

## MERENJA INFRACRVENE FLUORESCENCE – UTICAJ KALIBRACIONE FREKVENCE NA LONGITUDINALNO *IN VITRO* MERENJE SA KaVo DIAGNOdent™-OM

### INFRARED FLUORESCENCE MEASUREMENTS – THE INFLUENCE OF CALIBRATION FREQUENCY ON LONGITUDINAL *IN VITRO* MEASUREMENTS WITH KaVo DIAGNOdent™

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#### Kratak sadržaj

S obzirom na široku primenu fluorida u današnje vreme, promenila se i sama priroda zubne šupljine (kaviteta). Čvršća i otpornija zubna gleđ često prekriva subpovršinsku destrukciju i napredovanje karijesa, za jedan duži vremenski period, uz nisku aktivnost i sporu progresiju. KaVo DIAGNOdent™ je laserski fluorescentni uređaj napravljen za detekciju karijesa i kvantifikaciju, kao pomoć vizuelnom istraživanju i radiografskom ispitivanju. Cilj ove *in vitro* studije je bio da ispita stabilnost instrumenta za longitudinalno merenje. Istraživanje je sprovedeno u dve povezane etape gde su merenja obavljena u dve serije, "sa" i "bez" kalibracije. Materijal u prvoj etapi studije sačinjavalo je 30 izvađenih zuba sa različitim stupnjevima karijesnih oštećenja koja su merena pomoću DIAGNOdent™ aparata. U drugom delu, korišćena su dva uređaja da bi se utvrdila njihova ujednačenost a merenje obavljeno na 6 fluorescentnih standarda sa ciljem minimiziranja lažno pozitivnih očitavanja. Prva serija u istraživanju, sa samopočetnom kalibracijom, vremenom je pokazala značajne promene: linearni trend koji se kretao ka nižim vrednostima ( $p < 0.001$ ). U drugom serijalu, sa učestalom kalibracijom, nije se mogao uočiti značajniji linearni trend ( $p = 0.09$ ). Rezultati iz drugog dela pokazali su značajnu sistematičnu prekovremenu razliku između faktora "bez" i "sa" kalibracijom ( $p = 0.0023$ ) nezavisno od toga koji je uređaj korišćen. Na osnovu ove *in vitro* studije zaključeno je da učestala kalibracija DIAGNOdent™-a treba da bude sprovedena radi dobijanja komparativnih rezultata za longitudinalno praćenje.

**Ključne reči:** infracrveno, laser, fluorescentnost, kalibracija, DIAGNOdent™

#### Abstract

With today's widespread use of fluoride, the nature of cavities has changed. Harder, and therefore more resistant enamel can many times conceal sub-surface decay and the caries disease progresses, in many cases, for a prolonged period with low activity and slow progression. KaVo DIAGNOdent™ is a laser fluorescence device developed for caries detection and quantification as an adjunct to visual inspection and radiographic examination. The aim of this *in vitro* study was to investigate the stability of the instrument for longitudinal measurements. The study was carried out in two subsequent parts where measurements were performed in two series, with and without calibration. The material in part I of the study comprised 30 extracted teeth with various stages of carious lesions measured with one DIAGNOdent™ device. In part II, two devices were used to determine their unanimity and measurements were performed on six fluorescence standards in order to minimise false positive readings. The first series in study part I, with only one initial calibration, showed a significant change over time: a linear trend with drifting towards lower readings ( $p < 0.001$ ). In the second series, with frequent calibrations, no significant linear trend over time could be demonstrated ( $p = 0.09$ ). Results from part II showed a significant systematic over-time difference between the factor "without" and "with" calibration ( $p = 0.0023$ ) independent of which device that was used.

From this *in vitro* study it was concluded that frequent calibration of DIAGNOdent™ should be performed in order to obtain comparable data for longitudinal monitoring.

**Key words:** infrared, laser, fluorescence, calibration, DIAGNOdent™

## Uvod

Utvrđeno je da stopa progresije karijesa kod industrijske populacije opada u toku poslednje dve decenije, i fluorid paste su prihvaćene kao jedan od najvažnijih faktora za smanjenje rasprostranjenosti karijesa.<sup>1,2</sup>

Jedan od efekata široke primene fluorid suplemenata bila je promena obrazaca bolesti karijesa, sa istaknutom redukcijom razvoja oštećenja na mestima kojima fluorid ima direktan pristup, kao što su glatke površine, dok okluzalne površine pokazuju najmanju redukciju.<sup>3</sup> U populacijama sa manjom karijes prevalencom, proporcija okluzalnog karijesa se povećava.<sup>4,5</sup> Veliki broj kvarova se pojavljuje oko već postojećih restauracija i/ili na okluzivnim površinama zuba. U današnje vreme detekcija i kvantifikacija okluzivnih kaviteta predstavlja izazov pošto je bolest inicirana kao mikroskopski poremećaj u zubnoj gleđi čvrste površine, i može da se odmah proširi u mekšu zubnu strukturu ispod klinički intaktne površine gleđi, takozvani skriveni karijes.<sup>6,7</sup> Okluzalni skriveni karijes je tip oštećenja gde su tradicionalni dijagnostički metodi kao vizuelno ispitivanje, X-zračenje i sondiranje u velikoj meri ograničeni.<sup>4,8,9,10</sup> Kliničari su primetili poteškoće pri dijagnostikovanju okluzalnog karijesa samo na osnovu vizuelnog ispitivanja,<sup>11</sup> a upotreba radiografa može da bude problematična pri ranoj dijagnozi karijesa pošto demineralizacija može biti prikrivena radio-gustinom od obližnje zdrave gleđi.<sup>12</sup> Upotreba zubnog pretraživača je, takođe, kritikovana zbog mogućnosti da se mikroorganizmi prenese sa jednog mesta na drugo, što može da prouzrokuje ireverzibilna traumatska oštećenja kod ranih demineralizovanih površina.<sup>12,13</sup>

