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Evaluation of the DIAGNOdent method for detection and quantification of carious lesions - in vitro and in vivo studies

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To my parents and my wife

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Abstract

The sites most susceptible to dental caries are the occlusal surfaces and the margins of existing restorations. These are also the sites at which lesion detection by conventional methods is most unsatisfactory. There is a need in general dental practice for simple efficient methods of detecting and recording quantitative data about the presence and severity of caries at the most susceptible sites and monitoring lesion response to intervention.

Aim

The aim of the present thesis was to evaluate a new laser fluorescence-based device, DIAGNOdent, for detection and quantification of carious lesions on occlusal surfaces and around the margins of restorations under both *in vitro* and *in vivo* conditions.

Methods

Part I: DIAGNOdent and occlusal caries (papers I and IV)

The performance of the DIAGNOdent instrument was compared with the Electronic Caries Monitor (ECM), a method based on electrical conductance measurement, for *in vitro* detection and quantification of occlusal caries. In paper IV, DIAGNOdent readings on teeth scheduled for extraction were recorded before and after extraction, and during storage of the extracted teeth for three months. Lesion depth was determined by histopathological analysis in both papers.

Part II: DIAGNOdent and secondary caries (papers II and III)

Restored teeth were measured with DIAGNOdent along the margin of the restoration, under laboratory and clinical conditions. The restorations were then removed and visual-tactile examination was conducted by two observers. For the *in vitro* study, the teeth were hemisectioned and examined under a microscope.

Results

In vitro evaluation of DIAGNOdent for occlusal caries detection showed that DIAGNOdent had high reproducibility (ICC=0.97) and higher correlation with histopathological examination (r=0.93) than the ECM. For the clinical study on occlusal caries, t-test for dependent samples did not indicate any statistically significant differences between DIAGNOdent readings obtained intraorally, and after extraction, and from extracted teeth stored in thymol saline up to 3 months. The correlation coefficient ranged from 0.59 to 0.73.

For secondary caries detection, the clinical performance of DIAGNOdent in terms of sensitivity/specificity was lower than that in the *in vitro* study, (0.60/0.81 versus 0.77/0.81). Regarding ROC analyses, the A_z values were 0.89 and 0.78, for DIAGNOdent under *in vitro* and *in vivo* conditions, respectively.

Conclusions

The results suggest that DIAGNOdent may be a valuable adjunct to conventional methods for detection of occlusal and secondary carious lesions. If properly applied and correctly interpreted, this technique would facilitate the detection of carious lesions on occlusal surfaces and around the margins of restorations, and allow the clinician to make more well-informed treatment decisions.

List of Papers

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals:

I. **Bamzahim M**, Shi X-Q, Angmar-Månsson B. Occlusal caries detection and quantification by DIAGNOdent and Electronic Caries Monitor: *in vitro* comparison. Acta Odontol Scand 2002; 60: 360-364.

II. **Bamzahim M**, Shi X-Q, Angmar-Månsson B. Secondary caries detection by DIAGNOdent and radiography: a comparative *in vitro* study. Acta Odontol Scand 2004; 62: 61-64.

III. **Bamzahim M**, Aljehani A, Shi X-Q. Clinical performance of DIAGNOdent in detection of secondary carious lesions. Acta Odontol Scand 2005; 63: 00-00 (in press).

IV. Zhang DQ, **Bamzahim M**, Yuan Y, Shi X-Q. *In vivo* and *in vitro* validation of DIAGNOdent for detection and quantification of occlusal caries. Caries Res (submitted).

Papers I, II and III have been reproduced with kind permission from Taylor & Francis AS.

Introduction

The dramatic improvements in the prevalence and incidence of dental caries and the changes in distribution and pattern of the disease over the past thirty years are well-documented (Marthaler, 1990, 2004). Epidemiological studies confirm continuing overall low caries prevalence in developed countries, and have identified population groups with high disease levels or high caries risk. However, the disease has not been eradicated and although less widely distributed in the dentition and less acute in terms of lesion progression, caries persists in the general population.

Recent studies confirm active caries in adolescents and young adults, even in countries with well-established and progressive oral health services *e.g.* Sweden (Mejàre *et al*., 2004) and the Netherlands (Poorterman *et al*., 2003). When initial, arrested and slowly progressive lesions are taken into account, very few adolescents and adults are truly unaffected by the disease. This has important implications for the general dental practitioner, whose role is to provide management strategies appropriate to varying levels of caries risk in individual patients (Pitts, 2004).

As well as dramatic changes in the manifestations of the disease itself, in recent years there have also been major advances in our understanding of the mechanisms underlying the development of clinical stages of the disease. In clinical dental practice, this new knowledge has led to a pronounced change in interpretation of signs of possible hard tissue damage due to caries at individual tooth sites. At site level, the initial effect of the disease on the enamel is clinically undetectable subsurface demineralisation and net loss of tooth mineral as the result of a mineral imbalance between plaque fluid and tooth surface (Fejerskov, 1997). At this stage the damage is reversible and the affected area can remineralise. Support for the dynamic nature of the disease, with the equilibrium maintained by episodes of de-

and remineralisation, is provided by histopathological studies (Holmen *et al*., 1987). In the context of gradual disease progression, as described below, this potential for remineralisation has important implications for the clinical management of the disease.

Caries is now regarded as a chronic, endemic disease. The chronic nature of the disease is reflected in slow lesion progression and this offers a window of opportunity for intervention, to reverse the loss of mineral or arrest lesion progression, before the development of irreversible damage to the dental hard tissues. In clinical dental practice, the decrease in the rate of lesion progression has led to modification of thresholds for restorative intervention and a change towards a less invasive approach to management of the disease.

Despite our improved understanding of the disease process and the availability of effective intervention, many lesions, particularly in occlusal fissures, still progress to the stage where tooth structure is compromised and invasive intervention and restoration are required.

On the basis of these current concepts of the disease process, lesion detection and early intervention, the primary goals of modern clinical management of caries are: to inhibit the initiation of new lesions, to arrest the progression of established lesions and to enhance the natural process of lesion repair by remineralisation (Featherstone, 2004). A further goal, reflecting the reality of decision-making in general dental practice, is to recognize irreversible loss of tooth structure early, to restore it using minimally invasive therapy and to prevent the recurrence of secondary caries around such restorations.

It is clear that key factors in implementing treatment based on these principles are the accurate detection of carious lesions and quantification of lesion progression over time. Interpretation of these data, as part of the

overall diagnosis, allows the clinician flexibility in selecting intervention appropriate to the individual patient.

There is growing awareness that conventional methods of caries detection are inadequate for this modern approach to clinical caries management. Traditional methods allow detection of advanced, irreversible lesions, but are inadequate for detecting the non-cavitated occlusal lesion and recurrent caries around the margins of restorations.

As a complement to these conventional methods, new techniques and equipment have been developed over the last ten to fifteen years, intended to meet the need for objective quantitative methods for accurate detection and monitoring progression. Not all the new techniques have proved applicable to clinical conditions and may be very few could meet the requirements for an ideal method described below.

In the studies on which the present thesis is based, an infra-red fluorescence device was evaluated, with special reference to its application at two sites at which carious lesions are very difficult to detect with conventional methods: occlusal fissures and along the margins of existing restorations.

