatment on the histology of enamel[44]; 2) Possible excessive accumulation of fluorescent dye in voids between the resin sealant and enamel surfaces[43]; and 3) in the case of a complete lesion infiltration by sealant resin, the accuracy of the lesion's depth measurement.[43]

A single application direct delivery, non-fluoride releasing, visible light-polymerizing resin, opaque dental sealant was selected for the following reasons: 1) non-fluoride releasing dental sealant is expected to cause minimum effect on caries detection device readings and histological vlaidation[23]; 2) a visible light polymerizing resin sealant has been recommended by a meta-analysis[15] and a literature review;[23] 3) opaque dental sealants are recommended for clinical use because they are easier to identify and monitor;[23,24] and 4) a single dose package of direct delivery system was sought to offer a uniformed quality.

SAMPLE SIZE AND PREPARATION

Eight *in-vitro* studies that have evaluated detection of caries on occlusal surfaces of human permanent teeth, cited in this thesis, had a sample size range from 38-163 with a mean sample size of 86 human teeth and 2 examiners on average.[55,58,61,72-74,76,77] Both parts

of the study had a sample size of 60 surfaces each, but Part I had three examiners and Part II had only two examiners. Both groups of teeth were selected and prepared in very similar conditions except for the acid etch treatment of the surfaces of Group 2, which could have an effect on the histological validation process.[43,44] Histologically, it was found that lesions of histological score 0 were significantly less in Group 2 (N = 9) than Group 1 (N = 15), where an increase was observed in histological score 1 for Group 2 (N = 17) than those for Group 1 (N = 10) (Table XXII and Table XXIII). This could reflect the true image of selected specimens, but also could be explained, as described by Gwinnett,[44] due to surface demineralization of the sound specimens by the phosphoric acid, which could resemble a caries lesion of the outer half of the enamel (histology score-1) and thus scored as such.

For the storage medium, the effect of thymol is minimal on lesions subjected to laboratory demineralization/remineralization challenge.[115] Specimens of this study were not subjected to any demineralization/remineralization challenge; thymol was the initial storage medium before it was replaced with DI, and tap water was only used during the sectioning process of histological examination. The effect of thymol on the readings of the Canary System was a concern later expressed by the device manufacturer; however, this could not be verified or eliminated for certain in this study. Thymol as a storage medium could have contributed to the low performance of the Canary System in comparison with ICDAS, Inspector Pro and QLF-D Illuminator 2. An additional investigation would be necessary to assess this issue.

HISTOLOGICAL VALIDATION

Ideally, the histological validation process (Gold Standard) should relate to the parameters that the detection method is evaluating. When considering the caries process, it is more logical to think about measuring the extent of mineral loss of the lesion, or the entire volume of the lesion, rather than the depth of lesion.[41] However, lesion depth remains the most used parameter,[41] which makes it a standard for comparison between methods.

While the caries process occurs on a continuous scale, most detection methods try to distinguish stages of the process. Different stages of lesion depth have been correlated with treatment options.[73,75] But for validating methods, a dichotomous standard is used, which means reducing the scale to a binary decision. Whether to have the threshold between sound and early caries stage (histological score 0 and 1) or to place the threshold at the middle of dentin (histological scores 2 and 3) is debatable and hard to defend. The early threshold signifies the stage where preventive treatment should start, where the later threshold could be used to justify a restorative approach.[73,75]

For ICDAS, it is a matter of choice for adapting a cutoff point, where both thresholds can be justified depending on what is being evaluated. However, for the other methods in this study, Inspektor Pro, QLF-D Biluminator 2 and The Canary System, the manufacturers only provide distinction between the sound and the caries state (histological scores 0 and 1). For both QLF devices, more than 5-percent average mineral loss (ΔF) marks the transition from sound to the early caries stage. For the Canary System®, a Canary Number of 20 mark the same transition. None of the manufacturers has provided a guideline on which threshold to use to mark the transition between a preventive and

restorative approach. Therefore, it was logical for this study to use the early threshold (histological scores 0 and 1) as a base of comparison between methods.