Još jedna promena kod karijesnog procesa, i dodatni efekat upotrebe proizvoda koji sadrže fluorid, jeste da karijes poseduje nisku aktivnost i sporu progresiju. U većini slučajeva kliničari se bave otkrivanjem prvih najranijih oštećenja. Detekcija bi trebalo da se odigra na mikroskopskom nivou gde je remineralizacija ostvarljiva. Važnost ranog otkrivanja zubnog

## Introduction

It is well established that the rate of caries progression in industrialised nations has declined over the last few decades, and fluoride dentifrice is accepted to be one of the most important factors of the decrease in caries prevalence.<sup>1,2</sup>

One effect of the widespread use of fluoride supplements has been the change of the pattern of the caries disease, with a pronounced reduction in lesion development at sites that are readily accessible to fluoride, such as smooth surfaces, while occlusal surfaces show the least reduction.<sup>3</sup> In populations with decreasing caries prevalence, the proportion of occlusal caries has increased.<sup>4,5</sup> Much of the decay now occurs around existing restorations and/or on occlusal surfaces of the teeth. Detection and quantification of occlusal cavities is today more challenging as the disease is initiated as microscopic defects in the hard surface enamel, and readily can spread into the softer tooth structure beneath the clinical intact enamel surface, so called hidden caries.<sup>6,7</sup> Occlusal hidden caries is a type of lesion where traditional diagnosing methods such as visual examination, X-rays and probing with an explorer are highly limited.<sup>4,8,9,10</sup> Clinicians have reported difficulties in diagnosing occlusal caries by visual examination alone,<sup>11</sup> and the use of radiographs can be problematic in early caries diagnosis since a demineralisation can be masked by the radiodensity of the surrounding sound enamel.<sup>12</sup> The use of a dental explorer has also been criticised due to the possibility of transferring micro organisms from one site to another, and that it can cause irreversible traumatic defects in early demineralised areas.<sup>12,13</sup>

Another change in the caries process, and an additional effect of the use of fluoride-containing products, is that the caries disease progresses with low activity and slow progression. The clinicians are now, in most cases, dealing with the detection of the early primary lesions. The detection should take place on a microscopic level where the remineralisation is possible. The importance of early

karijesa naglašena je činjenicom da se početno oštećenje može zaustaviti. Prednost bi imao dijagnostički sistem koji objektivno sakuplja uporedne i precizne longitudinalno praćene podatke o oštećenjima koje izaziva karijes, kako bi se odredila aktivnost karijesa i izvršila procena preventivnih tretmana i njihovih ishoda, i za kliničara i za pacijenta.

Nedavno je bilo pokušaja da se usavrši pouzdanost tradicionalnih dijagnostičkih metoda karijesa<sup>4,8,14,15</sup> i da se razviju nove tehnologije za rano i kasnije otkrivanje i kvantifikaciju karijesa.<sup>16</sup> Uloga objektivnog detekcionog metoda je da podrži određene kliničke odluke kod svakog individualnog oštećenja: da li treba primeniti invazivni ili konzervativniji, neinvazivni pristup.<sup>17</sup> Kao deo strategije suzbijanja karijesa, bazirane na odsustvu rizika, potreba za novim detekcionim metodom koji može pouzdano da odredi stepen kvara ispod površine često je citirana.<sup>15,18,19,20,21</sup>

Laserski fluorescentni uređaj, DIAGNOdent2095<sup>TM</sup> (KaVo, Biberach, Germany) je neinvazivni instrument koji se koristi za detektovanje i kvantifikaciju zubnog karijesa na glatkim i okluzalnim površinama, i komercijalno je dostupan za kliničku primenu. (Slika 1) Zub je osvetljen pomoću svetlosti lasera ( $\lambda=655\text{nm}$ ) apsorbirane organskim i neorganskim molekulima u zubnoj supstanci i putem metabolita iz oralnih bakterija.<sup>22,23</sup>

detection of dental caries is emphasised by the fact that an incipient lesion can be arrested. An advantage would be a diagnostic system that objectively collects and records comparable and accurate longitudinally monitored data of carious lesions, to determine caries activity and to evaluate a preventive treatment and its outcome, both for the clinician as well as for the patient.

In recent years there have been attempts to improve the reliability of traditional caries diagnostic methods,<sup>4,8,14,15</sup> and to develop new technologies for early and advanced caries detection and quantification.<sup>16</sup> There is a role for an objective detection method, to support appropriate clinical decisions about management of the individual lesion: whether invasive therapy or a more conservative, non-invasive approach is indicated.<sup>17</sup> As part of a caries management strategy based on risk assessment, the need for new detection methods that can reliably establish the extent of the subsurface decay is frequently cited.<sup>15,18,19,20,21</sup>

The laser fluorescence device, DIAGNOdent 2095<sup>TM</sup> (KaVo, Biberach, Germany) is a non-invasive instrument used for detection and quantification of dental caries on smooth and occlusal surfaces, available commercially for clinical application. (Fig. 1) The tooth is illuminated with laser light ( $\lambda = 655 \text{ nm}$ ), which is absorbed by both inorganic and organic molecules in the tooth substance and by

*Slika 1. DIAGNOdent 2095<sup>TM</sup> (KaVo, Biberach, Germany). Aparat za infracrvenu lasersku fluorescencu, dizajniran za otkrivanje i kvantifikaciju koja se odnosi na gleđ i dentin, i to na okluzalnim površinama kao i na glatkim površinama, i koji se koristi kao dodatak pri kliničkom pregledu i radiografskom ispitivanju. Leva dva digitalna broja na displeju pokazuju vrednost trenutnog merenja, a desna dva digitalna broja (raspon od 0-99) pokazuju maksimalne vrednosti koje označavaju dubinu lezije*

