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Laboratory Research

Comparative *In Vitro* Validation of VistaProof and DIAGNOdent Pen for Occlusal Caries Detection in Permanent Teeth

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Clinical Relevance

Laser/light fluorescence devices were highly reliable for occlusal caries diagnosis in permanent teeth but not superior in accuracy to visual methods.

SUMMARY

Purpose: Current caries diagnostic tools are neither very accurate nor very reliable for the detection of carious lesions of different depths. Thus, the development of new devices and techniques is needed. The aim of this *in vitro* study was to validate a newer fluorescence device, VistaProof (VP), and compare it with DIAGNOdent Pen (DP), direct visual (DV) and indirect visual methods (IDV), with respect to accuracy and reliability for the detection of occlusal caries in permanent teeth.

Methods and Materials: One hundred seven sites on 41 occlusal surfaces of recently extracted premolars were selected and classified into lesion categories according to Ekstrand's clinical criteria, by direct and indirect visual examination. The fluorescence of the sites was also measured by the two devices, and the teeth were ground through the sites for histological evaluation of their lesion depth. One calibrated examiner of high reliability (intraclass correlation coefficient [ICC]>0.85) made all of the evaluations. Sensitivity, specificity, and accuracy of each detection method were estimated based on histological examination as the reference method, estimated using cut-off limits calculated on the basis of best agreement between the devices' values and histological examination. McNemar tests and receiver operating characteristic (ROC) curve

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analyses were used to compare the validity measures of all detection methods at α=0.05, while the ICC was used to test the reproducibility of the methods based on a second measurement one week after the first.

Results: There was no statistically significant difference ($p>0.05$) between the accuracy of DP and VPs for both enamel and dentin lesions. The areas under the ROC curves (AUC) for the two devices were also found not to be different ($p>0.05$). The reliability of DP was statistically significantly better than VP ($p<0.05$).

Conclusion: The validity of both fluorescence devices were not found to be significantly different and not better than visual methods for the detection of noncavitated carious lesions.

INTRODUCTION

Despite the reduction in the prevalence of tooth decay,[1] the pattern of the reduction is not uniform for all dental surfaces, and the occlusal surface still remains the most susceptible site for caries development.[2,3] The diagnosis of occlusal caries, especially the noncavitated type, is a difficult and problematic task because of the superficial remineralization potential that can delay cavitation and because of the extensive use of fluorides that can slow the progress of the lesion, strengthen occlusal enamel, and mask dentinal caries.[4,5]

In the past decade, numerous studies have been published to improve existing dental caries detection techniques and to seek new noninvasive ones that could quantify the depth of carious lesions.[6-9] New nonharmful devices that measure the fluorescence of the hard dental tissues illuminated by light of specific wavelengths have been developed and recommended as caries detection aids.[10,11]

In 1998, a chairside portable device that uses a diode laser to measure differences in fluorescence intensity between normal and demineralized enamel tissue in smooth and fissured surfaces, DIAGNOdent (DIAGNOdent 2095, KaVo, Bibberach, Germany), was introduced for caries diagnosis. A new model, DIAGNOdent Pen (DIAGNOdent 2190, KaVo, Bibberach, Germany), with smaller-diameter tips has been developed for fissured, smooth, and approximal surface caries detection.[11-13] The device emits a red laser beam at a wavelength of 655 nm (1 mW maximum power) and measures the lesion's fluorescence (at 720-750 nm), produced by microbial metabolic products, the porphyrins.[14] Although the device functions with the same principle as the old one, its main difference is at the tip, which is rotatable around its long axis to facilitate use in approximal areas. A laser light is sent to the tooth through a single sapphire fiber tip, which at the same time collects the reflected light and filters the ambient and the fluorescent light entering the tip. In DIAGNOdent 2095, the light is transported to the angulated tip through a central fiber while fluorescence light is collected through additional fibers that are concentrically arranged around this central fiber. Results of *in vivo*[13,15] and *in vitro*[11,12,16] studies in permanent teeth have shown that this new model has better sensitivity but worse specificity than the previous one on occlusal surfaces and that the validity of the device to detect caries in relation to their lesion depth was very good.

An intraoral fluorescence camera (VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) that illuminates teeth with a violet light (405 nm) and captures the reflected light as a digital image was recently developed.[17] The reflected light is filtered for light below 495 nm and contains the green-yellow fluorescence of normal teeth with a peak at 510 nm as well as the red fluorescence of bacterial metabolites with a peak at 680 nm. Special software quantifies the green and red components of the reflected light on a scale from 0 to 3 as a ratio of red to green, showing the areas with a higher than healthy tooth ratio. A detailed description of this camera is given by Rodrigues and others.[11]

This device uses the same principle as the QLF device (Inspektor Research Systems BV, EG Amsterdam, The Netherlands) but presents differences from it. The illumination light of the QLF has an average wavelength of 380 nm (290-450 nm), and the reflected light is filtered at 520 nm, allowing only the fluorescence above this wavelength to be recorded. QLF has more sophisticated software that allows the user to select and analyze areas of interest, even those of low autofluorescence (highly light-scattering areas).

