

Jonas de Almeida Rodrigues

**DETECÇÃO DE LESÕES DE CÁRIE OCLUSAL POR  
MEIO DE MÉTODOS BASEADOS NA MEDIÇÃO DA  
FLUORESCÊNCIA INDUZIDA PELA LUZ**

Tese apresentada ao Programa de Pós-Graduação em Ciências Odontológicas – área de Odontopediatria, da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista, para obtenção do título de Doutor em Odontopediatria.

Orientadora: Prof<sup>a</sup>. Dr<sup>a</sup>. Rita de Cássia  
Loiola Cordeiro  
Co-orientador: Prof. Dr. Adrian Lussi

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Araraquara, 28 de abril de 2008

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## Dedicatória

A todos que, de alguma forma, se fizeram presentes na minha vida durante esses anos fora do país, as mais insignificantes demonstrações de preocupação são as mais importantes quando se está distante de tudo e de todos...

"Ando devagar porque já tive pressa e levo esse sorriso porque já chorei demais

Hoje me sinto mais forte, mais feliz quem sabe

Eu só levo a certeza de que muito pouco eu sei e nada sei

Conhecer as manhas e as manhãs

O sabor das massas e das maçãs

É preciso amor pra poder pulsar

É preciso paz pra poder sorrir

É preciso chuva para florir

Penso que cumprir a vida seja simplesmente compreender a marcha

E ir tocando em frente como um velho boiadeiro levando a boiada

Eu vou tocando os dias pela longa estrada

Eu sou

Estrada eu vou

Todo mundo ama um dia

Todo mundo chora

Um dia a gente chega

Um outro vai embora

Cada um de nós compõe a sua história

E cada ser em si carrega o dom de ser capaz

De ser feliz...

(*Tocando em frente* – Composição Renato Teixeira)

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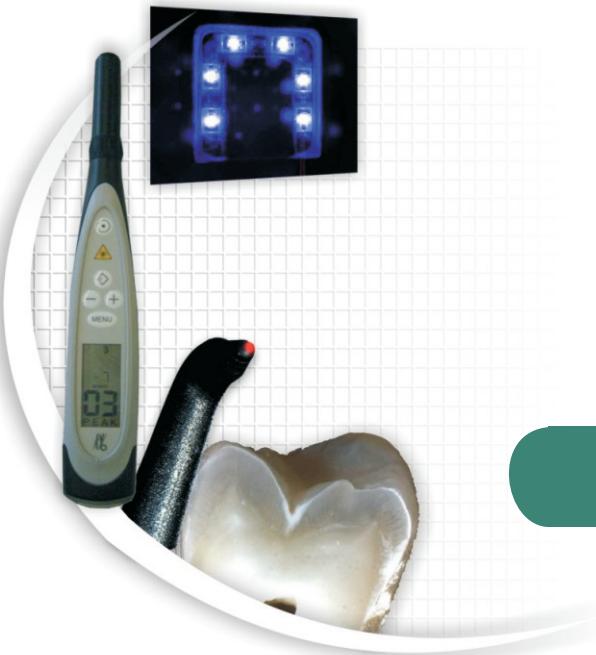
Muito obrigado!

*“Nunca deixe que lhe digam que não vale a pena acreditar nos sonhos que se têm  
ou que os seus planos nunca vão dar certo...  
ou que você nunca vai ser alguém...”*

*Renato Russo*

## *Sumário*

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## RESUMO

Rodrigues JA. Detecção de lesões de cárie oclusal por meio de métodos baseados na medição da fluorescência induzida pela luz [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2008.

O objetivo do presente estudo foi avaliar in vitro o desempenho dos métodos baseados na medição da fluorescência induzida pela luz para detecção de lesões de cárie oclusal em dentes permanentes. Para tanto, foram realizadas três pesquisas: (1) O objetivo dessa pesquisa foi avaliar a influência da subtração do valor de fluorescência zero no desempenho de dois aparelhos que induzem fluorescência na detecção de cárie. Desse modo, foram utilizados 119 molares permanentes, nos quais três áreas da superfície vestibular (cúspide, central e cervical) das porções mesial e distal foram selecionadas. Além disso, uma lesão de cárie oclusal por dente foi escolhida como “sítio teste”. Dois examinadores mensuraram tanto as áreas nas superfícies vestibulares quanto as lesões oclusais utilizando o DIAGNOdent 2095 (DD, KaVo, Alemanha) e o DIAGNOdent 2190 (DDpen). Foi observada influência da subtração do valor de fluorescência zero no desempenho do DD, com diminuição da sensibilidade. Pode-se concluir que as mensurações com o DD podem ser realizadas sem a subtração do valor de fluorescência zero. No entanto, para o DDpen esse procedimento não pode ser eliminado. (2) Foram selecionados 119 dentes com o objetivo de comparar o desempenho na detecção de lesões de cárie oclusal de três aparelhos que induzem fluorescência, exame radiográfico convencional e sistema ICDAS II. Uma lesão

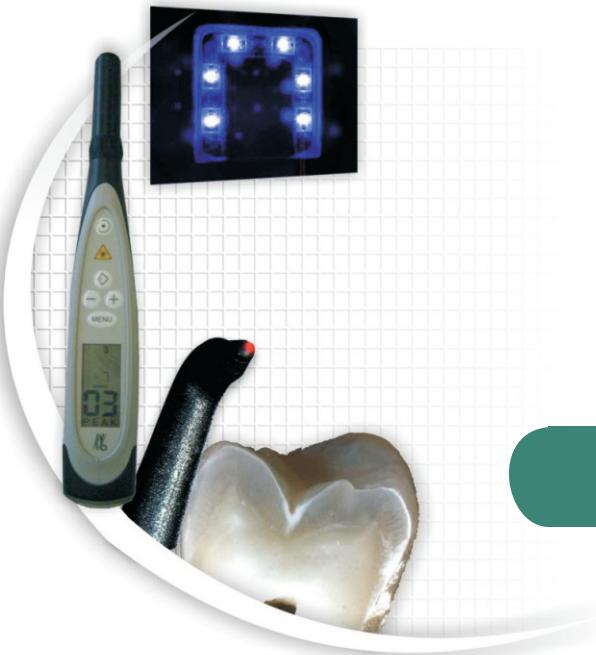
de cárie por dente foi escolhida como “sítio teste”. Dois examinadores realizaram duas medidas independentes utilizando o DD, DDpen, câmera VistaProof (VP, Dürr Dental, Alemanha), além de exame radiográfico convencional e exame visual através do sistema ICDAS II. Conclui-se que cada aparelho variou seu desempenho de acordo com os valores de sensibilidade e especificidade. O sistema ICDAS II combinado ao exame radiográfico convencional apresentou melhor desempenho na detecção de lesões de cárie na superfície oclusal. (3) Essa pesquisa objetivou avaliar a influência de selantes de fossas e fissuras sobre os valores de fluorescência obtidos por três aparelhos (DD, DDpen e VP). 160 molares permanentes foram selecionados e uma lesão de cárie por dente foi escolhida como “sítio teste” e mensurada utilizando os aparelhos descritos acima. Os dentes foram divididos aleatoriamente em quatro grupos e as superfícies oclusais foram seladas de acordo com os seguintes materiais: Delton Clear e Opaco (Dentsply, Alemanha), Helioseal Opaco (Ivoclar Vivadent, Liechtenstein) e um selante experimental Clear com nanopartículas (Voco, Alemanha). Depois de seladas, as lesões foram novamente mensuradas. Os dentes foram termociclados (1000 ciclos;  $5 \pm 2$  e  $55 \pm 2^\circ\text{C}$ ) e as mensurações repetidas. Foi observado que os selantes de fossas e fissuras influenciam os valores de fluorescência, podendo aumentá-los ou diminuí-los de acordo com material empregado. Pode-se concluir que o DD pode ser utilizado para monitorar superfícies oclusais sob selante “clear” e sem carga. Através dessa pesquisa, nota-se o importante papel dos métodos auxiliares de detecção das lesões de cárie. No entanto, a proposta do sistema ICDAS II, que detalha o exame visual convencional e a

*Resumo*

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sua associação ao exame radiográfico ainda deve ser priorizada antes de se optar por um terceiro método, salvo quando a visualização direta da lesão for impossibilitada. Estudos *in vivo* devem ser desenvolvidos com o intuito de avaliar o desempenho desse sistema e dos métodos baseados em fluorescência.

Palavras-chave: Fluorescência; diagnóstico; cárie dentária.



## ABSTRACT

*Abstract*

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Rodrigues JA. Light-induced fluorescence in detecting occlusal caries lesions [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2008.

The aim of this in vitro study was to asses the performance of light-induced fluorescence as auxiliary to conventional methods is detecting occlusal caries lesions in permanent teeth. For this reason, three studies were carried out: (1) The aim of this study was to evaluate the influence of zero value subtraction on the performance of two laser fluorescence devices for detecting occlusal caries. 119 permanent molars were selected. Three areas (cuspal, middle and cervical) of both mesial and distal portions and one occlusal site were assessed by two examiners using the DIAGNOdent 2095 (LF, KaVo, Germany) and DIAGNOdent 2190 (LFpen) devices. It was observed an influence of the zero value subtraction in the LF performance: the sensitivity decreased. However, because of the trend, it could be concluded that the LF readings could be performed without the zero value subtraction. Despite of that, it does not imply in wrong results clinically. For the LF pen, the zero value subtraction influences the performance though and should however not be eliminated. (2) This in vitro study compared the performance of fluorescence-based methods, radiographic examination, and ICDAS II system on occlusal surfaces. 119 molars were independently assessed twice by two experienced dentists using the laser fluorescence (LF and LFpen) and fluorescence camera (FC, Dürr Dental, Germany) devices, ICDAS II system and bitewing radiographs (BW). It can be concluded that the performance of each method changes according to the

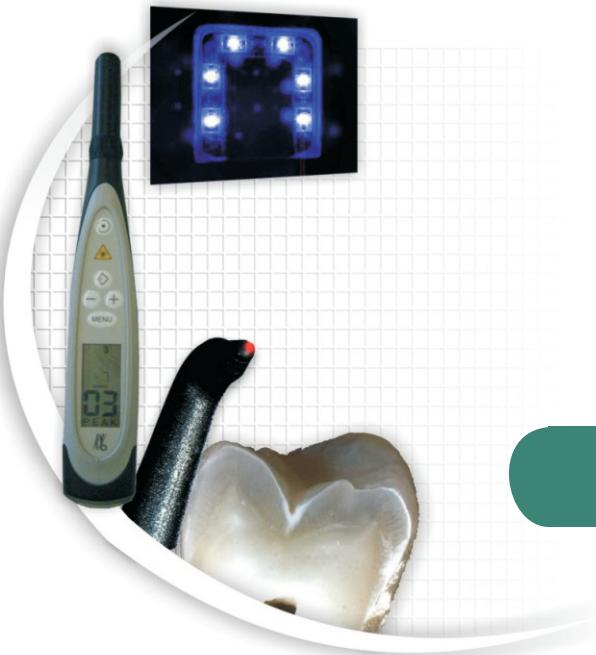
*Abstract*

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sensitivity and specificity. The ICDAS II combined to BW showed the best performance and it is the best combination to detect caries on occlusal surfaces.

(3) The aim of this *in vitro* study was to evaluate the influence of pit and fissure sealants on fluorescence readings. 166 permanent molars were selected and randomly divided into four groups for according to the following materials: Delton Clear and Opaque (Dentisply, Germany), Helioseal Opaque (Ivoclar Vivadent, Liechtenstein) and experimental nanofilled clear (Voco, Germany). The teeth were independently measured twice by two experienced dentists before and after sealing using the devices: LF, LFpen and FC. Subsequently, they were thermocycled (1000 cycles;  $5 \pm 2$  and  $55 \pm 2^\circ\text{C}$ ) and measured again. It was shown that pit and fissure sealants have influence on the fluorescence readings, with increase or decrease of the values according to the material used. In conclusion, the LF device could be useful as adjunct to monitor surfaces under clear unfilled sealants. From this study, the important role of the auxiliary methods in detecting occlusal caries lesions could be observed. Nevertheless, the purpose of the ICDAS II system, which is more detailed than the usual visual inspection, associated to the radiographic examination should have priority before using a third method, except when the lesion's visual examination was not possible. Further *in vivo* studies should be carried out in order to assess the performance of this system as well as the fluorescence-based methods.

**Keywords:** Fluorescence; diagnosis; dental caries.



## INTRODUÇÃO

Durante as últimas décadas, um significativo declínio na prevalência das lesões de cárie tem sido observado na população mundial. O desenvolvimento de novos materiais e tecnologias, assim como o avanço no conhecimento científico sobre a etiologia, progressão e prevenção da doença têm sido relatado como os principais responsáveis por esse declínio. Por esse motivo, a filosofia de imediata intervenção restauradora tem dado lugar à filosofia de detecção precoce e de controle da doença<sup>2,6</sup>.

A anatomia irregular muitas vezes profunda do sistema de fossas e fissuras favorece o acúmulo de placa bacteriana e dificultam a visibilidade e o acesso para uma higiene adequada, fatores determinantes para o início do processo de desmineralização. O primeiro sinal clínico indicativo da ocorrência deste processo é a mancha branca. Nas superfícies oclusais, a mancha branca pode também estar associada a outras alterações, como pigmentações e microcavidades<sup>3,8,28</sup>.

Atualmente, com o advento dos fluoretos e devido a sua capacidade de remineralização, as lesões de cárie em superfícies oclusais têm apresentado características peculiares. Essas lesões muitas vezes não apresentam uma cavidade evidente, mas sim uma descontinuidade da superfície de esmalte podendo se estender em dentina, e são dificilmente visíveis a olho nu. Tais são as chamadas lesões de cárie oculta<sup>22,34</sup>. No entanto, a ação preventiva dos fluoretos no sistema de fossas e fissuras parece ser limitada<sup>10,27</sup>. Por esse motivo, pode-se afirmar que a superfície oclusal apresenta uma susceptibilidade significativa ao desenvolvimento de lesões de cárie.

Como procedimento preventivo, pode-se optar pelo uso dos selantes de fossas e fissuras. O selamento dessa superfície é capaz de inibir a penetração dos nutrientes bacterianos e com isso reduzir significativamente a quantidade de microrganismos viáveis. Alguns estudos têm mostrado a eliminação desses microrganismos sob os selantes ou restaurações com margens seladas<sup>12,15,16,26</sup>. No entanto, o uso incorreto do material pode resultar em posterior infiltração, favorecendo novamente a penetração dos microrganismos por baixo do selante e a consequente recidiva de cárie. Por esse motivo, as superfícies seladas devem ser constantemente avaliadas e reparadas quando necessário<sup>9</sup>. Dessa forma, a detecção das lesões de cárie sob selantes é difícil quando se dispõe apenas dos exames visual e radiográfico<sup>32</sup>, já que a opacidade do material pode interferir na visualização da lesão. O uso de um selante transparente poderia facilitar a detecção dessas lesões.

Como descrito anteriormente, a perda mineral observada em decorrência do processo carioso provoca alterações visuais na superfície do dente, iniciando em estágio subclínico e progredindo para o desenvolvimento de mancha branca e subsequente cavitação. Por esse motivo, quanto mais precoce forem detectadas as lesões de cárie, maiores são as chances de essas lesões serem remineralizadas, paralisadas ou seladas.

Diante disso, pode-se assumir que a detecção das lesões de cárie e a avaliação da extensão dessas lesões desempenham um papel importante no processo de diagnóstico. Da mesma forma, a detecção das lesões sob selantes de fossas e fissuras também é de extrema importância. Por isso, a utilização dos métodos para detecção das lesões de cárie tem sido bastante estudada.

