Article:
Ghouth, N, Duggal, MS, Kang, J orcid.org/0000-0002-2770-1099 et al. (1 more author) (2019) A Diagnostic Accuracy Study of Laser Doppler Flowmetry for the Assessment of Pulpal Status in Children's Permanent Incisor Teeth. Journal of Endodontics, 45 (5). pp. 543-548. ISSN 0099-2399

https://doi.org/10.1016/j.joen.2019.01.017

A diagnostic accuracy study of laser Doppler flowmetry for the assessment of pulpal status

Abstract

Aim: To assess whether laser Doppler flowmetry is more accurate than the conventional pulp sensibility tests (Electric pulp test and ethyl chloride) in assessing the pulp status of permanent anterior teeth in children and to identify the LDF's Flux cut-off threshold.

Methodology: A cross-sectional cohort diagnostic accuracy study with randomisation was carried out in 8-16 year old children. Participants had one maxillary central or lateral incisor with either a completed root canal treatment or an extirpated pulp and a contra-lateral tooth with vital pulp. The outcome measures included the sensitivity, specificity and predictive values as well as the repeatability of all tests. Statistical analysis included the use of the Receiver Operating Characteristic (ROC) curve and contingency 2x2 tables. Kappa scores were used to assess the repeatability of EPT and ethyl chloride while inter-class correlation was used for LDF.

Results: The study included 74 participants as determined by sample size calculation. A significant difference between the Flux values for teeth with vital and non-vital pulps was found. The best cut–off ratio for LDF was 0.6 yielding a sensitivity of 54 % and a specificity of 32 % which were lower than the values of electric pulp test (Sensitivity = 83.8 – 94.6 %, Specificity = 89.2 – 97.6 %) and ethyl chloride (Sensitivity = 81.1 – 91.9 %, Specificity = 73 – 81.1 %). The repeatability of LDF, EPT and ethyl chloride were 0.85, 0.86 and 0.81, respectively.

Conclusion: Laser Doppler flowmetry was unable to differentiate between teeth with vital and non-vital pulps in children between the ages of 8-16 years, with an acceptable level of confidence. The results of this study showed that there was a high probability for false results. Further development of LDF in assessing pulpal blood flow would be required before it could be recommended for clinical use especially in children.

Introduction

The ability to diagnose the health of the pulp following dental trauma is a crucial part of treatment planning in dental traumatology. The most accurate method of evaluating the degree of inflammation or the presence of pulp necrosis is the histological assessment of the pulp (Andreasen 1989), which is of little value to clinicians who are faced with making clinical decisions regarding pulpal status following dental trauma.

The use of the conventional pulp sensibility tests, such as electric pulp testing (EPT) and cold tests, is primarily subjective and relies on the patient's response to the stimulus. Children's anxiety and cooperation are two major confounders in the use of such tests especially following traumatic dental injuries (TDIs), which introduce further unreliability of the tests. It is possible that no response is detected to sensibility tests after TDIs even though blood circulation may have been restored (Ohman 1965, Bhaskar and Rappaport 1973, Crona-Larsson *et al*. 1991). The use of such tests could result in false responses, especially when used in the child population (Cooley and Robison 1980, Peters *et al*. 1994). Therefore, a more reliable objective diagnostic tool would be a valuable diagnositic aid in order to assess pulp vitality.

Laser Doppler flowmetry (LDF) was developed for the assessment of pulpal blood flow, rather than the assessment of the pulp‘s sensory response derived from the innervation of the pulp (Gazelius *et al*. 1986). The Doppler Effect was the principle used in developing LDF technology whereby the laser light is aimed at the pulp through a fibre optic probe, which interacts with red blood cells causing backscattered light (Toman 1984). The backscattered light consists of Doppler-shifted and un-shifted light waves, is then captured by an afferent fibre within the same probe and directed to photodetectors in the flowmeter. The received signal is computed with a pre-set process in the LDF machine producing a signal termed the Flux (Roeykens and De Moor 2011).

