Research Square

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DIAGNOSTIC ACCURACY IN SELECTIVE CARIES REMOVAL: An In Vitro Comparison Between DIAGNOcam Vision Full HD Fluorescence Imaging and Caries Detector Dyes Using Histological Validation

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Abstract

Objectives

To compare the diagnostic performance and interobserver agreement of visual examination, fluorescence evaluation using DIAGNOcam Vision Full HD, caries detector dye, and post dye fluorescence combined evaluation for detecting residual carious tissue following selective caries removal, and to assess associations with histological lesion extension.

Materials and Methods

Forty-four extracted human premolars and molars with carious lesions were assessed on four cavity walls by two calibrated observers using the four methods and a standardized 0–100 ordinal percentage scale. Histological lesion extension was measured microscopically after sectioning. Interobserver agreement was assessed using quadratic weighted Cohen's kappa. Diagnostic scores were compared using the Friedman test and Bonferroni-adjusted Wilcoxon signed-rank tests. Baseline and post-dye fluorescence were compared and correlated using Spearman's correlation. Associations between histological lesion extension and diagnostic scores were evaluated using linear regression and ordinal mixed-effects models.

Results

Overall interobserver agreement was substantial ($\kappa w = 0.686$), with almost perfect agreement for fluorescence ($\kappa w = 0.861$) and combined evaluation ($\kappa w = 0.873$), moderate agreement for visual examination ($\kappa w = 0.563$), and fair agreement for dye ($\kappa w = 0.392$). Diagnostic scores differed significantly among methods for both observers ($p < 0.001$). Baseline and post-dye fluorescence scores also differed significantly (adjusted $p < 0.001$) but remained positively correlated ($\rho = 0.499$ and 0.644; both $p < 0.001$). Greater histological lesion extension was associated with higher scores for all methods, with no significant method-by-lesion-extension interaction ($p = 0.075$)

Conclusion

DIAGNOcam fluorescence demonstrated high reproducibility for assessing residual carious tissue. Caries detector dye significantly altered subsequent fluorescence measurements. Histological lesion extension was positively associated with diagnostic scores across methods.

Clinical Relevance:

The findings support the use of DIAGNOcam fluorescence imaging as a reliable assessment tool during selective caries removal, potentially helping clinicians assess residual carious tissue more consistently than visual assessment alone. However, clinicians using both fluorescence techniques should distinguish between baseline and post dye fluorescence when making decisions about residual carious tissue. These findings may help support more standardized clinical assessment while avoiding unnecessary additional dentin removal during minimally invasive caries management.

1. Introduction

Dental caries remains one of the most prevalent chronic diseases worldwide and continues to represent a major challenge for restorative dentistry despite advances in preventive strategies (1). The current understanding of dental caries as a biofilm-mediated, dysbiotic disease influenced by behavioral and dietary factors has shifted operative treatment away from the traditional surgical concepts introduced by G.V. Black toward the principles of Minimal Intervention Dentistry (MID**)** (2, 3). This contemporary approach emphasizes maximum preservation of healthy dental tissues while maintaining long-term tooth vitality through conservative management of carious lesions.

Within the MID philosophy, selective caries removal has become the preferred treatment for deep dentinal lesions because it significantly reduces the risk of accidental pulp exposure without compromising clinical outcomes (4). This approach is based on the biological distinction between infected dentin, which is irreversibly demineralized, heavily contaminated by bacteria, and lacks remineralization potential, and affected dentin, which is only partially demineralized, retains an intact collagen matrix, and can be remineralized (5–9). Consequently, complete removal of infected dentin while preserving affected dentin has become the primary objective of conservative operative dentistry.

Despite its widespread acceptance, selective caries removal remains highly dependent on the clinician's ability to distinguish between these two dentin substrates. In routine clinical practice, excavation is primarily guided by visual characteristics, dentin color, and tactile sensation (5). However, these criteria are subjective and poorly reproducible because dentin discoloration does not necessarily reflect bacterial contamination, while tactile assessment depends on operator experience and the force applied during excavation (6, 7, 10). As a result, clinicians may either overexcavate, unnecessarily removing remineralizable dentin and increasing the risk of pulp exposure, or underexcavate, leaving infected tissue beneath the restoration.