These four fluorescence devices (DIAGNOdent 2095, DIAGNOdent 2190, QLF, and VistaProof) have a similar function as they can analyze the fluorescence of porphyrins in bacterial waste. However, the first two collect data for the fluorescence of the lesion directly and in contact with the lesion, while the other two, being cameras, collect data indirectly from the fluorescence image map of the lesion. Several studies have reported on VistaProof,[11,17-23] and three of them compared it with

Table 1: *Criteria Used for Direct (DV) and Indirect (IDV) Visual Examination, Fluorescence Devices, and Histological Examinations*

Score	DV[a]	IV[a]	DP+	VPs+	VPm−	Histology
D0 *Sound*	No caries	No indication of enamel lesion	<9	<1.3	<1	No enamel demineralization
D1 *Early enamel caries*	Opacity or discoloration visible after air drying	Opacity or discoloration visible	9-24	1.30	1.0-1.49	Demineralization limited to the outer half of the enamel
D2 *Deep enamel caries*	Opacity or discoloration visible without air drying	Opacity or discoloration larger than the fissure width	25–44	1.41	1.5-1.99	Demineralization extending to the inner half of the enamel
D3 *Early dentin caries*	Grayish discoloration from the underlying dentin	Grayish discoloration from the underlying dentin	≥44	≥1.59	2.0-2.49	Demineralization involving the outer half of dentin
D4 *Deep dentin caries*	Cavitation exposing the dentin beneath	Cavitation exposing the dentin beneath			≥2.5	Demineralization involving the inner half of dentin

+, Cutoffs estimated from the data; −, cutoffs suggested by the manufacturer.
[a] Criteria based on the classifications from Ekstrand and others.[6]

DIAGNOdent.[11,22,23] Studies comparing QLF and DIAGNOdent devices are limited,[24-26] and no study has yet published results on a comparison of VistaProof and QLF.

Only two *in vitro* studies comparing VistaProof to other diagnostic methods have been published.[11,17] For permanent teeth, results showed VistaProof to have similar sensitivity (0.86) to DIAGNOdent Pen (0.78) and visual examination (0.73) but lower specificity (0.63) than all other methods.[11] On primary teeth, the study of De Benedetto and others[23] showed no differences between VistaProof and DIAGNOdent Pen (intraclass correlation coefficient [ICC]vp=0.85, ICCdp=0.85) in intraexaminer reliability. No validity estimations were made in this study. Therefore, more research is needed to clarify its validity in detecting enamel and dentin lesions.

Visual examination is usually used as a control to compare different examination methods.[27,28] Visual inspection aided by magnification has been shown to increase the *in vitro* sensitivity for caries,[29] while indirect visual examination through photographs[30] or digital images[28,31] seemed to have greater potential for the detection of caries.

The aim of this *in vitro* study in permanent teeth was to investigate the reliability and accuracy of VistaProof and DIAGNOdent Pen in enamel and dentin occlusal caries detection and to compare them with that of classical visual methods. The null hypotheses were therefore that there are no differences in the reliability and accuracy of the two devices or between the devices and the control methods for occlusal caries detection in permanent teeth.

METHODS AND MATERIALS

Sample Selection and Preparation

Forty-one premolars macroscopically sound or with initial (noncavitated) occlusal caries lesions[1] were selected upon visual inspection with a magnifying loupe 2.5× from a pool of recently extracted human teeth that were stored in tap water from the day of their extraction so that each caries category could be equally represented. Teeth were cleaned with a rubber cup and an air-water syringe and dried for 5 seconds using compressed air, and then the sites were selected carefully to represent lesions according to the criteria of Ekstrand and others[6] (see Table 1). Teeth with open occlusal cavities (D4), hypoplastic fissures, occlusal restorations, occlusal fissure sealants, extensive occlusal staining, and approximal caries close to the marginal ridge were excluded from the sample. Two sites on the same surface but distinctly separate were selected on each upper premolar (pits in proximal grooves) and three on each lower (pits in proximal grooves and the central pit) with the separation being at least 2 mm apart. The teeth yielded a total of 107 examination sites on their occlusal surfaces. Figure 1 shows a lower