LDF is often described as an objective, painless and non-invasive test that has the advantage of being a quantitative method (Gazelius *et al*. 1986). However, caution has been advocated in the interpretation of the results due to the inability of the device to measure the blood flow in absolute units. Other limitations include the cost of the equipment which is

considered high when compared to other relatively inexpensive pulp tests (Ames *et al*. 1993, Vongsavan & Matthews 1993a,b). There are also technical limitations affecting the results that have been reported which include patients/apparatus movement and contamination from blood flow to the surrounding tissues (Ikawa *et al*. 1999).

LDF has been reported to be more accurate, in differentiating between teeth with vital and non-vital pulps, than other dental pulp tests (Ghouth *et al*. 2018). Clinical studies have shown that LDF had higher sensitivity (81.8-100%) and specificity (100%) when compared to other pulp tests (Ingolfsson *et al*. 1994, Evans *et al*. 1999, Karayilmaz and Kirzioglu 2011). Despite the reports of higher accuracy of LDF in assessing pulp vitality, these data are based on studies with a high level of bias, and major shortfalls in study designs using methodologies that may have resulted in over estimation of the diagnostic accuracy of LDF (Mejare *et al*. 2012, Ghouth *et al*. 2018). Also, there has been inconsistency among studies regarding the Flux cut-off threshold used, below which the pulp could be considered as non-vital.

Therefore, the aim of this study was to evaluate the accuracy of LDF when compared to conventional pulp sensibility tests such as EPT and cold test (ethyl chloride) using a methodologically recommended diagnostic accuracy study design, methods and statistical analysis. In addition, the study aimed to determining the most accurate LDF Flux threshold below which a tooth could be identified as diseased (non-vital pulp). The null hypothesis was that LDF is as accurate as the conventional methods (EPT and ethyl chloride) in assessing the pulp status of permanent anterior teeth in children.

Materials and Methods

Ethical approval

Ethical approval was obtained from the National Research Ethics Service (NRES) committee, North West, Greater Manchester East – UK (Ref # 15/NW/0583). The study was reported in accordance with The Standards for Reporting of Diagnostic Accuracy Studies (STARD) (Cohen et al., 2016). The study protocol was registered at the International Standard Randomised Controlled Trial Number (ISRCTN) registry (ISRCTN12547356). Informed consent was

obtained from all parents/people with parental responsibilities for the children to take part in the study.

A cross-sectional cohort diagnostic accuracy study with randomisation of children and young adults was conducted at Leeds Dental Hospital, School of Dentistry, University of Leeds, UK. Study participants were recruited into the study when they fulfilled the following inclusion criteria:

- Aged between 8-16 years.
- Medically fit and well (ASA I, II).
- Children and their parents/people with parental responsibilities understood English language and were able to understand instructions.
- Showed an acceptable level of cooperation.
- Had one maxillary central or lateral incisor with root canal treatment or pulp extirpation and a minimal restoration covering less than half the labial crown surface.
- Had one anterior tooth (ideally contralateral tooth) with:
    - Vital pulp with no history of dental trauma,
    - No signs/symptoms of pulp inflammation/infection such as pain, tenderness to percussion, and/or associated sinus tract, and no radiographic signs such as periapical radiolucencies or root resorption.
    - No radiographic evidence of pulp canal obliteration.
    - A history of positive responses to sensibility testing for the past six months.
    - A minimal restoration covering less than half the labial crown surface of all teeth assessed.

Study participants with any of the following exclusion criteria were not recruited into this study:

- Learning disabilities.
- A history of moderate and significant behaviour management problems
- Heavily restored teeth (restorations covering more than half the labial surface).
- Routine analgesics, antidepressants or antihypertensive drugs.

- Teeth with necrotic pulps that had grey discolouration of the crown or treated with regenerative endodontic techniques.
- Teeth with vital pulps showing any of the following:
  - No consistent response to EPT and ethyl chloride pulp tests during the past six months.
  - Abnormal colour.
  - Tenderness to percussion.
  - Any radiographic sign of loss of vitality
  - Pulp canal obliteration.

Randomisation

Following consent/assent, participants were randomly assigned to two groups; Test = LDF, or Control = EPT and ethyl chloride, using a computer-generated random list made by an independent person. The independent person concealed the allocation sequence in sequentially numbered, opaque, and sealed envelopes. Each participant chose one envelope prior to commencing the chosen test(s).