As described below, the occlusal surfaces are the most caries susceptible and now account for a high proportion of carious lesions (Lussi, 1991; Mejàre *et al*., 1998; Ekstrand *et al*., 2001). Much of the restorative work undertaken in general dental practice comprises replacement or repair of defect fillings with recurrent caries (Kelsey *et al*., 1981; Özer and Thylstrup, 1995; Mjör, 1997).

Primary occlusal caries and conventional detection methods

The term primary caries is used to differentiate lesions on unrestored surfaces from those that develop adjacent to fillings, which are commonly referred to as recurrent or secondary caries.

The occlusal surface has a complex invaginated anatomy forming a series of pits and fissures. Some pits are flask-shaped, presenting an ideal habitat for bacterial colonization (Galil and Gwinnett, 1975). Owing to the minute dimension of the pits and fissures, a tooth brush bristle cannot access the area to disturb the micro-organisms (Ekstrand *et al*., 2001). It is not surprising that the majority of initial carious lesions in enamel occur on the inaccessible walls of the fissure or at its base (Juhl, 1983).

Occlusal caries detection has become further complicated by the phenomenon of so- called hidden or occult caries (Sawle and Andlaw, 1988; Pitts, 1991; Kidd *et al*., 1993), characterized on radiographs by a radiolucent area in the dentine underlying an ostensibly intact occlusal fissure system. In addition, such factors as the presence of plaque, stain and anatomical variation complicate visual-tactile detection of occlusal caries.

Although probing is still widely practiced, several studies have reported that it may damage the enamel surface of subsurface lesions and even cause cavitation, thereby increasing the rate of progression of carious lesions (Ekstrand *et al.*, 1987; van Dorp *et al.*, 1988).

Bite-wing radiographs disclose occlusal caries only at an advanced stage, *i.e*. lesions that have progressed well into dentin (Wenzel *et al*., 1991; Lussi, 1993; Ie and Verdonschot, 1994). Progression of such advanced lesions may be difficult to arrest.

In recent years, the traditional clinical routine for detection of occlusal caries by visual inspection, tactile examination by probing and bitewing radiographs has been the subject of critical scrutiny in several European countries.

A review of this model has disclosed low sensitivity, but high specificity (Lussi *et al.*, 2001). The sensitivity usually ranged from approximately 60 % to 90 %, and the specificity was usually greater than 80 %, when visible

fissure cavities were present (Lussi, 1996). However, for occult caries, *i.e.* dentin caries beneath macroscopically intact surfaces, the sensitivity was usually much lower, with reported sensitivities as low as 12 % (Wenzel *et al*., 1991; Lussi, 1991, 1993; Ie and Verdonschot, 1994). Therefore occlusal lesions were frequently not detected by visual examination and bite wing radiographs.

A further disadvantage of conventional methods, that they are based primarily on subjective evaluations, which may lead to large diagnostic variations among different examiners. Furthermore, as these methods are qualitative, they are of limited value for monitoring lesion development and evaluating the effectiveness of preventive procedures.

Secondary caries and conventional detection methods

The Fédération Dentaire Internationale (1962) defined secondary caries as a positively diagnosed carious lesion which occurs at the margins of an existing restoration. The lesion usually consists of two carious regions: an outer lesion formed in the enamel similar in histology to a primary lesion, and the wall lesion which is a narrower defect in the enamel and/or dentin along the cavity wall restoration interface (Grieve, 1978; Kidd *et al.*, 1992).

Despite the improvement in restorative material quality and the orientation of dental health care towards prevention, secondary caries remains an unresolved problem in dentistry and has become an important issue in daily dental practice, (Fontana and Gonzalez-Cabezas, 2000).

Among a number of factors that contribute to restoration failure, secondary caries is cited as a major reason for restoration replacement (Kelsey *et al.*, 1981; Mjör, 1997), accounting for 40-70 % (Özer and Thylstrup, 1995).

In clinical practice, visual-tactile examination and bitewing radiographs are used in various combinations for the detection of secondary caries. However, colour change around a restoration is difficult to interpret, and it

is not a reliable indicator for secondary caries (Kidd, 1991; Kidd *et al.*, 1995). Sharp explorers should be used with care in detection of secondary caries around a restoration, because probing pressure at the tooth/restoration interface may damage the tooth or the filling material at the margin (Kidd *et al.*, 1992). In addition, a catch on a restoration is not synonymous with caries because an explorer will catch in any crevice (Kidd *et al.*, 1992). Radiography is of limited value in detection of secondary caries because the restorative material tends to obscure the defective margin (Espelid and Tveit, 1991; Tveit and Espelid, 1992).

In summary, detection by conventional methods of the most common lesions, primary occlusal caries and secondary caries, is unreliable unless the lesion is relatively advanced, with significant destruction of hard tissue. It is therefore important that methods introduced as clinical aids to detection of dental caries should be evaluated specifically with respect to detection of such lesions.

Requirements of an ideal diagnostic method

The ideal diagnostic method should

1. Be safe for patients and users;
2. Enable detection of a lesion at an early stage, *i.e.* it should have a high sensitivity for diagnosing incipient lesions. At the same time it should have a low proportion of false diagnoses of sound teeth, *i.e.*, a high specificity. For a quantitative method, a high correlation coefficient is necessary between the outcome and a so-called gold standard;
3. Be objective and quantitative. The conventional methods for caries detection described above are applicable only for subjective, qualitative assessments of carious lesions and cannot be applied objectively for longitudinal studies. In epidemiological studies, objective methods for caries diagnosis could simplify the traditional calibration of observers;

4. Be reliable. In other words, measurements should be reproducible and have high inter- and intra-observer agreements;
5. Preferably be non-invasive, or less invasive than conventional methods;
6. Be cost effective and user friendly.

New methods for caries detection

Several new diagnostic techniques have been introduced during the past decade, such as electrical conductance measurement, digital radiography, light scattering, and methods based on laser/light fluorescence, (Angmar-Månsson and ten Bosch, 1993; Angmar-Månsson *et al*., 1996; Pine and ten Bosch, 1996; Ekstrand *et al*., 1997; Huysmans *et al*., 1998a,b; Wenzel, 1998; Verdonschot *et al*., 1999).

In the present thesis, an infra-red fluorescence method, DIAGNOdent (KaVo, Biberach, Germany), was evaluated in a series of *in vitro* and *in vivo* studies, with special reference to the detection and quantification of occlusal and secondary caries. In the first study DIAGNOdent was also compared with another relatively new device, Electric Caries Monitor, (ECM, Lode Diagnostic, Groningen, the Netherlands), for *in vitro* detection and quantification of occlusal caries.

Electrical conductance methods - Electronic Caries Monitor

Detection of carious lesions can be based on electrical conductance methods such as Electronic Caries Monitor, ECM (Lode Diagnostic, Groningen, The Netherlands). The electrical conductivity of a tooth changes with demineralisation, even when the surface remains macroscopically intact. The change in the electrical conductivity has been explained by the fact that the porosities, formed during demineralisation, fill with water and soluble electrolytes to form conductive pathways. Therefore the high electrical resistance of sound dental tissue decreases by the carious demineralisation process.

