

Article

Laser Doppler Flowmetry and Continuous Tissue Oxygenation Monitoring: Best of Vitality Tests?

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Abstract

This study aimed to explore the added value and usage of laser Doppler flowmetry (LDF) in conjunction with continuous tissue (arterial) oxygen saturation (SO₂) monitoring, electrical pulp testing (EPT), cold stimulation (CS) testing, and apical X-rays (RX). LDF data were evaluated in relation to three different scenarios. LDF records of all four upper incisors from 30 randomly selected patients aged 21–40 were analysed in relation to the following scenarios: (a) simultaneous SO₂ measurements using a pre-manufactured splint handled by an experienced LDF dentist, (b) EPT, and (c) CS. A total of 120 teeth were analysed, of which 11 were non-vital (7 denervated and 4 traumatised). Data assessment showed the following mean LDF values: vital teeth: 23.6 Perfusion Units (PU), SD 6.3 and SaO₂ of 88.7%, SD 17.1. For non-vital teeth, the mean LDF value was 16.1 PU (SD 11.8) and the mean SO₂ value was 70.8% (SD 31.9). The standard deviation was found to be twice as high for non-vital teeth as for vital teeth. No direct relationship was found between LDF and SO₂ values at low SO₂. For vitality discrimination, the ROC curves showed an area under the curve of 0.799 for LDF and 0.643 for SO₂. EPT data assessment showed a mean value of 18.1 (SD 19.7) out of a possible score of 0–80. This was distributed as follows: seven non-vital teeth (80/80); 109 vital teeth; and four undecided teeth. This was compared to the LDF and SO₂ results. The data assessment showed nine non-vital teeth, 108 vital teeth, and three undecided teeth in comparison to LDF and SO₂ results. Conclusion: LDF and SO₂ do not complement each other sufficiently in detecting non-vital teeth when the selection criteria are applied. While LDF clearly contributes, the vital or non-vital classification still depends on a combination of X-ray, sensitivity, and vitality tests.

Keywords: laser Doppler flowmetry; continuous tissue oxygen monitoring; oxygen saturation; vitality test; sensitivity test



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1. Introduction

Distinguishing between near pulp necrosis and revascularisation is challenging. Cold stimulation (CS), the heat test (HT), and electrical pulp testing (EPT), in combination with

mobility, percussion, and radiographic examination, are currently considered the gold standard for diagnosing pulpal health and tooth inflammation [1,2].

Since haemoglobin oxygen saturation correlates with vascular volume density, electrical sensitivity and the volume density of myelinated nerve fibres can also be assessed through histological analysis of the dental pulp [3]. This correlation indicates that pulpal blood flow is a reliable determinant of pulp vitality and tooth health [4].

Because sensitivity tests reflect the subject's perception, they are regarded as more subjective than histological findings. Therefore, diagnostic accuracy can be improved by measuring pulpal blood parameters [5].

The vascularity of the dental pulp is the only true indicator of pulp vitality. Several non-invasive methods have been developed for this purpose, including photoplethysmography (PPG) [6], pulse oximetry (PO) or blood oxygen saturation testing [7], ultrasound Doppler imaging (UDI) [8], laser speckle imaging (LSI) [9], laser Doppler flowmetry (LDF) [10], and continuous tissue oxygenation (SO₂) monitoring. These methods are all non-invasive and objective, providing semi-quantitative results, with LDF considered the most appropriate [11,12].

In addition to these measurements, patient-related parameters such as age, tooth colour, medical condition (e.g., blood pressure, fever), and medication must be taken into account. For accurate interpretation, the patient should be free of fever, not under the influence of blood flow-modifying medication, and placed in a semi-supine position during assessment [13–16].

Furthermore, diurnal variations have been documented in facial skin and gingival tissue, and more recently in dental pulp, highlighting the need for standardised follow-up when time-dependent measurements are required [17–19].

All vascularity tests are also influenced by external factors such as gingival blood flow, the position of the probe relative to the tooth surface, ambient light, and probe parameters. Therefore, the use of a stabilisation splint with multiple-teeth isolation (MTI) is universally recommended, along with consideration of the time of day (circadian rhythm) [20,21]. MTI stabilises the probe, producing an objective and reproducible LDF signal [22,23]. Other factors, such as laser wavelength, fibre diameter and separation, LDF bandwidth, and correct probe positioning, also affect LDF measurements [19,24–29].