Several adjunctive diagnostic methods have been proposed to improve the objectivity of selective caries removal. Caries detector dyes which typically consist of acidic dyes, such as basic fuchsin, 1% acid red, or acid green, dissolved in a propylene glycol or polyethylene glycol vehicle (11) identify altered dentin by staining disrupted collagen matrices and have been widely incorporated into clinical practice because of their simplicity and low cost (12). Nevertheless, previous studies have demonstrated that these dyes may penetrate affected dentin, producing false-positive results that can lead to unnecessary tissue removal (9, 13). Fluorescence-based technologies have emerged as a promising alternative by detecting

optical changes associated with bacterial metabolites, providing an objective and non-ionizing method for identifying residual carious tissue (14–16). Although encouraging results have been reported, evidence directly comparing fluorescence imaging with caries detector dyes during selective caries removal under standardized experimental conditions remains limited.

Furthermore, little is known about whether the application of caries detector dyes influences fluorescence measurements or whether the histological extension of a carious lesion affects the ability of different diagnostic methods to identify residual carious tissue. Addressing these questions may contribute to the development of more objective and reproducible protocols for selective caries removal.

Therefore, this in vitro study aimed to compare the diagnostic performance of conventional visual examination, DIAGNOcam Vision Full HD fluorescence imaging, caries detector dye, and the combined use of both methods for detecting residual carious tissue following selective caries removal, using histological evaluation as the reference standard. In addition, histological measurements of caries lesion length were used to investigate whether lesion extension influences the ability of each diagnostic method to detect residual carious tissue.

The null hypothesis was that there would be no significant diagnostic differences among the four diagnostic methods, application of the caries detector dye would not significantly alter fluorescence based diagnostic scores and histological lesion extension would not be significantly associated with diagnostic scores, and that this association would not differ among the four diagnostic methods.

2. Materials and Methods

This in vitro experimental study was approved by the Ethics Committee of the Hospital Clínico San Carlos, Madrid, Spain. With the following protocol approval number: 26/478-IC_P_CE and the following approval date: 02/06/2026.

To ensure donor confidentiality and anonymity, each tooth was randomly assigned a numerical identification code immediately after collection, making it impossible to trace any specimen back to the identity or clinical history of the donor. Informed consent was obtained from all donors prior to specimen use.

This study followed an exploratory laboratory design. A sample size calculation was not performed prior to the study. The number of specimens was based on availability of extracted teeth that met the inclusion criteria and is comparable to similar studies. Future investigations incorporating power analysis are recommended

A total of 44 extracted human premolars and molars presenting carious lesions on occlusal or proximal surfaces clinically classified as ICDAS 4 or ICDAS 5 were collected. Teeth with endodontic treatment, restorations, fractures or abnormal dental anomalies were excluded. The sample size was determined by

specimen availability and was comparable to previous in vitro studies evaluating caries detection methods.

2.1 Sample Preparation and Standardization

Following extraction, teeth were disinfected in 70% ethanol, cleaned using periodontal scalers and prophylaxis brushes to remove soft tissue remnants and calculus, and stored in distilled water at room temperature until use.

Furthermore, each tooth was individually embedded in a polymethyl methacrylate (PMMA) block with the crown positioned vertically and parallel to the longitudinal axis of the block to ensure stability during the optical evaluations.

Each tooth underwent a standardized periapical radiographic examination to assess the depth of the carious lesion and to confirm its suitability for inclusion. The ICDAS classification (scores 4 or 5) was determined by visual clinical examination according to the International Caries Detection and Assessment System (ICDAS II), with radiographic findings used as an adjunct to support lesion assessment.

2.2 Selective Caries Removal

The excavation procedure was designed to simulate a clinical selective caries removal protocol, reproducing the decision making process and clinical endpoints used during routine minimally invasive caries management.

Initial access to the cavity was achieved using standardized fine-grit round diamond burs (Komet, Lemgo, Germany) of the same size under high-speed rotation with continuous water cooling.

To maintain cutting efficiency and minimize variability associated with bur wear.

throughout the excavation procedure, the diamond bur was replaced after every four teeth.

Throughout the excavation procedure, a periodontal probe was periodically used to assess the consistency of the remaining dentin and to determine whether further carious tissue removal was required according to the predefined clinical endpoint.