ECM is a method based on electrical conductance measurement. ECM is a battery-powered machine (Fig. 1) with an alternating current output at a frequency of approximately 21 Hz. It has a conductance scale with values ranging from -1.00 to 13.00, which is inversely related to resistance and indicating increasing degrees of demineralization. The conductance measurements are made between a specially designed probe tip and a hand-held connector. ECM is fitted with an airflow meter so that the effect of air flow on conductance readings can be controlled. A minimum airflow of 7.5 L/min is required for acceptable diagnostic accuracy. ECM has been tested and found to be a useful diagnostic technique for fissure lesions (Lussi *et al*., 1995; Ie *et al*., 1995; Ricketts, 1996; Huysmans *et al*., 1998b).

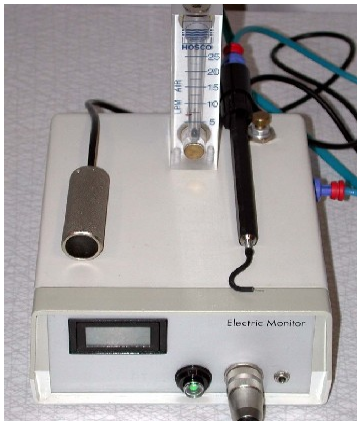

Fig. 1. ECM

The near infrared fluorescence method - DIAGNOdent

Fluorescence is a well-known phenomenon in science and technology. In simple terms, light at one wavelength (excitation wavelength) is absorbed by the tissue and emitted at a second longer wavelength (emission wavelength). The fluorescence of dental hard tissues has been known for a very long time (Benedict, 1928). Dental hard tissues possess the

characteristics of fluorescence and this natural fluorescence is called autofluorescence. The cause of baseline fluorescence in sound teeth is still unclear. It might be the result of combining the inorganic matrix with absorbing organic molecules (Hibst *et al*., 2001). Red light, as well as infrared fluorescence radiation, is less absorbed and scattered by enamel than light of shorter wavelengths, so that it penetrates the tooth more deeply (Hibst *et al*., 2001). It is therefore possible to measure fluorescence from underlying carious dentin.

Hibst and Gall (1998) found that red light induced fluorescence could differentiate between sound and carious tooth tissue. Fluorescence spectroscopic investigations revealed considerable contrast between sound and carious tooth tissues when excited by red light, 655 nm (Hibst *et al*., 2001). Fluorescence was found to be more intense in carious tissue compared with sound tissue (Fig. 2).

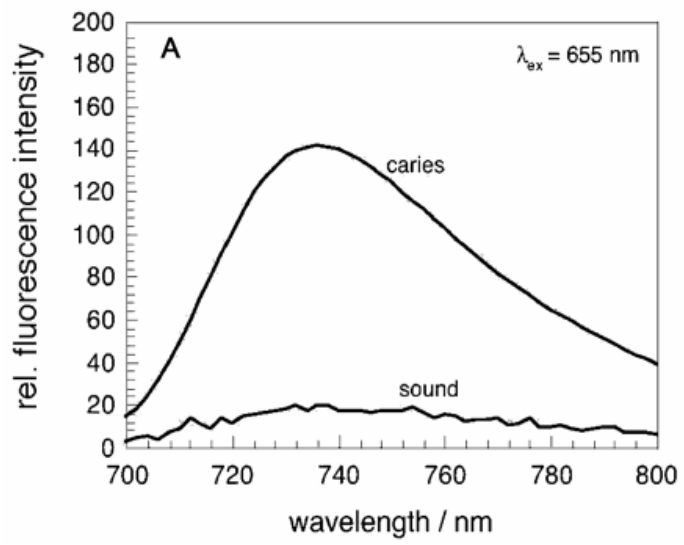

Fig. 2. Fluorescence spectra of sound and carious tooth areas when excited by red light, 655 nm, (Lussi *et al*., J Dent Res 2004).

On the basis of these findings, DIAGNOdent, a laser fluorescence-based instrument was introduced in 1998 as a complement to conventional methods for the detection and quantification of carious lesions.

The DIAGNOdent device consists of a main control unit and a hand-held probe (Fig. 3a).

Fig. 3a. DIAGNOdent

Fig. 3b. DIAGNOdent probe tips

The main control unit measures 12 x 15 x 11 cm and is connected to a hand probe by a cable, with descendant and ascendant optical glass fibers. The probe comes with 2 attachments, one with the tapered tip “A”, intended for occlusal surfaces, and the other with the flat tip “B”, intended for smooth surfaces (Fig. 3b).

The main DIAGNOdent unit contains a laser diode (655nm, modulated, 1 mW peak power) as the excitation light source, and a photo diode combined with a band pass filter (transmission > 680 nm) as the detector. Laser light is generated by the main unit and transmitted through the excitation optical fiber to the tip of the hand piece. Once the tip is in contact with a tooth surface, the laser energy penetrates the tooth surface and is absorbed by the surrounding tooth material, and fluorescence within the infra-red spectrum occurs. The emitted fluorescence, as well as backscattered ambient light, is collected by the tip and carried back to a photo diode detector in the main

unit via the detection fibers. The band pass filter absorbs the backscattered excitation and other short wavelength ambient light and transmits the long-wavelength fluorescence radiation. To eliminate the long-wavelength ambient light also passing through the filter, the laser diode is modulated, and only light showing the same modulation characteristic is registered by the main unit and displayed as nominal values ranging from 0 to 99, where 0 indicates minimum and 99 maximum fluorescence. The principle of DIAGNOdent is presented in figure 4.

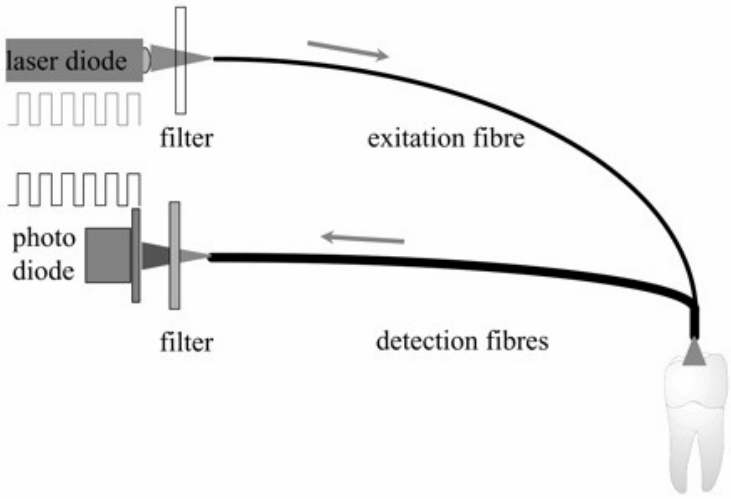

Fig. 4. The DIAGNOdent principle (Lussi *et al*., J Dent Res 2004).

The underlying mechanism is that carious tissue emits stronger fluorescence than sound tissue in the red and infrared part of the spectrum (λ= 655 nm), (Hibst and Gall, 1998; Hibst *et al.*, 2001). Thus, the fluorescence from a carious region, greater than that from sound tissue, is expressed as a higher numerical readout by the device. The exact mechanism of detection has not been fully clarified, however, one assumption is that the device measures the fluorescence of bacterial products within carious tissues, possibly porphyrins (Hibst *et al.*, 2001).

