In vitro evaluation of laser fluorescence devices for caries detection through stereomicroscopic imaging

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Abstract
The aims of this study were to determine the diagnostic accuracy and reproducibility of the laser fluorescence device (LF), and the relationship between laser fluorescence readings taken at the entrance of the fissure, ICDAS visual examination caries detection system and the histological depth of the lesion. Two hundred and forty teeth (122 human third molars and 118 bicuspids) were selected from 62 patients enrolled in the study. Visual and LF examinations (Diagnodent, Kavo, Biberach, Germany) of the occlusal surfaces were performed in vivo. After tooth extraction, histological sections were evaluated by stereomicroscopy in vitro. Statistical analysis was performed using multiple statistical methods (SPSS ver. 17). Intra-examiner reproducibility for the LF measurements was excellent: intra-class-correlation coefficient (ICC) for LF was 0.957. Kappa values for each examiner’s reproducibility were 0.74–0.82. The diagnostic performance of the LF device gave a good overall diagnostic accuracy according to ICDAS II codes and histological values as indicated by the area under the ROC curve of 0.707 and 0.709 respectively. The results of the study showed acceptable diagnostic accuracy for the laser fluorescence device. This supports the view that dentists can be site specific in applying fluorescence-based devices to multiple discrete sites within the same surface. In conclusion, these diagnostic methods have different characteristics, indications and limitations for use. In order to detect caries on occlusal surfaces thoroughly, a combination of methods would be the best practice moderated by clinical knowledge and experience.

Keywords: laser fluorescence, occlusal caries, stereomicroscopy, caries detection, diagnostic accuracy.

Introduction
Early caries detection is a difficult task since occlusal incipient lesions have become difficult to detect due to the widespread use of fluorides and their superficial remineralization potential that seems to delay cavitation. Occlusal surfaces of posterior teeth raise a specific diagnostic problem for the dentist; the invaginated anatomy in pits and fissures means that the lesions begin at the entrance to the fissure or on its walls and spread laterally into the dentine.

Topical fluoride makes the superficial enamel harder and more resistant to collapse, so that large undermining dentine lesions can occur with relatively minor visual changes at the surface.

Occlusal caries have therefore been the focus of much research into caries-detection methods. As the clinical diagnosis concerns, it is mandatory to introduce new methods that will improve the classic procedures. Current demands in the modern diagnosis of dental caries, as well as the way in which the decisions have to be made when assessing the carious activity, call for a strong technical background. One has always to choose the appropriate diagnosis method such not to exceed the initial carious lesion phase. The study of complex dental structures by the means of classic diagnosis methods is limited. Knowledge regarding the modern diagnostic technology is nowadays mandatory. A great drawback for the clinician regarding the caries management strategies based on the risk assessment is the lack of methods to certainly determine the degree of profound dental tissues decay. It is possible to detect the lesions in an early phase, before the emergence of cavity lesion by using the modern methods. This is of particular importance in order to take adequate preventive measures in due time [1].

Dentists have several options for the clinical detection of occlusal dental caries, including visual tactile examination, radiographs, fluorescence methods and electrical conductance measurements [2, 3]. While visual inspection is the primary method of detection and diagnosis of dental caries, it must be kept in mind that caries detection by eyesight is better at an advanced stage than early [4].

Meticulous visual inspection with a good operation light, a dry tooth and a sharp probe can render good sensitivity and specificity values of approximately 80% [5, 6]. Three systems are currently known for meticulous visual inspection: the Nyvad criteria [7] and International Caries Detection and Assessment System (ICDAS) [8] and UniViSS [9].
Besides the visual examination, the most widespread exam to improve the initial carious lesion diagnosis and also the only one used in most dental offices is the radiographic examination [10, 11]. Although the bitewing radiographic examination is thought to be important in the detection of proximal caries, this type of exam shows poor results in the detection of enamel occlusal caries [12]. Several new methods were introduced in the early 1990s, some of them as research tools, while others were used in the dental offices. These were intended to increase the reliability of occlusal caries detection in addition to the visual examination and the radiographic methods. FOTI, DIFOTI, ECM, QLF, DIAGNOdent and D-Carie are some of them [13–15].