The procedure was completed once the operator reached the following endpoints:

- Firm dentin on the cavity walls for ICDAS 4 lesions.
- Affected dentin with a soft or leathery consistency preserved over the floor for ICDAS 5 lesions, while avoiding iatrogenic pulp exposure.

2.3 Diagnostic Recording Methodology (100-Point Percentage Ordinal Scale)

To ensure standardized data collection, a 100-point percentage ordinal scale (0–100) was used.

to estimate the proportion of each cavity wall affected by carious tissue. The score represented the estimated extent of carious tissue within each individual cavity wall and was not intended to represent a histological measurement or an exact mathematical percentage.

Each of the four cavity walls (distal, mesial, palatal/lingual, and buccal) was independently assessed by two calibrated examiners using each of the four diagnostic methods. The extent of carious tissue identified on each wall was recorded using the following scale:

The scale was defined as follows:

- **0–25**: Carious tissue involving approximately 0–25% of the cavity wall.
- **25–50**: Carious tissue involving approximately 25–50% of the cavity wall.
- **50–75**: Carious tissue involving approximately 50–75% of the cavity wall.
- **75–100**: Carious tissue involving approximately 75–100% of the cavity wall.

2.4 Blinded Sequential Diagnostic Evaluation

A blinded sequential assessment design was implemented for the four diagnostic methods:

1. **Visual-tactile Examination**: The cavity wall was assessed visually and by tactile examination and assigned an ordinal score.
2. **Fluorescence Evaluation (DIAGNOcam Vision Full HD, KaVo, Biberach, Germany)**: Digital image acquisition under standardized lighting conditions after briefly drying the cavity with the three-way air-water syringe.
3. **Caries Detector Dye Evaluation**: Passive application of the dye (Kuraray Kurashiki, Japan), for 30 seconds, followed by thorough rinsing with water for 10 seconds and complete drying before recording residual staining. During scoring, only areas showing visible dye staining were recorded as positive findings. Areas in which carious tissue was clinically present but did not exhibit dye staining were not recorded as positive by the dye method.
4. **Combined Evaluation**: Fluorescence images were acquired after dye application to assess possible chromatic interference or synergistic effects on the optical signal.

2.5 Data Collection

For each specimen, both calibrated examiners independently assessed the mesial, distal, buccal, and palatal/lingual cavity walls using each of the four diagnostic methods in the predefined sequence. Each cavity wall received a score according to the previously described 100-point percentage ordinal scale (0–25, 25–50, 50–75, and 75–100), and the scores were recorded separately for each diagnostic method.

The two examiners were blinded to each other's scores throughout the study.

2.6 Histological Sectioning

Following completion of the diagnostic assessments, all specimens were processed for histological analysis, with sectioning oriented along the axis corresponding to the greatest clinical lesion extension.

Each specimen was sectioned vertically using a water-cooled diamond saw (EXAKT 400 EXACT Advances Technologies, Norderstedt, Germany.).

Sections were polished with silicon carbide papers (grit up to 1,200) under water irrigation.

Sections were examined under transmitted light microscopy at 8× magnification (LEICA MZ 12 LEICA, Wetzlar, Germany), and caries length was measured in micrometers using an image analysis system.

2.7 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics. A two-sided p-value < 0.05 was considered statistically significant. Because each cavity wall was evaluated independently, the four cavity walls were treated as separate observational units.

Descriptive statistics were calculated for each diagnostic method and observer, with median and interquartile range (IQR) reported for ordinal diagnostic scores. Interobserver agreement was assessed using quadratic weighted Cohen's kappa (κw) with 95% confidence intervals.

Differences among the four diagnostic methods were assessed separately for each observer using the Friedman test. Significant overall differences were followed by pairwise Wilcoxon signed-rank tests with Bonferroni adjustment. Rank-biserial correlation (r) was reported as the effect size.

Baseline and post-dye fluorescence scores were compared using the Wilcoxon signed-rank test, and Spearman's rank correlation coefficient (ρ) was used to assess their association.