The use of stereomicroscope or the light microscope in validating histological depth remains the most common method.[41,42] Rodrigues et al.[42] reported good agreement between the use of enhancing dye (Rhodamine B) with stereomicroscopy and the use of stereomicroscopy without staining (0.67 on weighted kappa). Also Rodrigues et al.[42] reported that the use of stereomicroscope without the use of coloring tends to overscore the depth of the lesion. In this study, agreement was found almost perfect between stereomicroscopy without staining and with stereomicroscopy with Rhodamine B (0.90 to 0.93 on weighted kappa) (Table XXVIII). On the other hand, and contrary to findings by Rodrigues et al.,[42] the use of Rhodamine B as enhancing dye slightly overscored the depth of the lesion (Table XXVIII). A possible explanation can be related to the application of dye technique; in Rodrigues et al., Rhodamine B solution was brushed and immediately washed of the specimens, while specimens in this study were immersed in diluted Rhodamine B dye overnight and then rinsed off. In conclusion, the use of Rhodamine B did not have any significant benefit over the use of stereomicroscopy alone.

Finally, sample distribution for the validation process should represent the target population of the disease distribution.[41] Sample distribution is usually designed to represent the whole spectrum of measurement included for the detection methods being evaluated. For instance, the sample selection in this *in-vitro* study contained sound surfaces and non-cavitated lesions in the range of ICDAS scores 0 thru 4, with slightly equal distribution. However, in a dichotomous histological scale, of a threshold between histological scores 0 and 1, the sample becomes not well distributed, which may yield to

unrealistic performance of sensitivity, specificity and even AUC.[41] Following the histological validation process, Group 1 teeth had a 3:1 ratio of caries to sound lesion distribution. In a way, this process gives greater weight to sensitivity than specificity in calculating accuracy (% correct and AUC), which translates into higher performance for methods that overscore lesions. Huysmans and Longbottom[41] suggest the use of a continuous scale for histological validation rather than the current four to five stages. Another solution would be to include a sample size of sound specimens comparable in number to the caries specimens, which is a big challenge under *in-vitro* conditions.

REPEATABILITY AND AGREEMENT

Management of dental caries has shifted toward a more conservative approach with emphasis on secondary preventive intervention to induce lesion remineralization at early stages.[18,19] This trend requires early caries detection devices that are accurate and valid.[18,19,45,46] But for successful longitudinal monitoring, which is vital for assessing the success of preventive intervention, reliability becomes as important as accuracy itself. High intra-examiner repeatability is essential for longitudinal monitoring, while a high inter-examiner agreement would facilitate communication and collaboration among the dental community.

Routinely, ICDAS agreements are reported by means of kappa; however, ICC is considered superior to kappa in multilevel measures.[47] This is the case for scale measures such as the Canary System, Inspektor Pro and QLF-D Biluminator 2. ICC also can be used for categorical scale such as those used for ICDAS and histological scores; therefore, it would be more practical to use the same correlation coefficient system ICC for all

detection methods in this study. Finally, ICC allows computing agreement for all examiners at once, rather than kappa's limitation to a pair of examiners each time.

A rough guide for classifying the examiner agreement of a diagnostic test using kappa, as suggested by Landis and Koch[48] and Fleiss,[49] for a diagnostic test is as follows: excellent, above 0.75; good, between 0.60 and 0.75; fair, between 0.40 and 0.59; and poor, below 0.40. However, ICC depends on the measurement that is being made. Acceptable ICCs for ICDAS are quite a bit lower than acceptable ICCs for QLFs, because ICDAS is a subjective assessment and inherently harder to repeat. There are no published kappa or ICC agreement values for the Canary System.

GROUP 1

Inter-examiner agreement, using ICC, was high for QLF-D Biluminator 2 (0.91 to 0.99); acceptable for ICDAS (0.72) and the Inspektor Pro (0.73 to 0.85); and was found not acceptable for the Canary System (0.48).