Sample size determination

Sample size calculation was determined using an online software (http://www.stat.ubc.ca/~rollin/stats/ssize/) based on a pilot study conducted in our clinic, using the same LDF device used in this study (Nazzal *et al*. 2014). As a result, the number of participants required to achieve a power of 80%, at 95% significance difference, with an effect size of 25% (LDF 87.5% vs EPT 62.5%) using one-sided test, was determined to be 37 participants per group, which meant a total of 74 in total were required.

Pulp assessment

Pulp assessment using all three tests were carried out by a single operator.

Test group (LDF)

A dual channel Moor VMS-LDF 2 (Moor Instruments, Axminster, UK) with a 2.5 mW max output power, 785 nm ± 10 nm wavelength and 15 KHz probe frequency filter was utilised. Two probes with 1.5mm diameter, each with two fibres of a diameter of 200 μm and a fibre separation of 500 μm were used.

At the start of each session, the device was calibrated as per the manufacturer's instructions and a LDF splint was constructed using Vinyl Polysiloxane impression material (UnoDent, Essex, England) (Fig. 1). Small holes were drilled into the splint labially at the level of the middle third of all teeth assessed using a tungsten carbide round bur with a slow speed handpiece in order to accommodate and stabilise the LDF probes. Participants were asked to rest for a few minutes while the splints were prepared for intra-oral use and before the start of LDF recordings. Teeth were isolated using a small piece of rubber dam (UnoDent, Essex, England) after which the splint was fitted over the rubber dam. The LDF probes (2 probes) were passed through the labial holes of the splint with each probe placed against each tooth tested allowing simultaneous recordings for both teeth. Movement of the participant or the probes was avoided as much as possible and a 30-second stable LDF recording was achieved. Two successive recordings were obtained.

Control group

Pulp sensibility was assessed in the control group using EPT followed by ethyl chloride. Prior to sensibility assessment of the tested teeth, a detailed explanation of the test procedure was given to the participant followed by a trial test of a sound lower anterior tooth for the child to experience the sensation.

EPT

Teeth assessed were isolated with cotton rolls and dried with air spray. Each participant was asked to hold the metal end of the EPT's probe. The EPT probe was placed in contact with the middle of the labial surface of the tooth assessed using conduction medium (Aquagel medium, Fabricado por, ECOLAB, Leeds, UK). Once a tingling sensation was felt, participants were asked to let go of the probe. Two recordings were obtained per tooth.

During the first recording, the rate of voltage change was set to 5 and then increased to 8 during the second recordings. Any sensation felt by participants at any time before EPT reached the maximum voltage of 80 on the scale was considered positive. An unreliable EPT response was recorded when different responses were obtained, i.e., if one recording was positive while the other was negative.

Ethyl chloride

All teeth were re-dried. A cotton pledget was sprayed with ethyl chloride until saturation, the excess was removed by shaking, and then applied twice to the teeth examined for 5 to 8 seconds with a 2-minute break between the two positive applications. A dry un-sprayed cotton pledget was used to assess false responses between the two positive applications. Each participant was asked to raise their hand when feeling a cold sensation. An overall unreliable response was recorded when disagreement in responses between the first and third applications occurred and/or a positive response to the dry cotton pledget.

Outcome measures

Accuracy outcomes of all tests were defined as follows (Petersson *et al*. 1999):

- Sensitivity is ‘’*the ability of a test to identify teeth that really are diseased. Diseased teeth = necrotic pulp. The sensitivity was calculated according to the formula: True Positive / (True Positive + False Negative)’’.*

- Specificity is ‘’*the ability of a test to identify teeth without the disease. Without disease = teeth with vital pulp. The specificity was calculated according to the formula: True Negative / (True Negative + False Positive)’’.*

- Positive predictive value is ‘’*the probability that a positive test result really represents a diseased tooth’’. The positive predictive value was calculated according to the formula: True Positive / (True Positive + False Positive).*

- Negative predictive value is *‘’the probability that a tooth with a negative test result really is free from disease. The negative predictive value was calculated according to the formula: True Negative / (True Negative + False Negative)’’*

Repeatability as a secondary outcome measure was defined as ''*the variation in repeat measurements made on the same subject, at least two measurements per subject, under identical conditions''* (Bartlett and Frost 2008).