*Figure 1. DIAGNOdent 2095<sup>TM</sup> (KaVo, Biberach, Germany). Infrared laser fluorescence device designed for detection and quantification defined to the enamel and the dentine, on occlusal surfaces as well as on smooth surfaces, intended to be used as an adjunct to the clinical inspection and radiographic examination. The left two-digit number on the display shows the current measured value and the right two-digit (range from 0-99) showing the maximum value, which indicates the lesion depth*



Infracrvena fluorescentnost se pojavljuje i detektuje, sakupljena i prosleđena putem instrumenta i prezentovana operateru u vidu digitalnog brojanja na displeju, što pokazuje dubinu oštećenja. Veruje se da fluorescentnost potiče od porfirina, jednog od elemenata u respiratornom lancu mitohondrija i verovatno su molekuli glavni odgovorni za apsorpciju plave i crvene svetlosti. DIAGNOdent™ je pokazao dobru sposobnost za detektovanje okluzalnih i karijesa glatkih površina *in vitro*<sup>24,25,26</sup> i *in vivo*<sup>27,28,29</sup> i smatra se da je veoma vredan alat za longitudinalno praćenje razvoja karijesa i za procenjivanje ishoda preventivne intervencije<sup>24,27</sup>. Ono što važi za sve uređaje u vezi s njihovim preciznim merenjem jeste stabilnost i reproduktivnost u toku dužeg vremenskog perioda da bi se osigurala bezbedna primena instrumenta. Da bi se postigla stabilnost DIAGNOdent™ instrumenta, proizvođači preporučuju kalibraciju kad god je potrebno izvršiti precizno merenje u toku dužeg vremenskog perioda (od 3 do 12 meseci), kada se sonde redovno zamenjuju, steriliziraju ili dezinfikuju. Kalibracija se sprovodi da bi se nadoknadile potencijalne varijacije sistema pomoću standarda poznate i stabilne fluorescenčne predaje. (Slika 2)

metabolites from oral bacteria.<sup>22,23</sup> Infrared fluorescence occurs and is detected, collected, and processed by the instrument and presented to the operator as a digital number on a display, which indicates the lesion depth. The fluorescence is believed to originate from porphyrins, one of the elements in the mitochondria's respiratory chain and are probably the molecules chiefly responsible for the absorption of blue and red light. The DIAGNOdent™ has shown good performance and reproducibility for detection of occlusal and smooth surface carious lesions *in vitro*<sup>24, 25, 26</sup> and *in vivo*<sup>27, 28, 29</sup> and has been reported to be a valuable tool for longitudinal monitoring of the carious process over time and for assessing the outcome of preventive interventions.<sup>24, 27</sup>

As for all medical devices and their accurate measurements, stability and reproducibility over a longer period is required to ensure a safe application of the instrument. To achieve a stability of the DIAGNOdent™ instrument, calibration is recommended by the manufacturer whenever accurate measurements over an extended period of time (3 to 12 months) are required, when probes are replaced or sterilised or disinfected regularly. Calibration is carried out in order to compensate for potential variations of the system by a standard of known and stable fluorescence yield. (Fig. 2)



Slika 2. Kalibracija. U kalibracione svrhe kasete uključuje standard poznatog i stabilnog fluorescentnog polja radi kompenzacije potencijalne varijacije sistema. Sonda se drži naspram keramičkog standarda sa preregistrovanom vrednošću. Displej mora da se poklapa sa standardnim vrednostima uz toleranciju od  $\pm 3$

Figure 2. Calibration. For calibration purposes, the cassette includes a standard of known and stable fluorescence yield to compensate for potential variations of the system. The probe is held against the ceramic standard with a preset value. The display must match the standard value with a tolerance of  $\pm 3$

Cilj ovog *in vitro* istraživanja bio je da se ispita stabilnost laserskog fluorescentnog sredstva – DIAGNOdent™ za longitudinalno merenje sa i bez frekventne kalibracije.

## ***Materijal i metode***

Ova studija sprovedena je u dva sukcesivna dela. U prvom delu studije materijal je sačinjavalo 30 prekutnjaka, izvađenih mladim adolescentima; petnaest zuba sa vizuelno netaknutim glatkim površinama ili različiti stupnjevi nekavitetnih karijes oštećenja, i petnaest zuba koji su prošli kroz različite stupnjeve nekavitetnih okluzalnih karijes oštećenja, ili koji su naizgled bili zdravi. Strane naslage su pažljivo očišćene pomoću Robinsonove četkice i vode. Obavljene su dve odvojene serije merenja sa i bez kalibracije sa prvom generacijom DIAGNOdent (DD I), koja je plasirana na tržište 1998. godine. Pre početka prve serije merenja, izvršena je standardizovana instrumentalna kalibracija korišćenjem keramičkog standarda koji je obezbedio proizvođač, i prema uputstvu za operativnu primenu. Zubna površina je isušena u roku od 5 sekundi pomoću sabijenog vazduha pre samog merenja. Za svaki zub su određene bazične vrednosti na zdravom delu zubne gleđi da bi se omogućila unutrašnja varijacija fluorescentne adaptacije individualnih uzoraka. Potom su izvršena dvadeset osam merenja u periodu od dvadeset osam dana bez ikakve dalje kalibracije (na bazičnoj liniji, i jednom u toku 1 sata u trajanju od 12 sati, zatim svaki treći sat u narednih 12 sati, pa na svakih 6 sati u narednih 12 sati, i jedno merenje 4, 7, 10, 14, 21. dana i završno merenje 28. dana). U toku druge etape merenja, izvršena je standardizovana kalibracija pre svakog merenja. Isti protokol korišćen je za drugo merenje kao i za prvo. Pre i u toku eksperimenta zubi su čuvani u saturisanom thymol rastvoru u frižideru na temperaturi od +8°C. Kapacitet baterije ovog uređaja i sobna temperatura određeni su pre početka svakog merenja. Pribavljene su i digitalne slike ispitivanih površina da bi se

The aim of this *in vitro* study was to investigate the stability of a laser fluorescence device the DIAGNOdent™ for longitudinal measurements with and without frequent calibration.