O desempenho dos métodos de detecção das lesões de cárie pode ser avaliado considerando dois parâmetros: a reproducibilidade e a validade. Um teste reproduzível é aquele cujas medidas repetidas mostram resultados semelhantes<sup>29</sup>, ou seja, mostra a concordância entre dois exames realizados em épocas diferentes ou por examinadores diferentes, utilizando a mesma amostra. A reproducibilidade pode ser avaliada pelo teste Kappa, pelo cálculo do coeficiente de correlação intraclass (ICC, do inglês *Intraclass Correlation Coefficient*) ou pelo método de Bland e Altman.

A validade reflete a capacidade do método em avaliar o que ele realmente se propõe a avaliar. É determinada pela proporção de resultados corretos levando em consideração o padrão-ouro<sup>29</sup>. Utilizando esses resultados, a validade de um método pode ser calculada em termos de sensibilidade e especificidade<sup>29,31</sup>. A porcentagem total de acertos considerando tanto a presença quanto a ausência da doença é representada pela acurácia.

Rotineiramente, para detecção das lesões de cárie oclusal e proximal, utilizam-se na clínica odontológica os exames visual e radiográfico convencional. No entanto, o exame radiográfico só é capazes de detectar as alterações nas superfícies dentárias em estágios avançados<sup>30</sup>. O exame visual deve ser realizado em ambiente bem iluminado e após a secagem da superfície do dente, para melhor visualização das lesões. Entretanto, por se tratar de um método subjetivo e depender da experiência clínica do cirurgião-dentista, esse método tem apresentado baixa reproducibilidade<sup>11</sup>. Por esse motivo, a dificuldade na detecção precoce e na determinação da profundidade

das lesões de cárie tem motivado o desenvolvimento de novos métodos que possam auxiliar nesse processo.

O sistema ICDAS (*International Caries Detection & Assessment System*) foi desenvolvido recentemente por pesquisadores e profissionais com o objetivo de estabelecer um critério internacional para detecção visual e determinação da atividade de lesões de cárie a ser utilizado em estudos laboratoriais, clínicos, levantamentos epidemiológicos e monitoramento de pacientes durante a prática clínica pública e privada. Em 2003, foi criado o sistema ICDAS I, cujo princípio fundamental era a realização de um exame visual da superfície dentária limpa, sem presença de placa bacteriana, além de secagem cuidadosa para que até as lesões iniciais pudessem ser identificadas. O uso da sonda periodontal de ponta romba poderia ser considerado como auxiliar em alguns casos. Mais recentemente, esse critério foi modificado e a ordem dos escores foi alterada para assegurar que o aumento do escore também refletisse a progressão da severidade das lesões, sendo então denominado ICDAS II. As poucas pesquisas publicadas utilizando esse sistema têm mostrado resultados de reprodutibilidade variando de bom a excelente e que o sistema é válido também para determinação da atividade das lesões de cárie<sup>7,14</sup>.

Com o intuito de auxiliar os métodos convencionais, alguns métodos têm sido recomendados para identificar e quantificar as lesões de cárie precocemente em superfícies oclusais e lisas<sup>25</sup>. Alguns desses métodos são baseados no fenômeno físico da fluorescência, já que os tecidos cariados

emitem fluorescência em intensidade diferente dos tecidos sadios quando estimulados pela luz com comprimentos de onda específicos<sup>1,5,13,33</sup>.

Um desses métodos foi proposto em 1998 e foi denominado DIAGNOdent 2095 (Kavo, Biberach, Alemanha). Este se baseia na captação da fluorescência emitida pelos componentes orgânicos dos tecidos cariados quando iluminados por um laser diodo (alumínio, gálio, índio e fósforo – AlGaInP) de comprimento de onda de 655nm, situado no âmbito vermelho do espectro visível. Os principais responsáveis pelo aumento da fluorescência após excitação com a luz nesse comprimento de onda parecem ser metabólitos bacterianos, provavelmente porfirinas<sup>13</sup>. O laser diodo chega à superfície do dente através de uma guia luminosa central contida em uma haste óptica flexível. A fluorescência emitida pelos componentes orgânicos dos tecidos cariados é captada por nove fibras arranjadas concentricamente à guia luminosa central e transformada em valores numéricos que variam de 0 a 99<sup>20</sup>. A luz refletida e a luz ambiente são eliminadas por um filtro que bloqueia comprimentos de onda abaixo de 680nm. Esse filtro absorve a excitação dispersada e outros comprimentos de onda mais curtos e transmite a fluorescência com comprimento de onda mais longa. O fabricante fornece dois tipos de pontas de fibra óptica, uma para superfície oclusal (ponta A) e outra para superfície lisa (ponta B). É um método quantitativo e não-destrutivo, além de portátil (600g), de fácil utilização e funcionar com cinco baterias (7,5V). Além disso, tem apresentado bons resultados na detecção de lesões oclusais de cárie<sup>2,13,20,21,24</sup>.

Recentemente, um modelo compacto denominado DIAGNOdent 2190 ou DIAGNOdent *pen* (Kavo, Biberach, Alemanha) foi introduzido no mercado com o objetivo de facilitar o manuseio pelo clínico e melhorar o desempenho na detecção de cárie. O novo modelo funciona utilizando o mesmo princípio do anterior. Para isso, o fabricante condensou os componentes do aparelho em uma única peça e modificou a estrutura das pontas utilizadas. As pontas utilizadas nesse novo modelo são feitas de fibra de safira e tanto o laser diodo emitido quanto a fluorescência captada pelo aparelho percorrem os mesmos feixes de fibras, em sentidos opostos, porém em comprimentos de onda diferentes<sup>20,23</sup>. O fabricante fornece duas pontas, uma para superfície oclusal e outra para superfície proximal. O aparelho pesa 140g e funciona com apenas uma bateria (1,5V).

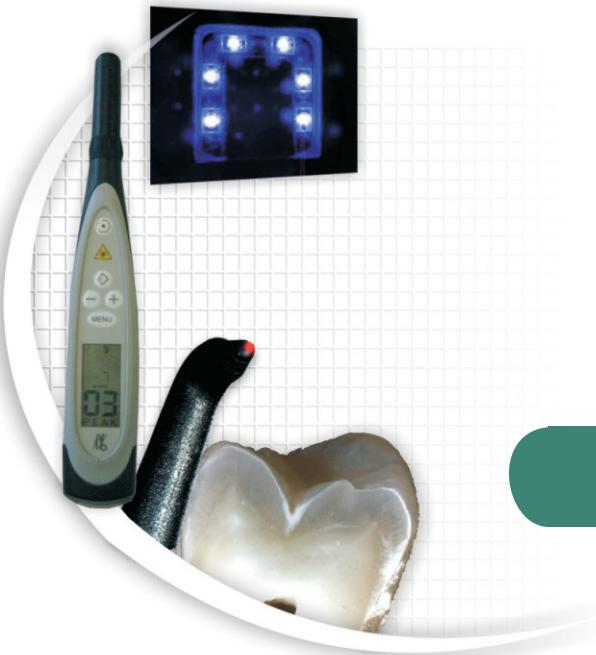
Ambos os aparelhos acima descritos devem ser calibrados antes do uso. Depois de selecionada a ponta a ser utilizada, realiza-se a calibração em um padrão cerâmico fornecido pelo fabricante cujo valor de fluorescência é conhecido e estável<sup>13</sup>. O fabricante recomenda que, em seguida, o aparelho seja calibrado em alguma superfície livre de cárie, pressionando o anel que circunda a haste do aparelho, com o objetivo de subtrair eletronicamente o valor de fluorescência zero (calibração individual). No entanto, alguns autores optaram por realizar a medida da superfície livre de cárie e subtrair esse valor manualmente após a mensuração da lesão<sup>17,18,19,20,23</sup>. Braun et al.<sup>5</sup>, medindo quatro diferentes regiões da superfície vestibular de molares permanentes, observaram valores 6 unidades menores quando realizada a calibração utilizando o DIAGNOdent 2095. Os autores sugerem que o procedimento deva

ser realizado, no entanto, não avaliaram a influência desse procedimento no desempenho do aparelho e nem mencionam qual a região da superfície vestibular deve ser utilizada. Já Braga et al.<sup>4</sup> utilizando o mesmo aparelho medindo a região central de molares decíduos não observaram influência da calibração no desempenho do aparelho em detectar lesões oclusais de cárie. Diante desses resultados contraditórios e devido a falta de pesquisas utilizando o DIAGNOdent *pen*, permanece a dúvida se esse procedimento é mesmo necessário quando ambos os aparelhos são utilizados para detecção de cárie oclusal.

Ainda considerando o fenômeno da fluorescência induzida pela luz para detecção de lesões de cárie, outro aparelho foi recentemente introduzido no mercado. Trata-se de uma câmera intra-oral (VistaProof, Dürr Dental, Bietigheim-Bissingen, Alemanha) com seis lâmpadas LEDs na sua extremidade capazes de emitir luz com comprimento de onda de 405nm, no âmbito azul do espectro visível. Essa câmera é capaz de digitalizar a imagem da superfície dentária no momento da emissão da fluorescência utilizando um sensor CCD (charge-coupled device). Nessas imagens podem ser observadas as diferentes regiões da superfície dentária que fluorescem em verde (aproximadamente com 510nm de comprimento de onda) até o vermelho (aproximadamente com 680nm de comprimento de onda). Para a digitalização e análise das imagens utiliza-se o software DBSWIN (Dürr Dental, Bietigheim-Bissingen, Alemanha) que traduz em números a relação entre a fluorescência verde e a vermelha, representada pela quantidade de pixels das imagens. Segundo o fabricante, os números mostrados pelo software estariam relacionados com a extensão da

lesão. No entanto, não existe evidência científica sobre os pontos de corte ideais que deva ser utilizado para determinação da extensão das lesões de cárie oclusal.

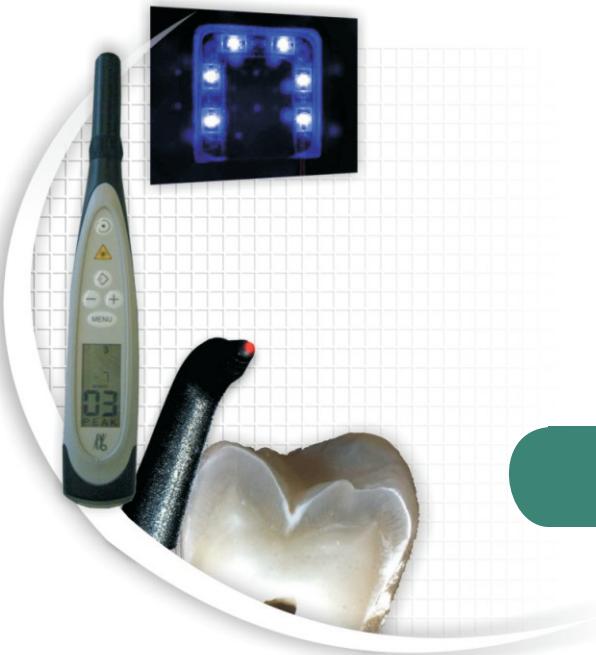
Os três métodos baseados em fluorescência descritos (DIAGNOdent 2095, DIAGNOdent *pen* e VistaProof) parecem contribuir de forma importante no processo de detecção de lesões de cárie, auxiliando os métodos convencionais. No entanto, ainda existem algumas dúvidas no que concerne ao desempenho desses métodos quando utilizados na superfície oclusal. O processo de calibração dos dois modelos do DIAGNOdent, bem como a validade e a reproduzibilidade desses novos métodos tem sido pouco estudada. Além disso, esses métodos baseados em fluorescência podem auxiliar também no processo de detecção e monitoramento de lesões de cárie sob selantes. Por esse motivo, através da presente pesquisa, procurou-se responder algumas dessas dúvidas com o intuito de auxiliar o clínico no processo de diagnóstico e tratamento das lesões de cárie oclusal.



## PROPOSIÇÃO

Avaliar in vitro o desempenho dos métodos baseados na medição da fluorescência induzida pela luz para detecção de lesões de cárie oclusal em dentes permanentes. Para isso, foram realizadas três pesquisas científicas intituladas:

1. “The influence of zero value subtraction on the performance of two laser fluorescence devices for detecting occlusal caries in vitro”
2. “Performance of fluorescence methods, radiographic examination and ICDAS II on occlusal surfaces in vitro”
3. “The influence of pit and fissure sealants on infrared fluorescence measurements”



**ARTIGO I**

**The influence of zero value subtraction on the performance of two laser  
fluorescence devices for detecting occlusal caries in vitro**

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## Abstract

**Background.** The aim of this study was to evaluate the influence of zero value subtraction on the performance of two laser fluorescence devices for detecting occlusal caries. **Methods.** One hundred and nineteen permanent molars were selected. Three areas (cuspal, middle and cervical) of both mesial and distal portions and one occlusal site were assessed using the LF and LFpen devices. For each tooth the value from cuspal, middle and cervical areas in the buccal surface were subtracted from the value measured in the respective occlusal site.

**Results.** Difference among the readings could be observed for both devices in the areas in the buccal surface as well as for with and without the zero value subtraction. When the zero value subtraction was not performed, statistically significant difference was found for sensitivity and accuracy for the LF. For the LFpen, specificity increased, and sensitivity decreased significantly.

**Conclusions.** For the LF device, the zero value subtraction decreased the sensitivity. For this reason, it could be concluded that the LF readings could be performed without the zero value subtraction. For the LF pen, the absence of the zero value subtraction changed both the sensitivity and specificity and should however not be eliminated.

**Clinical Implications:** For the LF the zero value subtraction might not be performed. However, for the LF pen the zero value subtraction should not be eliminated.

**Keywords:** Occlusal caries, DIAGNOdent, DIAGNOdent pen, Laser fluorescence, Zero value.

## Introduction

Occlusal caries detection is a challenging task for clinicians, since conventional methods are more specific than sensitive.<sup>1,2</sup> In recent years, new detection methods have been developed to help the dentists deciding for the best treatment and preventive management of dental caries.

It has been reported that caries lesions emit stronger fluorescence than sound tissues when stimulated by a red laser visible light with wavelength of 655nm.<sup>3</sup> Several studies have examined the performance of a laser device (LF; DIAGNOdent 2095, KaVo, Biberach, Germany) based on the principle of fluorescence, it has shown good to excellent results for occlusal caries detection.<sup>1,2,3,4,5</sup>

Recently, a new laser fluorescence device (LFpen; DIAGNOdent 2190, KaVo, Biberach, Germany) has been introduced on the market and supposed be used for approximal and occlusal caries detection. Because of the difference between the LFpen tips and its new design, approximal surfaces can also be assessed. The fluorescence runs the opposite direction from excitation in the same optical path of propagation.<sup>5,6,7,8</sup>

For both devices, in accordance with the manufacturer's instructions, it is necessary to perform a standard calibration on a porcelain object with known fluorescence (standard calibration). An additional calibration against a sound spot on the buccal surface should also be performed, allowing the automatic subtraction of the zero value from the site of interest (individual calibration)<sup>3,9,10</sup>. This step has been reported as a time-consuming procedure in the clinical practice<sup>9,11</sup> and has not been followed by practicing dentists. However, some

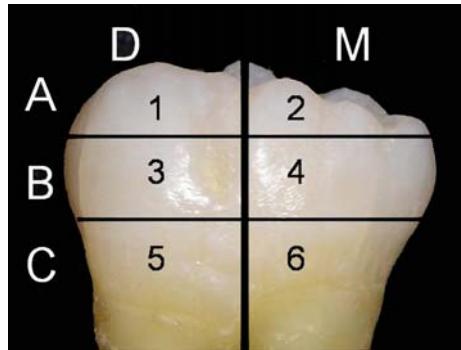
authors<sup>5,6,7,8</sup> decided to measure a sound spot on buccal surface to obtain the zero value of fluorescence and perform, after the lesion assessment, the subtraction manually (zero value subtraction). Preliminary studies showed higher values when occlusal sites were assessed after individual calibration, comparing to the results obtained after the subtraction of the zero value. This means that the values obtained from the buccal surface and automatically subtracted by the device are even lower than the values manually subtracted.