Intra-examiner repeatability, using ICC, was high for QLF-D Biluminator 2 for all parameters (0.94 to 1.00). For ICDAS, repeatability was found acceptable as well (0.81 to 0.87), whereas repeatability for the Canary System was not acceptable (0.33 to 0.63). On the other hand, Inspektor Pro repeatability varied among examiners and parameters; repeatability for Inspektor Pro ΔF, the parameter that is mostly used for caries detection,[74,86,104] was not acceptable for two of the examiners, Ex.2 (0.51) and Ex.3 (0.49), while it was high for the third examiner Ex.1 (0.97).

For ICDAS, Diniz et al.[73] reported similar agreements using ICCs. For Inspektor Pro, this study reports findings lower than those reported by Yin et al.[96] (0.68; 0.80) for

inter-examiner and intra-examiner, respectively, and significantly lower than those reported for smooth surfaces by Tranaeus et al.[104] (0.93 to 0.99). During calibration, examiners' repeatability was significantly higher for all examiners (0.84 to 0.95) and inter-examiner agreement was acceptable at 0.68. However, in the actual study with the exception for examiner (Ex.1), the other two examiners were not as able to repeat their scores. Repeatability agreement variation could be explained by the challenging use of the QLF software for Inspektor Pro. As illustrated in Figure 6, results can be somewhat influenced by the analyst; findings agree with Yin and co-workers'[96] recommendation of using a single trained analyst for analyzing images of Inspektor Pro, especially if longitudinal monitoring is needed. Given that the disagreements were mostly on ΔF, it was most likely due to the analyses portion rather than the image capturing. Agreements and repeatability for QLF-D Biluminator 2 were almost perfect; however, one must consider that the images for Inspektor Pro were taken using the handheld intraoral camera, while the QLF-D Biluminator 2 were taken using a stand, providing little room for difference in the capturing of the images both among examiners and within examiners. Reliability findings demonstrate a challenge to the Canary System in regards to agreements and repeatability, which also uses a hand-held device. It is likely that the setting used for the Inspektor Pro and the Canary System is more representative of a clinical situation. On the other hand, QLF-D Biluminator 2 utilizes an extraoral camera that is designed to be used differently than the other two methods; in this regard, taking an image in a clinical setting would be accomplished either directly for accessible surfaces or indirectly via an intraoral mirror for occlusal surfaces of posterior teeth. Practically, it was not possible for the examiner to hold the QLF-D camera and the tooth at the same time;

therefore, teeth were mounted in wax and the QLF-D Biluminator 2 camera was mounted on a tripod to allow examiners a precise control over imaging geometry and image focusing. Nevertheless, more studies are needed to assess the reliability of QLF-D Biluminator 2 and The Canary System.

GROUP 2

For ICDAS, it was only performed prior to sealant placement. Inter-examiner agreement and intra-examiner repeatability were acceptable. These findings were similar to Part I (Table X).

For Inspektor Pro, inter-examiner agreement and intra-examiner repeatability were significantly lower for all parameters after the placement of the sealant. For instance, after the placement of the sealant, inter-examiner agreement for ΔF decreased from 0.78 to 0.29 and intra-examiner repeatability for ΔF decreased from 0.98 to 0.24 (Table XI). Similarly to Part I, intra-examiner repeatability varied between the two examiners before the placement of the sealant; however, after the placement of the sealant, inter-examiner agreement and intra-examiner repeatability were not acceptable for both examiners (Table XI). Needless to say, after placement of the sealant the identification of where the lesion was and locating the sound margins became more subjective and is more likely the cause of the higher disagreements after the sealant placement.

For QLF-D Biluminator 2, after the placement of the sealant, inter-examiner agreements were significantly lower for all parameters as well. However, agreement and repeatability for ΔF and ΔQ remained acceptable after the placement of the sealant, 0.74 for agreement and (0.80 to 0.84) for repeatability (Table XI). Again, this could be more a

reflection of the fixed set up of the QLF-D Biluminator 2 camera (Figure 15) than of the examiners' agreement.

For the Canary System®, inter-examiner agreement and intra-examiner repeatability were not acceptable before the sealant placement (Table XI). Following the sealant placement, inter-examiner agreement was almost absent (0.01), while intra-examiner repeatability was found to be unacceptable (0.22 to 0.47). Because the Canary System® measurements do not appear to agreeable or repeatable, from before sealant placement or findings from Part I (Table X), it is difficult to say whether the low ICCs were due to differences in the measurements caused by the presence of the sealants or simply due to the poor repeatability of the method. It was also clear that this method has a steep learning curve as it can be seen that examiners' agreement improved with each phase (Table VI, Table VII and Table VIII).