## ***Material and methods***

The present study was carried out in two subsequent parts. The material in part I comprised 30 premolar teeth, extracted from young adolescents on orthodontic indications; fifteen teeth with visually intact smooth surfaces or various stages of non-cavitated carious lesions, and fifteen teeth that had various stages of non-cavitated occlusal carious lesions, or that were visually sound. Extrinsic deposits were gently cleaned with a Robinson brush and water. Two separate series of measurements were performed with and without calibration - with a first generation DIAGNOdent™ (DD I), introduced on the market in 1998. Before the start of the first series of measurements, standardised instrument calibration was performed using the ceramic standard provided by the manufacturer and according to the operating instruction manual. The tooth surfaces were air dried for 5 s with compressed air prior to the measurement. A baseline value for each tooth was measured on a sound enamel spot, to allow for intrinsic variation in fluorescence of individual specimen adaptation. Twenty-six measurements were then subsequently performed during twenty-eight days without any further calibration (at baseline, and once an hour for 12 h, then every third hour for the next 12 h, then every sixth hour for the next 12 h, and one measurement day 4, 7, 10, 14, 21 and a final measurement on day 28). During the second series of measurements, standardised instrument calibration was performed before every measurement session. The same time protocol as for the first series of measurements was used for the second one. Before and during the experiment the teeth were kept in saturated thymol saline under refrigeration, +8°C. The battery capacity of the device and room temperature were determined before each session of

olakšala reorijentacija onih mesta predviđenih za longitudinalno merenje.

U drugom delu tekuće studije korišćena su dva DIAGNOdent™ aparata, prva generacija (DD I) i druga generacija (DD II) plasirana 2001. godine. Merenje je izvedeno na 6 fluorescentnih standarda, uzoraka boja, napravljenih od poliestera koji su sadržali različite količine titanijum-dioksida (0,1% – 1,0% TiO<sub>2</sub>). Fluorescentni standardi korišćeni su da bi se eliminisali eksterni izvori greške kao što su prisustvo fleka, kalkulus i plak – prerekviziti potrebni da bi se smanjio broj lažnih pozitivnih rezultata. Izvedene su dve odvojene serije, "sa" i "bez" kalibracije, a pre početka prve serije merenja, samo je jednom sprovedena standardna instrumentalna kalibracija. Bazične vrednosti, adaptacije svakog uzorka, su pribavljene uz 26 merenja koja su jedna za drugim sprovedena u periodu od 28 dana bez ikakve dalje kalibracije. U toku drugog serijala, pre svakog merenja obavljena je standardna kalibracija. Isti onaj protokol koji je korišćen u prvoj etapi takođe je korišćen u oba merenja druge etape. Pre i u toku eksperimenta fluorescence standardi su čuvani u mračnoj sobi izloženi jedino sijalici od 60W. Kapacitet baterije ovog uređaja i sobna temperatura su određeni pre svakog merenja.

### ***Intra-operativno usklađivanje***

Intra-operativno ponavljanje je urađeno ponovnim merenjem dva karijes oštećenja odabrana metodom slučajnog uzorka (jedna okluzivna i jedna glatka površina) merena pod identičnim uslovima, 20 puta svaki, sa jednim DIAGNOdent aparatom. Merenja su obavljena u intervalima od 20 minuta i operator je napuštao sobu u toku svake pauze.

### ***Statistički metodi***

Dvosmerna ANOVA sa ponovljenim merenjima na dva faktora korišćena je da bi se analizirali podaci u prvoj i drugoj studiji.

To facilitate reorientation of the sites for the longitudinal measurements, digital images were obtained of the surfaces under investigation.

In Part II of the present study were two DIAGNOdent™ devices used, a first generation (DD I) and a second generation (DD II) introduced in 2001. Measurements were performed on six fluorescence standards, samples of colour, made of polyester containing different amounts of titanium dioxide filler (0,1% – 1,0% TiO<sub>2</sub>). The fluorescence standards were used in order to eliminate external sources of error such as storage media, presence of stains, calculus and plaque – prerequisites required to reduce the number of false positive readings. Two separate series, with and without calibration, were performed and before the start of the first series of measurements, standardised instrument calibration was carried out only once. Baseline values, an adaptation of each specimen, were obtained followed by twenty-six measurements subsequently performed during 28 days without any further calibration. During the second series of measurements, standardised calibration was performed before every measurement session. The same time protocol used in study part I was used for both series of measurements in part II. Before and during the experiment were the fluorescence standards kept in a dark room only exposed by a 60W light bulb. The battery capacity of the device and room temperature were determined before each session of measurements.

### ***Intra-operator agreement***

The intra-operator repeatability was determined by repeated measurements of two randomly selected carious lesions (one occlusal and one smooth surface) measured under identical conditions, 20 times respectively with one DIAGNOdent device. The measurements were performed with a 20 minute interval in between, and the operator left the room during every intermission.