Since its introduction in 1998, DIAGNOdent has generated a great deal of research interest. Many studies evaluating DIAGNOdent have been published during the past few years, but most comprise *in vitro* evaluations for detection and quantification of carious lesions on the occlusal surfaces of extracted permanent or deciduous teeth (Lussi *et al*., 1999; Shi *et al*., 2000; Attrill and Ashley, 2001; Alwas-Danowska *et al*., 2002; Costa *et al* 2002; Ouellet *et al* 2002; Baseren and Gokalp 2003; Cortes *et al*., 2003 ; Francescut and Lussi 2003; Lussi and Francescut, 2003; Kordic *et al*., 2003; Fung *et al*., 2004; Mendes *et al*., 2004).

A few *in vivo* studies have addressed the performance of DIAGNOdent for detection and quantification of occlusal carious lesions (Lussi *et al*., 2001; Heinrich-Weltzien *et al*., 2002; Anttonen *et al*., 2003; Rocha *et al*., 2003). Other studies have addressed further possible clinical applications: the detection of secondary caries, residual caries, root caries, caries under sealants, and the detection of subgingival calculus (Takamori *et al*., 2001; Lennon *et al*., 2002; Wicht *et al*., 2002; Boston, 2003; Krause *et al*., 2003; Ando *et al*., 2004).

In these studies, the sensitivity and specificity of DIAGNOdent has been compared with that of other diagnostic devices or traditional methods of caries detection. Under *in vitro* conditions, validation is usually by histopathology. For *in vivo* studies, however, validation is generally limited to clinical inspection after opening of the cavity *i.e*. only when the lesion is considered to have reached to the stage of requiring operative intervention. Most notable is the pronounced variation in the cut-off points or the thresholds (the score at which the caries was identified). However, the overall results indicate that DIAGNOdent may be a reproducible and accurate detection tool.

The above overview presents current concepts of dental caries as a chronic disease, the advent of interventions intended to arrest or reverse progression

of early lesions, and changes in the threshold for invasive intervention, with special reference to implications for the general dental practitioner, whose role is to make appropriate treatment choices for the individual patient. The shortcomings of conventional methods for detection and monitoring of carious lesions are highlighted, particularly in relation to two major challenges for the general practitioner, occlusal caries and recurrent caries. Recently introduced techniques to aid in lesion detection are described, with special reference to methods appropriate to the clinical setting. A brief review of published studies evaluating the laser fluorescence-based instrument DIAGNOdent, discloses general agreement that it may be a valuable clinical tool for detection and quantification of carious lesions

However, to ensure optimal performance under daily general practice conditions, several issues with respect to the use of DIAGNOdent require clarification. For equipment intended for use in general dental practice, it is important that careful objective evaluation is conducted by independent investigators. It is in this context that the studies underlying the present thesis were undertaken.

Aims

General aim

The aim of the present thesis was to evaluate a new laser fluorescence-based device, DIAGNOdent, for primary and secondary caries detection.

Specific aims

1) To test the reliability of DIAGNOdent in retrieving locations of occlusal lesions (Paper I).
2) To compare the reproducibility of DIAGNOdent and ECM for quantification of occlusal caries (Paper I).
3) To compare the validity of DIAGNOdent and ECM for detection and quantification of occlusal caries (Paper I).
4) To validate DIAGNOdent *in vitro* and *in vivo* for detection of secondary carious lesions (Paper II, III).
5) To compare the diagnostic accuracy of DIAGNOdent with radiography in terms of sensitivity/specificity, and ROC analysis for detection of secondary carious lesions *in vitro* and *in vivo* conditions (Paper II, III).
6) To study how DIAGNOdent readings alter when the environment changes from *in vivo* to *in vitro* conditions (Paper IV).
7) To validate DIAGNOdent for *in vivo* detection and quantification of occlusal caries using histopathological examination as gold standard (Paper IV).
8) To compare clinical performance of DIAGNOdent with visual inspection for occlusal caries detection using histopathology as gold standard (Paper IV).

Material and Methods

All the four studies were approved by the Ethics Committee at Huddinge University Hospital, Huddinge, Sweden (124/02, 101/03, 102/03, 103/03).

Teeth

Teeth used in this thesis are summarized in Table 1.

Table 1. Teeth used in the four studies.

Study number	***Study type***	***No of teeth***	***Type of teeth***	***Examination sites***
I	*In vitro*	87	premolar teeth	occlusal surface
II	*In vitro*	66	restored premolar and molar teeth	restoration margins
III	*In vivo*	51	restored premolar and molar teeth	restoration margins
IV	*In vitro* and *in vivo*	50	premolar and 3rd molar teeth	occlusal surface

For studies on extracted teeth (Papers I and II), the teeth were rinsed thoroughly under tap water, the examination sites were carefully cleaned with a toothbrush and calculus was carefully removed with a scaler before all measurements. The teeth were then numbered and stored under refrigeration in thymol-saturated saline, in individual plastic containers.

In Paper I, the teeth were recently extracted, less than six months, from young adolescents on orthodontic indications. The occlusal surfaces were visually sound or had non-cavitated lesions.

In Paper II, the sample comprised 48 teeth restored with amalgam and 18 with composite material. The teeth were recently extracted and the restoration margins were either visually sound or had suspicious sites of

secondary caries. The teeth were randomly mounted in plaster blocks in groups of three or four, with proximal contact, simulating their anatomical positions. Teeth with class II restorations were deliberately placed in the middle of the 3 or 4 teeth on each tooth block.

In Paper III, the material comprised 51 posterior teeth with amalgam restorations in 21 subjects aged between 19 to 45 years. Teeth in which amalgam restorations were indicated for removal for various reasons were included in this study (Table 2).

Table 2. Reasons for removal of amalgam restorations in the teeth in Paper III.

Reasons for amalgam removal	***Number of teeth***	***Premolar teeth***		***Molar teeth***	
		Class I	***Class II***	***Class I***	***Class II***
Secondary caries	19	2	5	5	7
Esthetics	15	5	4	3	3
Defective restoration	12	1	2	5	4
Other reasons	5	0	0	2	3
Total	51	8	11	15	17

In paper IV, 50 teeth were obtained from 50 young adults, 31 males and 19 females, ranging in age from 20 to 31 years. Among the 50 teeth, 11 were premolars and 39 were third molar teeth. The subjects were selected from patients attending a public dental clinic in Harbin, China and gave their informed consent before enrolling in the study. The subjects were scheduled for extraction of third molars, or premolars on orthodontic grounds. The occlusal surfaces of the teeth were visually sound or macroscopically intact with suspicion of presence of caries.

Equipment

DIAGNOdent

DIAGNOdent (KaVo, Biberach, Germany) was used for detection and quantification of primary occlusal carious lesions under *in vitro* and *in vivo* conditions (Papers I, IV) and detection of secondary carious lesions under laboratory and clinical conditions (Papers II, III). The handpiece with a cone-shaped tip was used for measurements on occlusal surfaces (Papers I, IV) and at the margins of restoration margins (Papers II, III).