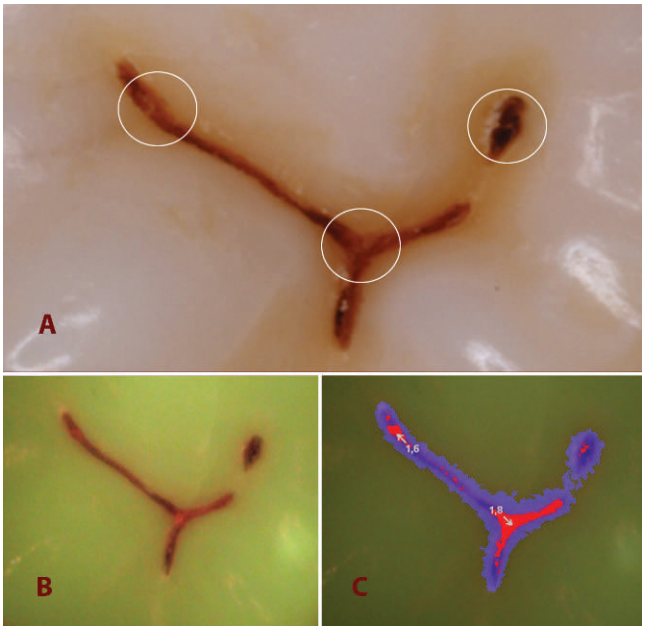

Figure 1. *(A): Original photo image of a second lower premolar with three selected occlusal sites. (B): Fluorescence image of the same occlusal surface. (C): Processed image for fluorescence analysis.*

premolar with three distinctly separate sites on its occlusal surface.

Examination sites on each tooth were recorded in digital photographs (3264 × 2448 pixels) using an Olympus digital camera at a magnification of 2×. A sketch of the surface with the selected areas was drawn on tracing paper for each tooth to assist the accurate histological preparation of the sites.

Examination Methods

The teeth were continuously stored in tap water and were removed and air dried for two to three seconds for examination. Four different examination methods were employed; two fluorescent and two visual. To reduce bias, calibration of the examiner was performed prior to the examination, each tooth was selected randomly and blindly for each method, and no standard sequence in examination method was followed. The examiner was assisted by a second trained person, who provided the samples.

Direct Visual Examination, DV (Control 1)—The selected sites were evaluated visually according to the caries scoring system shown in Table 1, by one calibrated examiner, under standard lighting conditions from a dental unit light and an observation distance of 30 cm, without the use of any visual aids.

Indirect Visual Examination, IDV (Control 2)—For the indirect visual examination, digital photographs of all occlusal surfaces were evaluated randomly on a computer screen (1280 × 1024) and scored using the same criteria as for the DV examination (Table 1). The initial photos were taken with an Olympus digital camera (E-500, Olympus Corp., Tokyo, Japan) and an Olympus digital 50-mm macro lens (plus 2× teleconverter) at a magnification of 2× (jpeg, 24-bit color, 3264 × 2448 pixels size) and viewed on a monitor screen (HPL1950) at a magnification of 8×, for a final magnification of 16×.

DIAGNOdent Pen Device, DP—The device was used according to the manufacturer's instructions, using the cylindrical tip suitable for occlusal surfaces. Calibration of the device was performed separately for every tooth to obtain precise measurements, Calibration against the reference occurred after every 10 teeth to minimize calibration shift. The tip was placed perpendicularly to the occlusal site and was rotated around its long axis to record the highest value. Three consecutive recordings were taken for each examination site, and their mean value was recorded as the final value for that site.

VistaProof device, VP—The device was employed using the long-distance spacer for optimum image quality of the occlusal surface according to the manufacturer's instructions. The optic sensor was facing downward, and the spacer was vertical to the occlusal surface. The video signal was digitized by the software, and a picture of 720 × 576 pixels with a resolution of 72 pixels/inch was created. The software (DBSWIN, Dürr Dental, Bietigheim-Bissingen, Germany) shows the pit and fissure areas that emit fluorescence and quantifies the red and green components of fluorescence (Figure 2). The analyzed pictures were saved to the connected computer, and the corresponding fluorescent values for each site were recorded.

Histological Examination, HIS (Gold Standard)—Following laser/light fluorescence measurements, teeth were mounted in transparent acrylic resin blocks covering the whole tooth surface. Each block was ground longitudinally in a buccolingual direction on a polishing machine (ECOMET III grinder, Buehler Ltd, AG, Uzwil, Switzerland) using silicon carbide paper of increasing grit number (wet-or-dry Tri-M-ite: 180- to 1200-grit in sequence) until the first examination site was reached. At the examination site, the specimen was polished using polishing cloths (DP/OP polishing cloths, Struers A/S, Ballerup, Denmark) with α-alumina polishing suspension (5 μm, Struers A/S, Ballerup, Denmark) to achieve a very smooth surface for evaluation. Each polished cut was examined under a stereomicroscope (Leitz Elvar, Esselte Leitz GmbH & Co KG, Stuttgard,