Faktori su bili kalibracija (sa nivoima bez kalibracije i/sa kalibracijom) i stanja merenja u toku vremenskog perioda (sa različitim nivoima). Interakcija u ANOVI odnosi se na statistički test da li se efekat jednog faktora, pošto je izmeren na osnovu razlika u prosecima odgovora, razlikuje za različite nivoe od ostalih faktora. U slučaju značajne interakcije, ispitani su jednostavni efekti npr. efekat jednog faktora koji drži drugi faktor fiksiran. Kada je F-ratio za merenje faktora stanja u toku vremena postao značajan, obavljena su planirana poređenja između tretmana. P-vrednosti su onda ispravljene na osnovu Bonferonijeve procedure. Takođe je ispitan i kontrast za linearni trend. Ako korelacije između svakog para ponovljenog merenja nisu iste (test na Sfernost), stepen slobode (d.f.) F testa povezanog sa faktorom merenja stanja u toku vremena, smanjen je uvećavanjem svakog od d.f. Greenhouse-Geisser epsilon. Ponovljena merenja ANOVA primenjena su da bi se analizirala konzistentnost između oba sredstva DD I i DD II. Za procenu ponavljanja, srednja vrednost, standardna devijacija, srednja i maksimalna vrednost su primenjene podjednako na glatka i okluzalna karijes oštećenja.

## Rezultati

### Prvi deo

Slika 3a pokazuje podatke dobijene na osnovu svih 30 izvađenih zuba i dvadeset šest uzastopnih merenja sa samo jednom početnom kalibracijom. Slika 3b pokazuje dvadeset šest merenja sa kalibracijom pre svakog merenja u toku 28 dana. Rezultati su pokazali značajnu promenu u toku vremena: linearni trend koji se kreće ka nižim stanjima u prvom delu studije sa samo jednom početnom kalibracijom ( $p < 0.001$ ). U drugom delu merenja, sa čestim

## Statistical methods

A two-way ANOVA with repeated measures on two factors was used to analyse the data in part I and II of the study. The factors were calibration (with the levels without calibration and with calibration) and measurement readings over time (with different levels). The interaction in the ANOVA refers to the statistical test of whether the effect of one factor, as measured by differences in the response averages, is different for different levels of the other factor. In case of significant interaction, simple effects were examined, i.e. effects of one factor holding the other factor fixed. When the F-ratio for factor measurement readings over time was significant, planned comparisons were performed between the treatments. The p-values were then corrected according to the Bonferroni procedure. Contrast for linear trend was also investigated. If the correlations between each pair of repeated measures were not the same (test of Sphericity), the degrees of freedom (d.f.) of the F-tests associated with the factor measurement readings over time were reduced by multiplying each of the d.f. by the Greenhouse-Geisser epsilon. Repeated measures ANOVA were applied to analyse the consistency between the two devices DD I and DD II. For assessment of repeatability, mean value, standard deviation, mean and max value were applied for smooth and occlusal carious lesions, respectively.

## Results

### Part I

Fig. 3a shows data obtained from a total of 30 extracted teeth and twenty-six subsequent measurements with only one initial calibration. Fig. 3b shows twenty-six measurements with calibration before every measurement, during 28 days. The results showed a significant change over time: a linear trend with drifting towards lower readings in the first study series with only one initial calibration ( $p < 0.001$ ). In the second series of measurements, with frequent cali-

kalibracijama, nije se mogao uočiti značajniji linearni trend ( $p=0.09$ ) u toku vremenskog perioda. Klinički bitne razlike u srednjoj vrednosti između serija merenja su vremenom postale uočljive (bez učestale kalibracije 8.57). Srednja vrednost rezultata iz ove serije sa stalnom kalibracijom je otprilike bila za jednu jedinicu viša u toku čitave studije, a pojedinačna posmatranja bila su viša i za šest jedinica.

### Drugi deo

Ponovljena merenja ANOVA pokazala su značajnu sistematsku razliku između faktora "sa" i "bez" kalibracije ( $p=0.0023$ ) a razlika je bila nezavisna od uređaja koji je korišćen ( $p=0.67$ ). Nije bilo značajnijih razlika između uređaja DD I i DD II ( $p=0.14$ ). Interakcija, vremenska kalibracija bila je značajna

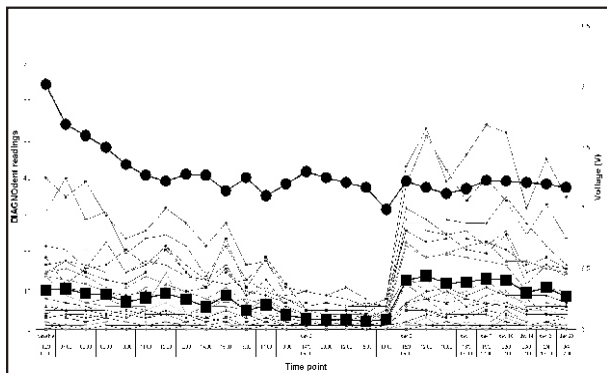


Figure 3a.

Slika 3a. DIAGNOdent očitavanje bez kalibracije. Podaci koji su dobijeni na 30 izvađenih zuba i 26 očitavanja za vreme 28 dana, sa samo jednom početnom kalibracijom. Pronađene su značajne promene u tom periodu sa linearnim trendom i pomeranja prema nižim vrednostima ( $p<0.001$ ). Tamnija tačkasta kriva na vrhu dijagrama pokazuje kapacitet voltaže baterije, koji opada postepeno za vreme prvog dana kao i kontinuirano opadanje vremenom. Donja naznačena kriva sa kvadratićima pokazuje srednje vrednosti sa očitavanjima nižim, približno za jednu jedinicu, kroz celu seriju studije ( $\bar{x}=7.64$ ) u poređenju sa serijama sa kalibracijom.