In the case of optical based methods for detecting carious lesions, the light interacts with hard dental tissues in different ways: it can be reflected, scattered, transmitted or absorbed. Fluorescence, in which the electrons having a lower energy level move to a higher level, is a possible consequence of absorption. When they reach back the initial level, the energy is emitted as light, which is known as fluorescence. In other words, fluorescence is the result of interaction between the electromagnetic radiation and molecules in the tissue. It is still not clear what causes the enamel fluorescence. The most part of fluorescence is induced by organic components, protein chromophores, but a portion is probably due to apatite. Dentine fluorescence has been suggested to be caused by inorganic complexes, as well as by organic components. In healthy enamel, wave lengths are large, with a high probability that photons will hit a chromophore. Thus, fluorescence is relatively intense. Demineralization of dental hard tissue, enamel or dentine leads to the loss of auto-fluorescence, the natural fluorescence. Several factors may contribute to the decreased fluorescence of early carious lesions. Four possible mechanisms have been proposed: the light scattering in the lesion causes the light path to be much shorter than in the healthy enamel: light absorption per volume is much lower in the lesion, so the fluorescence is weaker; light scattering within the lesion acts as a barrier for excitation light to reach the underlying fluorescent dentine and for fluorescent light in the dentine to reach the surface; fluorescence is ended by a switch in molecular environment of chromophores; protein chromophores are removed by dental caries evolution. The QLF method – light-induced quantitative fluorescence measures the fluorescence induced after use of green-blue laser light at approximately 488 nm wavelength, quantifying demineralization and severity of lesion. There are two optical QLF methods to determine quantitative loss of minerals: QLF I – quantitative laser-induced fluorescence and QLF II – quantitative light-induced fluorescence. In the case of QLF I, the light source is argon laser, while QLF II uses xenon arc lamp. The surface is illuminated with green-blue light with a wavelength of 488 nm from argon laser or with blue light from a xenon arc lamp. The device for intraoral camera has an orange filter for laser and a yellow one for lamp; images are captured by the device, data are collected, stored and analyzed by custom-made software (Inspektor Research Systems BV, Amsterdam, The Netherlands). This method depends on the clinician’s ability to capture the images that will show: healthy enamel – bright fluorescent; dematerialized areas – dark stains. Fluorescent radiance of caries lesions viewed by QLF is lower than that of healthy enamel. In order to allow for the calculation of fluorescence loss in carious lesions, the fluorescent radiance of healthy tissue at the lesion site is reconstructed by interpolation of radiance of healthy tissue surrounding the lesion. The difference between the measured and reconstructed values is the loss of resulting fluorescence in the lesion. Three quantities are obtained: lesion area (\( \text{mm}^2 \)), \( \Delta F \) (average change of fluorescence, in \%), and \( \Delta Q \) (volume lesion). QLF II is influenced by dehydration; being a phenomenon caused by light absorption and reflection by the enamel chromophores, diffusion is increased and fluorescence decreased in early lesions. Effects are higher in vivo than in vitro, were a humid environment exists [16].

The QLF method has been tested in several in vitro, in situ and in vivo studies for caries lesions of tooth surfaces. This method has high in vivo repeatability and reproducibility, which means it can monitor the effects of preventive programmes [17–19].

A laser fluorescence device (LF; DIAGNOdent, KaVo, Biberach, Germany) was introduced some years ago for caries detection. More recently, a pen-type LF device (LF pen) was developed and has been tested. Studies by Hibst R and Gall R showed the red light induced fluorescence (638–655 nm) could differentiate between healthy and carious tooth tissue [20, 21].