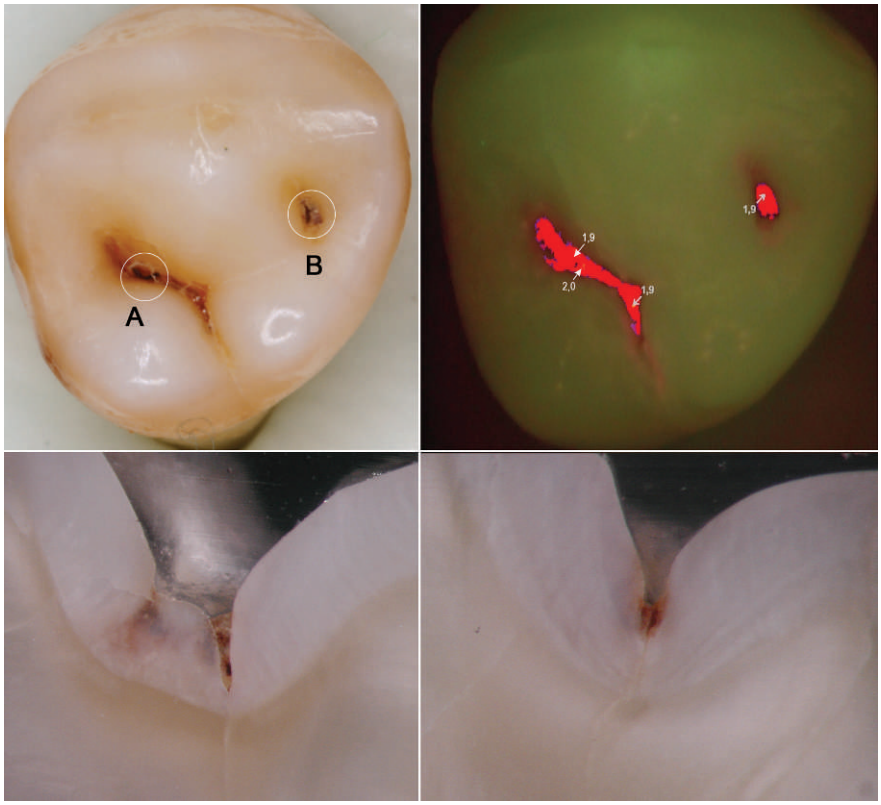

Figure 2. *(Upper left): Original photo image of a first lower premolar with two selected occlusal sites. (Upper right): Processed image of the surface for fluorescence analysis. (Lower left): Histological section of site A. (Lower right): Histological section of site B.*

Germany) at magnifications of 12.5× and 20× and photographed with a digital camera (Coolpix 990, Nikon Corp, Tokyo City, Japan). The depth of the lesions was evaluated microscopically and classified according to a five-grade caries scoring scale by the same single calibrated examiner (Table 1).

Cutoff Limits—To estimate the validity of the fluorescence devices, the original values obtained were transformed to a five-grade caries scoring scale as for all other methods. Cutoff points were calculated on the basis of the best agreement (Cohen's Kappa) between histological examination and measured values for each device (Table 1). For the VistaProof device, to determine the cutoffs that best agree with the lesion depth as found histologically, two different cutoffs for enamel and dentin involvement were used: the ones presently calculated (VPs) and those proposed by the manufacturer (VPm; Table 1).

Statistical Analysis

The validity (sensitivity, specificity, and accuracy) of the VP and DP devices was calculated with the statistical package SPSS version 15 (SPCC Inc, Chicago, IL, USA) using the histological examination as the reference method (gold standard). Differences between the accuracy of the examination methods were detected using McNemar's test at a 0.05 significance level. Comparison of validity among all diagnostic methods was made by estimating the receiver operating characteristic (ROC) curves and the area under the curve (AUC) in MedCalc version 9.0.1.1 (MedCalc Software, Broestraat 52, Mariakerke, Belgium).

Two measurements with all methods, separated by a one-week period, were used to estimate the reliability (reproducibility) of the methods using the ICC.

RESULTS

Intraexaminer Reliability

Calibration of the examiner was achieved by blind repeated measurements of 25 sites from teeth not included in the sample. The level of agreement was very good to high for all diagnostic methods.

Sample Distribution

The distribution of the 107 test sites for each diagnostic method according to their caries scoring

Table 2: *Distribution of the Examination Sites Into the Ekstrand 5-Caries Score Groups According to the Detection Methods*

Caries Score	DV	IDV	DP	VPm	VPs	HIS
D0	23	27	35	5	29	24
D1	31	35	29	50	18	37
D2	31	31	20	48	13	26
D3	22	14	23	4	47	20
Total	107	107	107	107	107	107

Abbreviations: DV, direct visual; IDV, indirect visual; DP, DIAGNOdent Pen; HIS, histological examination; VPm, VistaProof using manufacturer's cutoffs; VPs, VistaProof using calculated cutoffs.

is presented in Table 2. Regarding deep caries into dentin (D4), only two sites were detected histologically and thus were combined with the D3 lesions and are presented as D3.