These considerations are equally relevant to SO₂ assessments, since tooth position affects probe design, requiring different tips for the upper and lower jaws and for anterior versus posterior teeth. To address this, a universal pulse oximeter probe holder has been developed [30]. Moreover, pulse oximetry (PO) measures arterial blood oxygenation, whereas SO₂ monitoring accounts for both arterial and venous blood. Compared to EPT, CS, and the HT, PO is considered the most accurate method for diagnosing pulp status [5].

A simultaneous assessment of LDF and tissue oxygenation has been proposed as the optimal follow-up test in cases of trauma, with both flux and SO₂ measured using a single stainless steel probe [31].

To the best of our knowledge, simultaneous measurement of LDF and arterial blood oxygen saturation for evaluating blood flow in human dentition has not yet been investigated. Therefore, the aim of the present study was to determine whether LDF, when combined with SO₂ monitoring, EPT, and CS, could provide a more accurate diagnosis of pulpal health.

2. Materials and Methods

This protocol was approved under reference numbers EC2019/1804, BC-06546, and B.U.N. B670201942308.

2.1. Patient Selection

Thirty patients aged 21–40 years (mean age: 28 years; SD: 8 months) were randomly selected by a first master student from fifty respondents to a flyer distributed at the entrance of the Ghent University Hospital dental clinic via a sealed envelope system. The flyer, entitled “The Simultaneous Use of Oximetry and Laser Doppler Flowmetry in Dentistry”, invited individuals for a pulp vitality assessment alongside their annual dental examination. Participants were allocated a treatment only after consenting to join the trial, at which point the corresponding envelope was opened and its contents revealed, providing allocation concealment and thus preventing bias in the study.

Sixteen males and fourteen females underwent screening for sensitivity using cold and electrical pulp tests, and for vitality using laser Doppler flowmetry combined with continuous tissue oxygenation monitoring. Two lateral and two central maxillary incisors were examined in each patient, resulting in a total sample of 120 teeth.

To minimise the influence of extra-pulpal factors such as room temperature, ambient light, drug intake, and posture (semi-supine), all assessments were performed under standardised conditions. All measurements were conducted by one experienced clinician (HR), and each patient was required to be healthy and drug-free at the time of testing.

2.2. Diagnostic Protocol

Following the IADT guideline-based protocol, each assessment began with a cold test (application of a $-50\text{ }^{\circ}\text{C}$ cold pellet, Miracold® Plus, Hager & Werken, Duisburg, Germany), followed by a heat test (application of a $160\text{ }^{\circ}\text{C}$ gutta-percha stick), an electrical pulp (EP) test (Elements™ Diagnostic Unit, SybronEndo, Kerr Corporation, 1717 West Collins Street, Orange, CA, USA; sensitivity range: $0\text{--}80\text{ }\mu\text{A}$), colour assessment using a VITA shade guide, mobility assessment using Miller’s scoring system (MOB), percussion testing by tapping on the incisal edge (Perc), and an intraoral periapical radiograph (VistaScan Perio Plus, Dürre Dental, Bietigheim, Germany) (RX).

A waiting time of 60 s was allowed between each sensibility test. These were then followed by the fabrication of an MTI polyvinyl polysiloxane splint (Exaflex, GC America Inc., Alsip, IL, USA) with four drilled, right-angled shafts to position the LDF/SO₂ probe 2 mm from the cemento-enamel junction, centrally on the buccal surface of each central and lateral maxillary incisor (Figure 1).



Figure 1. Photo of a splint. A polyvinyl polysiloxane splint with 4 drilled right-angled shafts 2 mm from the enamel–cement border, central on the buccal surface of each central and lateral upper incisor.

2.3. Pulpal Blood Flow Registration

Measurements were performed using a Moor VMS-LDF 2 (Moor Instruments, Axminster, UK, serial no. 098) diode (785 nm) class 1 laser. The device’s power output was at least 1.0 mW, ranging from 0.5 to 1.5 mW at the probe tip (diameter = 1.65 mm). The manufacturer

recommends a 3 kHz bandwidth (broad-spectrum, low blood volume) for measurements of tooth vitality. Values were recorded every 0.1 s but displayed at a frequency of at least 40 Hz, twice every 30 s [32].

Oxygen saturation was measured simultaneously using a Moor VMS-OXY (Moor Instruments, Axminster, UK, serial no. 003) white light LED with a wavelength range of 500–650 nm and a spectral resolution of 2 nm. The maximum optical power of the device was 6 mW, with an integration time of 0.1 s, displayed at a frequency of 40 Hz over the same interval as the LDF analysis.