Statistical analysis

Descriptive statistics were used in reporting the demographics and clinical characteristics of the participants. Independent samples t-test was used to assess the difference in age between the test and control groups, while Fisher's exact test was used to assess the difference in gender and tooth type. Chi-square was used to assess the difference in the type of trauma and stage of root development. Paired t-test was used to assess the difference in Flux values between vital and non-vital pulps.

Using Receiver Operating Characteristic (ROC) curves, the Flux cut-off value and the ratio (Flux of teeth with non-vital pulps/ Flux of teeth with vital pulps) showing the best combination of sensitivity and specificity values were chosen. Sensitivity analysis was used to assess the outcomes of EPT and ethyl chloride when study participants provided unreliable results. The positive and negative predictive values for LDF and the accuracy outcomes for EPT and ethyl chloride were calculated using the traditional 2X2 (Akobeng 2007a).

Sensitivity analysis was carried out to assess the unreliable responses provided with EPT and ethyl chloride for different assumptions as if the unreliable responses were positive first indicating a positive patient response. Then the unreliable responses were assessed as if they were negative, and finally the unreliable responses were excluded. The ranges of all the values obtained were reported.

Kappa scores were used to assess the repeatability of EPT and ethyl chloride while inter-class correlation was used to measure the repeatability of LDF. The data was analysed using IBM SPSS (Statistical Package for Social Science) statistics version 23.

Results

The study included 74 participants with a mean age of 12.4 +/- 2.0 years, (range: 8-16 years). There was no significant difference between the two groups in terms of participants' age, gender

distribution, or the type of dental trauma sustained ($P > 0.05$). The tooth type and root development stage of the teeth used as control (teeth with vital pulps) were also not significantly different between the two groups ($P > 0.05$) (Table 1).

LDF

Paired t-test showed a significant difference between Flux values of the teeth with vital pulps , 10.24 (SD = 5.6), and non-vital pulps, 6.88 (SD = 5.4), $P < 0.05$ (Table 2).

There was no ideal cut-off value with high sensitivity and specificity (Fig. 2). The best cut-off value identified was 6.3 Flux with a sensitivity of 43.2% and a specificity of 21% with an area under the ROC curve equal to 0.24. Similar results were obtained when assessing the cut-off ratios (Flux of teeth with non-vital pulps/ Flux of teeth with vital pulps), as no ideal ratio was identified (Fig. 3). The best cut–off ratio identified was 0.6 with a sensitivity of 54 % and a specificity of 32.4% and an area under the curve equal to 0.25 (Table 3). The positive and negative predictive values are presented in Table 3. Re-calculating the ROC curves for both values and ratios after removing the outliers showed no difference in the outcomes. The repeatability of LDF was found to be 0.85.

Control group (EPT and ethyl chloride)

EPT showed a sensitivity of 83.8 – 94.6 %, specificity of 89.2 – 97.6 %, positive predictive value of 89.7 – 96.9 % and negative predictive value of 85.7 - 94.3 % (Table 5). Ethyl chloride showed a sensitivity of 81.1 – 91.9 %, specificity of 73 – 81.1 %, positive predictive value of 77.3 – 81.1 %, and negative predictive value of 81.1 – 90 % (Table 5). The repeatability of EPT and ethyl chloride were 0.86 and 0.81, respectively.

Discussion

The sensitivity and specificity of LDF in the present study were shown to be less than the reported values in previous studies (Ghouth *et al*. 2018, Mainkar and Kim 2018). This could be

attributed to the robust study design used in the present study to overcome some of the limitations seen in previous studies.

A recent systematic review of the LDF's accuracy outcomes in comparison to other sensibility and vitality tests highlighted some serious flaws in the study designs of the studies included in the review, with a lack of high-quality evidence supporting the reported LDF's superior accuracy over other sensibility and vitality tests. The authors concluded that further assessment of the LDF's accuracy using a more robust study design was needed (Ghouth *et al*. 2018). Therefore, this study adopted a cross-sectional study design, consistent with the recommended diagnostic accuracy study designs with random allocation of study participants and allocation concealment (Rutjes *et al*. 2005)**.** Randomisation and allocation concealment were missing in all previously reported LDF studies (Ingolfsson *et al*. 1994, Evans *et al*. 1999, Chen and Abbott 2011, Karayilmaz and Kirzioglu 2011). The authors acknowledge that the use of an independent assessor, blinded to the teeth assessed under the splint, would have further improved the study design somewhat, however, this was not deemed to be logistically achievable