ECM

ECM (Electronic Caries Monitor, Lode Diagnostic, Groningen, The Netherlands) was employed for detection and quantification of primary occlusal carious lesions (Paper I).

Radiographic methods

Film radiographs were obtained of all restored teeth, using Kodak Ektaspeed Plus film exposed with a Prostyl Intradental unit (Planmeca, Finland) at 70 kV and 8mA, with an exposure time of 0.25 s (Papers II, III).

Measurement with DIAGNOdent (Papers I, II, III, IV)

Before all the measurements, each tooth was documented with digital photography. The measuring sites of each tooth were carefully cleaned and then air-dried with compressed air prior to measurement. In accordance with the manufacturer's instructions, a conical tip was used for all the measurements. The device was calibrated against a ceramic standard before each measurement session. Before each measurement, the standard value for each tooth was calibrated by measuring at a sound site.

In Paper I, the occlusal surface of each tooth was carefully scanned with DIAGNOdent to locate the site of the highest reading. This reading was recorded and the site was indicated as a dot on the photograph. After an interval of two weeks, the same procedure was repeated by the same

operator, under identical conditions, without access to the data from the previous session. The photographs of each tooth from the two measurement sessions were then superimposed, to test the match of the measuring sites. The sites were considered to match when the distance between two dots was < 3 mm on the paper copy, or < 1 mm in reality, which corresponds to the diameter of the conical tip. By comparing the two sets of photographs indicating lesion sites, the percentage of teeth for which maximum readouts from the two sessions corresponded with matching sites on the photographs was calculated.

In Paper II, the margin between the restoration and the tooth was carefully scanned with DIAGNOdent by placing the DIAGNOdent tip directly on the tooth/restoration margin. The highest reading and its corresponding site were recorded on the digital picture by one operator.

In Paper III, two operators participated. The margin between the restoration and the tooth was carefully scanned with DIAGNOdent by the first operator. The highest reading and its corresponding site were recorded on the digital picture by the second operator. The DIAGNOdent reading was unavailable to the first operator as well as to the other two examiners who performed the validation.

In Paper IV, the measuring site on each occlusal surface was determined based on visual inspection. The measuring sites that were indicated in the images served as a guide for repositioning for the follow-up measurements after the teeth were extracted. One operator performed all the measurements with DIAGNOdent according to the instructions from the manufacturer. However, the data registration was done by operator 2 and kept unavailable to the first operator. After the teeth were extracted, they were cleaned under tap water, air-dried for 5 s and re-measured by DIAGNOdent on the same sites, determined from the pictures. Then the teeth were kept in thymol saturated saline under 4-degree refrigeration. The same measuring

procedure was repeated by the same two operators at three days, one week, two weeks, one month, and three months after extraction.

Measurement with ECM (Paper I)

The teeth with matching sites for maximum readouts of DIAGNOdent from the two sessions were monitored twice with ECM, by the same operator, at an interval of two weeks. Both the tooth and the reference electrode of ECM were held in the same hand without direct contact with each other. A measurement was made in accordance with the manufacturer's instructions by touching the occlusal site indicated on the photograph with the instrument probe. The air-flow was 7.5 L/min. Data shown on the front panel of the instrument were registered.

Radiographic examination (Papers II, III)

In paper II, each tooth block was exposed in a bucco-lingual direction, with the longitudinal tooth axes parallel to the surface of the x-ray film. A plastic compound, equivalent to a 15-mm layer of soft tissue, was placed in front of the blocks to simulate soft tissue. In Paper III, bitewing radiographs were taken of all teeth according to the standard clinical protocol, unless the patient had bitewing x-ray taken less than 6 months earlier.

In both studies, the focus-to-film distance was 20 cm. All films were developed in a standardized manner. The radiographs were examined independently by five experienced dentists twice at different time intervals. On the first occasion, the observers were instructed to classify the tooth surface at the margin of the restorations as sound or carious. On the second occasion the observers were instructed to select one of five ratings to present his or her level of confidence that a secondary carious lesion was present or not at the margins of the restorations. The following scale was used:

1) Definitely not caries;

2) Probably not caries;
3) Questionable;
4) Probably caries;
5) Definitely caries.

The films were viewed on a light box at a magnification of 2x.

Visual examination (Papers II, III, IV)

In Papers II and III, the site determined by DIAGNOdent was examined visually under conventional clinical lighting and the marginal integrity of the restoration at this site was recorded according to one of three categories: as clinically intact (restoration closely adapted to the tooth structure), ditching (a visible gap along the margin, no caries discernible), and caries. The colour of the tooth structure at the margin of the restoration was noted as stain-free or stained.

In Paper IV, an initial visual inspection was performed to enroll suitable subjects according to the following criteria and classification:

1) No changes in enamel translucency after prolonged air drying (sound surface);
2) Opacity or slight discoloration visible after prolonged air drying (enamel lesions);
3) Opacity or slight discoloration, distinctly visible without air-drying (dentinal lesion).

Validation

Table 3 summarizes the validation methods for primary and secondary carious lesions.

Table 3. Validation methods for primary and secondary carious lesions.

	Samples	***Validation method***	***Observers***	***Lesion evaluation***
Primary occlusal caries *(Papers I and IV)*	300 μm thick slices	Histopathology (microscope, 16x magnification)	Two	5-point scale
Secondary caries *(Paper II)*	hemisections	Histopathology (microscope, 16x magnification, and probe)	Two	yes/no
Secondary caries *(Paper III)*	tooth cavity	Clinical examination after removal of restoration (visually and by a probe)	Two	yes/no

For validation of primary occlusal caries in Paper I and IV, teeth were embedded in plastic and sectioned into slices approximately 300 μm thick in a bucco-lingual direction, perpendicular to the occlusal surfaces, using a water-cooled saw, at the sites indicated in the photographs. When the photographs indicated that the slice deviated from the test sites, two or more slices were prepared. Both sides of the tooth slices were examined carefully under a microscope at 16x magnification by two observers, independently. When discrepancy occurred, consensus was reached by performing the histological analyses together. The lesions were defined by the extension of

a whitish demineralized zone or a brown zone in the occluso-pulpal direction.

The sites were classified according to the following five-point scale:

1) Sound;
2) Enamel caries limited to the outer half of enamel;
3) Caries extending into the inner half of the enamel but not to the dentino-enamel junction (DEJ);
4) Caries penetrating the DEJ but limited to the outer half of the dentin (D_3);
5) Caries involving the inner half of the dentin.

For secondary caries validation in Papers II and III, the restorations were removed and two observers examined all cavities visually and by probing. For the *in vitro* study (Paper II), the teeth were also sectioned, perpendicular to the occlusal surfaces, using a water-cooled saw, and examined under the microscope at 16x magnification.

Statistical analyses

Reliability and Agreement

The reproducibility of DIAGNOdent and ECM was studied by Intra-class correlation coefficient, ICC (Paper I).

Agreement between DIAGNOdent measurement and radiographic examination with respect to true and false diagnoses was analysed by Cohen's kappa statistics (Paper II).

T-test for dependent samples was applied to analyze changes in DIAGNOdent readings over time: clinical DIAGNOdent readings followed by readings on the same teeth after extraction and then at scheduled intervals of storage in thymol saturated saline (Paper IV).