Figure 3a. DIAGNOdent readings without calibration. Data from a total of 30 extracted teeth and twenty-six subsequent measurements during 28 days, with only one initial calibration. A significant change over time was found, with a linear trend drifting toward lower readings ( $p<0.001$ ). The bold, dotted curve at the top of the diagram demonstrates the battery voltage capacity, which falls considerable during the first day, only to continuously decrease very slowly over time. The lower bold, square-marked curve shows the mean value, with readings approximately one unit lower throughout the study series ( $\bar{x}=7.64$ ) compared to the series with calibration.

brations, no significant linear trend over time could be demonstrated ( $p=0.09$ ). Clinically relevant differences in mean value between the series of measurements were seen over time (without frequent calibration 7.64 – with frequent calibration 8.57). The mean value of readings from the series with frequent calibrations was approximately one unit higher throughout the study, and single observations were one to six units higher.

### Part II

Repeated measures ANOVA showed a significant systematic difference between the factors "with" and "without" calibration ( $p=0.0023$ ) and the difference was independent of which device that was used ( $p=0.67$ ). There was no significant difference between the devices, DD I and DD II ( $p=0.14$ ). The

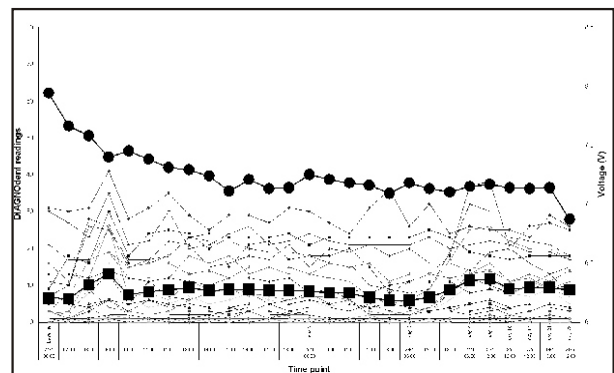


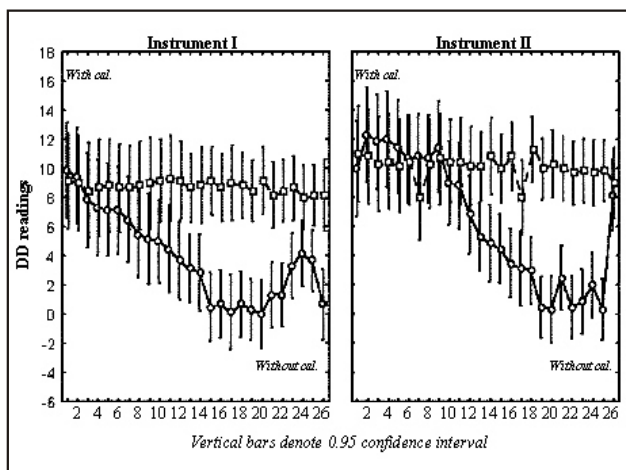
Figure 3b.

Slika 3b. DIAGNOdent očitavanje sa kalibracijom. Standardizovana kalibracija je urađena pre svakog merenja u skladu sa istim vremenskim protokolom kao za prvu seriju merenja. Nije uočen signifikantni linearni trend u opisanom vremenu ( $p=0.09$ ). Kapacitet baterijske voltaže, pokazan kao crna tačkasta kriva, sa vrha pada zanemarljivo malo u opisanom vremenskom periodu, sa 7.5 V na 6.75 V. Donja crna kriva sa kvadratićima pokazuje srednje vrednosti sa očitavanjima približno jedne jedinice više kroz celu seriju u studiji ( $\bar{x}=8.57$ ) u poređenju sa serijom bez kalibracije.

Figure 3b. DIAGNOdent readings with calibration. Standardised calibration was performed before every measurement session according to the same time protocol as for the first series of measurements. No significant linear trend over time could be demonstrated ( $p=0.09$ ). The battery voltage capacity, demonstrated as a bold, dotted curve at the top, falls negligible over time from 7.5V to 6.75V. The lower bold, square-marked curve shows the mean value, with readings approximately one unit higher throughout the study series ( $\bar{x}=8.57$ ) compared with the series without calibration.



( $p=0.000$ ) sa stabilnim dijagnozama u toku vremenskog perioda "sa kalibracijom", dok su se rezultati u periodu "bez kalibracije" kretali u pravcu nižeg stanja od prvog dana pa nadalje. (Sl. 4)



interaction, time-calibration, was significant ( $p<0.000$ ) with stable readings over time in the period "with calibration", while the readings in the period "without calibration" was drifting towards lower readings from day one and onward. (Fig. 4)

Slika 4. Uticaj kalibracione frekvence na merenje standardne fluorescence, sa DD I i DD II. Dvadeset šest merenja urađena su za 28 dana u seriji između faktora sa i bez kalibracije standardne fluorescence, sa dva instrumenta. Značajna razlika između "sa kalibracijom" i "bez kalibracije" je uočena ( $p=0,0023$ ), nezavisno upotrebljenog od aparata ( $p=0,67$ ). Rezultati su pokazali da nema signifikantne razlike između aparata DDI i DDII ( $p=0,14$ )

Figure 4. The influence of calibration frequency on measurements of fluorescence standards, with DD I and DD II. Twenty-six measurements were performed during 28 days in series between the factors with and without calibration on fluorescence standards, with two instruments. A significant difference between "with calibration" and "without calibration" was seen ( $p=0.0023$ ), independent of which device used ( $p=0.67$ ). The results showed no significant difference between the devices, DD I and DD II ( $p=0.14$ ).

### Intra-operativno usklađivanje

Ponovljena merenja jednog okluzalnog karijes oštećenja, putem jednog operatora, dalo je srednju vrednost od 13.85 sa SD od 2.24 (min 11, max 18). Za glatke površine, odgovarajući podaci iznosili su 8.15 za srednju vrednost, a za SD 0.67 (min 7, max 9).