Both LDF and SO_2 measurements were obtained using the same VP3 blunt-end needle probe (CP3-500, Moor Instruments, Axminster, UK), which contained two afferent fibres and one efferent fibre. These fibres were housed in a hypodermic stainless steel tube with an external diameter of 1.65 mm and a length of 20 mm. Each fibre had a diameter of 200 μm and was separated by 0.5 mm. Calibration was carried out in accordance with the manufacturer's instructions (Figure 2).

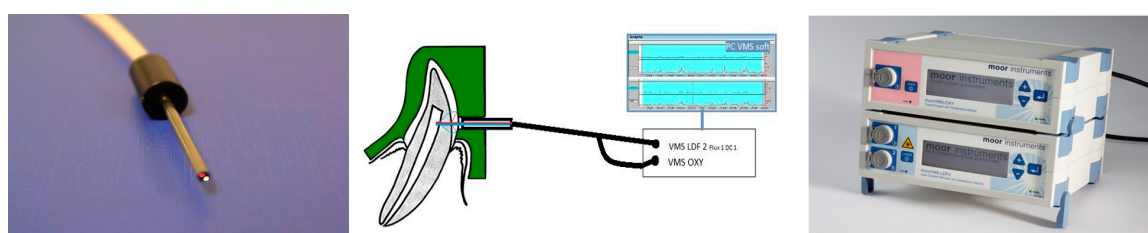


Figure 2. One probe for simultaneous LDF and SO_2 measurements.

2.4. Statistical Analysis

A patient sample size of 30 was calculated using Kane's calculator with a confidence level of 95%, $\alpha = 0.05$, $\beta = 0.05$, and a study power of 0.95, resulting in a mean study group of 120 teeth within a population of 152 ± 48 teeth [33]. Inclusion criteria were as follows: adult patients aged 21–40 years old, both sexes, with present upper incisors, in good general health, without drug intake. Exclusion criteria were as follows: aged younger than 21 or older than 40 years old, drug intake, and bad general health.

All criteria (EPT, SO_2 , and LDF) and their interrelationships were examined using descriptive statistics and graphical representations, alongside assessments of plausible vitality. ROC curves were used to evaluate the diagnostic information of each criterion with respect to vitality.

Threshold values were then determined for these criteria. A discriminant analysis was performed for LDF. However, the inherently skewed distribution of SO_2 did not permit discriminant analysis. Even after transformation, the considerable saturation effect at 100%—a natural phenomenon—prevented transformations from approximating normality.

All analyses were performed using IBM SPSS Statistics, version 18.0.1.1.

3. Results

All 120 teeth were presumed vital, regardless of the information provided by patients prior to assessment. After sensitivity testing and intraoral radiographs, several teeth were identified in which a single diagnostic feature (RX, cold, EP, MOB, or percussion) suggested a possible dental health problem within this convenience sample. With non-vitality as the only criterion, the diagnostic contribution was distributed as follows: RX (9), cold (3), EP (2), MOB (0), and percussion (3).

Radiographic assessment (RX) contributed most to identifying compromised dental health (9/120), revealing seven endodontically treated teeth and two cases of apical pathology. The mobility test contributed nothing, which was expected since mobility was a case

selection criterion. All other tests provided unique diagnostic information for two or more teeth (Table 1).

Table 1. Description of the convenience sample with respect to indicators of dental health.

Criterion	RX	Cold	EP	MOB	Perc	Hot	Total
Non-vital	17 (14.2%)	13 (10.8%)	11 (9.2%)	0 (0.0%)	5 (4.2%)	17 (14.2%)	24 (20.0%)
Presumed vital	103 (85.8%)	107 (89.2%)	109 (90.8%)	120 (100.0%)	115 (95.8%)	103 (85.8%)	96 (80.0%)
Unique feature	9	3	2	0	3	2	

For each criterion, the property ‘unique feature’ was determined according to whether it was presumed to be vital or non-vital. The periapical X-ray was found to be the best indicator of dental health issues.

For the purposes of this study, a tooth was considered vital if the periapical radiograph showed no pathology (apical lesion), if no trauma was present (e.g., root fracture), if percussion was negative, and if mobility was less than 1 mm. Cold and heat tests were excluded because loss of sensitivity may indicate either loss of vitality or nerve damage in an otherwise healthy tooth.

3.1. LDF Versus SO_2 in Determining Vitality

Vital and non-vital teeth differed in mean LDF value as well as mean SO_2 . The mean LDF value for vital teeth was 23.6 PU (SD 6.3 PU), and the mean SO_2 was 88.7% (SD 17.1%). For non-vital teeth, the corresponding values were 16.1 PU (SD 11.8 PU) and 70.8% (SD 31.9%), respectively (Table 2). Among endodontically treated teeth, the mean LDF value decreased to 7.3 PU (SD 8.1 PU), and the mean SO_2 to 32.5% (SD 31.9%).