The study participants were from a younger age group to that reported in studies in the literature to specifically assess the accuracy of dental pulp tests in a child population. In the present study, the researchers wanted to directly investigate the issue of unreliability of pulp testing methods which is an issue of concern and of direct relevance to clinical practice of traumatology and endodontics in children. Only one previous study (Karayilmaz and Kirzioglu 2011) assessed the diagnostic accuracy of LDF in teenagers and young adults aged 12-18 years old while most other studies used a wide age range from 6.5-74 years (Ingolfsson *et al*. 1994, Evans *et al*. 1999, Chen and Abbott 2011).

Maxillary central incisors are the most likely teeth to be affected by traumatic dental injuries (Pitts *et al.* 2013) and were the teeth that were mostly included in the present study. For assessment of LDF ratios and specificity of the tests employed, assessment of vital teeth was important. The authors acknowledge that some of the teeth considered non-traumatised with vital pulps might have been involved in the trauma at the time the trauma was sustained. However, the use of strict inclusion criteria such as no evidence of trauma at time of assessment, lack of signs and symptoms of pulpal damage and positive response to sensibility tests for a minimum of six months prior to recruitment should have minimised any such effect. The choice of a tooth from the opposing arch was considered as a possibility, however, that would have introduced another variable in the interpretation of the results.

The electrical and cold stimulation to the dental pulp have two different mechanisms of action according to the hydrodynamic theory. Consequently, the application of cold testing appears to have no effect on electrical stimulation on the pulp. As a result, the sequence of pulp tests has not been found to affect the results of the tests when EPT and ethyl chloride were reversely used (Trowbridge *et al*. 1980, Pantera *et al*. 1993, Fuss *et al*. 1986). The application of EPT followed by thermal testing is a common sequence of pulp testing (Peters *et al*. 1994). Cold application of five to eight-second has been shown to be sufficient to determine the responsiveness of the teeth in the majority of the cases (White and Cooley 1977).

Choosing an ideal reference standard is fundamental in diagnostic accuracy studies. The reference standard is the best available method to establish the presence or absence of a disease to which the test results could be compared. The use of an inappropriate reference standard can cause an error in diagnoses (classification bias) and can result in under/over estimation of the performance of the test (Rutjes *et al*. 2006). The present study included a composite reference standard for teeth with vital pulps which was based on clinical and radiographic examinations. The use of a composite reference standard can sometimes be used when there are several tests to diagnose a condition and which combines the results of the tests to present a better indicator of true disease status (Alonzo and Pepe 1999), similar to previous studies (Evans *et al*. 1999, Karayilmaz and Kirzioglu 2011, Ingolfsson *et al*. 1994). With regards to the necrotic (pulpless) teeth, unlike other studies where the reference standard was the presence of necrotic tissue or blood upon root canal treatment (Ingolfsson *et al*. 1994, Evans *et al*. 1999, Chen and Abbott

2011), which is subjective, a standaridised reference standard of either pulpal extirpation or a completed root canal treatment was used in the present study. Polat *et al*. (2004) showed that there was no significant difference in LDF recordings between empty and filled root canals.

Laser penetration and reflection have been shown to be affected by crown restorations (Chandler *et al*. 2014, Chandler *et al*. 2010). Therefore, the inclusion of heavily restored teeth was avoided. For standardisation purposes, included teeth were non-discoloured with restorations covering less than half-crown labial surfaces in order to allow LDF's and EPT's probes as well as ethyl chloride's cotton pledget placement at the middle third of the crown in contact with sound tooth structure.

The use of rubber dam in addition to the splint is supported by studies in the literature and have been shown to reduce non-pulpal contamination of the surrounding tissues (reduce mean blood flow by 56-82 %) (Hartmann *et al*. 1996, Soo-ampon *et al*. 2003, Kijsamanmith *et al*. 2011). The use of a rubber dam and splint was utilised in the present study.