Validity Estimation

Sensitivity, Specificity, and Accuracy—Sensitivity, specificity, and accuracy for all diagnostic methods based on histological examination as the reference method are shown in Table 3 and are reported for enamel (D1, D2, D1+D2), for dentin (D3), and for all caries categories (D1+D2+D3).

Comparing the two fluorescence devices for enamel lesions (D1, D2, or D1+D2), VPs had slightly higher numerical accuracy than DP, but for dentin lesions (D3), the opposite was found. However, there was no statistically significant difference ($p>0.05$) between the accuracy of DP and VPs for both enamel and dentin lesions.

Among the different examination methods, the highest accuracy both for enamel and dentin caries was found with IDV. Comparing IDV to the fluorescence devices, IDV showed statistically significantly higher accuracy for dentin lesions (D3; $p<0.05$).

ROC Analysis—The AUC for the different examination methods at all caries levels was estimated, and data are shown in Table 4. Figures 3 and 4 show the ROC curves of all methods in detecting enamel (Figure 3) or dentin lesions (Figure 4). All diagnostic methods had a significantly smaller AUC than histological examination ($p<0.001$) at all caries categories. Comparing the other diagnostic methods,

Table 3: *Sensitivity, Specificity, and Accuracy of the Diagnostic Methods, Based on Histological Examination, as the Reference Method*

Lesion Category		DV	IDV	DR	VPm	VPs
D1	sens	0.459	0.514	0.432	0.568	0.351
	spec	0.800	0.771	0.814	0.586	0.929
	accu	0.682[a]	0.682 [a]	0.682 [a]	0.579 [a]	0.729 [a]
D2	sens	0.500	0.692	0.308	0.462	0.269
	spec	0.778	0.840	0.852	0.556	0.926
	accu	0.710 [a]	0.804 [a]	0.720 [a]	0.553 [b]	0.766 [a]
D1+D2	sens	0.730	0.825	0.540	0.905	0.429
	spec	0.636	0.682	0.659	0.068	0.909
	accu	0.692 [a]	0.766 [a]	0.589 [b]	0.561 [b]	0.626 [ab]
D3	sens	0.750	0.650	0.550	0.000	0.950
	spec	0.919	0.989	0.862	0.954	0.678
	accu	0.888 [ab]	0.925 [a]	0.804 [bc]	0.776 [bc]	0.729 [c]
D1+D2 +D3	sens	0.879	0.879	0.795	0.976	0.855
	spec	0.542	0.708	0.750	0.125	0.708
	accu	0.804 [a]	0.841 [a]	0.785 [a]	0.785 [a]	0.822 [a]

Abbreviations: DV, direct visual; IDV: indirect visual; DP: DIAGNOdent Pen; VPm: VistaProof using manufacturer's cutoffs; VPs: VistaProof using calculated cutoffs.
Note: Same superscript letters on accuracy indicate no statistically significant difference at $\alpha=0.05$, among accuracy values of the same line, based on MacNemar's test.

IDV had the highest AUC for enamel lesions, and DV for dentin lesions, mostly but not always statistically significant with the other methods. Regarding the two fluorescence devices (DP and VPs), no statistically significant differences were found between their AUCs at all caries categories ($p>0.05$).

Comparisons of VP Methods With Different Cutoff Limits—The validity and the AUC of the VistaProof device based on the two different sets of cutoff limits, those given by the manufacturer (VPm) and those

Table 4: *Comparison of Receiver Operating Characteristic Curves of the Different Methods, Based on Histological Examination*