Validity

In Paper I, the relationship between the gold standard, determined by histopathology, and DIAGNOdent and ECM readings, respectively, was studied by regression analysis. Correlation coefficients between the two methods and the lesion depth were calculated. Subsequently the sensitivity and specificity of caries detection at D_3 level were calculated for each method.

In Papers II and III the diagnostic performances of DIAGNOdent and radiography for secondary caries detection were evaluated in terms of receiver operating characteristic (ROC) curve and sensitivity/specificity. The majority answer out of five observers was used to calculate the sensitivity and specificity for radiographic examination. ROC curve is a plot of the true positive fraction, TPF (sensitivity), against the false positive fraction, FPF (1- specificity), for the different possible cut-off points of a diagnostic test. The resulting curve illustrates how sensitivity and specificity vary along the whole diagnostic range. Receiver operating characteristic curves, ROC, were plotted for the radiographic examination for each observer based on five score of confidence level and for the DIAGNOdent reading. To make the data comparable between radiographic examination and DIAGNOdent measurement when performing ROC analysis, the DIAGNOdent readings were classified into the following five-point scale:

1) Values ranging from 0 to 10;
2) Values ranging from 11 to 20;
3) Values ranging from 21 to 30;
4) Values ranging from 31 to 40;
5) Values above 40.

The areas under the curve were calculated for DIAGNOdent as well as radiographic examination from each of the five observers. For DIAGNOdent, the cut-off point for the presence of secondary dentinal

caries was chosen based on our results by comparing DIAGNOdent values with the gold standard in order to balance sensitivity and specificity, preferably with specificity > 0.80.

In Paper IV, the correlation between the DIAGNOdent reading and the lesion depth using histopathology as the gold standard was evaluated by Spearman's rank correlation coefficient. Sensitivity and specificity of DIAGNOdent and visual inspection under *in vivo* conditions were calculated at D_2 and D_3 levels.

Results

The distribution of teeth with respect to lesion depth in the four studies is listed in Table 4.

Table 4. Result of validation, in terms of number (n) and percentage (%) of sound and carious teeth.

	Paper I		***Paper II***		***Paper III***		***Paper IV***	
	No	***%***	***No***	***%***	***No***	***%***	***No***	***%***
Sound	17	22.1	32	48.5	26	51	5	10
Enamel caries	40	51.9	-	-	-	-	35	70
Dentine caries	20	26	34	51.5	25	49	10	20
Total	77	100	66	100	51	100	50	100

Reliability and Agreement

In Paper I, 77 pairs of photographs were found to have matching sites, *i.e.* for 89 % of the sample, the highest DIAGNOdent readings on two separate measurement occasions were recorded at matching sites. The intra-class correlation coefficient was based on these readings. The intra-class correlation coefficients for DIAGNOdent and ECM readings were 0.97 and 0.71, respectively.

In Paper II, Cohen's kappa statistic disclosed moderate agreement 56 %, between DIAGNOdent measurements and radiographic examinations.

In Paper IV, t-test for dependent samples did not indicate any statistically significant differences between DIAGNOdent readings obtained intraorally and from the teeth after extraction. However, the measurement made immediately after extraction was significantly higher than the other sets of *in vitro* measurements.

Validity

Sensitivity and specificity of DIAGNOdent, and the cut-off points chosen for detection of dentinal carious lesions in the four studies are summarized in Table 5.

Table 5. Sensitivity and specificity of DIAGNOdent, and the cut-off points chosen for detection of dentinal caries in the four studies.

	Paper I	***Paper II***	***Paper III***	***Paper IV***
Cut-off point	18	20	30	30
Sensitivity	0.8	0.77	0.60	0.60
Specificity	1.00	0.81	0.81	0.80

In Paper I, the correlations with histopathology were r = 0.93 and 0.83, for DIAGNOdent and ECM, respectively. For detection of D3 lesions, the sensitivity and specificity were 0.8 and 1 for DIAGNOdent, and 0.75 and 0.88 for ECM.

In Paper II, the sensitivity and specificity for DIAGNOdent and conventional radiography were 0.77/0.81 and 0.65/0.81, respectively. For DIAGNOdent, the cut-off point for secondary dentinal caries was 20, considering both sensitivity and specificity. Regarding ROC analyses, the A_z values were 0.89 and 0.72 for DIAGNOdent and radiography, respectively. Logistic regression analysis showed that Log $_{\text{DIAGNOdent readings}}$ best explained the presence of secondary caries by the model including the variables of DIAGNOdent, radiographic examination, the marginal integrity as well as staining of the tooth surface. The effect of the presence of secondary caries on DIAGNOdent readings was significant (P=0.0005).

In Paper III, for DIAGNOdent, the best cut-off point for secondary dentinal caries was 30 considering both sensitivity and specificity. The sensitivity

and the specificity for DIAGNOdent and conventional radiography for detection of secondary caries were 0.60/0.81 and 0.56/0.92, respectively. Sensitivity and specificity of visual inspection of the marginal site were 0.44 and 0.96, respectively. The areas under the ROC curves were 0.78 and 0.69 for DIAGNOdent and radiography, respectively.

In Paper IV, DIAGNOdent showed better sensitivity and specificity values than visual inspection for caries detection at D_2 and D_3 levels under clinical conditions. The sensitivity and specificity for DIAGNOdent and visual inspection at D2 were 0.75/0.76 and 0.41/0.75, respectively, and the corresponding values at D3 were 0.60/0.80 and 0.10/0.98, respectively. For DIAGNOdent, the cut-off points chosen at D_2 and D_3 were 20 and 30, respectively.

The correlation between the gold standard and groups of DIAGNOdent readings ranged from 0.59 to 0.73. In general, the correlation was around 0.65. When stained samples were excluded the correlation coefficient improved by about 10 percent.

Discussion

The general dental practitioner is trained in clinical cariology, to assess the extent of existing caries, to predict the risk of future disease and to develop an appropriate disease management strategy for the individual patient. It is clear that reliable, detailed information about the extent and severity of existing caries will enhance the likelihood of a successful treatment outcome.

Unfortunately, the sites most susceptible to dental caries, the occlusal surfaces and at the margins of existing restorations, are also the sites at which lesions are most difficult to detect by conventional methods. Therefore, evaluation of new clinically applicable methods to aid caries detection at these sites is of importance.

The studies on which this thesis is based were designed to evaluate the application of a new laser fluorescence technique to aid the detection of occlusal and secondary caries. The method was evaluated with special reference to its application in general dental practice, where advances in knowledge and understanding of the disease process underlying the development of carious lesions have led to a paradigm shift in treatment philosophy towards early, non-invasive intervention. A primary goal is to arrest lesion progression before there is irreversible damage, requiring replacement of lost tooth structure by restoration. Thus for appropriate treatment decisions, the clinician requires reliable methods for detecting early lesions.

Because caries is a dynamic process, there is a need for quantitative, continuous data which reflect the dynamics of the disease. This would enable the clinician to monitor changes in lesions over time and to evaluate the effect of interventions. The clinician could then make informed

decisions, tailoring intervention appropriate to the individual patient's disease activity and allowing greater flexibility in treatment options.