There is an inconsistency in the literature with regards the optimum duration of LDF recording. Furthermore, it is well established that movement artefacts, whether related to the patient or apparatus itself, affect LDF recordings (Ramsay *et al*. 1991, Hartmann *et al*. 1996). Therefore, allowing sufficient time to obtain a stable Flux recording has been recommended (Jafarzadeh 2009). Valid and correct acquisition requires a complex technique, which includes the precise positioning of the probe as well as relaxation and absence of any movement in order to avoid artefacts. A stable 30-second interval, as free as possible from movement artefacts, was used to calculate the Flux values for each patient. Miron *et al.* (2010) found that there was no statistically significant difference between Flux measurements from six 30-second stable time interval LDF outputs.

The Flux values of teeth with non-vital pulps were higher than the values of teeth with vital pulps in a few recordings. Roebuck *et al.* (2000) reported similar findings where they assessed the vitality of anterior teeth. Most of the different probe design combinations used resulted in at least one recording where a Flux value of a non-vital pulp was higher than the vital pulp. This may be an additional limitation of the use of LDF which adds to the difficulty in

interpreting the results. Moreover, fluctuations and heterogeneity of Flux values have been observed in the present data. Which is similar to another study where LDF results showed non-interpretable Flux values (Roy *et al.* 2008).

One of the most important and crucial factors in using LDF is the use of a cut-off threshold to aid in the diagnosis of non-vital pulps. Currently, there is no consensus as to the LDF's cut-off threshold despite few suggestions which are based on low-quality research (Ghouth N *et al*. 2018). Different cut-off thresholds have been used and reported in the literature. The use of cut-off ratios below which the pulp is considered non-vital (diseased pulp Flux/ known healthy pulp Flux) of 0.1 and 0.6 have been used in two studies (Chen and Abbott 2011, Karayilmaz and Kirzioglu 2011). The cut-off ratios used by Chen and Abbott (2011) was based on the work by other researchers (Ingolfsson *et al*. 1994, Roebuck *et al*. 2000), despite the inherent and serious limitations of the two studies on which these were based (Ghouth N *et al,* 2018). The rationale behind the 0.1 ratio used by Karayilmaz and Kirzioglu (2011) was also not clear. The current study showed that a cut off ratio of 0.6 produced the best combination of sensitivity and specificity. However, these accuracy values are too low for a diagnostic tool to be used with confidence and to be clinically acceptable.

The use of a cut-off value, rather than ratio, of 7.0 PU was used by one study showing sensitivity and specificity of 100% (Evans *et al*. 1999). It was however unclear what the authors' rationale was behind the use of this particular value. In addition, no power calculation or randomisation was performed in that study. Applying this value, 7.0 PU, to the data in the present study showed poor sensitivity and specificity of 35% and 27 %, respectively. Applying an arbitrary cut-off value/ratio to analyse LDF recordings would result in overestimation of the true accuracy.

The ROC curve is a graphical technique for assessing the ability of a test to distinguish between diseased and non-diseased subjects. This technique helps in the determination of the cut-off threshold which results in the best sensitivity and specificity that may be attained (Akobeng 2007b). The ROC analysis used in the present study showed that the cut-off ratio of non-vital pulp/healthy pulp $\geq$ 0.6 to yield the best possible combination of sensitivity and specificity. In addition, a perfect test would have an area under the ROC curve of 1.0, while a value less

than 0.5 indicates a completely unusable test with the results likely obtained by chance (Zou *et al*. 2007, Akobeng 2007b). The area under the curve in the present study for both LDF Flux values and ratios was much lower than 0.5 which confirms the results as having low sensitivity and specificity.

The sensitivity and specificity of EPT and ethyl chloride, in the present study, are in agreement with those reported in the literature (Fuss *et al*. 1986, Villa-Chavez *et al*. 2013, Petersson *et al*. 1999, Evans *et al.* 1999), while those of the LDF were much lower than those reported in the literature (Evans *et al*. 1999, Karayilmaz and Kirzioglu 2011).

The authors of the present study are fairly certain that these results, although somewhat unexpected, are a consequence of the more stringent study conditions used in the present study conducted with a rigorous study design in conformity with that required for a cross-sectional cohort diagnostic accuracy study with randomisation (Rodger *et al.* 2012). Some of the attributes carefully introduced into the study design were power calculation, participants randomisation, the use of a younger age group, exclusion of teeth with large restorations, and the use of a combination of rubber dam and splint to reduce non-pupal signals.