Evidently, the placement of opaque resin sealant has significantly lowered all agreements for all methods, but it had a lesser effect on QLF-D Biluminator 2 (Table XI). Sealant thickness had no impact on methods agreements; correlation between readings before and after sealant was very low (Table XIII), which indicates the presence of the sealant itself is the contributing factor to the lower agreements.

METHODS' PERFORMANCE

For assessing performance of caries detection methods, no single parameter can be used in lieu of all others. A methods that maintains a balance in sensitivity, specificity, % correct and AUC would be the preferred method of choice.[45] A method of higher sensitivity and lower specificity could lead to more preventive treatment or more

restorative treatment depending on the treatment decision. Either way, there is the chance of over treatment.[75]

The most important value of any detection method is aiding in forming a diagnosis and subsequent treatment decision, provide a mean for longitudinal monitoring, and/or predicting lesion progression and regression. Most treatment decisions are made during the visual examination; however, preventive treatments are not usually initiated until a later time.[39,73,75,76] Zandona et al.[79] described the potential of using ICDAS combined with Inspektor Pro in predicting lesions that are more likely to progress. Similarly, Jablonski-Momeni et al.[76] found that the use of additional methods increases the accuracy of treatment planning. On the other hand, Pereira et al.[75] reported a substantial increase in invasive treatment when multiple detection methods are combined. But as reported by numerous studies, other detection methods should be used as an adjunct to visual examination, not as a replacement.[18,19,56,73-76,82,102]

A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system: 0.90 to 1 = excellent; 0.80 to .90 = high; 0.70 to .80 = acceptable; 0.60 to .70 = poor; and below 0.60 = unacceptable.

GROUP 1

Figure 38 combines ROC curves for all detection methods together, which allows rough visual comparison of methods accuracy. Comparing all parameters, the performance of ICDAS was substantially high for sensitivity, specificity, % correct, and AUC, ranging from 0.82 to 0.87 (Table XIV). Inspektor Pro had comparable sensitivity, % correct, and AUC of those of ICDAS; however, specificity was poor (0.60). This is mostly related to

the set cutoff point (threshold) of 5% ΔF. From the ROC curve (Figure 28), a more favorable cutoff point for ΔF would be 7 percent. By shifting the Inspektor Pro ΔF to 7 percent rather than 5 percent, specificity would significantly increase to 0.82, while sensitivity would slightly decrease to 0.87, while % correct would increase to 0.86 without changing the accuracy AUC (Table XVIII). At this suggested threshold of 7% ΔF, Inspektor Pro would have substantially high performance and be comparable to ICDAS.

For QLF-D Biluminator 2 at the current threshold of 5% ΔF, sensitivity and AUC were excellent, 0.96 and 0.94 respectively (Table XIV). However, specificity was poor (0.57). Similarly from ROC curve (Figure 29), a more reasonable cutoff point for ΔF is suggested at 7 percent. By doing so, specificity would increase 0.89 while sensitivity would slightly decrease to 0.84, while % correct would decrease insignificantly to 0.85 without changing the accuracy AUC (Table XVIII).

Of course, changing the cutoff points for the QLF methods requires more investigation to find out whether these new cutoff points are limited to the conditions of this *in-vitro* study or if they can be generalized. Gomez et al.[74] found similar findings and they used 8% ΔF for Inspektor Pro as a cutoff point for sound lesion *in vitro*. Essentially, the sample selection criteria in the Gomez et al.[74] study were very similar to the selection criteria adopted in this study. For both studies, the sample distribution ranged from ICDAS scores 0 thru 4 of permanent posterior teeth, and sample teeth were selected from a pool of teeth collected by the Oral Health Institute of IUSD. Therefore, it cannot be verified if 7% ΔF or 8% ΔF cutoff points are specific to these conditions or could be generalized.