KaVoDIAGNOdent (KaVo, Biberach, Germany), based on the studies of Hibst and Gall, is a laser-based instrument, developed for the detection and quantification of tooth caries on smooth and occlusal surfaces. It operates with light from a diode laser at a wavelength of \( \lambda \) 655 nm and 1 mW peak power. Light is transmitted through a descendent optical fiber to a hand-held probe, having an oblique tip that is applied on the dental surface. The device comes with two optical fiber active heads, one angular for occlusal surfaces and one straight for smooth surfaces. Calibration of DIAGNOdent rods is made on healthy enamel. Both organic and inorganic molecules in the tooth substance absorb the light and the fluorescence appears in infrared spectrum. The emitted fluorescence, as well as the scattered light, are captured and passed through the ascending fibers to a photodiode detector. Backscattered excitation and short wavelength ambient light are absorbed through a filter in front of the photo-diode detector. In order to discriminate the fluorescence form the ambient light, the laser diode is modulated. By amplifying only the modulated fraction of the signal, ambient light is suppressed. The signal is finally processed and presented on a screen as an integer between 0 and 99, depending on maximum value and time; an audio signal is emitted. In order to collect fluorescence form the maximum extension of carious lesions on occlusal surfaces, the
instrument has to be tilted on the inspected surfaces. Variations in the output power of the laser should be regularly compensated by calibrating the instrument against the standard fluorescence, according to manufacturer’s instructions. Fluorescence increases in the presence of carious tooth substance. The origin of fluorescence is still on debate, but proto- and mesoporphyrins, bacterial metabolites in the mitochondrial respiratory chain, play a major role. Increased fluorescence in the carious lesions is most probably due to porphyrin, especially protoporphyrin IX, which is synthesized by oral microorganisms. The DIAGNOdent device has been assessed in several in vitro and in vivo studies [21–24].

The aims of this in vitro study were: firstly, to determine the diagnostic accuracy and reproducibility of the laser fluorescence device – DIAGNOdent, and of the ICDAS-II visual classification system for occlusal caries detection and to determine the relationship between the two methods of detection, and secondly, to determine the relationship between laser fluorescence readings taken at the entrance of the fissure and the histological depth of the lesion.

Materials and Methods

Sample selection

The working protocol for the present study was approved by the Ethics Committee of the “Victor Babes” University of Medicine and Pharmacy, Timișoara, Romania. All experiments were conducted according to the European and Romanian law.

Subjects were recruited after one independent examiner assessed their caries status and need for orthodontic treatment, and filled all information regarding medical and dental history.

Inclusion criteria were: subjects aged 12–20 years, who needed extraction of premolars and impacted third molars for orthodontic purposes, having no occlusal restorations or fissure sealants on at least one molar or bicuspid, and having at least one untreated molar or bicuspid presenting an ICDAS score of 0–5.

Subjects had to be healthy and willing to sign the “Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research” form. There were no gender restrictions.

Subjects were excluded from the study if they were suffering from systemic diseases, had a significant past medical history of conditions that may affect oral health (i.e., diabetes, HIV, heart conditions that require antibiotic prophylaxis), or were taking medications that may affect the oral flora (e.g., antibiotic use in the past three months).

Informed consent was obtained from patients or legal guardians of patients who were selected to participate in the study.

Sixty-two patients aged between 12–20 years, mean age 15.81±2.469, comprising 41 females and 21 males, were recruited to participate in the study.

Two hundred and forty teeth, including 122 permanent human third molars and 118 premolars, were selected from the 62 patients enrolled in the study. The study involved only teeth that need to be extracted for orthodontic purposes because histological evaluation and stereomicroscopy is used for caries assessment. Only one occlusal site per tooth was selected for the laser fluorescence measurements and visual examination.

Preliminary preparation

Before evaluating the occlusal surfaces, the teeth were cleaned with a sodium bicarbonate powder-cleaning tool (Prophyflex) for 5 to 10 seconds per tooth and then carefully rinsed to remove the powder remnants from the fissure with an air-water spray. Cotton roles were placed and the occlusal surface was shortly air-dried (three seconds per tooth) immediately before performing an assessment.

Investigation of caries lesion

Visual and laser fluorescence examination (DIAGNOdent, Kavo, Biberach, Germany) of the occlusal surfaces were performed in vivo. After the extraction of the teeth, the histological sections were evaluated through stereomicroscopy in vitro. Since the major aim of using the DIAGNOdent device is the detection of caries in their early stages of development when they can be treated only by preventive methods, only ICDAS II codes up to five (non-cavitated lesions) were included in our study.

Visual examination

All sites were visually examined by two calibrated investigators using the International Caries Detection and Assessment System (ICDAS-II) [25]. The visual classification system ICDAS-II (International Caries Detection and Assessment System) was developed to provide clinicians, epidemiologists, and researchers with an evidence-based method for standardized data collection in different settings and better comparison between studies [26].