The relationship between histological lesion length and diagnostic scores was assessed using regression and mixed-effects ordinal models, accounting for ICDAS category, diagnostic method, cavity-wall location, observer, and within tooth clustering. An interaction between lesion length and diagnostic method was included to determine whether the association between lesion extension and diagnostic scores differed among the four methods.

3. Results

3.1. Study Sample and Diagnostic Scores

The analysis included 44 teeth, with each tooth evaluated across four cavity walls, resulting in 176 tooth wall observations. Four diagnostic methods were assessed independently by two observers, resulting in 1,408 observer level diagnostic scores. The recorded ICDAS categories were 4 in 37 teeth (84.1%), 5 in 7 teeth (15.9%).

The four diagnostic methods consisted of visual examination, fluorescence evaluation using DIAGNOcam, caries detector dye, and combined evaluation using both DIAGNOcam and caries dye. Score distributions were non-normal for all combinations according to the Shapiro Wilk test (all $p < 0.001$). Therefore, medians and interquartile ranges were emphasized. The median score was 25 for all four methods and both observers as shown in Table 1.

Table 1
Descriptive Statistics for Diagnostic Scores by Method and Observer

Method	Observer	n	Median	IQR	Mean ± SD	Range
Visual examination	1	176	25	0–25	26.1 ± 23.1	0–100
Visual examination	2	176	25	0–50	25.1 ± 29.1	0–100
Fluorescence evaluation	1	176	25	0–50	28.6 ± 27.0	0–100
Fluorescence evaluation	2	176	25	0–50	28.7 ± 29.9	0–100
Caries detector dye	1	176	25	0–25	17.2 ± 18.7	0–100
Caries detector dye	2	176	25	0–50	33.2 ± 31.0	0–100
Combined evaluation	1	176	25	25–50	37.8 ± 25.8	0–100
Combined evaluation	2	176	25	25–50	41.3 ± 29.5	0–100

For Observer 1, mean scores were 26.1 ± 23.1 for visual examination, 28.6 ± 27.0 for fluorescence, 17.2 ± 18.7 for caries detector dye, and 37.8 ± 25.8 for combined evaluation. For Observer 2, corresponding scores were 25.1 ± 29.1, 28.7 ± 29.9, 33.2 ± 31.0, and 41.3 ± 29.5. All methods had a range of 0–100. The combined evaluation yielded the highest mean scores for both observers, whereas the largest difference between observers was observed for the caries detector dye.

3.2. Interobserver Agreement

Overall exact agreement was 68.6%, with substantial interobserver agreement ($\kappa w = 0.686$; 95% CI, 0.620–0.747).

Agreement was highest for combined evaluation ($\kappa w = 0.873$; 95% CI, 0.814–0.917) and fluorescence evaluation ($\kappa w = 0.861$; 95% CI, 0.790–0.911), followed by visual examination ($\kappa w = 0.563$; 95% CI, 0.384–0.717) and caries detector dye ($\kappa w = 0.392$; 95% CI, 0.271–0.508). Exact agreement was 79.0%, 73.9%, 63.6%, and 58.0%, respectively.

Across cavity wall locations, κw ranged from 0.604 to 0.793. The highest method-by-wall agreement was observed for combined evaluation at the distal wall ($\kappa w = 0.896$; 95% CI, 0.745–0.983), while the lowest was observed for caries detector dye at the palatal wall ($\kappa w = 0.133$; 95% CI, − 0.041–0.378).

3.3. Effect of Caries Detector Dye on Fluorescence Based Scores

To evaluate whether application of the caries detector dye influenced the fluorescence based diagnostic assessment, fluorescence evaluation scores were compared with fluorescence scores obtained after dye application.

Baseline and post-dye fluorescence scores differed significantly for both observers (Observer 1: adjusted $p < 0.001$, $r = -0.505$; Observer 2: adjusted $p < 0.001$, $r = -0.682$).

Spearman's correlation showed a significant positive association between baseline and post-dye fluorescence scores for Observer 1 ($\rho = 0.499$, $p < 0.001$) and Observer 2 ($\rho = 0.644$, $p < 0.001$). Thus, although scores changed following dye application, higher baseline scores tended to remain associated with higher post-dye scores.