For The Canary System, sensitivity was high (0.85) along with acceptable % correct (0.74) and AUC (0.79). However, specificity was not acceptable for the Canary System (0.43) (Table XIV). From the ROC curve (Figure 30), an optimum cutoff point of the Canary Number (25) yields slightly better specificity (0.64), but still remains poor (Table XVIII). As discussed earlier with such sample distribution, % correct and AUC are expected to be inclined toward higher sensitivity.

The Canary System, under the limitations of these *in-vitro* study conditions, was more likely to identify sound lesions as caries (Tables XVIII and XXV), which might lead to overtreatment. The effect of thymol on the readings of the Canary System as raised by the device manufacturer, could not be verified nor eliminated for sure in this study; therefore, the Canary System low performance obtained here may have been caused by using thymol as the initial storage medium. The Canary System is still considered very recent and further investigation into its performance is highly recommended.

GROUP 2

Following the placement of opaque sealant (Delton DDS), accuracy for all methods was reduced significantly as evident in Figure 39 for Inspektor Pro, Figure 40 for QLF-D Biluminator 2 and from Figure 41 for The Canary System. Specificity for ΔF was abolished for both QLF methods, Inspektor Pro and QLF-D Biluminator 2 (0.03 and 0.00), respectively (Table XVI). Therefore, comparing performance values before and after the sealant is deemed inappropriate, because the methods were unable to correctly identify sound surfaces. Moreover, no optimum cutoff point that brings sensitivity and specificity above (0.60) could be identified using ROC curve for Inspektor Pro ΔF (Figure 33). On

the other hand, QLF-D Biluminator 2 had fair accuracy AUC under the sealant (0.70), and from the ROC curve a cutoff point (threshold) of 12.5% ΔF was suggested (Figure 35), which yields 0.69 for sensitivity, 0.70 for specificity and 0.69 for % correct (Table XVIII). Such performance is a borderline between poor and acceptable; however, it was significantly higher than reported in this study for Inspektor Pro and the Canary System or for those reported for DIAGNOdent[34] and Spectra.[35]

For The Canary System, all values of performance were not acceptable for sensitivity, specificity, % correct and AUC following the placement of the sealants (Table XVII). Before sealant placement sensitivity was very high while specificity was very low. However, following the placement of the sealant, sensitivity (0.54) and specificity (0.50) were similar to those of random probability. This may suggest that the placement of the opaque sealant may have precluded the caries detection ability of the Canary System under the sealant.

Previous reports have indicated that the Canary System can detect caries under the opaque sealant. Abstracts published by the manufacturer[36,37] proclaim its ability to perform well under the opaque sealant including Delton opaque sealant,[36] similar to the one used in this study. Manufacturer's reported sensitivity and specificity after the placement of the sealant were 0.83 and 0.79, respectively,[37] which was not confirmed in this study.

SUMMARY AND CONCLUSIONS

Although dental caries is preventable, it remains the most prevalent chronic disease of children in the US. Intervention that starts at the stage of cavitation is simply considered inadequate. For a timely preventive intervention, early diagnosis of lesions that are more likely to progress is unequivocally crucial. Placement of dental sealants has been shown to be effective in preventing new lesions and in arresting early lesions. However, there is no reliable method that can monitor lesion status under opaque sealants. Therefore, the development and testing of valid methods that detect the early stages of caries is valuable to the dental community and the general population.

Treatment decisions are largely carried during the visual examination. Recent visual examination methods, such as The International Caries Detection and Assessment system, ICDAS, have demonstrated the ability to detect early caries. While performance and agreements tend to be moderate to high, ICDAS requires training and calibration, and it also has the limitation of not being able to detect caries under opaque sealants.

This study evaluated in two parts the *in-vitro* performance of several of the most recent early caries detection methods. The evaluated methods were ICDAS, two Quantitative Light-induced Fluorescence QLF (Inspektor Pro and QLF-D Biluminator 2) and Photothermal Radiometry and Modulated Luminescence, PTR/LUM, (The Canary System). In the first part, methods were evaluated on their ability to detect early caries on occlusal surfaces of posterior permanent human teeth. In the second part, non-conventional methods (QLFs and PTR/LUM) were evaluated on their ability to detect

caries lesions under an opaque sealant on occlusal surfaces of posterior permanent human teeth.