The chosen sites were recorded by two examiners blinded to each other, according to the ICDAS standardization as:

- 0 – sound;
- 1 – first visible sign of non-cavitated lesion seen only when the tooth is dried;
- 2 – clinically visible non-cavitated lesion seen when wet and dry;
- 3 – microcavitation in enamel;
- 4 – non-cavitated lesion extending into dentine seen as an undermining shadow;
- 5 – small cavitated lesion with visible dentine: less than 50% of surface.

ICDAS-II code 6 represents large cavitated lesions with visible dentine in more than 50% of the surface; therefore, teeth with this code were not included in the examinations.

After independently scoring for ICDAS II, the examiners discussed their findings and agreed on one ICDAS II score per different areas of the tooth.
Examination of the occlusal surfaces with the laser fluorescence device (LF)

The laser fluorescence measurements were carried out with the DIAGNOdent 2095 (KaVo, Bieberach, Germany). The measurements, as well as visual examination, were performed three times by two calibrated examiners observing a one-week interval between the evaluations. The probe tip selected for the DIAGNOdent was the cone-shaped one (Type A), indicated for pits and fissure areas. Dry, clean surfaces were inspected by direct visualization and under excellent lighting, without probing. Prior to the laser fluorescence measurements, for each individual tooth, the device was calibrated against a ceramic reference (standard calibration) according to the manufacturer’s specifications, and, afterwards, the zero value of fluorescence was obtained from a sound part of the buccal surface. The probe tip was placed perpendicularly on the test site and moved until the maximum value (peak) was reached, and this value was recorded. From the peak value, the zero value fluorescence was subtracted. This peak value was then correlated with the definitions of a scale supplied by Lussi A and Hellwig E [27], which corresponds to the absence or presence of a carious lesion, as well as to its degree of progression, as follows: D0: 0–7, sound; D1: 7.1–14, caries up to halfway through the enamel; D2: 14.1–24, caries in the inner half of enamel; D3>24, caries in the dentine.

Stereomicroscopic evaluation of the carious lesion

Stereomicroscopy allows the study of three-dimensional images. These qualities are based on large fields of interpretation and the large distances between 92 mm and 286 mm, with a magnification from ×1.95 to ×225. For the optical study in stereomicroscopy an Olympus microscope SZX 7 and an Olympus camera with 2.5× digital zoom and 3× optical zoom and polarized light were used.

For validation of the results, the teeth were sectioned for histological examination. Teeth were hemi-sectioned in a mesial–distal direction through the fissure pattern with a high-speed drill and fine diamond bur. The specimens were submerged in water and examined on the microscope.

The Downer histological classification system [28] was used to record caries severity at each investigation site: 0 – No enamel demineralization or a narrow surface zone of opacity (edge phenomenon); 1 – Enamel demineralization limited to the outer 50% of the enamel layer; 2 – Demineralization involving the inner 50% of the enamel, up to the enamel–dentine junction; 3 – Demineralization involving the outer 50% of the dentine; 4 – Demineralization involving the inner 50% of the dentine.

A histological score was given to each section corresponding to each investigation site and the worst/deepest score was taken as the definitive score for further analysis. All the sites were investigated by each examiner blind to each other.

Data management and statistical evaluation

Statistical analysis was performed using multiple statistical methods (SPSS ver. 17). For the laser fluorescence (LF) measurements, intra-examiner reproducibility was calculated using the Intraclass Correlation Coefficient (ICC).

The distribution of the laser fluorescence measurements taken on the tooth surface was plotted according to ICDAS-II codes and histological measurements using box plots. Diagnostic accuracy of the laser fluorescence device was calculated as the area under the ROC curve (AROC) according to ICDAS and histological scores.

Results

Two hundred and forty investigation sites were visually examined, scored with the laser fluorescence device (DIAGNOdent, Kavo, Bieberach, Germany) and the corresponding sections were available for histological examinations. The distribution of the readings is shown in Figures 1 and 2.

The intra-examiner reproducibility for the laser fluorescent measurements was calculated using the intra-class-correlation coefficient (ICC) and the Kappa coefficient. The resulting laser fluorescence (LF) scores were plotted according to ICDAS II scores and histological values using box plots. ROC curves were generated using sensitivity and specificity values. The relationship between the non-parametric measurements was assessed using Spearman’s rank correlation coefficient.
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Intra-examiner agreement

The intra-examiner reproducibility for the laser fluorescent measurements was excellent: intra-class-correlation coefficient (ICC) for LF was 0.957 (95% confidence interval: 0.942–0.967). Kappa values for each examiner’s reproducibility 0.74–0.82.