Furthermore, there is no mention of it in the literature about the correct area in which the zero value should be recorded when using the LF devices in occlusal surfaces. It can be assumed that the incorrect measurement or the lack of it might influence the assessment of the caries state and hence the treatment decision. The studies evaluating the individual calibration are controversial. While Braun et al.<sup>9</sup> suggested that the individual calibration should be performed because of a difference of 6 units in the LF assessments was found, Braga et al.<sup>10</sup> did not find influence of the calibration on the performance using primary teeth. Due to these diverging results, it was decided to carry out this *in vitro* investigation, before any *in vivo* use of the referred devices could be indicated, concerning the calibration process. Furthermore, the influence of the zero value subtraction on laser fluorescence performance has not yet been evaluated using the LFpen device for occlusal caries detection.

The aim of this study was to evaluate the influence of zero value subtraction on the performance of two laser fluorescence devices (LF and LFpen) for detecting occlusal caries *in vitro*.

## Materials and Methods

One hundred and nineteen extracted upper and lower third human molars suspected to have initial caries lesions on occlusal surface, frozen at -20°C until use were used. Preliminary studies in our laboratory showed that this method of storage does not significantly change the red fluorescence signal.<sup>12</sup> All teeth had been extracted by dental practitioners in Switzerland (no water fluoridation, 250 ppm F<sup>-</sup> in table salt). Before the extraction, the patients were informed about the use of the teeth for research purposes and their consent was obtained. Calculus and debris were removed using a scaler (Cavitron). The teeth were cleaned for 15s with toothbrush and water and then for 10s with Prophyflex with sodium bicarbonate (KaVo, Biberach, Germany). In order not to leave powder remnants in the fissure, the teeth were rinsed off with the 3-in-1 syringe for 10s.<sup>13</sup> Photographs of the occlusal and buccal surfaces were taken (Leica DC300, Leica, Heerbrugg, Switzerland; magnification of 6.25x), and the site giving the highest laser fluorescence value (DIAGNOdent 2095) on the occlusal surface from each tooth was chosen<sup>9</sup> (test site). Additionally, the buccal surfaces of the selected teeth were divided into six different non-caries areas (sites 1 to 6; figure 1) for the zero value measurements: three sites on mesial portion (M) and three on distal portion (D), which were called cuspal (A), middle (B) and cervical (C) areas.



**Figure 1:** Six sites on the buccal surface measured with the LF and LFpen.

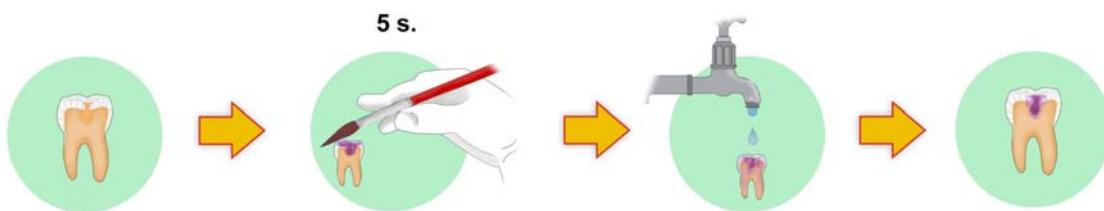
Before the LF measurements, the teeth were defrosted for 3 hours at room temperature. During the assessments they were stored in 100% humidity to prevent dehydration.

The mode of function of both laser fluorescence devices (LF and LFpen) has been described in detail previously.<sup>1,5,6</sup> The first LF system and the new one function on the same principle. They are based on the phenomenon of fluorescence, where absorption of light of a given wavelength by a fluorescent molecule is followed by the emission of light at longer wavelengths. These LF systems emit red light at wavelength of 655nm ( $\approx 1\text{mW}$  power). A photodetector quantifies the fluorescence light passing through the filter and displays digitally a real time (moment) and a maximum (peak) value. In the LFpen device the excitation and the emission of fluorescence follow the same solid fibre tip, but in opposite directions. This is the main difference to the first one, which has different fibres for light excitation and emission.

After standard calibration each test site in the occlusal and each delimited area in the buccal surface was independently assessed twice by two experienced dentists using the same tips for both surfaces according to their

respective device and the values were recorded. For the measurements, specific tips for occlusal caries detection were used: the tip A for LF and the cylindrical sapphire fibre tip for LFpen (with a diameter of 1.1 mm).

After the assessments the teeth were ground longitudinally on a Knuth-Rotor polishing machine using silicon carbide paper (60 $\mu\text{m}$  of grain size) while being cooled in running tap water. Progression of the grinding process was constantly checked under the microscope (magnification 6.25x). When the periphery of the site was reached, silicon carbide papers of grain size 30, 18, 8 and 5 $\mu\text{m}$  were used for further polishing. The cut surfaces were then colored with saturated rhodamine B (Fluka, Buch, Switzerland) and the examination was performed according to its penetration either into the enamel or both enamel and dentin tissues (Figure 2).



**Figure 2:** Drawing describing the Rhodamine B application.

The sites were assessed for caries extension (Leica DC300, Leica, Heerbrugg, Switzerland; magnification 10x) as caries free ( $D_0$ ), caries extending up to halfway through the enamel ( $D_1$ ), caries extending in the inner half of enamel ( $D_2$ ), caries in dentine ( $D_3$ ) and deep dentinal caries ( $D_4$ ). Subsequently, photographs were taken.

### *Statistical analyses*

The normal distribution of the values measured in the buccal as well as in the occlusal sites was rejected ( $p<0.0001$ ; MedCalc for Windows 9.3.0.0, Belgium). As the Wilcoxon test did not show any statistically significant difference between the mesial and distal areas of the buccal surface, the values from cuspal, middle and cervical of these two areas were combined. For each tooth the average between mesial and distal surfaces from cuspal, middle and cervical areas in the buccal surface were calculated and subtracted from the value measured in the respective occlusal site. The Wilcoxon test was performed to compare the values of fluorescence obtained without and with zero value subtraction. The areas ( $A_z$ ) under the Receiver Operating Characteristic (ROC) curve were calculated and compared for each threshold.<sup>14</sup> The performance were assessed according to the cut-off limits suggested by Lussi and Hellwig<sup>5</sup> (both with and without zero value subtraction), as follow: for the LF device: 0-7: sound; 7.1-14: caries in enamel; 14.1-24: caries in dentin-enamel junction; >24: caries in dentine. For the LFpen device: 0-6: sound; 6.1-13: caries in enamel; 13.1-17: caries in dentin-enamel junction; >17: caries in dentine. The values of sensitivity, specificity and accuracy were compared by performing the McNemar test. The significance level was set at  $p<0.05$ .

## **Results**

The average, standard deviation (SD) and the 95% confidence interval (CI) of the readings from each area using the LF and LFpen devices in the

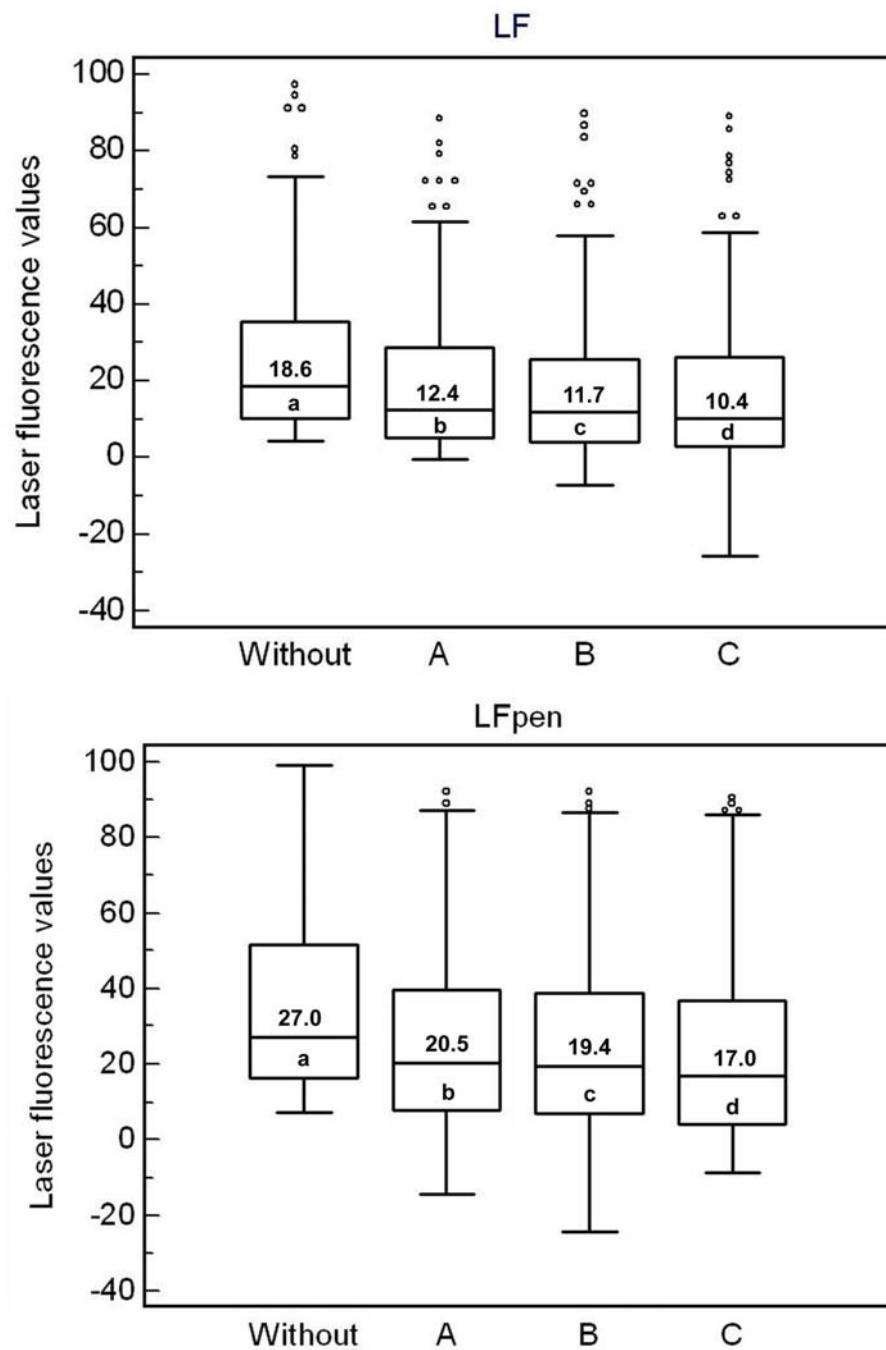
buccal surface are shown in table 1, where the increase of values can be observed from the area A (6.5 and 8.9) toward area C (9.4 and 13.0, respectively for LF and LFpen).

**Table 1:** Average, standard deviations (SD) and the 95% confidence interval (CI) of LF and LFpen readings for both devices in cuspal (A), middle (B), cervical (C), mesial (M) and distal (D) areas.

	Area	Average	SD	95% CI
<b>LF</b>	A	6.5 <sup>a</sup>	4.2	5.8 – 7.3
	B	7.8 <sup>b</sup>	5.1	6.9 – 8.8
	C	9.4 <sup>c</sup>	5.6	8.3 – 10.4
	M	8.0 <sup>b</sup>	5.3	7.1 – 9.0
	D	7.8 <sup>b</sup>	4.3	6.9 – 8.5
<b>LFpen</b>	A	8.9 <sup>a</sup>	7.2	7.7 – 10.2
	B	11.5 <sup>b</sup>	9.9	9.7 – 13.3
	C	13.0 <sup>c</sup>	9.0	11.3 – 14.6
	M	11.3 <sup>b</sup>	8.9	9.7 – 12.9
	D	10.8 <sup>b</sup>	8.1	9.3 – 12.2

Significant differences are represented by different lower case letters, within the column and considering each device separately (Wilcoxon, p<0.05).

For both devices, the Wilcoxon test showed a statistically significant difference (p<0.05) between the areas A, B and C but no difference between M and D. Furthermore, statistical difference was found while comparing the values obtained by the two devices. A statistically significant difference was observed when the procedures without and with zero value subtraction (subtracting the values from the areas A, B and C) were compared (figure 3).



**Figure 3:** Box plots for laser fluorescence values of the LF and LFpen devices measured in the occlusal surface without and with zero value subtraction (taking the areas A, B and C). Statistically significant difference were observed between the procedures (different lower case letters,  $p<0.05$ , Wilcoxon test). The median, first and third quartiles, minimum and maximum values (whiskers) and outliers (marked as dots) are shown.

Figure 4 shows three examples of occlusal sites and the values obtained in the different areas in the buccal surface and the values after zero value subtraction, as well as pictures taken initially and after histological assessments.

LF							
Teeth	Occlusal	A	Subtr.	B	Subtr.	C	Subtr.
1	8.3	3.5	4.8	4.0	4.3	6.5	1.8
2	38.8	5.5	33.3	7.5	31.3	7.5	31.3
3	50.5	6.0	44.5	6.0	44.5	7.0	43.5
LFpen							
Teeth	Occlusal	A	Subtr.	B	Subtr.	C	Subtr.
1	11.8	5.0	6.8	5.5	6.3	6.5	5.3
2	71.0	7.0	64.0	7.0	64.0	10.0	61.0
3	72.0	6.5	65.5	6.5	65.5	10.0	62.0

The figure consists of two rows of images. The top row shows three dental occlusal surfaces (Teeth 1, 2, and 3) with white circles indicating specific measurement points. The bottom row shows the corresponding histological sections of these teeth, with arrows pointing to the same areas as the circles above them.

**Figure 4:** Occlusal pictures and averaged laser fluorescence values (LF and LFpen) obtained in the different areas of the buccal and occlusal surface as well as the values after zero value subtraction. Histological assessments: teeth 1: score 2; teeth 2: score 3 and teeth 3: score 4.

The values of sensitivity, specificity, accuracy and area under the ROC curve ( $A_Z$ ) are shown in table 2.

**Table 2:** Performance of the LF and LFpen devices for occlusal caries detection without and with zero value subtraction (taking the areas A, B and C).