### Diskusija

Promena u obrascu po kome se karijes razvija zahteva i menjanje filozofije samog tretmana; originalna maksima "ekstenzija za prevenciju" izbegavana je i kod pristupa minimalnim intervencijama, iako je ovaj pristup efektivan samo ako se karijes otkrije rano. Netačna dijagnoza je uzrok nepravilne odluke u vezi tretmana koji će se primenjivati. U današnje vreme kada je nizak stepen kvara dominantan a progresija bolesti spora, potencijalni rizik nepotrebne restauracije veći je od rizika propuštanja ranog kvara. Kao dodatak konvencionalnim karijes dijagostičkim metodama kao što su vizuelni pregled i "bitwing" radiografija, potreba za objektivnim kvantitativnim metodama je od velike važnosti.

### Intra-operator agreement

Repeated measurements of one occlusal carious lesion, by one operator, showed a mean value of 13.85 with a SD of 2.24 (min 11, max 18). For the smooth surface, the corresponding findings were a mean of 8.15, SD 0.67 (min 7, max 9).

### Discussion

The change in pattern of the caries disease calls for a shift in treatment philosophy; the original maxim of "extension for prevention" has been eschewed for a minimal intervention approach, though this approach is only effective if caries is diagnosed at an early stage. Incorrect diagnosis results in incorrect treatment decisions. In current age of lower overall prevalence of decay and slow disease progression, the potential risk of unnecessary restorations is greater than the risk of missing early decay. As an adjunct to conventional caries diagnostic methods such as visual inspection and bitewing radiography, a need for objective quantitative detection methods is of high importance.

Laserski fluorescentni uređaj, KaVo DIA-GNOdent™, je neinvazivan instrument za otkrivanje i kvantifikaciju zubnog karijesa na glatkim i okluzalnim površinama, upotrebljava se u istraživanjima, koriste ga kliničari, zubari i zubni higijeničari, a zastupljen je i u kliničkoj primeni. Ova stoličasta baterijski kvantitativna laser dioda sa kompaktnim dizajnom i brzom procedurom merenja pokazala je obećavajuće rezultate kao dopuna konvencionalnim vizuelnim, taktilnim i radiografskim tehnikama, kao i za longitudinalno praćenje karijes oštećenja<sup>30</sup>. Dobra validnost<sup>31</sup> i dobra<sup>29,31</sup> ili skoro odlična reproduktivnost DIAGNOdent™-a objavljena je u štampanim *in vitro* istraživanjima, ali stabilnost ovog aparata još nije procenjena. Prema proizvođaču, kalibracija ovog uređaja je neophodna samo kada su potrebna precizna merenja u toku dužeg vremenskog perioda (od 3 do 12 meseci), kada se sonde redovno menjaju, sterilišu ili dezinfikuju. Trenutna *in vitro* istraživanja predstavljaju rezultate merenja sa značajnim linijskim kretanjem ka nižim rezultatima u serijalu bez kalibracije. Procena intra-operativne respektabilnosti je pokazala veoma dobre rezultate, čime se ističe da znatne varijacije u rezultatima najverovatnije nisu rezultat intra-operativnih varijacija. Ovo pokazuje da je neophodno obavljati stalnu kalibraciju ovog aparata da bi se dobili uporedni podaci pri longitudinalnom praćenju karijes oštećenja. Nije bilo značajne razlike između oba uređaja DD I i DD II, niti značajnije uređaj/kalibracija interakcije. Calculus, plak, kompozitni materijal za plombiranje, ostaci polir pasti i mrlje mogu da stvore fluorescencu i pritom prouzrokuju lažne pozitivne rezultate.<sup>24,26,30</sup> Zbog toga su drugi autori predložili da je neophodno izvršiti temeljno čišćenje zubne površine pre merenja.<sup>24,30,31</sup> Na osnovu rezultata ovog istraživanja, preporučuje se da se, takođe, u ove prekrizite uključi i frekventna kalibracija DIA-GNOdent™-a da bi se smanjio broj lažnih pozitivnih rezultata.

#### **Zahvalnost**

Istraživanje je potpomognuto i odobreno od strane švedskog Patentnog državnog fonda za istraživanje u preventivnom zubarstvu.

The laser fluorescence device, KaVo DIAGNOdent™, is a non-invasive instrument for detection and quantification of dental caries on smooth and occlusal surfaces, used for research purposes and by clinicians, dentists and dental hygienists, for clinical application. This chair side, battery powered quantitative laser diode with a compact design and fast measurement procedure, has shown promising results as a complement to conventional visual, tactile and radiographic techniques, and for longitudinal monitoring of carious lesions.<sup>30</sup> Good validity<sup>31</sup> and good<sup>29, 31</sup> to excellent reproducibility<sup>27</sup> of the DIAGNOdent™ has been reported in published *in vivo* studies, but the stability has not been evaluated. According to the manufacturer, calibration of the device is only needed whenever accurate measurements over an extended period of time (3 to 12 months) are required, when probes are replaced or sterilised or disinfected regularly. The present *in vitro* study demonstrated measurement readings with a significant linear drifting toward lower readings in series without calibration. Assessment of the intra-operator repeatability exhibited very good results, which indicates that the significant variations in readings were probably not caused by intra-operator variation. This indicates that frequent calibration of the device should be performed in order to obtain comparable data at longitudinal monitoring of carious lesions. There was no significant difference between the two devices, DD I and DD II, nor any significant device/calibration interaction. Calculus, plaque, composite filling materials, remnants of polish pastes and stains may produce fluorescence and could therefore cause false-positive readings.<sup>24, 26, 30</sup> Therefore, other authors have been suggested that a thorough cleaning of the tooth surfaces should be performed before the measurement.<sup>24, 30, 31</sup> Based on the findings from this study, a recommendation is to also include frequent calibration of the DIAGNOdent™ into these prerequisites required to reduce the number of false positive readings.