Stereomicroscopic evaluation

The results of histological examination showed that 25 teeth were sound, 10 had enamel caries and eight had dentinal caries. Out of the 192 surfaces with enamel carious lesions, histologically distinguished in stereomicroscopy, 58 presented values of 14–24, during the examination with the DIAGNOdent device. Out of 151 teeth, which showed histological Downer score 1, DIAGNOdent reading gave values of 14–24 for 15 teeth with DIAGNOdent. Histological dentin caries did not differ from the results of DIAGNOdent (Figures 3–5).

Distribution of DIAGNOdent readings according to ICDAS-II and histological codes

The box plots show the distribution of DIAGNOdent readings on the tooth surface according to ICDAS-II codes. The horizontal line gives the median reading for all LF readings in each group, the top of the shaded box gives the median of the upper readings and the bottom of the box the median of the lower readings. The whiskers give the minimum and maximum readings, which are no outliers. The box-and-whisker plots divide the readings into quartiles (Figure 6).

Diagnostic performance of laser fluorescence readings

Since the major aim of using the DIAGNOdent device is the detection of caries in their early stages of development when they can be treated only by non-invasive methods, we assessed the diagnostic performance for each ICDAS II score as well as histological values.

The diagnostic performance of the DIAGNOdent device gave a good overall diagnostic accuracy according to ICDAS II codes and histological values as indicated by the area under the ROC curve of 0.707 and 0.709, respectively (Figure 7).

Figure 3 – (A and B) Occlusal surface with deep fissures, dental plaque deposits, stereomicroscopy, ×32; (C) Morphometry of the occlusal surface with deep fissures, stereomicroscopy, ×32.

Figure 4 – (A and B) Bicuspid, cuspal area with multiple structural defects of the enamel, reactive hypermineralization at the limits of the lesion, stereomicroscopy, ×8.

Figure 5 – (A and B) Dentin caries, uneven advancing of the carious process, lysed dentinal matrix, sclerotic dentin areas, stereomicroscopy, ×32; (C) Stereomicroscopy of a carious lesion, diffused along the enamel–dentin junction, polytopic lysis of the enamel and dentin surrounding the caries, ×32.
Discussion

Caries lesions can occur at different sites on the occlusal surfaces of teeth and may differ in appearance and severity. The histological severity of caries can vary between investigations on the same occlusal surface, thus dentists need to be site-specific when diagnosing a tooth surface in order to decide individually on the appropriate treatment plan. This has been recently shown by Jablonski-Momeni A et al. for the use of the visual scoring system ICDAS-II, that even when using a detailed system such as the ICDAS-II, there is a degree of subjective interpretation, e.g., due to visual perception, lighting and potential bias, which may arise from other surfaces on the same teeth, other teeth or within other areas of the same surface [29, 30].

In previous studies, the performance of the laser fluorescence device DIAGNOdent has been related to cut off limits used in laboratory or clinical studies to determine sound sites from those with enamel caries and those with caries extending into dentine. Several cut-off limits have been suggested by different manufacturers and by *in vivo* and *in vitro* studies [31]. If the cut-off points described by Lussi A and Hellwig E [27] are compared to the results from these studies, it can be seen that over half of histologically sound sites have readings above the upper threshold of 7. This is echoed in the low specificity of 0.48 obtained at the D1 diagnostic threshold (readings between 0–7).

A systematic review of the literature has also shown that the majority of studies using the DIAGNOdent device *in vitro* obtain high levels of false-positive results or low specificity [14]. In a study by Lussi A et al. that...
compared traditional examination and treatment to concurrent use of DIAGNOdent device, good to excellent sensitivity and excellent reproducibility were reported. Reproducibility is high; the device is therefore used for the longitudinal monitoring of caries, for the differentiation between active and inactive lesions and for establishing the treatment plan [22]. Stained dental materials might affect DIAGNOdent readings and consequently result in false-positive diagnoses of secondary caries. Dental fillings should be polished prior to DIAGNOdent measurement [32]. As the DIAGNOdent accuracy concerns, it is influenced by the presence of bacterial plaque and dental calculus; thus, the professional hygiene prior to measurements is required. A prolonged drying will also modify the reading [33]. DIAGNOdent accuracy is superior to that of radiography, while its specificity is higher than that of ECM [34, 35]. The factors that can also influence in different ways the measurement results are: dehydration degree of dental tissue, the presence of sealing materials or professional hygiene [36–38]. An ideal diagnostic method should have high sensitivity and high specificity and should also be reliable and valid, with good intra- and inter-examiner agreement [39–44].