Of several recently introduced methods for detection of carious lesions, a quantitative laser method, KaVo DIAGNOdent has shown promising results in a number of studies.

In the present series of studies, the DIAGNOdent method was evaluated under both *in vitro* and *in vivo* conditions, for detection and quantification of carious lesions on occlusal surfaces and around the margins of restorations.

Reliability and Agreement

When evaluating a diagnostic method it is very important to test whether it provides consistent and standardized measurements between and within observers. For longitudinal application in the dental clinic, dentists require good reproducibility without the need to record the location of the site as a reference for follow-up measurements. The *in vitro* results indicate that the ability of DIAGNOdent to retrieve the location of a carious site was high (>89%).

High reproducibility of DIAGNOdent has previously been reported under both *in vitro* and *in vivo* conditions (Lussi *et al*., 1999, 2001; Shi *et al*., 2000; Alwas-Danowska *et al*., 2002; Heinrich-Weltzien *et al*., 2002; Anttonen *et al*., 2003; Francescut and Lussi 2003; Lussi and Francescut, 2003). This was confirmed in the present study: the reproducibility of DIAGNOdent, expressed as ICC, was high and superior to that of ECM, 0.97 versus 0.71. The high reproducibility of DIAGNOdent indicates consistent performance. Thus, the method may be suitable for longitudinal monitoring of carious lesions. This would allow assessment of caries activity and the outcome of preventive interventions.

DIAGNOdent was also investigated with respect to possible alterations in readings associated with a change in the environment from *in vivo* to *in vitro*

conditions (Paper IV). There were no systematic differences between the clinical DIAGNOdent readings and post-extraction readings over three months. Thus, DIAGNOdent readings did not change significantly when the environment changed from *in vivo* to *in vitro* conditions. However, the immediate post-extraction reading was higher than the clinical (pre-extraction) reading. The fluorescence measured by the device is attributed to the presence of bacterial metabolites in the carious tissue and it seems unlikely that conditions within the tooth or on the tooth surface would change so rapidly.

The increased reading is probably due to better accessibility from all angulations when holding a tooth in the hand, compared to intraoral conditions, especially for the third molar. After only three days, the DIAGNOdent readings were systematically lower than those immediately after extraction. This may indicate that substances contributing to the fluorescence signals were released into the surrounding thymol saline medium within the first three days. Measurements taken three days or more after extraction did not vary significantly.

Validity

An ideal diagnostic method should offer, among other characteristics, high sensitivity and high specificity. However, there are discrepancies between our *in vitro* and *in vivo* studies with respect to sensitivity and specificity and the cut-off points for DIAGNOdent. A review of studies by other authors disclosed similar discrepancies between studies. Table 6 gives an overview of the performance of DIAGNOdent with respect to sensitivity and specificity for detection of dentinal caries, and the cut-off points in our papers and in some previous studies.

Table 6. Overview of the sensitivity, specificity and cut-off points of DIAGNOdent as assessed in different studies.

	References	*Cut-off points*	*Sensitivity*	*Specificity*
In vitro studies	**Studies on occlusal surfaces, permanent teeth**			
	Paper I	18	0.8	1
	Lussi *et al.*, 1999	10	0.76 - 0.84	0.79 - 0.87
	Shi *et al.*, 2000	19 - 22	0.78 - 0.82	1
	Alwas-Danowska *et al.*, 2002	20 - 21	0.93 - 1	0.47 - 0.59
	Cortes *et al.*, 2003	23	0.84	0.67
	Baseren and Gokalp, 2003	20	1	0.92
	Francescut and Lussi, 2003	10	0.73	0.65
	Kordic *et al.*, 2003	10	0.91	0.76
	Costa *et al.*, 2002	21	0.79	0.89
	Fung *et al.*, 2004	30	0.44 - 0.66	0.81 - 0.94
	Studies on occlusal surfaces, deciduous teeth			
	Attrill and Ashley, 2001	18	0.79	0.81
	Francescut and Lussi, 2003	9	0.82	0.85
	Lussi and Francescut, 2003	13	0.82	0.85
	Mendes *et al.*, 2004	18	0.86	0.95
	Studies on restored teeth (secondary caries)			
	Paper II	20	0.77	0.81
	Boston, 2003	22	0.73	0.84
In vivo studies	**Studies on occlusal surfaces, permanent teeth**			
	Paper IV	30	0.6	0.8
	Lussi *et al.*, 2001	20	0.92	0.86
	Heinrich-Weltzien *et al.*, 2002	20	0.93	0.63
	Anttonen *et al.*, 2003	30	0.92	0.69
	Study on occlusal surfaces, deciduous teeth			
	Rocha *et al.*, 2003	21	0.73	0.95
	Study on restored teeth (secondary caries)			
	Paper III	30	0.6	0.81

The DIAGNOdent performance is partially dependent on the cut-off point which separates the numerical output into sound and caries scores. The cut-off values for DIAGNOdent readings were determined in the four studies to maximize the performance (highest sum of sensitivity and specificity), taking into consideration that specificity should be preferably > 0.80. Comparison of the cut-off values applied to define occlusal dentinal caries and secondary caries in the four studies disclosed that the clinical studies (Papers III and IV) had higher cut-off values than the *in vitro* studies (Papers I and II), (Table 6). Furthermore, the cut-off value for occlusal dentinal caries under clinical conditions (Paper IV) was also higher than that in most of the *in vitro* and *in vivo* studies (Table 6), and similar to two other studies (Anttonen *et al.,* 2003; Fung *et al.,* 2004). Choosing a higher cut-off point for detection of dentinal caries would reduce the sensitivity and increase the specificity. This would minimize false positive readings, decreasing the risk of overtreatment. This is an important consideration in low caries risk populations, such as those in most western countries. Therefore, the cut-off value of 30 for dentinal caries, as chosen in the present clinical studies, is recommended.

In Papers I and II, DIAGNOdent was evaluated under *in vitro* conditions. For *in vitro* detection of occlusal caries at D_3 level in deciduous teeth, sensitivity and specificity were higher for DIAGNOdent than for ECM. With respect to the correlation with the gold standard, DIAGNOdent showed higher correlation coefficients than ECM. The high performance of DIAGNOdent for detection of occlusal caries in terms of sensitivity/specificity found in this study is in agreement with the results of other *in vitro* studies on occlusal surfaces (Lussi *et al*., 1999; Shi *et al*.,

2000; Costa *et al*., 2002; Baseren and Gokalp, 2003; Cortes *et al*., 2003) (Table 6).

In comparison with conventional radiographic examination for *in vitro* detection of secondary caries, DIAGNOdent exhibited higher diagnostic accuracy in terms of sensitivity/specificity and ROC analysis. The results of this study indicated that DIAGNOdent may have a potential role for detection of secondary carious lesions and generally supported the results reported of other *in vitro* studies of secondary caries detection (Boston, 2003; Ando *et al*., 2004).

Our *in vitro* studies (Papers I and II) have shown promising results for primary and secondary caries detection by DIAGNOdent. Thus, the DIAGNOdent method has potential for clinical application for detection of primary and secondary caries.