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## LITERATURA / REFERENCES

1. Marthaler TM. Caries status in Europe and prediction of future trends. *Caries Res* 1990;24:381–96.
2. Marthaler TM, O'Mullane DM, Vrbic V. The prevalence of dental caries in Europe 1990-1995. *Caries Res* 1996;30:237–55.
3. Newbrun E. Preventing dental decays: Current and prospective strategies. *J Am Dent Assoc* 1992;123:68–73
4. Lussi A. Validity of diagnostic and treatment decisions of fissure caries. *Caries Res* 1991;25:296–303.
5. Mejäre I, Källestål C, Stenlund H, Johansson H. Caries development from 11 to 22 years of age: A prospective radiographic study. Prevalence and distribution. *Caries Res* 1998;32:10–6.
6. Weerheijm KL, Gruythuysen RJ, van Amerongen WE. Prevalence of hidden caries. *ASDC J Dent Child* 1992;59(6):408–12.
7. Ricketts D, Kidd E, Weerheijm K, de Soet H. Hidden caries: what is it? Does it exist? Does it matter? *Int Dent J* 1997;47(5):259–65.
8. Lussi A. Comparison of different methods for the diagnosis of fissure caries without cavitation. *Caries Res* 1993;27:409–16.
9. Lussi A. Impact of including or excluding cavitated lesions when evaluating methods for the diagnosis of occlusal caries. *Caries Res* 1996; 30:389–93.
10. Wenzel A, Larsen MJ, Fejerskov O. Detection of occlusal caries without cavitation by visual inspection, film radiographs, xeroradiographs, and digitized radiographs. *Caries Res* 1991;25:365–71.
11. Weerheijm KL, Kidd EAM, Groen HJ. The effect of fluoridation on the occurrence of hidden caries in clinical sound occlusal surfaces. *Caries Res* 1997; 31:30–4.
12. Dodds MWJ. Dental caries diagnosis toward the 21<sup>st</sup> century. *Nature Medicine* 1996;2(3):283.
13. Ekstrand K, Qvist V, Thylstrup A. Light microscope study of the effect of probing in occlusal surfaces. *Caries Res* 1987;21:368–72.
14. Pitts NB. Current methods and criteria for caries diagnosis in Europe. *J Dent Educ* 1993; 57:409–14.
15. Pitts NB. Advances in radiographic detection methods and caries management rationale. In: Stookey GK (ed). *Early Detection of Dental Caries*. Indianapolis, Indiana University, School of Dentistry 1996; 38–50.
16. Verdonshot EH, Angmar-Månsson B. Advanced methods of caries diagnosis and quantification. In: Fejerskov O, Kidd E (eds). *Dental Caries: The disease and its clinical management*. Blackwell Munksgaard 2003; 129–38.
17. Featherstone JDB. Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol* 1999; 27:31–40.
18. Featherstone JDB. Clinical implications of early caries detection: New strategies for caries prevention. In: Stookey GK (ed). *Early Detection of Dental Caries*. Indianapolis, Indiana University, School of Dentistry 1996; 285–93.
19. Pine CM, ten Bosch JJ. Dynamics of and diagnostic methods for detecting small carious lesions. *Caries Res* 1996; 30:381–8.
20. Stookey GK. Practical applications of early caries detection methods. In: Stookey GK (ed). *Early Detection of Dental Caries II*. Indianapolis, Indiana University, School of Dentistry 2000; 357–63.
21. ten Cate JM, van Amerongen JP. Caries diagnosis, conventional methods. In: Stookey GK (ed). *Early Detection of Dental Caries*. Indianapolis, Indiana University, School of Dentistry 1996; 27–37.
22. Hibst R, Gall R. Development of a diode laser-based fluorescence caries detector. *Caries Res* 1998; 32:294.
23. Hibst R, Paulus R. Molecular basis of red exited caries fluorescence. *Caries Res* 2000; 34:323.
24. Lussi A, Imwinkelried S, Pitts NB, Longbottom C, Reich E. Performance and reproducibility of a laser fluorescence system for detection of occlusal caries *in vitro*. *Caries Res* 1999; 33:261–6.
25. Shi X-Q, Tran'us S, Angmar-Månsson B. Validation of DIAGNOdent for quantification of smooth-surfaces caries: an *in vitro* study. *Acta Odontol Scand* 2001;59:74–8.
26. Shi X-Q, Welander U, Angmar-Månsson B. Occlusal caries detection with KaVo DIAGNOdent and radiography: An *in vitro* comparison. *Caries Res* 2000;34:151–8.
27. Lussi A, Megert B, Longbottom C, Reich E, Francescut P. Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. *Eur J Oral Sci* 2001;109:14–9.
28. Pinelli C, Serra MC, Loffredo LCM. Validity and reproducibility of a laser fluorescence system for detecting the activity of white-spot lesions on free smooth surfaces *in vivo*. *Caries Res* 2002;36:19–24.
29. Tran'us S, Lindgren LE, Karlsson L, Angmar-Månsson B. Evaluation of the *in vivo* performance of the DIAGNOdent device. In: Stookey GK (ed). *Early Detection of Dental Caries III*. Indianapolis, Indiana University, School of Dentistry, 2003, in press.
30. Hibst R, Paulus R, Lussi A. Detection of occlusal caries by laser fluorescence: Basic and clinical investigations. *Med Laser Appl* 2001;16:205–13.
31. Pinelli C, Serra MC, Loffredo LCM. Validity and reproducibility of a laser fluorescence system for detecting the activity of white-spot lesions on free smooth surfaces *in vivo*. *Caries Res* 2002;36:19–24.

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