3.4. Comparison of Diagnostic Methods

The Friedman test demonstrated statistically significant differences among the four diagnostic methods for both observers. For Observer 1, the Friedman statistic was $\chi^2(3) = 99.515$ ($p < 0.001$; Kendall's $W = 0.188$), while for Observer 2 it was $\chi^2(3) = 85.417$ ($p < 0.001$; Kendall's $W = 0.162$).

Bonferroni adjusted pairwise comparisons between diagnostic methods was done as shown in Table 2.

Table 2
Bonferroni Adjusted Pairwise Comparisons Between Diagnostic Methods

Obs.	Method 1	Method 2	n	Wilcoxon W	Adjusted p	Rank-biserial r	Significant
1	Visual examination	Fluorescence evaluation	176	2281.0	= 1.000	-0.132	No
1	Visual examination	Caries detector dye	176	2064.5	< .001	0.412	Yes
1	Visual examination	Combined evaluation	176	1472.0	< .001	-0.518	Yes
1	Fluorescence evaluation	Caries detector dye	176	1362.0	< .001	0.537	Yes
1	Fluorescence evaluation	Combined evaluation	176	1275.5	< .001	-0.505	Yes
1	Caries detector dye	Combined evaluation	176	241.0	< .001	-0.922	Yes
2	Visual examination	Fluorescence evaluation	176	1172.0	= .115	-0.277	No
2	Visual examination	Caries detector dye	176	1742.5	< .001	-0.408	Yes
2	Visual examination	Combined evaluation	176	743.0	< .001	-0.748	Yes
2	Fluorescence evaluation	Caries detector dye	176	2008.0	= .103	-0.250	No
2	Fluorescence evaluation	Combined evaluation	176	867.5	< .001	-0.682	Yes
2	Caries detector dye	Combined evaluation	176	1272.5	< .001	-0.453	Yes

For Observer 1, five of six pairwise comparisons were statistically significant, with no significant difference between visual examination and fluorescence evaluation (adjusted p = 1.000; r = − 0.132). For Observer 2, four of six comparisons were significant; visual examination did not differ significantly from fluorescence evaluation (adjusted p = 0.115; r = − 0.277) or caries detector dye (adjusted p = 0.103; r = − 0.250). All other significant pairwise comparisons are presented in Table 2.

3.5. Association Between Diagnostic Scores and Histological Lesion Extension

At the tooth level, the two observer scores were averaged for each tooth wall method combination, and the maximum score among the four cavity walls was selected for each tooth and diagnostic method.

This resulted in one aggregated diagnostic score per method for each of the 44 teeth. Four separate linear regression models were fitted using square root transformed histological measurements as the dependent variable, with ICDAS and the corresponding diagnostic score as predictors as shown in Table 3.

Table 3
Tooth Level Linear Regression Models Predicting Square Root Transformed Histological Measurements.

Diagnostic method	B per 25 points	HC3 SE	95% CI	p	Standardized β	Adjusted R^2	Residual Shapiro p
Visual examination	13.646	3.038	7.510–19.782	< .001	0.622	0.476	0.778
Fuorescence evaluatiion	12.014	2.838	6.283–17.744	< .001	0.632	0.460	0.032
Caries detector dye	10.994	4.224	2.465–19.524	= .013	0.473	0.305	0.908
Combined evaluation	10.688	4.002	2.606–18.771	= .011	0.511	0.305	0.191

Higher diagnostic scores were significantly associated with greater histological measurements for all four diagnostic methods. The regression coefficient per 25 point increase in diagnostic score was 13.646 for visual examination ($p < 0.001$), 12.014 for fluorescence evaluation ($p < 0.001$), 10.994 for caries detector dye ($p = 0.013$), and 10.688 for combined evaluation ($p = 0.011$). All corresponding 95% confidence intervals excluded zero.

The strongest standardized associations were observed for fluorescence evaluation ($\beta = 0.632$) and visual examination ($\beta = 0.622$). The adjusted R^2 values were 0.476 for visual examination, 0.460 for fluorescence evaluation, and 0.305 for both caries detector dye and combined evaluation. ICDAS was not an independent significant predictor in any of the four models.

3.6. Association Between Diagnostic Scores For each Method and Histological Lesion Extension

An ordinal cumulative mixed effects model was fitted to all 1,408 diagnostic scores, accounting for repeated measurements within teeth. The model included histological measurement, ICDAS score, diagnostic method, cavity wall and observer, with a random intercept for tooth. A second model additionally included the interaction between histological measurement and diagnostic method.