Under the limitations of the conditions of this study, the following were the findings for Part I;

1) ICDAS demonstrated high performance and agreement. Sensitivity, specificity, and AUC were 0.82, 0.86 and 0.87, respectively for examiners 1, 2 and 3. Intra-examiner repeatability ranged from 0.81 to 0.87, while inter-examiner agreement was 0.72. ICDAS was also found to highly correlate with the histological validation.

2) QLF-D Biluminator 2 as used in this study had an almost perfect repeatability, which makes it very reliable for longitudinal monitoring. The method has an excellent accuracy AUC, but poor specificity using the manufacturer recommended threshold of 5% ΔF. However, if the threshold is changed to 7% ΔF, the performance of this method would become comparable to ICDAS. Further investigation is recommended for this particular method.

3) Inspektor Pro specificity was significantly lower than ICDAS. On the other hand, there was no significant difference between the two methods in regards to sensitivity, % correct, and AUC. Repeatability of this method varied among examiners, which makes the method less reliable. Changing the threshold to 7% ΔF would make the performance of this method comparable to ICDAS but its repeatability remains lower than ICDAS. In summary, there was no advantage of using Inspektor Pro alone over the use of ICDAS.

4) The Canary System demonstrated unacceptable performance and repeatability. This method had high sensitivity on the expense of poor specificity. This makes this method unreliable and that it may lead to over treatment.

Following the placement of opaque sealants on the occlusal surfaces, and limited to the conditions of this study, the following were the findings for Part II:

1) Neither Inspektor Pro nor the Canary System has been able to detect caries under the opaque sealant. Moreover, ROC curves did not demonstrate any usable cutoff points (thresholds) to identify early enamel lesions (histological scores 0 and 1).

2) Under the standard thresholds of 5% ΔF for QLF-D Biluminator 2, the method was not able to detect caries under the opaque sealant due to abolished specificity. However, accuracy AUC was acceptable (0.70) and from ROC curve, a threshold of 12.5 % ΔF would yield an acceptable specificity (0.70) and borderline sensitivity (0.69). Considering that (1) agreement and repeatability of ΔF for this method were acceptably high and that (2) no other method has been able to detect caries under the opaque sealant, this method demonstrates a guarded potential in this field. However, more investigations are needed to determine validity of the method and whether this cutoff point (threshold) is unique to the conditions of this study or can be generalized.

3) There was no correlation between sealant thickness and performance of detection methods.

4) Acid etch treatment involved in placing the sealant may have altered the histological validation process by decreasing the number of sound lesions and increasing the number of specimens of histological score 1.

For histological validation methods, there was no significant advantage of using Rhodamine B as enhancing dye with stereomicroscopy over stereomicroscopy alone.

In conclusion and limited to the study's *in-vitro* conditions:

(1) ICDAS remains the method of choice for detection of early caries lesion due to its acceptably high accuracy and repeatability.

(2) QLF systems demonstrate potential in longitudinal monitoring due to an almost perfect repeatability of QLF-D Biluminator 2.

(3) The Canary System performance and repeatability were not acceptable as a valid method of early caries detection.

(4) None of the methods demonstrated acceptable ability in detecting of occlusal caries under the opaque sealant.

(5) However, QLF-D Biluminator 2, with limitation to these *in-vitro* conditions and Delton® opaque sealant, demonstrates a fair accuracy AUC (0.70) in detecting of caries under the sealant at an experimental threshold of 12.5% ΔF.

HYPOTHESES

Objective I

Null Hypothesis 1: From Table XXIX, in terms of sensitivity, QLF-D Biluminator 2 ΔF was significantly higher than ICDAS. In terms of specificity, ICDAS was significantly higher than all other methods. For AUC, QLF-D Biluminator 2 ΔF was significantly higher than ICDAS.

Based on this significance level ($p < 0.05$), there is a difference between the methods in the ability to detect caries. Therefore, the null hypothesis must be rejected; however, the alternative hypothesis cannot be accepted, either, due to specificity values.