Caries Score Diagnostic Methods	AUC	SE	95% CI LB-UB	z Statistic	*p* Value
D1 lesion					
DV-HIS	0.630[a]	0.058	0.531-0.721	6.382	<0.001
IDV-HIS	0.642[a]	0.058	0.544-0.733	6.202	<0.001
DR-HIS	0.623[a]	0.058	0.524-0.715	6.473	<0.001
VPm-HIS	0.577[a]	0.059	0.477-0.672	7.174	<0.001
VPs-HIS	0.640[a]	0.058	0.541-0.730	6.237	<0.001
D2 lesion					
DV-HIS	0.639[a]	0.065	0.540-0.730	5.538	<0.001
IDV-HIS	0.766[a]	0.059	0.674-0.842	3.990	<0.001
DR-HIS	0.580[b]	0.066	0.480-0.675	6.358	<0.001
VPm-HIS	0.509[b]	0.066	0.410-0.607	7.497	<0.001
VPs-HIS	0.598[b]	0.066	0.498-0.691	6.101	<0.001
D1+D2 lesion					
DV-HIS	0.683[a]	0.051	0.586-0.770	6.206	<0.001
IDV-HIS	0.754[a]	0.046	0.661-0.832	5.344	<0.001
DR-HIS	0.599[ab]	0.055	0.500-0.693	7.291	<0.001
VPm-HIS	0.514[b]	0.057	0.415-0.611	8.528	<0.001
VPs-HIS	0.669[a]	0.052	0.571-0.757	6.386	<0.001
D3 lesion					
DV-HIS	0.835[a]	0.058	0.751-0.900	2.836	0.005
IDV-HIS	0.819[a]	0.060	0.733-0.887	3.001	0.003
DR-HIS	0.706[ab]	0.070	0.610-0.790	4.208	0.001
VPm-HIS	0.477[b]	0.071	0.424-0.620	6.705	0.001
VPs-HIS	0.814[a]	0.061	0.727-0.883	3.055	0.002

Table 4: Comparison of Receiver Operating Characteristic Curves of the Different Methods, Based on Histological Examination (cont.)

Caries Score Diagnostic Methods	AUC	SE	95% CI LB-UB	z Statistic	*p* Value
D1+2+3					
DV-HIS	0.711[a]	0.055	0.615-0.794	5.293	<0.001
IDV-HIS	0.794[a]	0.046	0.705-0.866	4.531	<0.001
DR-HIS	0.773[a]	0.048	0.681-0.848	4.729	<0.001
VPm-HIS	0.550[b]	0.066	0.451-0.647	6.857	<0.001
VPs-HIS	0.782[a]	0.047	0.692-0.856	4.643	<0.001

Abbreviations: AUC, area under the curve; CI, confidence interval; DV, direct visual; IDV: indirect visual; DP: DIAGNOdent Pen; HIS, histological examination; LB, lower bound; SE, standard error; UB, upper bound; VPm: VistaProof using manufacturer's cutoffs; VPs: VistaProof using calculated cutoffs)
Note: The superscript letters above AUC values indicate comparisons among diagnostic methods (vertical values). Same superscript letters indicate no difference at α=0.05 level of significance (p>0.05).

calculated from the data of this study (VPs), are presented in Tables 3 and 4. The accuracy for VPs and VPm was not statistically significantly different for both enamel and dentin lesions. However, the AUC for VPs is statistically significantly greater than VPm for both enamel and dentin lesions. It should also be mentioned that VPm accuracy is misleading, as the VPm sensitivity for dentin lesions was zero. All of the above suggest that cutoffs calculated from the present data may be more accurate than the ones proposed by the manufacturer.

Reliability-Reproducibility of Diagnostic Methods

Assuming low intraexaminer variation (as demonstrated by the calibration sample), the reproducibility of the diagnostic methods was estimated by the reliability coefficient (ICC) of the two sets of repeated

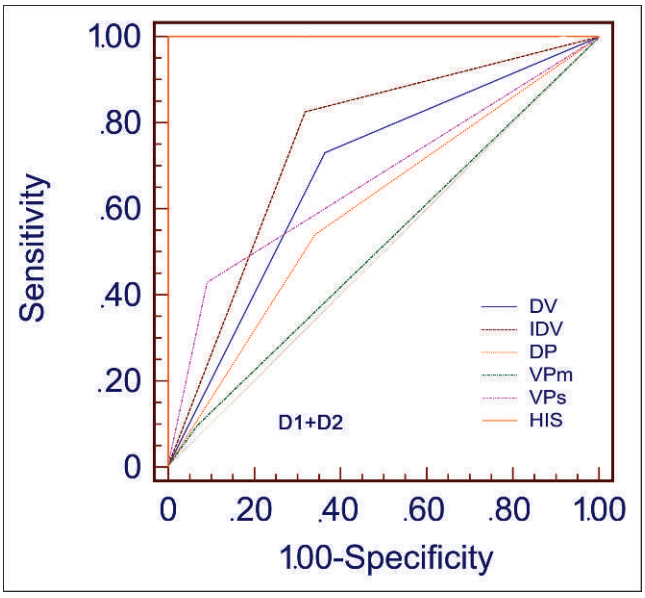

Figure 3. *Receiver operating characteristic curves of the examination methods for the detection of enamel (D1+D2) lesions.*