In the mixed effects analysis, histological lesion measurement was significantly associated with higher diagnostic-score categories (OR = 1.493; 95% CI, 1.321–1.687; $p < 0.001$). However, the histological measurement by diagnostic method interaction was not statistically significant ($p = 0.075$), indicating

that the strength of the association between lesion extension and diagnostic scores did not differ significantly among the four diagnostic methods.

4. Discussion

The present in vitro study evaluated four diagnostic approaches for the detection of residual carious tissue following selective caries removal. Overall, the findings indicate that the diagnostic scores were influenced by the method used, with significant differences observed among the four methods for both observers.

The findings were broadly consistent with previous studies evaluating fluorescence based approaches for the detection of residual carious tissue. Peskersoy et al. (17) and Koç Vural et al. (18) reported that fluorescence aided caries excavation (FACE) detected residual or partially removed carious areas more frequently than conventional visual–tactile or magnification assessment and caries detector dye. Sadasiva et al. (19) further compared visual-tactile assessment, caries detector dye, and laser fluorescence and investigated the findings against microbiological confirmation, supporting the use of fluorescence as an adjunct to conventional assessment. More recently, Abba et al. (20) compared visual–tactile assessment, caries detector dye, and DIAGNOdent in a randomized clinical trial and reported the highest diagnostic accuracy and agreement with bacteriological confirmation for DIAGNOdent. Together, these studies support the potential value of fluorescence based methods as adjunctive tools for identifying residual carious tissue. However, the present study differs from these investigations as it evaluated visual examination, caries detector dye and directly compared baseline fluorescence images with fluorescence images obtained after caries detector dye application.

The lower interobserver agreement observed for the caries detector dye in the present study is also consistent with the variability reported for dye based caries detection. Although caries detector dyes are intended to identify irreversibly damaged dentin, their staining response depends on the characteristics of the dentinal substrate and may extend beyond areas of active or irreversibly infected tissue which can result in removal of greater amounts of dentin than approaches based on tissue hardness or other diagnostic criteria (9, 21). In comparative studies, fluorescence based methods have also identified residual carious tissue that was not detected by caries detector dyes. Peskersoy et al. (17) for example, found that fluorescence assisted examination identified substantially more residual carious areas than caries detector dye after conventional excavation. These findings may help explain the lower reproducibility of caries detector dye scoring observed in the present study, particularly because the current protocol recorded only visibly stained carious tissue and did not classify unstained carious tissue as positive.

An important finding of the present study was the significant difference between baseline and post dye fluorescence scores observed for both observers. This indicates that application of the caries detector dye altered the fluorescence based diagnostic signal obtained with DIAGNOcam Vision Full HD. Previous studies have investigated fluorescence and caries detector dyes as complementary or alternative

approaches for detecting residual carious dentin, but their primary focus has been on diagnostic performance or the amount of residual caries identified rather than on the direct effect of dye application on a subsequent fluorescence measurement. For example, Sadasiva et al. (19) evaluated changes in laser fluorescence measurements during different stages of caries removal in conjunction with visual–tactile assessment and caries detector dye. Therefore, the present finding provides an additional consideration when these two diagnostic approaches are used sequentially. Although baseline and post dye fluorescence scores remained significantly and positively correlated, the significant difference between the two measurements indicates that the post dye fluorescence signal should not be interpreted as equivalent to the baseline fluorescence measurement. The mechanism underlying this alteration cannot be determined from the present study and may relate to optical changes produced by the presence of the dye within the dentinal substrate and for that further experimental studies specifically designed to characterize the optical interaction between caries detector dyes and fluorescence imaging are needed.