Table 2. Mean and standard deviation of LDF (PU) and SO_2 (%) measurements for vital and non-vital teeth.

		Mean		SD	N
LDF		22.1	+/−	8.2	
	non-vital	16.1	+/−	11.8	24
	vital	23.6	+/−	6.3	96
SO_2		85.1%	+/−	22.0%	
	not vital	70.8%	+/−	31.9%	24
	vital	88.7%	+/−	17.1%	96

No direct relationship was found between SO_2 and LDF results for saturation levels below 85% ($p < 0.05$). Interpretation was limited by the high frequency of oxygen saturation values near 100%. When considering only teeth with SO_2 below 100%, no significant relationship between LDF values and oxygen saturation was observed (Figure 3).

Further investigation is needed to determine whether LDF and oxygen saturation complement each other in detecting non-vital teeth when combined with the selection criteria. Threshold lines were established for the SO_2 –LDF relationship: a lower threshold at 12 PU for LDF and 40% for SO_2 , and an upper threshold at 24 PU and 80%, respectively (Figure 4).

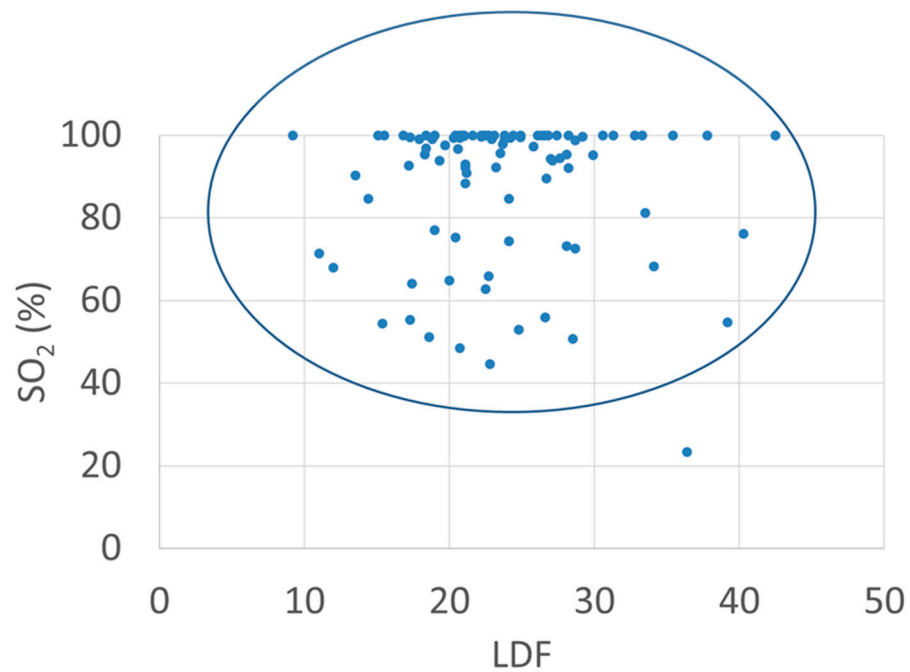


Figure 3. SO₂ (%) in relation to LDF (PU). When considering teeth not 100% oxygen saturated, there is no indication of a relation between LDF results and saturation—see oval surface. For an LDF point, no direct corresponding point related to SO₂.