Objective II

Null Hypothesis 2: There was no significant difference between Inspektor, QLF-D Biluminator 2 and the Canary System in terms of sensitivity, specificity or AUC; however, the ability of the methods to detect caries was decreased after the placement of opaque sealant. The null hypothesis was not rejected, but the alternative hypothesis could not be accepted, either, due to unacceptable performance under the opaque sealant.

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ABSTRACT

PERFORMANCE OF SEVERAL DIAGNOSTIC SYSTEMS ON DETECTION OF OCCLUSAL PRIMARY CARIES IN PERMANENT TEETH

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Detection of caries at an early stage is unequivocally essential for early preventive intervention. Longitudinal assessment of caries lesions, especially under the opaque preventive sealant, would be of utmost importance to the dental community.

OBJECTIVES: The aim of this two-part *in-vitro* study is to evaluate the performance of multiple detection methods: The International Caries Detection and Assessment System (ICDAS); two quantitative light-induced fluorescence systems QLF; Inspektor™ Pro and QLF-D Biluminator™2 (Inspektor Research Systems B.V.; Amsterdam, The Netherlands); and photothermal radiometry and modulated luminescence (PTR/LUM) of The Canary System® (Quantum Dental Technologies; Toronto, Canada).

All these are to be evaluated on their detection of caries on posterior human permanent teeth for 1) of primary occlusal lesions, and 2) under the sealant of primary occlusal lesions.

METHODS: One hundred and twenty (N = 120) human posterior permanent teeth, selected in compliance with IU-IRB "Institutional Review Board" standards, with non-cavitated occlusal lesions ICDAS (scores 0 to 4) were divided into two equal groups. The second group (N = 60) received an opaque resin dental sealant (Delton® Light-Curing Pit and Fissure Sealant Opaque, Dentsply, York, PA). All lesions were assessed with each detection method twice in a random order except for ICDAS, which was not used following the placement of the sealant. Histological validation was used to compare methods in regard to sensitivity, specificity, % correct, and the area under receiver-operating characteristic curve (AUC). Intra-examiner repeatability and inter-examiner agreement were measured using intraclass correlation coefficient (ICC).

RESULTS: 1) Of primary occlusal lesions, sensitivity, specificity, and AUC values were respectively: 0.82, 0.86 and 0.87 (ICDAS); 0.89, 0.60 and 0.90 (Inspektor Pro); 0.96, 0.57 and 0.94 (QLF-D Biluminator 2); and 0.85, 0.43 and 0.79 (The Canary System). Intra-examiner repeatability and inter-examiner agreement were respectively: 0.81 to 0.87: 0.72 (ICDAS); 0.49 to 0.97: 0.73 (Inspektor Pro); 0.96 to 0.99: 0.96 (QLF-D Biluminator 2); and 0.33 to 0.63: 0.48 (The Canary System). 2) Of primary occlusal lesions under the opaque dental sealants, sensitivity, specificity, and AUC values were respectively: 0.99, 0.03 and 0.67 (Inspektor Pro); 1.00, 0.00 and 0.70 (QLF-D Biluminator 2); and 0.54, 0.50 and 0.58 (The Canary System). Intra-examiner repeatability and inter-examiner agreement

were respectively: 0.24 to 0.37: 0.29 (Inspektor Pro); 0.80 to 0.84: 0.74 (QLF-D Biluminator 2); and 0.22 to 0.47: 0.01 (The Canary System).

CONCLUSION

Limited to these *in-vitro* conditions, 1) ICDAS remains the method of choice for detection of early caries lesion due to its adequately high accuracy and repeatability. QLF systems demonstrate potential in longitudinal monitoring due to an almost perfect repeatability of QLF-D Biluminator 2. The Canary System performance and repeatability were not acceptable as a valid method of early caries detection. 2) None of the methods demonstrated acceptable ability in detecting of occlusal caries under the opaque sealant. However, QLF-D Biluminator 2, with limitation to these *in-vitro* conditions and Delton opaque sealant, demonstrated a fair accuracy AUC (0.70) in detecting of caries under sealants at an experimental threshold of 12.5% ΔF.

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