		Specificity		Sensitivity		Accuracy		ROC ( $A_Z$ )			
		Area	D <sub>2</sub>	D <sub>3</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>2</sub>	D <sub>3</sub>	
<b>Without</b>											
LF	zero value subtraction	-	0.63 <sup>a</sup>	0.84 <sup>a</sup>	0.71 <sup>a</sup>	0.65 <sup>a</sup>	0.69 <sup>a</sup>	0.75 <sup>a</sup>	0.74 <sup>a</sup>	0.81 <sup>a</sup>	
	With zero value subtraction	A	0.74 <sup>a</sup>	0.89 <sup>a</sup>	0.52 <sup>b</sup>	0.45 <sup>b</sup>	0.57 <sup>b</sup>	0.68 <sup>a,b</sup>	0.73 <sup>a</sup>	0.81 <sup>a</sup>	
	With zero value subtraction	B	0.78 <sup>a</sup>	0.89 <sup>a</sup>	0.51 <sup>b</sup>	0.41 <sup>b</sup>	0.57 <sup>b</sup>	0.66 <sup>b</sup>	0.74 <sup>a</sup>	0.81 <sup>a</sup>	
LFpen	With zero value subtraction	C	0.81 <sup>a</sup>	0.90 <sup>a</sup>	0.49 <sup>b</sup>	0.44 <sup>b</sup>	0.56 <sup>b</sup>	0.68 <sup>a</sup>	0.74 <sup>a</sup>	0.80 <sup>a</sup>	
	<b>Without</b>										
	zero value subtraction	-	0.48 <sup>a</sup>	0.47 <sup>a</sup>	0.89 <sup>a</sup>	0.93 <sup>a</sup>	0.79 <sup>a</sup>	0.67 <sup>a</sup>	0.75 <sup>a</sup>	0.80 <sup>a</sup>	
LFpen	With zero value subtraction	A	0.67 <sup>a,b</sup>	0.59 <sup>b</sup>	0.71 <sup>b</sup>	0.78 <sup>b</sup>	0.69 <sup>b</sup>	0.67 <sup>a</sup>	0.73 <sup>a</sup>	0.77 <sup>a</sup>	
	With zero value subtraction	B	0.70 <sup>b</sup>	0.67 <sup>b,c</sup>	0.68 <sup>b</sup>	0.76 <sup>b</sup>	0.68 <sup>b</sup>	0.71 <sup>a</sup>	0.73 <sup>a</sup>	0.80 <sup>a</sup>	
	With zero value subtraction	C	0.78 <sup>b</sup>	0.73 <sup>c</sup>	0.67 <sup>b</sup>	0.74 <sup>b</sup>	0.69 <sup>b</sup>	0.73 <sup>a</sup>	0.72 <sup>a</sup>	0.77 <sup>a</sup>	

D<sub>2</sub>: D<sub>0</sub>D<sub>1</sub>=sound, D<sub>2</sub>D<sub>3,4</sub>=decayed;

D<sub>3</sub>:D<sub>0</sub>-D<sub>2</sub>=sound, D<sub>3,4</sub>=decayed

Significant differences are represented by different lower case letters, within the same column and considering each device separately (McNemar test, p<0.05).

For the LF device, the values of specificity did not show statistically significant difference but sensitivity and accuracy were higher when the zero value subtraction was not performed. For the LFpen device, the specificity increased and the sensitivity decreased significantly when the zero value subtraction was performed. However, only the accuracy at D<sub>2</sub> threshold

changed significantly. The area of the ROC curve varied from 0.73 to 0.81 for the LF device and from 0.72 to 0.80 for the LFpen. For both devices, no statistically significant difference was found comparing the areas under the ROC curves with and without the zero value subtraction.

## Discussion

As suggested by the manufacturer, a sound spot on the buccal surface should be used in order to obtain the value of fluorescence zero, which could be either automatically subtracted from the site of interest (individual calibration)<sup>9,10</sup> or manually after the lesion assessment (zero value subtraction).<sup>5,6,7,8</sup> This step of calibration is a somewhat time consuming procedure, which is not well followed in practice. Some variation in these measurements may change the LF values and consequently, the treatment decision. Some authors studying the LF device and assessing the buccal surface found values varying by up to 6 units over the four quadrants<sup>9</sup> while others did not find differences in LF readings without the zero value subtraction.<sup>10</sup>

The highest values of fluorescence in cervical areas can be explained by the mineralization levels observed in the different areas of the teeth<sup>15</sup> and by the reducing in thickness of enamel toward the cement-enamel junction, which allows high fluorescence measurements of the underlying dentine<sup>16</sup>. The high values of fluorescence found in the cervical area (C) for both devices could respond very similarly to dental caries on occlusal surface. However, when mesial (M) and distal (D) areas were evaluated, the values showed no statistically significant difference. This was most probably due to the fact that

the average among the areas A, B and C of both mesial and distal portion represents similar levels of mineralization.

Highest values for the LFpen were found when the same areas assessed by both devices were compared, with a statically significant difference (Wilcoxon,  $p<0.05$ ), as also found by Kühnisch and colleagues<sup>7</sup> assessing occlusal sites in vitro. By contrast, Krause and colleagues<sup>17</sup> has found lower fluorescence values for the LFpen in an in vivo study assessing occlusal surfaces. The authors<sup>17</sup> did however not mention the area used for the assessment of the buccal surface, and a median value of 5 units lower was obtained after zero value calibration. In the present study the values measured in the buccal surface ranged from 6.5 to 9.4 (LF) and 8.9 to 11.0 (LFpen), varying in the cuspal to the middle and cervical areas. In face of that, the influence of the area for the zero value subtraction on the LF and LFpen performance could be tested and confirmed. The smaller diameter of the cylindrical tip (1.1mm) and the different composition could be the responsible for the difference between the values found by LF and LFpen. The cylindrical tip used for the LFpen assessments in occlusal surfaces is made of a solid single sapphire fibre and the end of the tip is formed with a prismatic shape that deflects the beam of excitation and collects it laterally along its longitudinal axis. For the LF device, the whole probe is made of a fibre rod consisting of a bundle of singles fibres, each of them with a diameter of 40 $\mu\text{m}$ .<sup>5</sup> Furthermore, the high number of outliers observed in the figure 3 could be probably due to the non-normal data distribution.

In the present study, different areas for the zero value subtraction were tested to verify its influence on the performance of both devices. For the LF device, no influence was found when the values of specificity were compared with and without zero value subtraction. The zero value subtraction showed an influence on the performance, using the cut-off limits proposed by Lussi and Hellwig<sup>5</sup>, decreasing significantly the sensitivity and accuracy from cuspal toward to the cervical. Considering other parameters were maintained and the observed difference was not positive, this step could be suppressed for LF device. This study agrees with Braga and colleagues<sup>10</sup>, as they found higher LF readings from assessments performed without the individual calibration using the center of each tooth's buccal surface, but no influence on the LF performance assessing primary teeth. Some authors studying the same subject in permanent teeth obtained different results. They observed that the individual calibration influences the LF readings and indicate that this procedure should be performed before the diagnostic measurement.<sup>9</sup> However, it must be considered that in both studies<sup>9,10</sup> the individual calibration was performed and not the zero value subtraction and a comparison between both kinds of procedures could be suggested for further studies. The histological validation and the cut-off limits used in the previous studies were different from the present investigation, which could also explain the different results observed. In unpublished studies from our laboratory, it was observed good correlation between the microhardness and dye enhanced Rhodamine B.

For the LFpen device, influence of the zero value subtraction was observed when the specificity was analyzed, and difference was found by comparing these values to the different areas. However, higher values of sensitivity at D<sub>2</sub> and D<sub>3</sub> were observed when the zero value subtraction was not performed. The difference between the high sensitivity and low specificity would suggest that the zero value subtraction should not be eliminated and any area could be suggested for the zero value assessment. More research is needed, associating cut-off limits and zero value subtraction using the LFpen for occlusal caries detection.

The area under de ROC curve ( $A_z$ ) displays the good ability of both devices to detect occlusal caries lesions (sensitivity) as well as the absence of it (specificity) with and without the zero value subtraction. However, it must be taken into account that the present analysis considers several cut-off limits. It is not accounting for a stipulated value for the calculation<sup>19</sup>. The advantage of ROC analysis is that positive and negative predictive values are independent of the prevalence of the problem<sup>20</sup>.

It is important to point out that these results represent an in vitro situation and caution must be taken when these devices are used in clinical practice. Even though the setup of study was such that storage should not influence the LF values<sup>9</sup>, the use of LF devices should be considered a second opinion only<sup>6</sup>. Furthermore, the treatment decision also depends on other patients' variables, such as dietary and toothbrushing habits, caries activity and use of fluoride. Further in vivo studies using deciduous and permanent teeth should be carried out. The use of the LFpen device also needs to be analyzed further.

## Conclusion

It was observed an influence of the zero value subtraction in the LF performance: the sensitivity decreased. However, it could be concluded that the LF readings could be performed without the zero value subtraction. For the LF pen, the absence of the zero value subtraction changed both the sensitivity and specificity and should however not be eliminated.

## Acknowledgments

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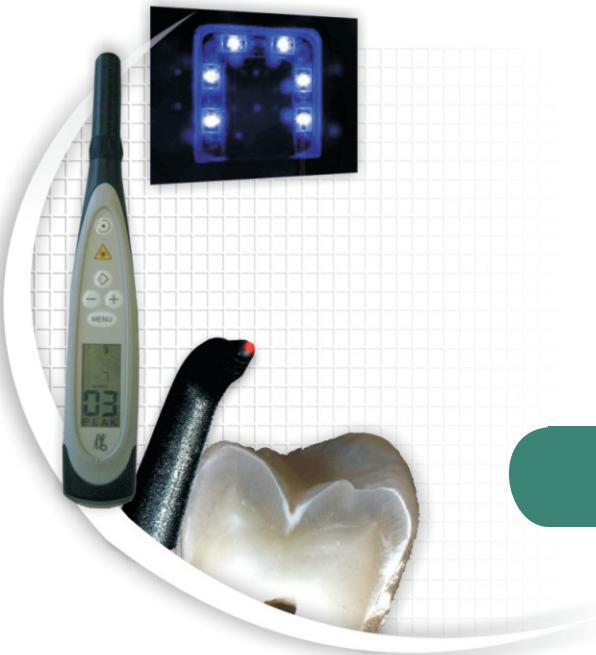
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## ARTIGO II

**Performance of fluorescence methods, radiographic examination and  
ICDAS II on occlusal surfaces in vitro**

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**Short title: Performance of methods on occlusal surfaces**

**Key Words:** Caries detection, Occlusal caries, Laser fluorescence, DIAGNOdent, DIAGNOdent pen, Fluorescence camera, ICDAS, Radiography.

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*Caries Research*

**Abstract**

This study compared the performance of fluorescence-based methods, radiographic examination, and ICDAS II on occlusal surfaces. One hundred and nineteen unstained permanent human molars were assessed twice by two experienced dentists using the laser fluorescence (LF and LFpen) and fluorescence camera (FC) devices, ICDAS II and bitewing radiographs (BW). After measuring, the teeth were histologically prepared and assessed for caries extension. The values of sensitivity were 0.86 (FC), 0.78 (LFpen), 0.73 (ICDAS II), 0.51 (LF) and 0.34 (BW). The values of specificity were 0.97 (BW), 0.89 (LF), 0.65 (ICDAS II), 0.63 (FC) and 0.56 (LFpen). BW presented the highest values of LR<sup>+</sup> (12.47) and LR<sup>-</sup> (0.68). Rank correlations with histology were 0.53 (LF), 0.52 (LFpen), 0.41 (FC), 0.59 (ICDAS II) and 0.57 (BW). The area under the ROC curve varied from 0.72 to 0.83. Inter- and intraexaminer ICC values were respectively 0.90 and 0.85 (LF), 0.93 and 0.87 (LFpen) and 0.85 and 0.76 (FC). The ICDAS II kappa values were 0.51 (inter-examiner) and 0.61 (intra-examiner). The BW kappa values were 0.50 (inter-examiner) and 0.62 (intra-examiner). The Bland and Altman limits of agreement were 46.0 and 38.2 (LF), 55.6, and 40.0 (LFpen) and 1.12 and 0.80 (FC), for intra- and inter-examiner reproducibilities. The post-test probability for dentine caries detection was high for BW and LF. In conclusion, LFpen, FC and ICDAS II presented better sensitivity and LF and BW better specificity. ICDAS II combined with BW showed the best performance and is the best combination for detecting caries on occlusal surfaces.

## Introduction

The detection of caries is a key element in the prevention and treatment of lesions, and a difficult task in dentistry [Bader and Shugars, 2004]. Occlusal surfaces are the most caries-affected sites in children and adults because of the special morphology of the pits and fissures and the difficulty of plaque removal. For this reason, the importance of early occlusal caries detection has grown in the last years [Sheehy et al., 2001; Rodrigues et al., in press]. Incipient occlusal lesions have become difficult to detect because of the wide-spread use of fluorides and their superficial remineralization potential that seems to delay cavitation [Rodrigues et al., in press]. Additionally, the changes in lesion morphology could lead to the presence of occlusal dentine caries under a fissure which seems intact to the naked eye [Lussi et al., 1999]. Visual inspection and radiographic examination have been commonly used in the clinical practice but they can detect caries lesions only at an advanced stage [Ricketts et al., 2002].

A new visual method, the International Caries Detection and Assessment System (ICDAS), was devised by an international group of researchers with the goal of designing an internationally accepted caries detection system that would also allow assessment of caries activity [Ekstrand et al., 2007]. In the ICDAS I, devised in 2003, the visual examination was carried out on clean, plaque-free teeth, after careful drying. Later, the criteria were modified and the ICDAS II created. The improvement consisted in an exchange of codes to ensure that the system would reflect increased severity [Ismail et al., 2007; Ekstrand et al., 2007].

Other new methods have been developed and recommended as diagnostic aids to identify and quantify early caries lesions on smooth and occlusal surfaces [Mendes et al., 2006]. Some of these methods are based on the phenomenon that caries lesions fluoresce more strongly than sound tissues when excited by lights at specific wavelengths [Hibst et al., 2001; Bader and Sugars, 2004; Braun et al., 2005, Thoms, 2006]. Both the first laser fluorescence device (LF) developed and a more recent pen-type LF device (LFpen) function on the same principle: they emit red light at 655 nm and measure fluorescence of bacterial metabolites in infected dentine [Hibst et al., 2001; Lussi and Hellwig et al, 2006; Lussi et al., 2006]. A recently devised fluorescence camera device (FC) emits blue light at 405 nm, and records fluorescence from the teeth as digital images. However, only limited data are available in the literature and the performance of this device, which is already on the market, has been not evaluated.

The aim of this in vitro study was to compare the performance of different fluorescence-based methods, radiographic examination and ICDAS II on occlusal surfaces

## **Materials and Methods**

### *Sample Selection*

One hundred and nineteen unstained permanent human molars (35 with microcavities and 84 non-cavitated, of which 18 were apparently sound) were selected from a pool of extracted teeth, which were stored frozen at -20°C until use. This storage method does not change the red fluorescence significantly

[Francescut et al., 2006]. All teeth had been extracted by dental practitioners in Switzerland (no water fluoridation; 250 ppm F<sup>-</sup> in table salt). Prior to extraction, the patients were informed about the use of their teeth for research purposes and their consent was obtained. The teeth were defrosted for 3 h and calculus and debris were removed using a scaler (Cavitron). They were cleaned for 15 s with water and toothbrush (Trisa ultra super-sensitive, BrushAbo, Switzerland) and for 10 s with a water-powder jet cleaner (PROPHYflex II, KaVo, Biberach, Germany) and sodium hydrogen carbonate powder. To remove powder remnants from the fissures, the teeth were rinsed with the 3-in-1 syringe for 10 s [Lussi and Reich, 2005]. During measurements, teeth were stored in 100% humidity. The occlusal surfaces were photographed at 6.25x magnification and one spot from each tooth was selected in the fissure surface (test site). All assessments were carried out twice by two experienced dentists, with a one-week interval between measurements.

#### *Assessments with the Laser Fluorescence devices (LF and LFpen)*

Both the LF system (DIAGNOdent 2095) and the new LFpen (DIAGNOdent 2190) were supplied by KaVo, Biberach, Germany.