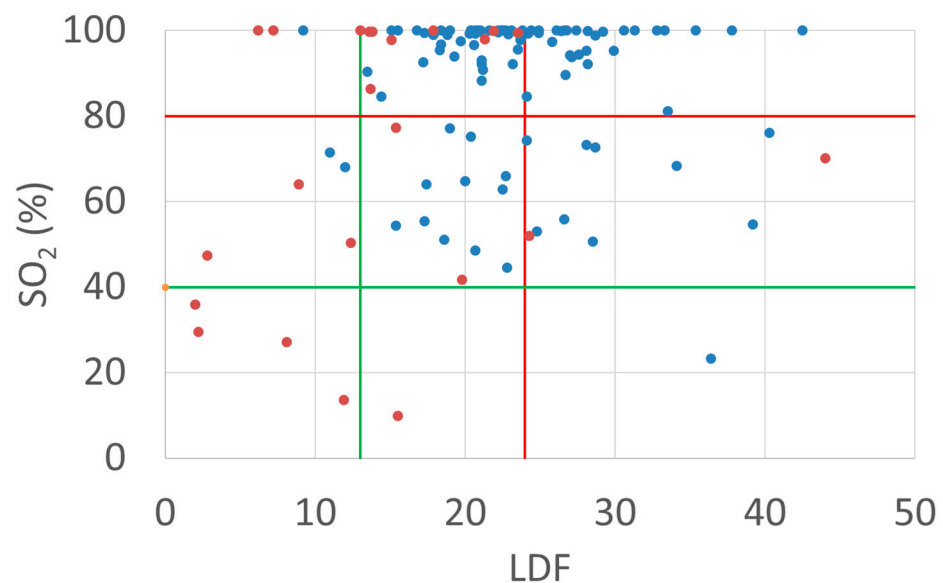


Figure 4. SO₂ (%) in relation to LDF (PU) with lower (green) and upper thresholds (red), with red dots referring to non-vital and blue dots to vital teeth.

3.2. Diagnostic Accuracy

Both LDF and SO₂ provided information about tooth condition, with areas under the ROC curve (AUCs) of 0.799 ($p = 0.000$) and 0.643 ($p = 0.046$), respectively. The difference between the AUCs of LDF and SO₂ was not statistically significant ($p = 0.080$). However, this result should be interpreted with caution due to the relatively small sample size ($n = 120$) and the limited number of non-vital teeth ($n = 24$) (Table 3).

Table 3. Area under the ROC curve: The test result variable(s): LDF, SO₂, and EP have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

Test Result Variable (s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Lower Bound	Confidence Interval Upper Bound
LDF	0.799	0.065	0.000	0.673	0.926
SO ₂	0.643	0.072	0.046	0.503	0.784
EP	0.773	0.064	0.000	0.648	0.898

^a. Under the nonparametric assumption. ^b. Null hypothesis: true area = 0.5.

The difference between the two techniques was more evident in Q–Q plots. In such plots, if two sets of quantiles are drawn from the same distribution, the data points align along a straight line. In this case, LDF demonstrated superior performance compared with tissue oxygenation.

3.3. LDF Versus Cold in Determining Vitality

The cold test is inherently binary: sensitive or not. When combined with LDF, however, an additional category, undecided, must be introduced, as some LDF values are too high for non-vital teeth yet too low for vital teeth.

In this sample of 120 teeth, nine teeth (7.5%) were non-sensitive to cold, with a mean LDF score of 11.5 PU (SD 11.6 PU). Among the cold-sensitive teeth ($n = 108$; 90% of the sample), the mean LDF value was 23.2 PU (SD 7.2 PU). Three teeth (2.5%) were classified as undecided, with a mean LDF value of 17.0 PU (SD 8.0 PU) (Table 4).

Table 4. Cold sensitivity related to LDF (PU).

LDF	n	%	Mean	SD
Not sensitive	9	7.5%	11.5	11.6
Sensitive	108	90.0%	23.2	7.2
Undecided	3	2.5%	17.0	8.0

With undecided: not a clear response to the test.

3.4. LDF Versus Electrical Pulp Testing (EPT)

In order to assess normality with a visual method, observed data were compared to a theoretical distribution like the normal distribution with Q–Q plots. The latter to highlight deviations. Q–Q plots demonstrated greater inconsistency for EPT compared with LDF and SO₂ (Figure 5a–c).

To clarify the relationship between EPT and LDF, threshold lines were drawn (Figure 6): the lower limit in green and the upper limit in red. For EPT (sensitivity range 0–80 units), the lower threshold coincided with zero, while the upper threshold was set at 55. Red dots in the plot indicate possible outliers. This scatterplot comparing LDF, EPT, and vitality revealed a key difference: at higher values (e.g., threshold 55), EPT (μ A) identified non-vital teeth, whereas LDF thresholding at 24 PU identified vital teeth. At lower values of both EPT and LDF, a mixture of vital and non-vital teeth was observed.

Although the areas under the ROC curves for LDF and EPT were similar (0.799 and 0.773 [95% CI: 0.648–0.898], respectively; Figure 7), LDF and EPT scores better visually than SO₂.