The significant association between histological lesion extension and higher diagnostic scores observed in the present study is also supported by previous investigations of fluorescence based caries detection. Mendes et al. (22) reported a strong correlation between DIAGNOdent readings and histological lesion depth in smooth-surface caries, with fluorescence measurements showing a stronger relationship with lesion depth than with mineral loss. Similarly, Lussi et al. (23) demonstrated that laser fluorescence readings were reproducible between examiners and showed a moderate correlation with histological lesion depth. More recently, Kreher et al. (24) reported a significant correlation between laser fluorescence values and lesion depth measured by micro-computed tomography in non cavitated root caries, with fluorescence values increasing as demineralization depth increased. These findings are consistent with the present observation that greater histological lesion extension was associated with higher diagnostic scores. However, the present study extends this observation by evaluating lesion extension in relation to four different diagnostic methods at the individual cavity-wall level. Importantly, the non-significant interaction between lesion extension and diagnostic method ($p = 0.075$) indicates that the strength of this relationship did not differ significantly among the four methods. Therefore, while increasing lesion extension was reflected by increasing diagnostic scores, the present data does not demonstrate that fluorescence, dye, or visual–tactile assessment was more strongly related to lesion extension than the combined method.

The findings of the present study have several potential clinical implications for selective caries removal. Determining the appropriate endpoint of caries excavation remains challenging because conventional visual–tactile assessment is subjective and may vary according to the operator’s interpretation of dentin characteristics (25). Fluorescence based approaches may provide clinicians with an additional, more standardized source of information when the endpoint of excavation is uncertain. Previous studies have shown that fluorescence assisted methods can identify residual carious tissue that may not be recognized by conventional visual–tactile assessment or caries detector dyes, supporting their use as an adjunct rather than as a replacement for clinical judgment (13–16).

In the present study, the high interobserver agreement obtained with both baseline and post dye fluorescence supports their potential value for improving consistency between clinicians. However, the significant change in fluorescence scores following dye application has an important clinical implication when a caries detector dye is applied before fluorescence assessment, the resulting measurement should be interpreted as a post dye signal rather than being directly equated with the baseline fluorescence value. This consideration may be particularly relevant when both methods are used sequentially during selective caries removal, especially in deep lesions where preservation of affected dentin and avoidance of unnecessary tissue removal are important for maintaining tooth structure and reducing the risk of pulpal exposure.

Several limitations should also be acknowledged. First, the study used an exploratory in vitro design with a relatively limited sample of 44 extracted teeth, and no a priori sample size calculation was performed. Therefore, the findings should be interpreted cautiously and require confirmation in larger studies. Second, extracted teeth cannot fully reproduce the clinical conditions encountered during caries removal, including saliva, biological variation, patient related factors, and differences in clinical visibility and accessibility. Third, the diagnostic scores were based on a predefined ordinal percentage scale with four categories, which provides standardized categorization but does not represent a continuous quantitative measurement of the amount of residual carious tissue. Fourth, the study evaluated lesion extension histologically, but the histological measurement was based on sectioning along the axis of greatest clinical lesion extension and therefore represents a two-dimensional assessment of a three-dimensional lesion. Finally, although the results demonstrated significant associations between lesion extension and diagnostic scores, the study was not designed to establish conventional diagnostic accuracy measures such as sensitivity, specificity, or area under the receiver operating characteristic curve. Consequently, the present findings should not be interpreted as demonstrating superiority of one diagnostic method in terms of diagnostic accuracy.

5. Conclusion

- Fluorescence based methods demonstrated the highest interobserver agreement among the evaluated diagnostic methods while caries detector dye showed the lowest interobserver agreement.
- Application of the caries detector dye significantly altered the DIAGNOcam fluorescence scores.
- Baseline and post-dye fluorescence scores remained significantly positively correlated.
- Greater histological lesion extension was associated with higher diagnostic scores for all four diagnostic methods.
- Further clinical studies with larger samples are needed to validate these findings.

Declarations

Disclosure Statement

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Author Contribution

V.V.G. contributed to the conception and design of the study, supervised the experimental procedures, and contributed to interpretation of the results. Y.S.E. contributed to the study design, data collection, statistical analysis, and drafting of the manuscript. B.H.D. contributed to data collection, experimental procedures, and interpretation of the findings. V.V.R. contributed to data collection, methodological procedures, and data organization. C.P.M.C. contributed to the experimental procedures and data collection. M.V.R. contributed to the interpretation of the results and critically revised the manuscript for important intellectual content. D.G.G. contributed to the statistical analysis, interpretation of the findings, and critical revision of the manuscript. All authors reviewed and approved the final manuscript and agree to be accountable for all aspects of the work.

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