The test sites were measured using both LF devices. No calibration training was performed and the examiners were informed about the device functioning. Both devices were first calibrated for every tooth using a ceramic standard, in accordance with the manufacturer's instructions. The fluorescence value of a sound part of the cuspal area on the buccal surface (zero value) was then recorded, to be later subtracted from the peak value. For measurements,

tip A (for the LF device) and a cylindrical sapphire fibre tip for occlusal surfaces (for the LFpen device) were used. The device was moved around the test site until the highest value was obtained. The peak values were recorded and the zero value of fluorescence was subtracted. For dentine caries level the concrete cut-off values were 24 for LF and 17 for LFpen [Lussi and Hellwig, 2006].

#### *Assessments with the Fluorescence Camera device (FC)*

The FC (VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) is a system that has been modified by exchanging the white LEDs of the camera with six blue GaN-LEDs emitting at 405 nm (optical power 60 mW). An optical long pass filter has been introduced into the beam path in front of the CCD-sensor to cut down the excitation light below 495 nm. DDview software (Dürr Dental, Bietigheim-Bissingen, Germany) was used to digitize the video signal to create the images of 720 x 576 pixels with 3 x 8 bit intensities of RGB-channels and resolution of 72 pixels/inch [Thoms, 2006]. These images were analyzed with the software, which quantified the red and green components of fluorescence. This software shows the region of the teeth that emits fluorescence varying from green (approximately 510 nm wavelength) to red (approximately 680 nm wavelength) and an outcome value, ranging from 0 to 3 corresponding to the lesion severity and calculated as the intensity ratio of the red and green fluorescence. Caries lesions were identified when the red/green-ratio was higher than that of sound tissue. The fluorescence ratio of caries lesions was taken as the maximum red/green-ratio recorded. Images of the teeth were taken using a prototype of the FC system, analyzed by the software,

stored in the computer and the values recorded for further analysis. However, no scale for interpretation of these numbers is available in the literature since this method was only recently developed and introduced into the market. For dentine caries level the cut-off value were determined by the highest sum of sensitivity and specificity.

#### *Visual Examination – ICDAS II System*

Visual examination was performed following the ICDAS II [Ismail et al., 2007; Ekstrand et al., 2007], with direct visualization of the teeth under illumination and coded as: (0) sound tooth surface, (1) first visual change in dry enamel, (2) distinct visual change in moist enamel, (3) localized enamel breakdown due to caries with no visible dentine or underlying shadow, (4) underlying dark shadow in dentine with or without localized enamel breakdown, (5) distinct cavity with visible dentine and (6) extensive distinct cavity with visible dentine. Score 3 represented the cut-off for dentine lesions. The teeth were examined in the same room with the aid of a light reflector and a 3-in-1 air syringe.

#### *Bitewing Radiographs (BW)*

Standardized bitewing radiographs were taken of all the teeth using an X-ray machine (HDX Dental EZ, USA) and double Kodak Insight films (22 x 35mm, Kodak, Rochester, Minn., USA) at 65kV, 7 mA and exposure time of 0.09 seconds. An automatic X-ray film developer XR 24 Pro (Dürr Dental, Bietigheim-Bissingen, Germany) was used to process the films. The

radiographs were then examined independently using an X-ray viewer (Imatec Röntgentechnik, Switzerland) and an X-ray film magnifier (magnification 2x) (Svenska Dental Instrument, Sweden) in a dark room to determine whether the occlusal surfaces under study showed: no radiolucency (0), radiolucency in enamel (1), radiolucency in the outer half of dentine (2) and radiolucency in the inner half of dentine (3). Score 2 represented the cut-off for dentine lesions.

### *Validation*

After assessment, the teeth were ground longitudinally on a Knuth-Rotor polishing machine using silicon carbide paper (60 µm grain size) cooled under tap water. Progress of grinding was constantly checked under the microscope (magnification 6.25x) and compared to the initial pictures of the test site. When the periphery of the site was reached, papers of grain size 30, 18, 8 and 5 µm were used. The occlusal cut surfaces were photographed to ensure that the caries lesion was not ground away. The tooth surfaces were then colored with saturated rhodamine B (Fluka, Buch, Switzerland) dissolved in water. Sites were histologically assessed for caries extension according to the rhodamine B penetration (magnification 10x) as: caries free ( $D_0$ ), caries extending up to halfway through the enamel ( $D_1$ ), caries extending into the inner half of enamel ( $D_2$ ), caries in dentine ( $D_3$ ) and deep dentine caries ( $D_4$ ). Subsequently, photographs were taken.

### *Statistical Analyses*

For FC, as no interpretation of the scale was available, the cut-off limits were determined by the highest sum of sensitivity and specificity at each threshold. Sensitivity, specificity, accuracy, area under the ROC curve ( $A_z$ ) and likelihood ratios ( $LR^+$  and  $LR^-$ ) for a positive and negative test were calculated (MedCalc for Windows, version 9.3.0.0, Mariakerke, Belgium) at  $D_3$  threshold for all methods. For the LF devices, the average among the four separated measurements was calculated. The cut-off limits described by Lussi and Hellwig [2006] were used to obtain the values of sensitivity and specificity. The McNemar test was used to compare the values of sensitivity, specificity and accuracy among the methods. Cross-tabulation and rank correlation (Spearman's coefficient) with histology were provided.

Using the separate  $LR^+$  values for each method, the post-test probability for combinations of the methods were calculated [Lussi et al., 1995] to assess the relative value of using the different methods separately and in combination. At threshold  $D_3$ , the pre-test odds was 1.02 and the prevalence of disease in the sample was 46%.

A nonparametric statistical test was used to assess the difference among the areas under the ROC curve ( $A_z$ ) [Hanley and McNeil, 1983]. The significance level was set at  $p < 0.05$ .

Intra-class correlation (ICC) and Cohen's unweighted kappa values were used to assess inter- and intraexaminer reproducibility [Lin, 1989]. The ICC was used for LF, LFpen and FC since they showed discrete values. The unweighted kappa was calculated for all of them, including ICDAS II and BW. For LF, LFpen

and FC, the Bland and Altman method was applied to identify systematic differences and the 95% limits of agreement were calculated [Fleis, 1981; Bland and Altman, 1986].

## Results

Histological examination revealed that of the 119 occlusal test sites, 8 were caries free ( $D_0$ ), 19 had caries extending up to halfway through the enamel ( $D_1$ ), 37 had caries extending into the inner half of enamel ( $D_2$ ), 35 had caries in dentine ( $D_3$ ) and 20 had deep dentine caries ( $D_4$ ). Cross-tabulation for LF, LFpen, ICDAS II and BW with the corresponding histology were given in the tables 1, 2, 3 and 4.

**Table 1:** Cross-tabulation for LF device with the corresponding histology (\*reference for the calculation of sensitivity, specificity, accuracy and LR<sup>+</sup> at  $D_3$  threshold)

<b>Histological score</b>	<b>LF cut-off values</b>					<b>Total</b>
	<b>0 - 7</b>	<b>7.1 - 14</b>	<b>14.1 - 24</b>	<b>*&gt;24</b>		
<b>0</b>	8					8
<b>1</b>	7	5	3	4		19
<b>2</b>	13	11	10	3		37
<b>3</b>	4	8	10	13		35
<b>4</b>	1	1	3	15		20
<b>Total</b>	33	25	26	35		119

**Table 2:** Cross-tabulation for LFpen device with the corresponding histology (\*reference for the calculation of sensitivity, specificity, accuracy and LR<sup>+</sup> at D<sub>3</sub> threshold)

Histological score	LFpen cut-off values					Total
	0 - 6	6.1 - 13	13.1 - 17	*>17		
0	7	1				8
1	5	4	1	9	19	
2	6	10	2	19	37	
3	1	6	3	25	35	
4		1	1	18	20	
<b>Total</b>	19	22	7	71	119	

**Table 3:** Cross-tabulation for FC device with the corresponding histology (\*reference for the calculation of sensitivity, specificity, accuracy and LR<sup>+</sup> at D<sub>3</sub> threshold)

Histological score	FC cut-off values					Total
	0 - 1.262	1.263 - 1.299	1.300 - 1.319	*>1.319		
0	7	1				8
1	11	1			7	19
2	17	1	2		17	37
3	5				30	35
4	2		1	17	20	
<b>Total</b>	42	3	3	71	119	

**Table 4:** Cross-tabulation for ICDAS II system and BW with the corresponding histology (\*reference for the calculation of sensitivity, specificity, accuracy and LR<sup>+</sup> at D<sub>3</sub> threshold)

Histological score	ICDAS II score							BW score				
	0	1	2	*3	4	5	6	0	1	*2	3	Total
0	8							8				8
1	3	6	7	3				19				19
2	1	2	13	21				35	2			37
3	2		6	24	3			27	3	4	1	35
4		5		9	3	3		5	1	11	3	20
<b>Total</b>	14	13	26	57	3	6		94	6	15	4	119

The optimal cut-off limits for FC device determined by the point in which the sum of sensitivity and specificity was maximal are shown in table 3. Specificity, sensitivity, accuracy,  $A_z$ ,  $LR^+$  and  $LR^-$  are shown in table 5.

**Table 5:** Specificity, sensitivity, accuracy, area under the ROC curve ( $A_z$ ) and  $LR^+$  of different methods at  $D_3$  threshold.

Method	Specificity $D_3$	Sensitivity $D_3$	Accuracy $D_3$	$A_z$ $D_3$	$LR^+$ $D_3$	$LR^-$ $D_3$
LF	0.89 <sup>a</sup>	0.51 <sup>a</sup>	0.74 <sup>a</sup>	0.809 <sup>a</sup>	4.65	0.55
LFpen	0.56 <sup>b</sup>	0.78 <sup>b</sup>	0.64 <sup>b</sup>	0.794 <sup>a,b</sup>	1.79	0.39
FC	0.63 <sup>b</sup>	0.86 <sup>b</sup>	0.72 <sup>a</sup>	0.752 <sup>a,b</sup>	2.28	0.23
ICDAS II	0.65 <sup>b</sup>	0.73 <sup>b</sup>	0.68 <sup>a</sup>	0.753 <sup>a,b</sup>	2.11	0.41
BW	0.97 <sup>c</sup>	0.34 <sup>c</sup>	0.63 <sup>b</sup>	0.715 <sup>b</sup>	12.47	0.68

$D_3$ :  $D_0-D_2$ =sound;  $D_{3,4}$ =decayed

Within columns, significant differences are represented by different superscript letters (McNemar test,  $\alpha = 0.05$ ).

At threshold  $D_3$ , the highest sensitivities were observed for FC (0.86), LFpen (0.78) and ICDAS II (0.73), with no statistically significant difference among them. However, BW and LF showed highest specificities (0.97 and 0.89, respectively). Rank correlations (Spearman's coefficient) with histology were 0.53 (LF), 0.52 (LFpen), 0.41 (FC), 0.59 (ICDAS II) and 0.57 (BW).

Table 6 gives an overview of the probabilities of correct detection when the methods are used independently or in combination.

**Table 6:** Probability of different methods and their combination for the detection of caries at D<sub>3</sub> threshold.

<b>Method</b>	<b>D<sub>3</sub></b>	
	<b>Post-test odds</b>	<b>Post-test probability</b>
<b>LF</b>	3.95	79.8%
<b>LFpen</b>	1.52	60.3%
<b>FC</b>	1.93	65.9%
<b>ICDAS II</b>	1.79	64.1%
<b>BW</b>	10.60	91.4%
	<b>LF</b>	8.34
<b>ICDAS II +</b>	<b>LFpen</b>	3.21
	<b>FC</b>	4.09
	<b>BW</b>	22.36
	<b>LF</b>	103.97
<b>ICDAS II + BW +</b>	<b>LFpen</b>	40.02
	<b>FC</b>	50.98

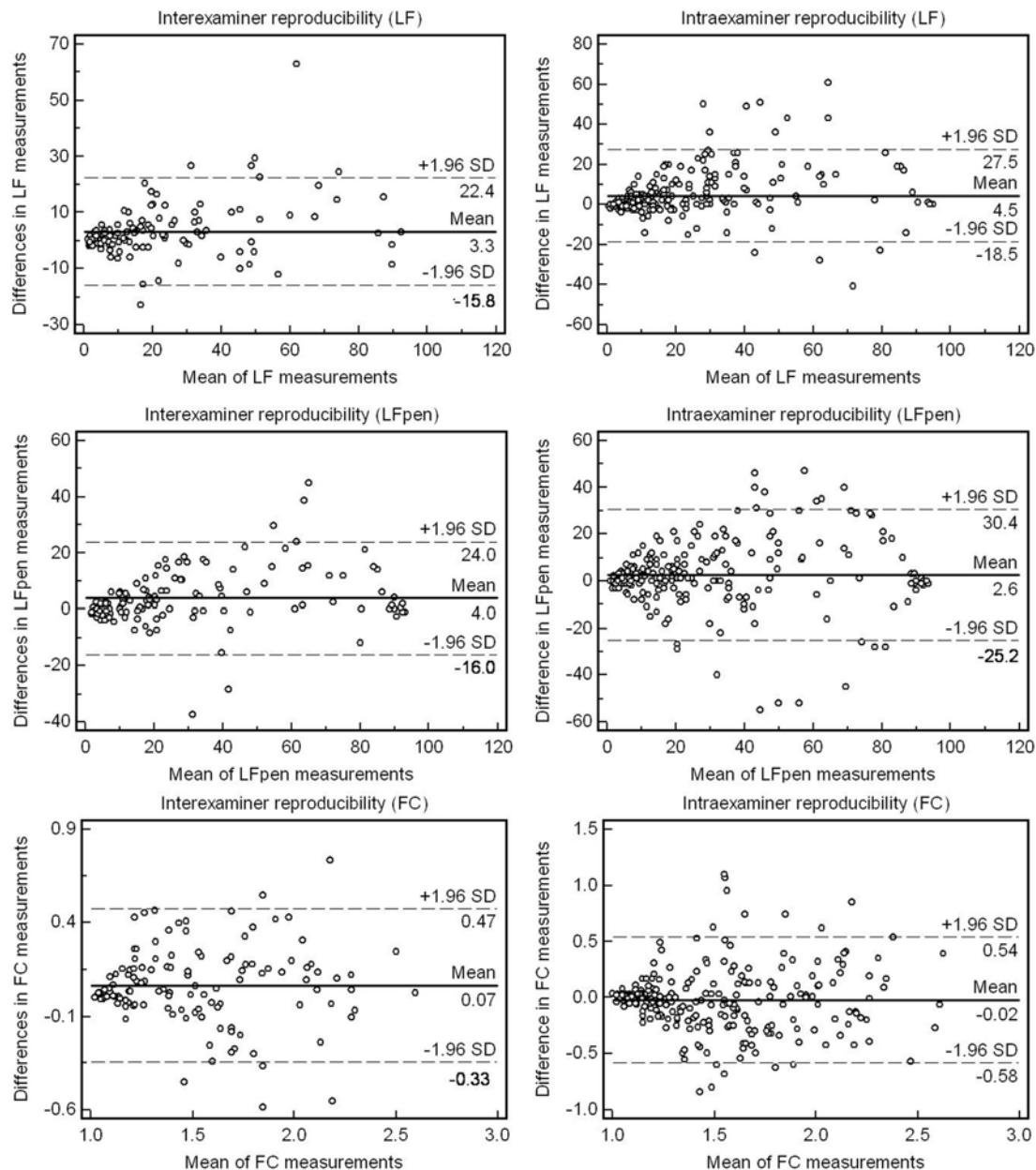
When ICDAS II was combined with BW at threshold D<sub>3</sub> the post-test probability was 95.7%. The combination of ICDAS II, BW and any third method did not increase the post-test probability significantly.