In our study, the efficiency of the DIAGNOdent, was compared with that of visual examination in permanent molars. DIAGNOdent showed higher specificity than did visual examination, in agreement with the findings of Reis A et al. [45]. However, in a meta-analysis, Bader JD and Shugars DA [14] reported higher sensitivity and lower specificity for this device when it was compared with the visual examination, and Alwas-Danowska HM et al. [43] did not find any statistically significant difference between them. Lussi A and Hellwig E [27], who stored the teeth frozen and performed the histological analysis using Rhodamine B, found higher values for sensitivity and lower ones for specificity. They found values of 0.96, 0.88 and 0.81 for sensitivity at threshold D1, D2 and D3 respectively. However, the values for specificity were 0.69, 0.69 and 0.79 (lower than ours).

The diagnostic performance of the laser fluorescence detection method gave a fair to good overall diagnostic accuracy, as indicated by the area under the ROC curve (0.707 in correlation with ICDAS and 0.709 with histology). This is in concordance with previous results that show that at the D1 diagnostic threshold, while sensitivity is high, specificity is low, indicating a high risk of false-positive results. This may be due to stained fissures impacting upon both techniques. These studies also proved that, as far as dentine caries are concerned, specificity is higher, but the sensitivity is low for the DIAGNOdent using the recommended cut-off reading of 0.24.

In accordance to the results of our study, the performance of the LF was also classified as fair to good both at D1 and D3 threshold (AUC 0.71–0.82) [46].

The thresholds set by Lussi A and Hellwig E [27] for enamel caries (8–24) would broadly be in agreement with the results from this study as approximately 75% of the readings fall into this range. There is a great deal of overlap of the box-and-whisper plots for LF readings taken on the tooth surface corresponding to ICDAS codes 0 to 5 (Figure 1). Thus, LF thresholds could not be set to differentiate between increasing visual severities of the carious lesions as documented by the ICDAS-II codes.

It might be argued that it is not only the histological depth of a lesion that is important in determining lesion severity; it might also be the degree of demineralization and the level of bacterial infection within the dentine lesion that affects the result [46, 47].

The distribution of the visual appearances determined by ICDAS-II and by the histological findings is relatively similar to the distribution of the sample in previous studies and the distribution of the caries-free sites reflects roughly the caries prevalence of the population where the teeth were collected [48–50].

The measurements with LF showed similar high correlation with histology, and visual examination (r=0.893, respectively 0.886). Rodrigues JA et al. [13] showed a rank correlation of 0.41 between the prototype of the fluorescence camera and histology that is lower than our findings. The authors discuss that the device showed difficulties in detecting enamel caries lesions. They showed a correlation of 0.52 between LF and histology, which is lower than our findings.

As regards reproducibility, the ICC calculated for the DIAGNOdent showed an excellent correlation for both intra- and inter-examiner agreement for primary and permanent teeth. This is supported in this study, as an excellent intra-class correlation coefficient, for intra-examiner repeatability, ICC 0.957 for LF. Additionally, kappa values confirmed the good reproducibility of the DIAGNOdent. These results are in agreement with those found by Kühnisch J et al., who observed excellent agreement using the DIAGNOdent [40]. Lussi A and Hellwig E [27] also observed excellent values for the ICC (>0.98) and kappa values varying from 0.83 for intra-examiner reproducibility, in agreement with those of this study. High ICC values were also observed by Alwas-Danowska HM et al., who assessed the reproducibility of the device [43]. The good reproducibility means that the device could also be used for monitoring the carious process.

**Conclusions**

The results of the study showed acceptable diagnostic accuracy for the laser fluorescence device DIAGNOdent for caries investigation. Different diagnostic methods have different characteristics, indications and limitations for use. In order to detect caries on occlusal surfaces thoroughly, a combination of methods would be the best practice moderated by clinical knowledge and experience.

**References**


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