Conclusion

The results of this study show a high probability of false results when using LDF in assessing the pulp blood flow/pulp vitality in children. Therefore, within the limitations of this study, the results suggest that LDF is unable to differentiate between teeth with vital and non-vital pulps in children between the ages of 8-16 years, with any acceptable level of confidence. Further assessment of the LDF with different parametres such as wavelengths and/or probe type and fibre distance is needed. In addition, further technical development may also be needed to allow the more convenient use of the device before it can be recommended for routine clinical use for the assessment of the dental pulp especially in the child population.

References

Akobeng AK (2007a) Understanding diagnostic tests 1: sensitivity, specificity and predictive values. *Acta Paediatrica* **96,** 338-341.

Akobeng AK (2007b) Understanding diagnostic tests 3: Receiver operating characteristic curves. *Acta Paediatrica* **96,** 644-647.

Alonzo TA, Pepe MS (1999) Using a combination of reference tests to assess the accuracy of a new diagnostic test. *Statatistics in Medicine* **18,** 2987-3003.

Andreasen FM (1989) Pulpal healing after luxation injuries and root fracture in the permanent dentition. *Endodontics and Dental Traumatology* **5,** 111-131.

Bartlett JW, Frost C (2008) Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. *Ultrasound in Obstetrics & Gynecology* **31**, 466-475.

Bhaskar SN, Rappaport HM (1973) Dental vitality tests and pulp status. *Journal of the American Dental Association* **86,** 409-11.

Chandler NP, Pitt Ford TR, Monteith BD (2010) Effect of restorations on pulpal blood flow in molars measured by laser Doppler flowmetry. *International Endodontic Journal* **43,** 41-46.

Chandler NP, Pitt Ford TR, Monteith BD (2014) Laser light passage through restored and carious posterior teeth. *Journal of Oral Rehabilitation* **41,** 630-634.

Chen E, Abbott PV (2011) Evaluation of accuracy, reliability, and repeatability of five dental pulp tests. *Journal of endodontics* **37,** 1619-1623.

Cohen JF, Korevaar DA, Altman DG et al. (2016) STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *British Medical Journal Open* **6,** 1-17.

Cooley RL, Robison SF (1980) Variables associated with electric pulp testing. *Oral Surgery, Oral Medicine, Oral Pathology* **50,** 66-73.

Crona-Larsson G, Bjarnason S, Noren JG (1991) Effect of luxation injuries on permanent teeth. *Endodontics and Dental Traumatology* **7,** 199-206.

Evans D, Reid J, Strang R, Stirrups D (1999) A comparison of laser Doppler flowmetry with other methods of assessing the vitality of traumatised anterior teeth. *Endodontics and Dental Traumatology* **15,** 284-290.

Fuss Z, Trowbridge H, Bender IB, Rickoff B, Sorin S (1986) Assessment of reliability of electrical and thermal pulp testing agents. *Journal of Endodontics* **12,** 301-305.

Ghouth N, Duggal MS, BaniHani A, Nazzal H (2018) The diagnostic accuracy of laser Doppler flowmetry in assessing pulp blood flow in permanent teeth: A systematic review. *Dental Traumatology.* [Online] URL: https://doi.org/10.1111/edt.12424 [Accessed on 3 Septemebr 2018]

Hartmann A, Azerad J, Boucher Y (1996) Environmental effects on laser Doppler pulpal blood-flow measurements in man. *Archives of Oral Biology* **41,** 333-339.

Ingolfsson, A. R., Tronstad, L., Hersh, E. V. & Riva, C. E. (1994) Efficacy of laser Doppler flowmetry in determining pulp vitality of human teeth. *Endodontics and Dental Traumatology* **10,** 83-87.

Jafarzadeh H (2009). Laser Doppler flowmetry in endodontics: a review. *International Endodontic Journal* **42,** 476-90.

Karayilmaz H, Kirzioglu Z (2011) Comparison of the reliability of laser Doppler flowmetry, pulse oximetry and electric pulp tester in assessing the pulp vitality of human teeth. *Journal of Oral Rehabilitation* **38,** 340-347.