Reproducibilities are represented in Table 7.

**Table 7:** Unweighted kappa values and ICC for inter- and intraexaminer reproducibility of different methods.

<b>Method</b>	<b>Interexaminer</b>		<b>Intraexaminer</b>	
	<b>Unweighted kappa</b>	<b>ICC</b>	<b>Unweighted kappa</b>	<b>ICC</b>
<b>LF</b>	0.58	0.90	0.60	0.85
<b>LFpen</b>	0.55	0.93	0.54	0.87
<b>FC</b>	0.58	0.85	0.61	0.76
<b>ICDAS II</b>	0.51	-	0.61	-
<b>BW</b>	0.50	-	0.62	-

The mean differences as well as the limits of agreement (mean  $\pm 1.96SD$ ) for both inter- and intraexaminer reproducibility for the LF, LFpen and FC can be observed in the Bland-Altman plots (figure 1).



**Figure 1:** The difference between fluorescence values plotted against their mean representing the Bland and Altman method of intra- and interexaminer reproducibility (LF, LFpen and FC). The range between the upper and the lower limits of agreement (dashed lines: mean difference  $\pm 1.96\text{SD}$ ) corresponds to the interval in which 95% of all measurements were reproducible.

The range between the upper and the lower limits of agreement was 46.0 and 38.2 for LF, 55.6, and 40.0 for LFpen and 1.12 and 0.80 for FC, for both intra- and inter-examiner reproducibilities.

## Discussion

Within the limitations of an in vitro study, each method showed different sensitivities and specificities. In agreement with our study, Attrill and Ashley [2001] observed lower sensitivity of radiographic examination for both enamel and dentine and found it a worst method for occlusal caries detection than LF or visual examination. Burin et al. [2005] reported that visual inspection is as valid an evaluation method as LF, which should be considered a better adjunct for occlusal caries detection than radiographic examination. Only BW showed a significantly lower value of accuracy than the other methods, which is in agreement with Ricketts et al., [2002].

Of the laser fluorescence devices, LF showed higher specificity and lower sensitivity and LFpen lower specificity and practically the same sensitivity as was found by Lussi and Hellwig [2006]. In a recent in vivo comparison of LF, visual and radiographic examination, it was concluded that LF may be a useful supplement to visual examination, and its diagnostic performance seems to be good for occlusal caries detection [Alkurt et al., 2007]. Other in vivo investigations demonstrated that LF provided good ability to detect dentine caries lesions [Diniz et al., in press]. The difference between the sensitivity and specificity of LF and LFpen could be due to the difference between both cut-off limits, since the fluorescence values obtained using both devices are also not similar.

FC showed a good performance for detecting dentine caries, if the sensitivity is analyzed. This method could represent a useful tool to aid the diagnostic process like the other methods tested. Its performance has been

assessed and the fluorescence spectrum at the white spot lesions has shown two dominant emission peaks at 640 and 700nm. Therefore, the role of the fluorophores compatible with porphyrins on the fluorescence spectrum could be assumed [Thoms, 2006]. Owing to the lack of a scale for the interpretation of FC values, we calculated optimal cut-off limits for the various thresholds from the maximum sum of sensitivity and specificity as a function of FC values. The prototype used showed cut-off values narrow to each other that make its use in the clinical practice difficult, if not impossible. Caution must be used when these in vitro cut-offs are used for clinical assessment, although in this study no change in the fluorescence values due to storage should be expected [Francescut et al., 2006]. Despite of the high value of sensitivity obtained for the FC device, the Spearman's coefficient showed a weak correlation with the histology, which could be also observed in the cross-tabulation. The difficulty of this device in detecting enamel caries lesions could be observed. The FC device also presented the lowest value of LR<sup>-</sup>, which means how much the odds of the disease decrease when a test is negative, considering the D<sub>3</sub> threshold. It is hoped that the FC device could be improved in order to provide spread cut-off values, since this device should not be a dichotomous instrument.

The same difficulty in detecting enamel lesions was observed when the BW was assessed. However, the ICDAS II showed the best correlation coefficient with the histology and combining the cross-tabulation results this method could be suggested as the best to detect changes in enamel.

A similar device (QLF - Quantitative Laser-induced Fluorescence) almost equal in design has the same excitation wavelength (peak at 405 nm) and a

closely similar cut-off filter (520 nm). The red fluorescence is detected and stored in the same way for both devices. The QLF device has been extensively tested and is reported to be a valuable tool for early detection, quantification and monitoring of non-cavitated caries lesions [Kühnisch and Heinrich-Weltzien, 2004].

The areas under the ROC curve ( $A_z$ ) confirmed the good performance of the methods in detecting either the presence or the absence of occlusal caries lesions. The advantages of ROC curve are: (a) it includes several cut-off points; (b) it shows the relationship between the sensitivity and specificity; and (c) it is not affected by the prevalence of disease [Obuchowski, 2003]. Burin et al [2005] did not find statistical difference in  $A_z$  between LF, visual and radiographic examination. In our study, the LF presented the highest  $A_z$  value when compared to the other methods. However, this value was statistically significant different from the BW  $A_z$  value.

The ICC values obtained for both intra- and inter-examiner reproducibilities agreed with those found by Kühnisch et al. [2007a], who observed high values for both LF and LFpen. Lussi and Hellwig [2006] also observed values of ICC (> 0.98) for both LF devices and kappa varying from 0.83 (LF) to 0.89 (LFpen) for intra-examiner reproducibility, which is in agreement with this study. High ICC was also observed by Alwas-Danowska et al. [2002] and Rodrigues et al. [in press] for LF. The good reproducibility means that both devices can be used for monitoring the caries process [Lussi and Hellwig, 2006].

The Cohen's kappa values for ICDAS II found in this study were lower than those found by Ekstrand et al., [2007] for both intra- and inter-examiner reproducibility. It should be considered that these results involve subjective aspects such as background knowledge and individual clinical experience of the examiners [Fung et al., 2004], which could explain the difference between both studies.

In Bland and Altman plots, there should ideally be no systematic deviation (mean difference = 0) and only a small range between the upper and the lower limits of agreement. The line showed in the plot for LF and LFpen (with deviation from 2.6 to 4.5 from the zero line) indicates the mean of the differences between two measurements. Those values would only be zero on an ideal situation where no differences between the measurements were observed. Kühnisch et al. [2007a] suggested that the range should not exceed  $\pm$  20 LF units. Although the present study showed ranges of 38.2 for LF and 40.0 for LFpen for interexaminer reproducibility, we could not say that these limits of agreement could be considered good and the reproducibility of LFpen seems to be lower. For LFpen intra-examiner agreement, the chevron effect was observed, where the concentration of points on the right (close to 99) and on the left corner (close to 0) shows that the area with better agreement is the one with the lowest and the highest fluorescence values. This arrangement of points in a diamond shape was already reported by Huysmans et al. [2005]. The left and the right corners on the zero line represent perfect agreement of extreme measurements. Data points on the top and bottom corner of the diamond represent disagreement of extreme measurements [Huysmans et al., 2005].

The disagreement between the measurements was observed in the range between 50 and 60 values of fluorescence. The ideal situation would be found in case of having the values close to each other and at the same time close to the zero line. It could also be observed for both LF devices that the pairwise differences were smallest for the lowest values of fluorescence. For FC, considering that the highest mean value obtained using this device was 2.6, the interval in which the measurements were more reliable was between 1 and 1.5.

As the main principle of the FC device is based on an intraoral camera function, the possibility of capturing images of the fluorescing carious teeth or with plaque and showing them to the patient make the device useful in the clinical practice. Additionally, the facility of picture storage is another advantage of this method, due to the possibility to follow the caries lesion's progress or arrest.

It is important to point out that both LF devices present some limitations. A high value of fluorescence may result from other sources than caries, such as the presence of stains, disturbed tooth development or mineralization [Sheehy et al.2001; Souza-Zaroni et al., 2006]. Such alterations could lead to some bias, increasing the sensitivity as a false-positive result. Nevertheless, in the present study, teeth with such alterations were not included in the sample. At the same time, the exclusion of stained teeth could have made the performance of the fluorescence-based methods appear better than it would if a random sample had been used.

As described earlier, the  $LR^+$  can be used to calculate the post-test odds of a test, which then become the pre-test odds for a second independent test

with a known  $LR^+$ , resulting in the post-test probability of their combination [Lussi et al., 1995]. The tests of combinations of two or three methods using  $LR^+$  confirmed the important role of conventional methods, such as visual and radiographic examination, when combined with adjunct methods. Such combinations seem to improve the process of pit-and-fissure caries detection.

Each method has different characteristics and specific modes of functioning, so vary in sensitivity and specificity. In the present study, some methods presented better sensitivity (LFpen, FC and ICDAS II) and others better specificity (LF and BW). However, the post-test probability for dentine caries detection was high for LF and BW. Therefore, a combination of methods would be the best choice in order to detect caries on occlusal surfaces, as also suggested by some authors [Shi et al., 2000; Rickets et al., 2002, Souza-Zaroni et al., 2006, Rodrigues et al., in press]. The ICDAS II combined with BW showed the best post-test probability and is the best combination to detect caries on occlusal surfaces.

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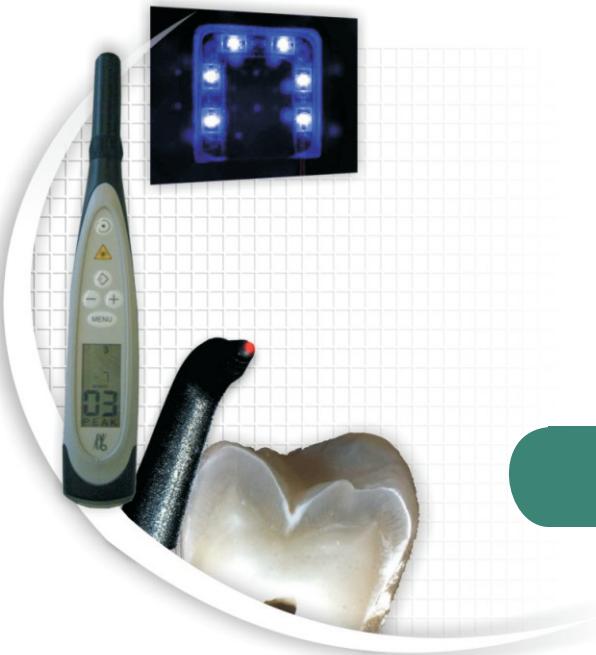
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## ARTIGO III

# **The influence of pit and fissure sealants on infrared fluorescence measurements**

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**Key words:** Pit and Fissure Sealants, Dental Caries, Diagnosis, Fluorescence

**Under review in:**

*Caries Research*

**Abstract**

The aim of this in vitro study was to evaluate the influence of pit and fissure sealants on fluorescence readings. One hundred and sixty-six permanent molars were selected and randomly divided into four groups for sealing (I Delton Clear; II Delton Opaque; III Helioseal Opaque; IV experimental nanofilled clear). The teeth were independently measured twice by two experienced dentists before and after sealing using the devices: DIAGNOdent 2095 (LF), DIAGNOdent 2190 (LFpen) and VistaProof (FC). Subsequently, they were thermocycled and measured again. Reproducibility was calculated (intra-class correlation and unweighted Kappa). Fluorescence values increased after clear and decreased after opaque material sealing placement. Wilcoxon test showed no statistically significant difference among the LF and LFpen values after and before sealing for the Delton Clear group. However, in this latter group LFpen values increased significantly after thermocycling. For FC values, there was statistically significant difference before, after sealing, and after thermocycling for the tested groups, except for the experimental clear sealant after sealing and after thermocycling. The ICC showed values ranging from 0.54 to 0.96 for inter- and 0.28 to 0.94 for intraexaminer reproducibility. The Kappa values for interexaminer reproducibility ranged from 0.55 to 0.77 and for intraexaminer from 0.51 to 0.82. It was shown that pit and fissure sealants have influence on the fluorescence readings, with increase or decrease of the values according to the material used. In conclusion, the LF device could be useful as an adjunct to monitoring surfaces under clear unfilled sealants.

## Introduction

In the last decades there has been a general decline in the prevalence of dental caries especially due to the use of various forms of fluoride [Marthaler, 2004]. In adolescents, occlusal surfaces have more caries than the other surfaces. Lesions in these areas are difficult to detect due to the surface morphology, anatomical fissure, and presence of plaque and stain, which can mask caries lesions. Recently, several methods have been studied which aim to improve the detection and to quantify early caries lesions on smooth and occlusal surfaces.

While pit and fissure sealants have demonstrated to be effective in occlusal caries prevention [Wendt et al., 2001], their efficacy may be related to the background caries prevalence in the population [Ahovuo-Saloranta et al., 2005]. The incorrect application of the sealant could result in leakage and microorganisms remain underneath sealants and lead to caries lesion development. Consequently, undesirable outcomes, such as loss of tooth structure, larger restorations and endodontic treatment can occur [Chapko, 1987]. Therefore, regular follow-up is required to ensure long-term success of the sealant treatment [Feigal, 2002]. However, as conventional methods are difficult to perform under pit and fissure sealants, adjunct methods must be used to improve the follow-up assessments and to increase the diagnostic accuracy.

Some of these methods are based on the fluorescence phenomenon. It has been shown that porphyrins present in the caries lesions fluoresce when stimulated at specific excitation wavelengths [Hibst et al., 2001; Bader and

Sugars, 2004; Braun et al., 2005]. The DIAGNOdent 2095 (LF; KaVo, Biberach, Germany) was the first laser fluorescence device introduced and used as an adjunct to detecting caries. Recently, a new laser fluorescence device DIAGNOdent 2190 (LFpen; DIAGNOdent pen, KaVo, Biberach, Germany) was developed and has been studied for occlusal as well as for approximal caries detection [Lussi and Hellwig, 2006; Lussi et al., 2006]. This new system and the first LF system are based on the same principle. The fluorescence camera device VistaProof (FC; Dürr Dental, Bietigheim-Bissingen, Germany) was recently devised and is capable of emitting light with 405nm of wavelength through six blue LEDs. This system captures and digitalizes images of the teeth while they are emitting fluorescence [Thoms, 2006]. However, to date this device has not been evaluated.

Several studies have evaluated the LF devices for occlusal caries detection with good results of validity and reproducibility as well in vivo as in vitro [Lussi et al., 1999; Shi et al., 2000; Sheehy et al., 2001; Lussi and Hellwig, 2006]. Nevertheless, the presence of composite filling materials might influence the LF readings leading to false-positive results [Lussi and Reich, 2005].

There are controversial data regarding the influence of pit and fissure sealants on laser fluorescence measurements. Previous studies have shown that fissure sealants do not affect LF readings and suggested that the device could be used during routine check-ups to detect caries under fissure sealants [Takamori et al.; 2001; Anttonen et al.; 2003; Deery et al., 2006; Krause et al., 2007]. However, other studies have shown contradictory results and problems with this practice [Hosoya et al., 2004; Gostanian et al., 2006]. Besides, no

study has evaluated the performance of LFpen and the fluorescence camera to detect caries under fissure sealants. Therefore, the aim of this in vitro study was to assess the influence of different fissure sealants, clear and opaque, on the fluorescence readings of three different devices (LF, LFpen and FC).