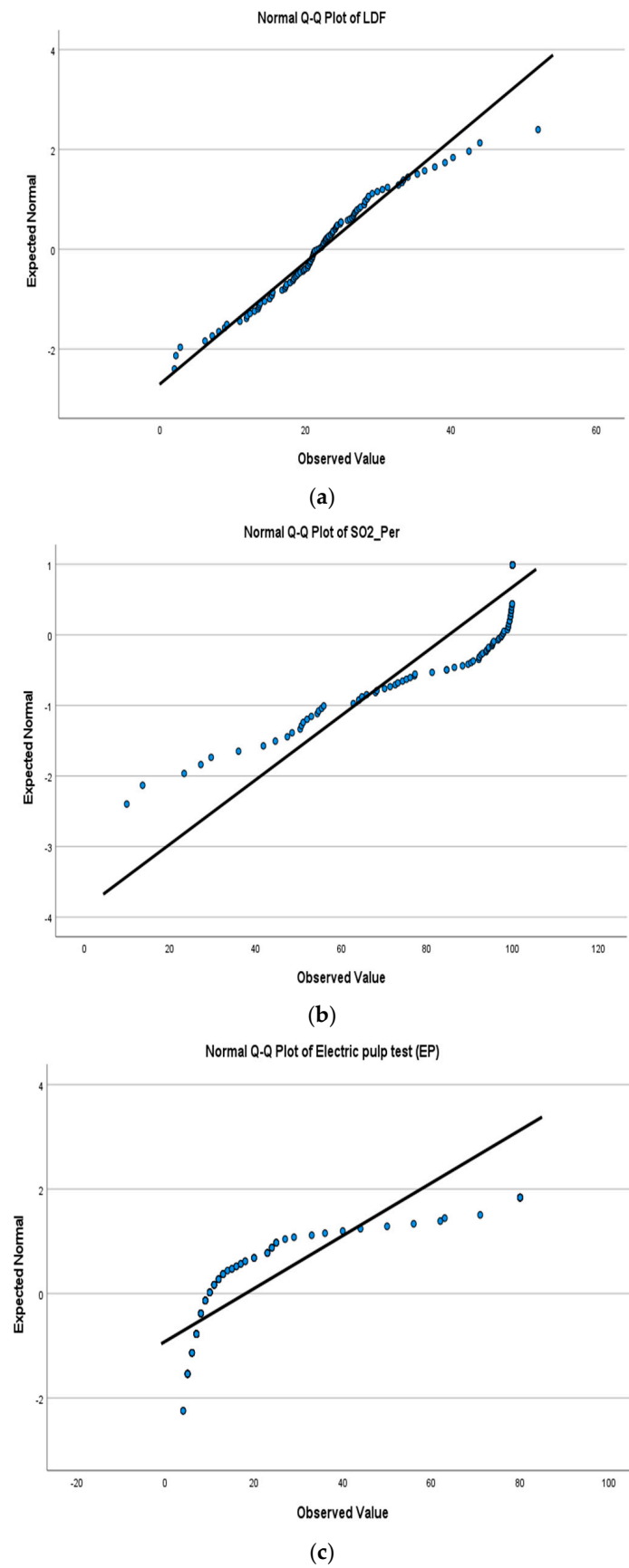


Figure 5. (a) Q-Q plot of LDF. (b) Q-Q plot of SO₂. (c) Q-Q plot of EPT.

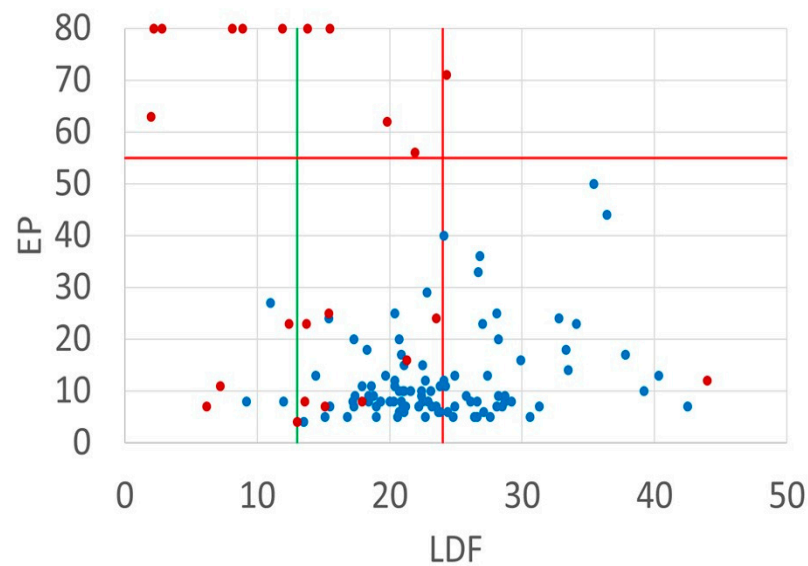


Figure 6. Electrical pulp test related to LDF (PU). Red dots in the plot indicate possible outliers.