Kijsamanmith K, Timpawat S, Vongsavan N, Matthews B (2011) A comparison between red and infrared light for recording pulpal blood flow from human anterior teeth with a laser Doppler flow meter. *Archives of oral biology* **56,** 614-618.

Mainkar A, Kim SG (2018) Diagnostic Accuracy of 5 Dental Pulp Tests: A Systematic Review and Meta-analysis. *Journal of Endodontics **44**, 694-702*

Mejare IA, Axelsson S, Davidson T *et al*. (2012) Diagnosis of the condition of the dental pulp: a systematic review. *International Endodontic Journal* **45,** 597-613.

Ohman A (1965) Healing and sensitivity to pain in young replanted human teeth. An experimental, clinical and histological study. *Odontologisk Tidskrift* **73,** 166-227.

Pantera EA, Anderson R W, Pantera CT (1993) Reliability of electric pulp testing after pulpal testing with dichlorodifluoromethane. *Journal of Endodontics* **19,** 312-4.

Peters DD, Baumgartner JC, Lorton L (1994). Adult pulpal diagnosis. I. Evaluation of the positive and negative responses to cold and electrical pulp tests. *Journal of Endodontics* **20,** 506-11.

Petersson K, Soderstrom C, Kiani-Anaraki M, Levy G (1999) Evaluation of the ability of thermal and electrical tests to register pulp vitality. *Endodontics & Dental Traumatology* **15,** 127-131.

Pitts N, Chadwick B, Anderson T (2015) Children's dental health survey 2013, report 2: dental disease and damage in children, england, wales and northern ireland. URL Http:// www.hscic.gov.uk/catalogue/pub17137/cdhs2013-report2-dental-disease.pdf [accessed September 2018].

Ramsay DS, Artun J, Martinen SS (1991) Reliability of pulpal blood-flow measurements utilizing laser Doppler flowmetry. *Journal of dental research,* **70,** 1427-1430.

Rodger M, Ramsay T, Fergusson D (2012) Diagnostic randomized controlled trials: the final frontier. *Trails,* **13,** 1-7

Roebuck EM, Evans DJ, Stirrups D, Strang R (2000) The effect of wavelength, bandwidth, and probe design and position on assessing the vitality of anterior teeth with laser Doppler flowmetry. *International Journal of Paediatric Dentistry* **10,** 213-220.

Roeykens H, De Moor R (2011) The use of laser Doppler flowmetry in paediatric dentistry. *European Archives of Paediatric Dentistry* **12,** 85-89.

Roy E, Alliot-Licht B, Dajean-Trutau DS, Fraysse C, Jean A, Armengol V (2008) Evaluation of the ability of laser Doppler flowmetry for the assessment of pulp vitality in general dental practice. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* **106,** 615-620.

Rutjes AW, Reitsma JB, DI Nisio M, Smidt N, Van Rijn J C, Bossuyt P M (2006) Evidence of bias and variation in diagnostic accuracy studies. *The Canadian Medical Association Journal* **174,** 469-76.

Rutjes AW, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PM (2005) Case-control and two-gate designs in diagnostic accuracy studies. *Clinical Chemistey* **51,** 1335-41.

Soo-Ampon S, Vongsavan N, Soo-Ampon M, Chuckpaiwong S, Matthews B (2003) The sources of laser Doppler blood-flow signals recorded from human teeth. *Archives of oral biology* **48,** 353-360.

Toman K (1984) Christian Doppler and the Doppler effect. *History of Geophysics* **65,** 7-10.

Trowbridge HO, Franks M, Korostoff E, Emling R (1980) Sensory response to thermal stimulation in human teeth. *Journal of Endodontics* **6,** 405-12.

Villa-Chavez CE, Patino-Marin N, Loyola-Rodriguez JP, Zavala-Alonso NV, Martinez-Castanon GA, Medina-Solis CE (2013) Predictive values of thermal and electrical dental pulp tests: a clinical study. *Journal of Endodontics* **39,** 965-969.

White JH, Cooley RL (1977) A quantitative evaluation of thermal pulp testing. *Journal of Endodontics* **3,** 453-457.

Zou KH, O'malley AJ, Mauri L (2007) Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation,* **115,** 654-7.