## **Material and methods**

One hundred and sixty-six extracted third permanent human molars from sound to carious (108 with microcavities and 36 non-cavitated, of which 22 were apparently sound) were selected from a pool of teeth, which were stored frozen at –20°C until use and during the experiments. Earlier studies showed that this method of storage does not change the red fluorescence significantly [Francescut et al., 2006]. All teeth were extracted by dental practitioners in Switzerland (no water fluoridation, 250 ppm F<sup>-</sup> in table salt). Prior to extraction, the patients were informed about the use of their teeth for researches purposes and their consent was obtained. The teeth were defrosted for 3 hours and the calculus and debris were removed using a scaler (Cavitron). They were cleaned for 15 s with water and toothbrush (Trisa ultra super-sensitive, Triengen, Switzerland) and for 10s with a water-powder jet cleaner (PROPHYflex II, KaVo, Biberach, Germany) and sodium hydrogen carbonate powder. In order not to have powder remnants in the fissure, the teeth were then rinsed off with a 3-in-1 syringe for 10s [Lussi and Reich, 2005].

Photographs of the occlusal surfaces were taken at a 6.25x magnification using a light microscope (Leica DC300, Leica, Heerbrugg, Switzerland) equipped with a video camera linked to a computer (Leica M420, Leica,

Heerbrugg, Switzerland) and the test site was marked. Before starting the experiments, teeth were defrosted at room temperature for 3 hours. All assessments were carried out twice by two experienced dentists observing a one-week interval between the measurements. The following devices were tested: DIAGNOdent 2095 (LF), DIAGNOdent 2190 (LFpen) and VistaProof (FC). During the measurements, the teeth were stored in 100% humidity. These examinations were conducted before sealing (baseline), after sealing, and after thermocycling.

#### *Baseline measurements*

The LF measurements were performed using the probe tip "A" developed by the manufacturer and the LFpen using the cylindrical sapphire fibre tip according to manufacturer's instructions. Before each measurement, the devices were calibrated with a ceramic standard and the zero value of fluorescence of a sound part of the cuspal area on the buccal surface was recorded. The tip was placed on the selected site and rotated around the vertical axis until the highest fluorescence reading was obtained. The peak values were recorded and the zero value of fluorescence was subtracted.

The FC device was used and the occlusal surfaces were illuminated with blue light emitted by GaN-LEDs at 405 nm. After this excitation, fluorescence emission was detected by collecting fluorescence images on a RGB camera chip and the images were analyzed by the software DDview (Dürr Dental, Bietigheim-Bissingen, Germany) with regard to the intensity ratio of the red and green fluorescence. Caries lesions were determined when the red/green-ratio

was higher than the red to green ratio of sound enamel. To calculate the fluorescence ratio of caries lesions, the maximum of the red to green ratio in the lesion was recorded [Thoms, 2006].

After measuring, in order to divide the teeth into groups, the average fluorescence values obtained with the LF device and the cut-off limits suggested by Lussi and Hellwig [2006] were used to form groups with the same number of sound teeth, enamel and dentine caries. The teeth were then randomly divided according to the material for pit and fissure sealing. The groups were arranged as follows:

- Group I - Delton Unfilled Clear (Dentsply, Konstanz, Germany);
- Group II - Delton Opaque (Dentsply, Konstanz, Germany);
- Group III - Helioseal Opaque (Ivoclar Vivadent, Schaan, Liechtenstein);
- Group IV - Experimental Nanofilled Clear (Voco, Cuxhaven, Germany).

#### *Sealant Placement*

Occlusal surfaces were etched with phosphoric acid gel 35% (Vococid etching liquid, Voco, Cuxhaven, Germany) for 60s. The etchant was gently stirred on the occlusal surfaces using a soft microbrush. Teeth were then rinsed with water/air spray for 15s and dried with an air syringe for 5s. The application of the sealant was performed directly onto the etched and dried surface with a round-ended applicator BR 06/08 (A. Deppeler SA, Rolle, Switzerland), taking care to not overfill the fissures and avoiding contact of the applicator with the enamel surfaces. The sealant was left undisturbed for 20s in order to allow for it to flow into the fissures system and etched surface. After that, the sealant was

light cured for 40s (Optilux 400, 300 mW/cm<sup>2</sup>, Demetron Research Corporation) [Celiberti and Lussi, 2007]. Photographs at a magnification of 6.25x were taken and the teeth were stored individually under 100% humidity at -20°C.

After sealing, the same examiners re-measured the teeth using the fluorescence-based devices as described above.

#### *Thermocycling Procedure*

The sealed teeth were thermocycled in deionized water for 1000 cycles between 5 ± 2 and 55 ± 2°C, with a dwelling time of 30s. Photographs at a magnification of 6.25x were taken again and measuring was repeated using the fluorescence-based devices as described earlier.

#### *Material Samples*

In order to assess the fluorescence values of the materials tested, two disc shaped samples of each material were light-cured for 60 seconds and polished until 0.5 and 1 mm of thickness. LF and LFpen were used and the values were obtained. The samples were thermocycled as described above and measured again.

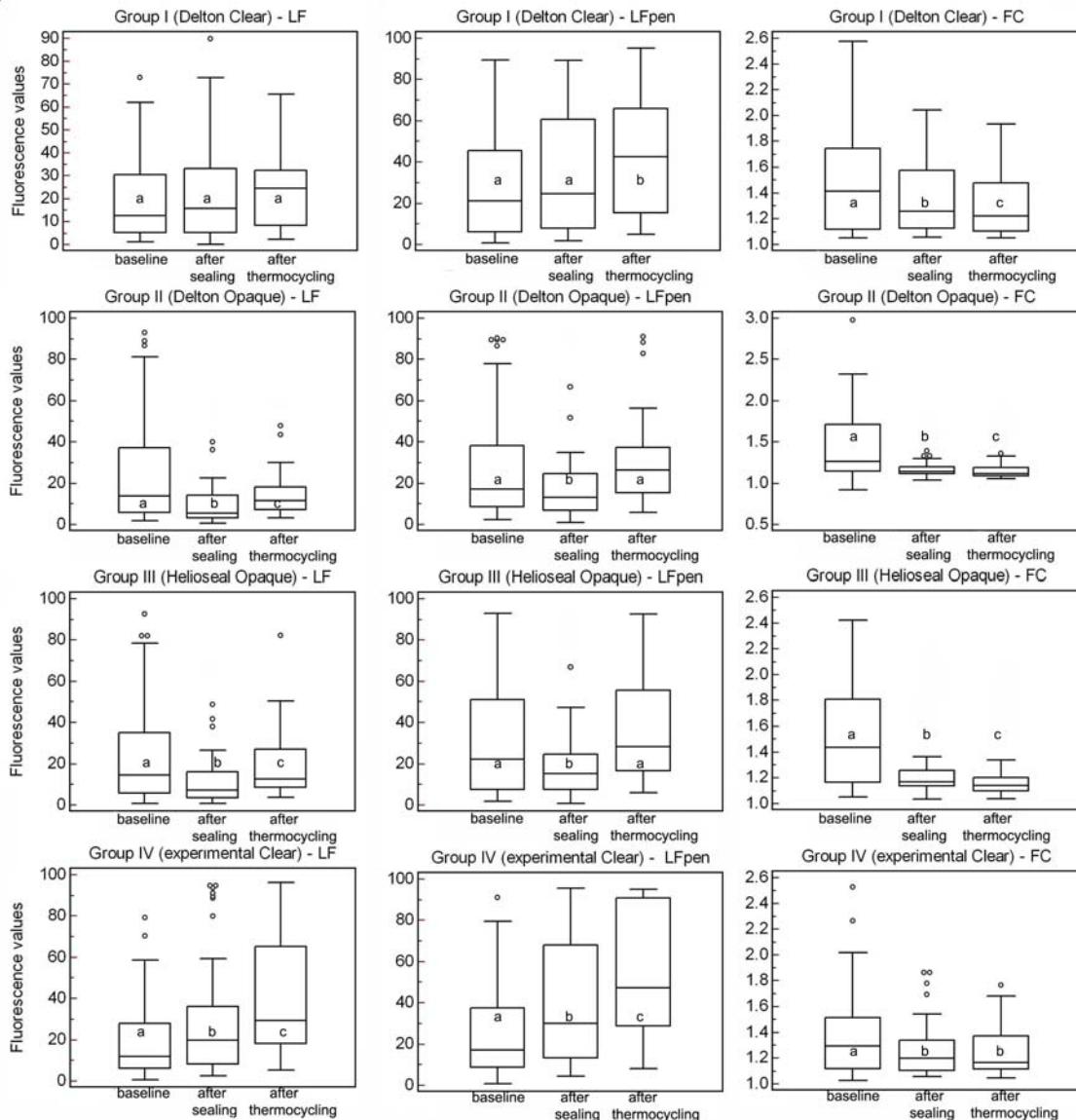
#### *Statistical Analysis*

For each method, the values obtained at baseline after sealing and after thermocycling were compared by performing the Wilcoxon test (nonparametric), because the data were not-normally distributed. The first and third quartiles, as well as the minimum and maximum values were calculated.

Intra-class correlation (ICC) as well as Cohen's unweighted kappa values were used to assess inter- and intraexaminer reproducibility [Lin, 1989]. Reproducibility indicates the closeness of the agreement between the results of measurements carried out under changed conditions of measurement. The significance level was set at  $p<0.05$ .

## Results

The fluorescence values obtained for LF, LF pen and FC are shown in the box plots in figure 1. Generally, fluorescence values increased after clear and decreased after opaque material sealing placement. No statistically significant difference between the LF, LFpen values after and before sealing was observed for the Delton Clear group. However, in this group LFpen values increased significantly after thermocycling. For FC values, there was statistically significant difference before and after sealing as well as after thermocycling for the tested groups, except for the experimental Clear sealant after sealing and after thermocycling.



**Figure 1:** Box plots for fluorescence values measured at baseline, after sealing and after thermocycling using the LF, LFpen and FC devices for the different groups. The median, first and third quartiles, minimum and maximum values and outliers (open circles) are shown. Different letters show statistical significant difference (Wilcoxon test;  $p<0.05$ ).

The reproducibility assessed by calculating the unweighted Kappa and ICC is represented in table 1. The ICC showed values ranging from 0.54 to 0.96 for inter- and 0.28 to 0.94 for intraexaminer reproducibility. The Kappa values

for interexaminer reproducibility ranged from 0.55 to 0.77 and for intraexaminer from 0.51 to 0.82.

**Table 1:** Intra-class correlation and Kappa values for intraexaminer and interexaminer reproducibility for the three fluorescence-based methods in the three phases.

	Baseline				After sealing				After thermocycling			
	Inter		Intra		Inter		Intra		Inter		Intra	
Method	Kappa	ICC	Kappa	ICC	Kappa	ICC	Kappa	ICC	Kappa	ICC	Kappa	ICC
<b>LF</b>	0.58	0.87	0.55	0.82	0.61	0.96	0.70	0.93	0.70	0.94	0.70	0.94
<b>LFpen</b>	0.72	0.92	0.60	0.87	0.55	0.94	0.51	0.90	0.77	0.94	0.64	0.92
<b>FC</b>	0.77	0.54	0.64	0.28	0.64	0.93	0.59	0.65	0.68	0.94	0.59	0.65

The fluorescence values of the samples tested with 0.5 and 1.0 mm of thickness using LF and LFpen at baseline and after thermocycling are shown in the table 2.

**Table 2:** Fluorescence values of the sealants tested with 0.5 and 1.0 mm of thickness using LF and LFpen.

	Baseline				After thermocycling			
	0.5 mm		1.0 mm		0.5 mm		1.0 mm	
	LF	LFpen	LF	LFpen	LF	LFpen	LF	LFpen
<b>Delton Clear</b>	2	1	7	6	2	1	4	5
<b>Delton Opaque</b>	5	4	5	5	5	4	5	5
<b>Helioseal Opaque</b>	2	2	2	1	1	2	1	1
<b>Experimental Clear</b>	2	1	1	1	1	1	1	1

## Discussion

To decide between treatment involving sealing a pit and fissure surface and following-up the caries lesion is often a challenge for dental practitioners. To obtain good sealing of the occlusal surface, it is necessary to get adequate bonding for the retention of the sealant materials and for prevention of marginal microleakage, avoiding bacteria growth and caries process development [Celiberti and Lussi, 2007]. Consequently, the monitoring of sealed surfaces must be effective during dental clinical practice.

LF, LFpen and FC readings were significantly lower ( $p<0.05$ ) after sealing for both opaque sealants, and the values increased after the thermocycling procedure. These low LF values have also been reported after opaque [Takamori et al, 2001; Hosoya et al., 2004; Krause et al., 2007] and glass ionomer sealants placement [Hosoya et al., 2004]. Some authors have suggested factors responsible for this observation such as the opacity of titanium dioxide used as a pigment in opaque resin composites, or even residual polishing paste left on the surface [Hosoya et al., 2004; Deery et al., 2006; Krause et al., 2007], absorbing either the light emitted by the devices, or the fluorescence emitted by the carious tissue. In this study, however, no polishing paste has been used. All teeth were cleaned with an air-polishing device to remove any calculus or plaque in the occlusal surfaces. In addition, Delton Opaque is composed of silicon dioxide and titanium dioxide, which are opacifier agents and probably are related to the higher values of the intrinsic fluorescence in this material [Gostanian et al., 2006]. Takamori et al. [2001] attribute the lower values of LF in opaque sealants to the fluorescence,

absorption and scattering of irradiation and reflected beams, as they are differently composed than the clear sealants.

Using the Delton Clear sealant, the LF as well as the LFpen did not show statistically significant differences after the sealing procedure; however, for FC this difference was observed. These results disagree with Deery et al. [2006], who showed lower LF measurements after sealing teeth stored in 1% aqueous thymol solution using the same material. However, Anttonen et al. [2003] in an in vivo study as well as Krause et al. [2007] reported that clear sealants did not affect LF measurements. Although in the present investigation the LF and LFpen values increased, no statistically significant difference was found after sealing using Delton Clear. Clinically, this must be considered since the interpretation of the readings depends on the cut-off limits used for both devices. A small variation in the measurements can influence the diagnosis. After thermocycling, LFpen presented higher and FC lower values when compared to the baseline. This might be due to color changes of the sealant materials or loss of marginal integrity between sealant and enamel (marginal failure) in the occlusal surfaces after thermocycling. The thermocycling procedure simulates the daily ingestion of hot and cold liquids and solids which are part of the normal diet, and its result is a thermal stressing between two materials with different coefficients of thermal expansion [Simmons et al., 1976]. After the sample's thermocycling, some cracks on the materials were observed which could have allowed more intensity of infrared light to reach the occlusal surface. This could be the reason for the LF and LFpen values increase after thermocycling.

Although some studies have shown that an unfilled sealant penetrates deeper into the fissure and is better retained [Rock et al., 1990; Simonsen, 2002], the development of the nanotechnology in dentistry have allowed considerable improvement of the physical and mechanical characteristics of dental materials. It was observed that the LF and LFpen values increased after sealing with the experimental nanofilled clear sealant and after thermocycling when compared with the baseline; however, for FC the values were lower. These results disagree with Krause et al. [2007], who found that experimental nanofilled clear sealant did not influence the LF readings. They also reported an increase in the fluorescence values after acid etching caused by a modified scattering pattern of the treated enamel. The difference in this present study's results might be due to the following factors. Before use the teeth were frozen. This method does not change the red fluorescence significantly [Francescut et al., 2006]. Furthermore, Krause et al. [2007] based their conclusions on a sample of 15 teeth (more than 1 site per tooth) stored in physiological saline solution and with an acid etching time of 30 s in the sealing procedure, whereas in the present study, an etching time of 60 s was used. The increase in the fluorescence values after sealing using the experimental nanofilled clear sealant was not due to the material intrinsic fluorescence. It could be explained by the translucency of the clear sealant, which allows the light to reach the etched surface. A further pilot experiment was carried out in order to observe whether the fluorescence values would change after sealing using the clear sealants without acid etching. A mean decrease of 3 units of fluorescence was observed, which confirms this study's conclusions.