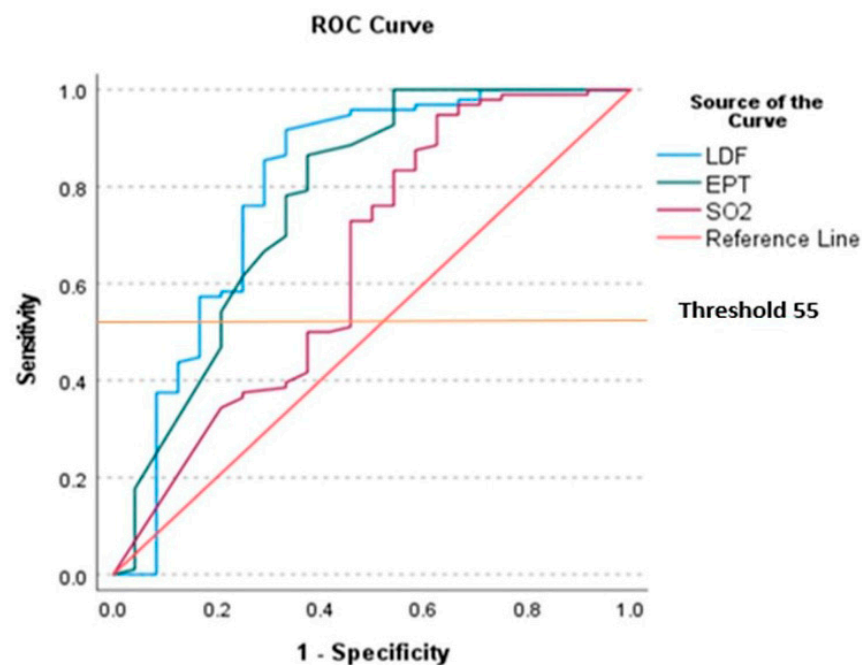


Figure 7. ROC vitality curves for LDF, SO₂, and EPT. LDF and EPT scores are visually better than SO₂. This result is not definite since the power is relatively low due to the limited sample size.

4. Discussion

Understanding blood oxygen levels requires three blood gas values: the partial pressure of oxygen (PaO₂), oxygen saturation (SO₂), and total oxygen content (CaO₂).

PaO₂ determines the oxygen saturation of haemoglobin (mm Hg). SO₂ represents the percentage of haemoglobin binding sites occupied by oxygen (%) and can never exceed 100%. CaO₂ is derived from SO₂ in combination with haemoglobin concentration (g/dL).

Although PaO₂ is the most important determinant of SO₂, other factors can also influence SO₂ at a given PaO₂. These include conditions that shift the oxygen dissociation curve to the left or right, such as temperature, pH, PaCO₂, and blood levels of 2,3-DPG. The latter provides additional information about the oxygen state at any given moment [34].

In this study, tissue oxygenation was continuously monitored in the 500–650 nm range, capturing both arterial and venous blood [35]. This differs from pulse oximetry (PO), which measures intermittently at 660 nm (red light) and 940 nm (infrared light) and relies

on tissue volume changes during the cardiac cycle [36]. LDF measurements were taken simultaneously using the same probe.

Reference values for pulp oxygen saturation as a diagnostic tool in endodontics have been reported as 84.94% (95% CI) for central incisors and 89.20% for lateral incisors, with a minimum of 77.52% [37]. For LDF measurements using the VMS-LDF 2 instrument (Moor Instruments), reported reference values are 20.3 PU (SD 2.6) for maxillary central incisors and 26.8 PU (SD 3.2) for maxillary lateral incisors in patients aged 21–40 years, for both vital and non-vital teeth. This demonstrates the contribution of standard deviation to the validity of an LDF signal [38].

A significant decrease of 46–57% in the initial LDF signal after pulp removal and replacement has also been documented [21,34]. SO_2 values have been reported as higher in maxillary lateral incisors than in central incisors [39]. Central incisors also showed greater sensitivity to EPT, with responses of $10.43 \mu A \pm 3.73$ versus $11.33 \mu A \pm 5.37$ for lateral incisors [40].

Periapical radiography as a test for the absence of apical lesions underestimates lesion prevalence by approximately 22% compared to cone beam computed tomography (CBCT) [41]. However, CBCT was not used in this study in accordance with the ALARP (As Low As Reasonably Practicable) principle for radiographic exposure. Here, cold and heat tests identified 108 of 120 teeth as sensitive and 12 as undecided, compared with periapical radiography, which indicated 111 of 120 teeth as vital. Only two cases of apical pathology were detected. Thus, periapical radiography once again proved useful in complementing standard vitality tests.