In Papers III and IV, performance of DIAGNOdent was evaluated under clinical conditions, in order to determine whether the results of the *in vitro* studies described above were reproducible under clinical conditions. However, clinical performance of DIAGNOdent for detection of secondary caries in terms of sensitivity/specificity was relatively lower than those obtained from the *in vitro* study, (0.60/0.81 versus 0.77/0.81). Regarding ROC analyses, the A_z values were 0.89 and 0.78 for DIAGNOdent under *in vitro* and *in vivo* conditions, respectively.

The relatively lower performance of DIAGNOdent under clinical conditions may be attributed to several factors. Firstly, performance could be influenced by oral environmental factors such as saliva, oral microflora, or body temperature, and is likely to be less consistent. Secondly, under *in vitro* conditions it was easier to clean the margins than in the clinical setting. Finally, under clinical conditions, DIAGNOdent was more difficult to

manoeuvre, especially for class II restorations, because the interproximal embrasures may be more limited *in vivo* than in artificial tooth blocks.

In Paper IV, the measurements made with DIAGNOdent under clinical conditions were eventually validated by histopathology. The performance of the DIAGNOdent for detection of occlusal carious lesions has previously evaluated under *in vivo* conditions in a number of studies (Lussi *et al*., 2001; Heinrich-Weltzien *et al*., 2002; Anttonen *et al*., 2003; Rocha *et al*., 2003). However, with respect to validation, a major drawback in these earlier clinical studies is that the gold standard usually applied is quite weak, because clinical examination based on visual inspection is very subjective. In addition, the tooth can only be validated operatively where it is diagnosed to have a carious lesion requiring operative intervention, and thus false negative results cannot be evaluated. Although the dentists were trained to clinically distinguish dentinal caries from enamel caries after excavation, it is still difficult to distinguish lesions extending into the inner half of the enamel from those extending to the outer half of the dentin.

Therefore in the present study, in which all teeth in the sample were scheduled for extraction, the performance of DIAGNOdent *in vivo* was validated by post-extraction histopathological examination. The validity of the method in terms of sensitivity/specificity and correlation coefficient with the gold standard, is somewhat lower than in the *in vitro* study (Paper I), as well as other *in vitro* studies using histopathology as the gold standard (Shi *et al*., 2000; Baseren and Gokalp, 2003).

The composition of the tooth sample, in terms of lesion depth, is an important factor to consider when analyzing sensitivity and specificity of a method. In Paper IV, frankly carious lesions detected by visual inspection were excluded and accordingly there were only 10 teeth with dentinal carious lesions, all limited to the outer half of the dentine and 70% of the tooth sample had enamel caries. As it is known from previous studies that

DIAGNOdent is more sensitive at detecting dentinal caries, the correlation would be lower in a sample of predominantly enamel lesions. However, DIAGNOdent showed better sensitivity and specificity values than visual inspection for caries detection at D_2 and D_3 levels.

DIAGNOdent readings were higher in the presence of staining (Papers II, III, IV). This implies that DIAGNOdent measurement is extremely sensitive to stains, even slight discoloration. Under clinical conditions, teeth with suspicious carious lesions are seldom without stain. Therefore, when assessing the performance of DIAGNOdent on stained sites it is important to be aware that the signal may be overstimulated.

The cone-shaped tip was used for the DIAGNOdent examination in all studies, even for secondary caries detection in Papers II and III, to enable access to the sample sites with ditching and to improve the sensitivity of caries detection (Reich *et al.*, 1998). However, the tip diameter was too big to reach the proximal margins. Therefore, a smaller tip might produce more accurate results.

With a relatively lower performance of DIAGNOdent under clinical conditions than under *in vitro* conditions, caries involving dentin determined solely on the basis of DIAGNOdent readings should not indicate immediate operative intervention. Furthermore, under clinical conditions it is inappropriate to apply the cut-off value rigidly when considering treatment decisions, because sound and carious sites are represented by overlapping ranges of DIAGNOdent values. If a cut-off value for dentinal caries > 30 is used, it makes little sense to treat lesions with scores of 30 or 31 differently without considering the factors which can affect this reading, like the presence of stains, deposits and calculus, or without applying an additional method of assessment.

It is therefore recommended that DIAGNOdent readings should serve as guidelines, and a treatment decision about an operative intervention should not be based on the DIAGNOdent values alone. However, in doubtful cases, after application of conventional methods, DIAGNOdent can be used for a "second opinion", provided due allowance is made for the various factors that can affect DIAGNOdent readings under clinical conditions.

DIAGNOdent is probably a valuable device for dental practitioners. It is relatively inexpensive, and more readily manipulated intraorally than other new devices like ECM. If properly applied and correctly interpreted, this technique would improve caries detection and diagnosis and, therefore, the selection of proper treatment.

However, some factors may influence and affect the DIAGNOdent readings obtained in the clinical setting. For optimal advantage, the following factors should be considered:

1) Calibration against a ceramic standard before every measurement session (Karlsson *et al*., 2004), and calibration of each tooth by measuring at a site of sound tissue.
2) The tooth surface should be cleaned before measurement because the instrument is very sensitive to the presence of deposits, plaque, and calculus: these may be falsely registered as a change in enamel or dentin.
3) Tooth staining. DIAGNOdent measurement is extremely sensitive to stains, even slight discoloration. Therefore, DIAGNOdent readings of discolored surfaces should be interpreted with caution. Thus, when assessing the performance of DIAGNOdent on stained sites, it is important to be aware that the signal may be overstimulated.
4) The tip of the probe of the instrument should be rotated and tilted around its axis during measurement. This ensures that the instrument

tip picks up the highest fluorescence reading from the measuring sites.

5) Fluorosis and hypomineralized tooth tissue could give false results. However, further research is required to validate this.

To improve performance of DIAGNOdent readings, factors like tip design, cut-off values, and the reduction of the effect of stains should be optimized.

Future applications

There is little information on the use of DIAGNOdent in teeth with fluorosis or hypomineralization. These teeth may be able to give false readings with DIAGNOdent. Therefore, further research is required to validate this fact.

To our knowledge, there are no previously published studies evaluating the DIAGNOdent system in clinical detection of secondary carious lesions. Because secondary caries is a frequent cause of restoration failure and detection by conventional means is difficult, further investigations into the application of DIAGNOdent to aid clinical detection of secondary caries are warranted.

Conclusions

Based on the four studies, it may be concluded that:

- The ability of DIAGNOdent to retrieve the site of a carious lesion is very good.
- The reproducibility of DIAGNOdent is excellent and superior to that of ECM.
- For *in vitro* detection of non-cavitated occlusal carious lesions, DIAGNOdent is superior to ECM in terms of both correlation with histopathology and specificity /sensitivity.
- DIAGNOdent may be a valuable adjunct to, but not a substitute for conventional methods for *in vitro* and *in vivo* detection of secondary caries.
- There were no statistically significant changes in DIAGNOdent readings performed *in vivo* and after extraction when followed up to 3 months. Therefore, we do not recommend the use of different cut off values for *in vivo* and *in vitro* studies.
- For detection of occlusal caries under clinical conditions, DIAGNOdent is superior to visual inspection. The instrument can thus be recommended as an aid to, but not as a substitute for conventional methods of caries detection.

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