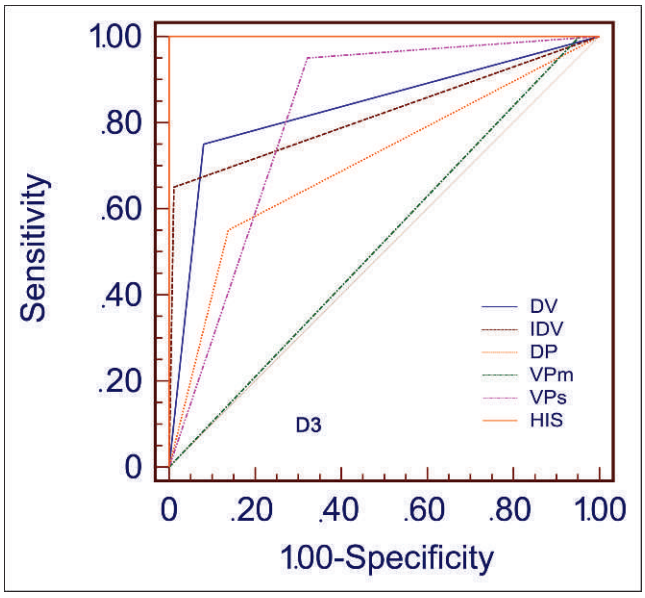

Figure 4. *Receiver operating characteristic curves of the examination methods for the detection of dentin (D3) lesions.*

Table 5: *ICCs of the Diagnostic Methods With the Lower and Upper Bounds of a 95% CI*

Diagnostic Method	ICC*	95% CI LB-UB
DV	0.996[a]	0,994-0,997
IDV	0.988[b]	0.982-0.992
DR	0.982[b]	0.971-0.988
VPm	0.887[c]	0.828-0.925
VPs	0.937[c]	0.905-0.958
HIS	0.975[b]	0.963-0.983

Abbreviations: CI, confidence interval; DV, direct visual; ICC, intraclass correlation coefficient; IDV: indirect visual; DP: DIAGNOdent Pen; HIS, histological examination; LB, lower band; UB, upper bound; VPm: VistaProof using manufacturer's cutoffs; VPs: VistaProof using calculated cutoffs.
** Average measures of ICC estimated under an absolute agreement definition, and differences were based on nonoverlapping 95% CIs. ICCs with the same superscript letter are not significantly different (p>0.05).*

measurements and was found to be excellent for all examination methods (Table 5), with the most reliable being DV. Comparing the reliability coefficient of the two fluorescence devices, DP was statistically significantly more reliable than VPs.

DISCUSSION

In this study, the two fluorescence devices were tested in permanent teeth to test, under *in vitro* conditions, their validity and reliability for occlusal caries detection, using histological examination as the gold standard. The accuracy of both devices was not statistically significantly different. DIAGNOdent Pen was, however, more reliable when compared with VistaProof, at a statistically significant level.

Regarding the design of the study, the visual caries scoring system used was based on the one proposed by Ekstrand and others[6] to allow for more comparisons with previous studies.

The selection of cutoff limits for both the DIAGNOdent Pen and VistaProof device for D1, D2, and D3 caries lesions was based on the best K-agreement of the devices' values with those from histological examination, a method previously used in caries studies.[32] The calculated cutoff limits of our study appear slightly higher than the ones used previously, mainly for lesions into dentin (D3), and may be attributed to differences in the cutoff selection method used, teeth storage medium, pit remnants, or drying time before each measurement.[11]

Cutoff limits for the DIAGNOdent Pen have been studied previously[32] and are close to the ones proposed by the manufacturer. For VistaProof, however, cutoffs have not been extensively studied, nor have the ones proposed by the manufacturer been tested.[11] Applying the manufacturer's cutoff limits in the present sample, no dentin lesions could be detected, suggesting that limits should be reevaluated, especially when detecting more advanced lesions. Discrepancies with the manufacturer's cutoffs have been also found by Rodrigues and others,[11] in which the calculated cutoffs for dentin lesions were smaller than the value of 2.0 proposed by the manufacturer. In our study, the cutoffs between D1-D2-D3 lesions have a narrow range, from 1.3 to 1.6 units, which makes proper discrimination between lesion categories difficult; thus, a more refined scale is needed for such discrimination. Until this is accomplished, its use would be more appropriate for discriminating D1 and D3 lesions. Cutoff limits found in this study by comparison to histological sections are thus specific to this sample and cannot be extrapolated to a clinical situation, indicating that further studies are needed to confirm or to reject the above limits. With cutoff limits differing widely between enamel and dentin lesions, clinical usage of fluorescence detection devices using a single compromise value may alter the calculated values and result in reduced accuracy. In clinical situations, this is even more difficult because of plaque presence, different bacteria metabolic by-products, and influence from the environment.