As the FC device is capable of digitalizing the image of the teeth while they are emitting fluorescence, the increase in scattering due to the acid etching could not be identified. For this reason, the fluorescence values observed after sealing decreased by the materials of all groups. Obviously, the fluorescence values decreased more with the opaque sealants than the clear sealants. This new fluorescence-based method may represent a useful tool, like the other methods, to aid the caries detection. Yet, this procedure could be affected by the lesion's being covered by pit and fissure sealants. The advantage of this device is that it can capture images of the fluorescing teeth with caries or plaque, which permits showing the patient and following-up the caries lesion progression.

The development of new methods of caries detection is based on the requirement that they present high validity and reproducibility to obtain reliable results. The inter- and intraexaminer Kappa values obtained with the three methods tested before sealing, after sealing and after thermocycling represented agreement ranging from 0.51 to 0.77. Similar in vivo and in vitro results have been reported for the LF with considerable agreement [Lussi et al., 1999; Shi et al., 2000; Sheehy et al., 2001] as well as for LFpen [Lussi et al., 2006]. The intraexaminer reproducibility revealed ICC values from 0.28 to 0.94 and from 0.54 to 0.96 for interexaminer reproducibility. It is important to point out that FC presented the lowest ICCs values of inter- (0.54) and intraexaminer (0.28) reproducibility before sealing. This could be explained with the way the fluorescence images of the teeth are captured. For FC, the entire occlusal surface is examined, different from LF and LFpen, which assess the exact test

site. However, there is to date no data available concerning FC reproducibility. Both LF and LFpen presented excellent ICCs values, as previously showed by Kühnisch et al. [2007].

In the study of Gostanian et al. [2006], the intrinsic fluorescence of the sealants (clear and opaque) showed a considerable effect on LF readings. In this case, the LF device registers the sealant's intrinsic fluorescence as well as the true caries fluorescence, which cannot be differentiated from each other. Furthermore, the possibility of false-positives results may lead to different outcomes. In the present study, when the sealant material thickness increased (from 0.5 to 1.0 mm), only the fluorescence readings of Delton Clear samples increased. Both opaque materials as well as the experimental nanofilled sealant did not change the fluorescence values. Although the thickness of opaque sealant does not play an important role in the fluorescence readings, it is important to note that in general dental practitioners perform the technique of sealant placement carefully in order not to over-fill the pit and fissures.

All possible invalidating factors as plaque, calculus, and remnants of toothpaste or prophylaxis paste [Lussi and Reich, 2005] were excluded in this *in vitro* study. In a clinical situation, those factors can influence the treatment decision, giving false-positive or false-negative results and can lead to under- or over-treatment. For clinical use, the clear sealants seem to be the best option to allow caries detection procedures, since they allow the visual examination of the lesion. Nevertheless, clinical studies are necessary to evaluate the influence of pit and fissure sealants on occlusal caries detection.

This in vitro study showed that pit and fissure sealants influence the fluorescence readings, with an increase or decrease of the values according to the material used. It can be concluded that the LF device is useful as an adjunct to monitoring surfaces under clear unfilled sealants.

### Acknowledgments

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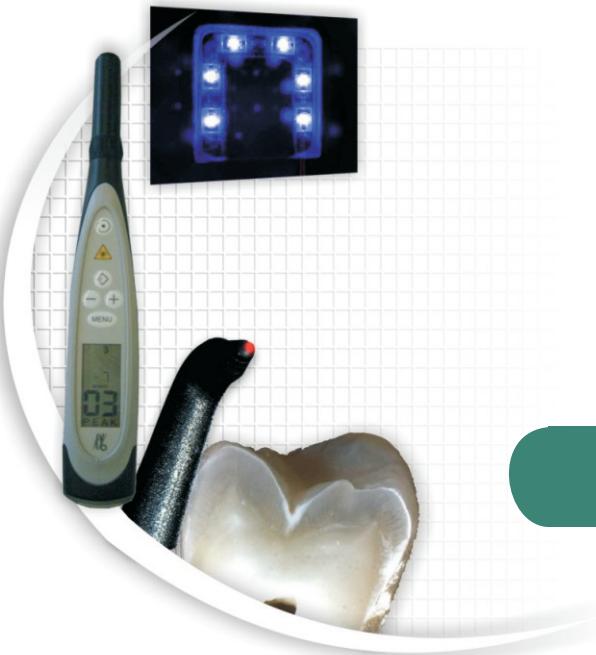
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## CONSIDERAÇÕES FINAIS

A detecção das lesões de cárie e a determinação da sua extensão é um dos fatores que devem ser considerados no processo de diagnóstico e tratamento dessa doença. Com o objetivo de auxiliar o exame visual e o radiográfico, novos métodos têm sido desenvolvidos, cujo desempenho tem sido pesquisado. No presente trabalho, o desempenho de dois métodos baseados no fenômeno de captação da fluorescência foi avaliado e alguns fatores relacionados a esses métodos foram investigados.

Avaliar o processo de calibração, ainda controverso na literatura, foi um dos objetivos da presente pesquisa. A mensuração e a subtração do valor de fluorescência zero utilizando os dois modelos do DIAGNOdent foram realizadas com o intuito de avaliar a influência desse procedimento no desempenho de ambos os métodos na detecção de lesões de cárie oclusal. Quando utilizado o DIAGNOdent 2095, pode-se sugerir que não há necessidade de mensurar a superfície hígida do dente a ser avaliado. O mesmo não pôde ser afirmado quando utilizado o DIAGNOdent 2190 ou *pen*, cujo desempenho foi influenciado pela falta da calibração.

O desempenho desses dois métodos na detecção de lesões de cárie oclusal também foi comparado ao da câmera VistaProof, sistema ICDAS II e exame radiográfico convencional. Cada um desses métodos apresenta características distintas e modos de funcionamento variados. Por esse motivo, cada um apresentou desempenho diferente, de acordo com os valores de sensibilidade e a especificidade. Enquanto o DIAGNOdent *pen*, a câmera VistaProof e o sistema ICDAS II apresentaram valores mais altos de

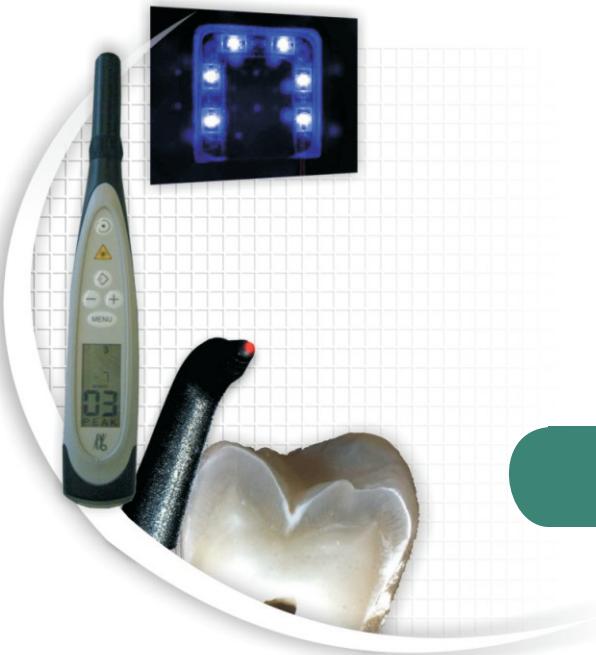
sensibilidade, o DIAGNOdent 2095 e o exame radiográfico convencional mostraram valores altos de especificidade e também maior probabilidade de diagnósticos corretos de lesões em dentina. Diante disso, pode-se afirmar que a combinação de alguns desses métodos tornaria mais acurada a detecção das lesões de cárie oclusal. Na presente pesquisa, a combinação do sistema ICDAS II com o exame radiográfico convencional foi a que apresentou maior probabilidade de diagnósticos corretos das lesões em dentina.

Além disso, a detecção de lesões de cárie sob os selantes de fossas e fissuras é difícil quando se dispõe apenas dos métodos convencionais, pelo fato do material utilizado interferir na visualização da lesão. Por esse motivo, a utilização de métodos auxiliares parece desempenhar um papel importante nesse processo. A influência de selantes de fossas e fissuras sobre os valores de fluorescência também foi testado na presente pesquisa. Dois selantes opacos e dois transparentes (“clear”) foram utilizados nas superfícies oclusais e foi observada interferência da opacidade do material nos valores de fluorescência obtidos. Foi observado que os selantes de fossas e fissuras influenciam as valores de fluorescência, podendo aumentá-los ou diminuí-los de acordo com material empregado. Pode-se concluir que o DD pode ser utilizado para monitorar superfícies oclusais sob selante “clear” e sem carga. Pode-se sugerir ainda que o uso do selante “clear” favoreceria a visualização das lesões.

Através dessa pesquisa, nota-se o importante papel dos métodos auxiliares de detecção das lesões de cárie. No entanto, a proposta do sistema ICDAS II, que detalha o exame visual convencional e a sua associação ao

exame radiográfico ainda deve ser priorizada antes de se optar por um terceiro método, salvo quando a visualização direta da lesão for impossibilitada. Outros estudos *in vivo* devem ser estimulados com o intuito de avaliar o desempenho desse sistema e dos métodos baseados em fluorescência.

Como já dito, o processo de diagnóstico de cárie depende de outros fatores além da detecção e da determinação da profundidade das lesões. Devem ser ainda avaliados os sintomas do paciente, a experiência passada de cárie, a capacidade tampão da saliva, bem como os fatores etiológicos da doença como o consumo de açúcar e o acesso ao fluor. Além disso, a atividade das lesões de cárie é um fator que também não pode deixar de ser avaliado, bem como o desempenho desses métodos em determinar a atividade dessas lesões.



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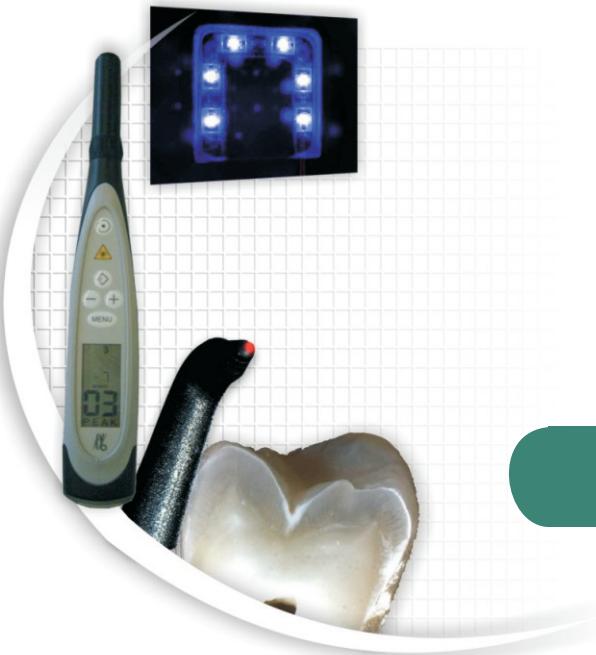
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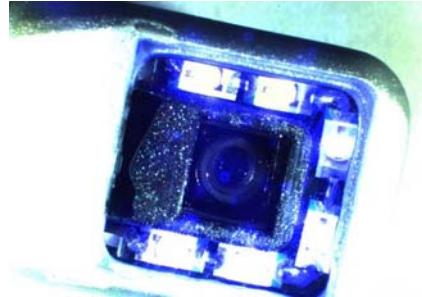


## ANEXOS

## Anexo A

### Aparelhos utilizados

Câmera VistaProof



DIAGNOdent 2190 ou *pen*



Ponta para superfície oclusal (cilíndrica)



### Calibração no padrão cerâmico



DIAGNOdent 2095



Ponta para superfície oclusal



### Calibração no padrão cerâmico



## Anexo B

### Artigo II

Exemplos de três dentes - fotos iniciais e obtidas utilizando a  
Câmera VistaProof

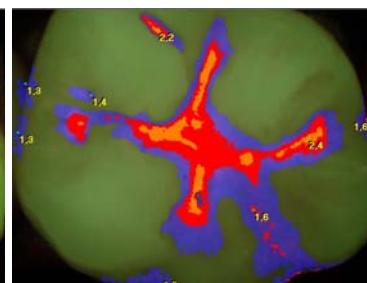
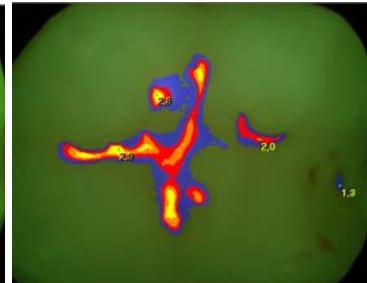
Foto inicial



Câmera VistaProof



DDview



## Anexo C

### Artigo III

#### Selantes utilizados



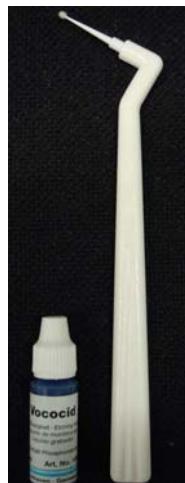
Grupo I

Grupo II



Grupo III

Grupo IV



Ácido fosfórico 35%, microbrush e casulos para o selamento das superfícies oclusais

### Identificação dos dentes para termociclagem



### Termociclagem



## Anexo D

### **Comprovantes de envio dos artigos**

#### ***Artigo I***

Dear Dr. Rodrigues:

Your manuscript entitled "***The influence of zero value subtraction on the performance of two laser fluorescence devices for detecting occlusal caries in vitro***" has been successfully submitted online and is presently being given full consideration for publication in the ***The Journal of the American Dental Association.***

We will consider your article for publication with the understanding that

- it has not been published previously;
- it has been submitted solely to JADA;
- each author has fully disclosed any financial, economic or other conflicting interests in products or services described in the article.

Your manuscript ID is 419-07.

A decision regarding the disposition of the manuscript will be made after critical evaluation and advisory comment by selected reviewers.

Thank you for submitting your manuscript to The Journal of the American Dental Association.

Respectfully,

The Journal of the American Dental Association Editorial Office

***Artigo II***

Ms. No. 200711018

Dear Dr. de Almeida Rodrigues,

Thank you for submitting a revised version of your Original Paper entitled "***Performance of fluorescence methods, radiographic examination and ICDAS II system on occlusal surfaces in vitro***" to "***Caries Research***". We shall inform you as soon as possible of the final decision reached by the editorial board.

For information regarding the status of your manuscript and for future submissions you can logon to the manuscript tracking system with your personal logon name and password as follows:

<http://www.karger.com/cre>

With kind regards,

R P Shellis  
(Editor-in-Chief, Caries Research)  
Division of Restorative Dentistry  
Bristol University Dental School, Bristol BS1 2LY, U.K.  
Fax. +44 117-928-4778  
Tel. +44 117-928-4328  
[r.p.shellis@bristol.ac.uk](mailto:r.p.shellis@bristol.ac.uk)

***Artigo III***

Dear Dr. de Almeida Rodrigues,

Thank you for submitting your manuscript "***The influence of pit and fissure sealants on infrared fluorescence measurements***" to "***Caries Research***"; the submission number is: 997. Your submission will now be checked by the editorial office, and you will receive a confirmation mail from the editorial office soon. This step will also activate your personal user-id and password, enabling you to login to the system to check the status of your manuscript.

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JONAS DE ALMEIDA RODRIGUES

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