A statistical design using randomisation based on a gold standard, such as tooth loss or induced trauma/pathology, is feasible only in animal models. However, inducing trauma or pathology without treatment in animal studies is not ethically defensible [42].

LDF was found to be a more reliable and effective method than PO and EPT for assessing pulpal status in human teeth [39]. It may be considered an objective, rapid, and non-invasive tool for measuring blood flow in teeth, particularly those with vascular compromise [32]. LDF and EPT provided a similar overall amount of diagnostic information, but their contributions were complementary: EPT was unreliable at lower values, while LDF was reliable in the mid-range (Figure 5).

Since PO has been reported to be clinically relevant for monitoring changes in pulp status [5,37], its simultaneous use with LDF is beneficial [32]. Unlike conventional PO, the method applied here used continuous tissue oxygenation monitoring with white light spectroscopy [35]. The Moor VMS-OXY instrument employs a bright white LED as its light source and continuously analyses scattered and reflected light across 500–650 nm. Tissue oxygenation (SO_2) is calculated by fitting the observed spectra to the ratios of oxygenated and deoxygenated haemoglobin spectra. These data directly reflect microcirculation, in contrast to pulse oximetry, which measures only arterial oxygenation, and transcutaneous partial pressure of oxygen ($TcPO_2$).

The design and (re)positioning of the probe in PO differ in continuous tissue oxygenation monitoring, as a single probe is used simultaneously for LDF with two pairs of glass fibres conducting light to and from the tooth: one efferent and one afferent. LDF operates at 785 nm. Because blood perfusion in teeth is minimal, this probe is used with multiple-teeth isolation (MTI) to shield against ambient light and to stabilise and reposition the probe when time-dependent recordings are required, such as during follow-up after trauma [24,38]. However, the design of the dental PO probe remains problematic; stabilising a single probe in different scenarios with a clamp-type design is not optimal [43].

ROC curves and Q–Q plots suggest that LDF measurements are more accurate than SaO_2 measurements. Similar findings have been reported in multiple studies and reviews

comparing LDF, PO, and EPT [30,39,40]. In this study, the SybronEndo Elements Diagnostic Unit was used for EPT. Vitality response values ranged from 1 to 80, with 1 representing high sensitivity and 80 indicating non-sensitivity. According to the manufacturer, the normal response range for vital incisors is 10–40, which was confirmed in this study (Figure 5).

Regarding threshold plots, more outliers appeared in the EPT plot ($n = 4$) than in the SO_2 plot ($n = 2$). This aligns with reports in the literature, which describe LDF and PO as more reliable than EPT for vitality quantification [30]. In this study, ROC curves indicated that EPT performed better than SO_2 evaluation, while Q–Q plots did not, suggesting a distinction between SO_2 and PO.

Nine percent of the teeth in this sample demonstrated lower blood flow than the level considered vital, and patients were unaware of the pulpal status of these teeth. Comparable findings have been reported in the literature and should serve as a warning to the dental profession, since untreated periodontal problems can compromise general health [21,44].

These findings emphasise the importance of vitality control in addition to sensitivity testing in all trauma cases. In this respect, LDF, SO_2 , and PO all have clinical value.

Pulpal blood flow is subject to diurnal and circadian rhythms. The range of variation is comparable to the difference between a vital and a non-vital pulp, where a tooth is diagnosed as non-vital if its values are 50–70% lower than those of an adjacent vital tooth. To minimise variability, all measurements in this study were performed at the same time of day—an important methodological consideration [38].

Therefore, combining multiple-teeth isolation with a silicone repositioning splint and adhering to a strict schedule for repeated measurements is strongly recommended. This approach enhances the validity of blood flow recordings and reduces interference.

5. Conclusions

Blood flow registration adds diagnostic value in determining true tooth vitality. As a non-invasive, objective, instant, and reliable semi-quantitative method, laser Doppler flowmetry (LDF) is the most appropriate technique. It supports accurate treatment decisions with pulp preservation in mind.

Combining LDF with oximetry does not improve discrimination between vital and non-vital teeth, as LDF performs better in terms of sensitivity and specificity. Nonetheless, LDF is not a stand-alone test and should always be used in conjunction with CS and EPT.

Although still limited in dental practice, this study demonstrates the benefits of LDF for vitality testing, particularly in trauma cases. The cold sensitivity test combined with LDF proved to be the most reliable approach. However, clinicians should be aware of the associated learning curve and costs.

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