The results of the present study indicated no validity differences between the two devices and for all caries categories, since their accuracy values and AUCs were not statistically significant different. No difference in validity between the two devices was found in a previous study on permanent teeth,[11] although that study was based on only D3 lesions. Comparing all four methods, the present study shows DV/IDV having significantly better accuracy than DP for enamel lesions and DV/IDV having significantly better accuracy than VPs for dentin lesions. These results suggest that fluorescence methods for the detection of occlusal caries in permanent teeth were not superior to, and in certain cases worse than, visual methods. This may be attributed to the effect of stain inclusion in the lesions as Reis and others[33] suggested, resulting in higher measurement values and consequently in higher rates of false-positives with both devices.

Systematic reviews indicated variability in the accuracy of visual[34] and light fluorescence methods.[35] Comparisons of DIAGNOdent with the visual method[35] showed the former to be more specific and less sensitive for detecting occlusal caries. This is evident in our study and consistent with the newer device, DiagnoDent Pen (DP), which also showed greater specificity and lower sensitivity compared with DV. Results of the present study and the lack of homogeneity of evidence that is offered in the systematic reviews[35,36] stress the need for studies with generally accepted standardized methodologies and evaluation criteria.

The high accuracy of indirect visual examination found in this study was also found previously for primary teeth,[28] and this may have clinical implications. Visual diagnostic methods, such as the use of high-resolution intraoral cameras, might be promising for caries diagnosis in the future, especially with the use of a dedicated image analysis software.[31] However, more research is required before a definite conclusion is drawn on their use.

Findings of this study suggest that VP performs better for dentin (high sensitivity) than for enamel lesions (high specificity). This can be explained by the following: the red fluorescence, monitored by the device as caries, is related to a more advanced or deeper carious lesion since this fluorescence comes from microbial metabolic products,[37] expected to exist in greater amounts in larger or deeper cavities. Furthermore, the scattering of fluorescence light in early demineralized enamel areas cannot be quantified by the device so it has a lower performance for early enamel lesions. The red fluorescence has been suggested during demineralization to derive from exposed tooth matrix elements that have interacted with mutans streptococci and have unmasked fluorophores exhibiting strong fluorescence in red[38]. This probably means that the device is not sensitive enough to monitor this red fluorescence, which is scarce in early lesions.

The low sensitivity of VistaProof (VPs) for enamel lesions and its moderate specificity for dentin lesions imply that it can be combined with indirect visual examination to increase its diagnostic validity for the detection of occlusal caries in permanent teeth, since IDV has high sensitivity for enamel and high specificity for dentin lesions. Although the present results cannot be extrapolated to *in vivo* conditions, such a combination could be helpful in clinical situations. A similar combination of IDV with DIAGNOdent has worked well for primary teeth.[39]

DIAGNOdent Pen showed an overall higher specificity and lower sensitivity both for enamel and dentin lesions, suggesting that it might be more useful for the detection of healthy sites. Most of the validity values calculated in this study for DIAGNOdent Pen are within the range of previous studies, except for the sensitivity of enamel lesions, which was found to be much lower.[11,13,15,40] This may be attributed to parameters such as sample selection (eg, normal distribution of the selected teeth in the caries categories and exclusion of teeth with D4 lesions), storage techniques (eg, teeth stored in tap water and not frozen), specimen preparation techniques (eg, only rubber caps for cleaning), reference methods used, and differences in histological evaluation (no stains used).

Reliability of both devices was very good, according to their ICC values. The clinical significance of the above finding suggests that both devices could be used for long-term monitoring of the carious process. Furthermore, DIAGNOdent Pen had a significantly higher ICC value than VistaProof, suggesting that it is more reliable than VistaProof. This finding is in agreement with the study of Rodrigues and others,[11] in which reliability was reported for only D3 lesions.

Results of this study suggest that laser fluorescence, at least *in vitro*, is not superior to the visual methods for occlusal caries detection in permanent teeth. The two fluorescence devices may have different discrimination ability in clinical situations between intact, enamel, or dentin lesions. It is important to point out that although both fluorescence devices have advantages over the commonly used detection methods, they also have limitations. Confounding factors for laser/light fluorescence measurements, such as plaque, stains, and defects in tooth development, were eliminated in this study; however, clinical conditions could not be simulated, underlying the limitations of an *in vitro* study and the need for further research on the performance of the devices *in vivo*.

CONCLUSIONS

1) There was no statistically significant difference in the accuracy and AUC between DIAGNOdent Pen and VistaProof for both enamel and dentin lesions.
2) The accuracy of direct and indirect vision was significantly higher than DIAGNODent Pen for enamel lesions (D1+D2) and than VistaProof for dentin lesions (D3).
3) Reliability (reproducibility) of both fluorescence devices was excellent, but the DIAGNOdent Pen

was higher than VistaProof. Direct vision presented the highest reliability among all methods and VistaProof the lowest.

4) VistaProof cutoffs proposed in this study (VPs) resulted in a better performance of the device for the detection of enamel and dentin lesions as compared with those proposed by the manufacturer (VPm).

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