The assessment of pulpal blood flow using laser Doppler flowmetry

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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My father, my mother and my wife, I dedicate this thesis to you…

Global abstract

Aims: The overall aim of this work was to study the use of Laser Doppler flowmetry for the assessment of the dental pulp in permanent teeth. The thesis is presented as four distinct studies; 1) A systematic review was carried out to assess the published evidence on the use of laser Doppler flowmetry in the assessment of the pulp status of permanent teeth, 2) A cross-sectional survey was carried out in order to understand the use of dental pulp tests by paediatric dentists and general dental practitioners in children with dental trauma in the United Kingdom, 3) The first clinical study aimed to assess whether laser Doppler flowmetry was 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 4) The second clinical study aimed to prospectively monitor pulp sensibility/vitality of traumatised teeth using laser Doppler flowmetry, electric pulp testing and ethyl chloride, and to prospectively investigate the accuracy of each test.

Methods:

Systematic review: A systematic literature search, using MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, www.clinicaltrials.gov and www.controlled-trials.com, in addition to citation and manual reference list searches, was conducted up to 15th January 2018. A risk of bias assessment was performed using the quality assessment for diagnostic accuracy studies tool (QUADAS-2) with all steps performed independently by two reviewers.

Survey: A cross-sectional study utilising an 18-item questionnaire that was developed using the Bristol Online Survey (BOS) tool and circulated electronically to the members of the British Society of Paediatric Dentistry between June and August 2017.

Clinical study 1: 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 pulp extirpation and a contra-lateral tooth with vital pulp. The outcome measures included the cut-off threshold for LDF and the sensitivity, specificity and predictive values as well as the repeatability of each test. The Receiver Operating Characteristic (ROC) curve and the contingency 2X2 table were used for analysis. Kappa scores were used to assess the repeatability of EPT and ethyl chloride while inter-class correlation was used for LDF.

Clinical study 2: Children who sustained dental trauma to an anterior permanent tooth with uncertain pulp vitality requiring monitoring for a minimum of 12 months were included in the study. Recordings of dental pulp tests were carried out at baseline and at the end of the follow-up period.

Results

Systematic review: Only four studies all with a high risk of bias were included in the final systematic review for analysis. Laser Doppler flowmetry was reported to be more accurate in differentiating between teeth with normal pulps and pulp necrosis with a sensitivity of (81.8-100%) and

specificity of 100 % in comparison to other vitality tests such as pulp oximetry (sensitivity = 81.3 %, specificity = 94.9 %) and sensibility tests such as electric pulp testing (EPT) (sensitivity = 63.3 – 91.5 %, specificity = 88 – 100 %).

Survey: One hundred and forty-one respondents, both, paediatric dental specialists (56%) and GDPs (44%) were included in the analysis. Almost all specialists (93.7%) reported using sensibility tests routinely in comparison to 80.6% of GDPs. Child perception and cooperation were the most commonly reported barriers. GDPs mainly used cold testing, while specialists used cold and electric pulp tests equally. Inconsistencies in recording as well as documentation the results varied among respondents. Only a few specialists reported having some experience in using laser Doppler flowmetry.

Clinical study 1: There was a significant difference between the Flux values for teeth with vital and non-vital pulps. 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.

Clinical study 2: The study included a convenience sample size of 15 participants with a mean age of 10.7 years (SD=1.66), age range 8-14 years. The mean follow-up period was 7.29 months (SD 1.9) with a range of 6-12 months. All traumatised teeth remained vital at the end of follow-up except one tooth. The specificity of LDF at baseline was 80% compared to

66.6% and 60-73.3% for EPT and ethyl chloride, respectively. At the end of the follow-up period, LDF showed lower specificity (71.4 %) than EPT (78.5 – 85.7 %) and ethyl chloride (71.4 – 78.5 %).

Conclusion: Despite the high reported sensitivity and specificity of laser Doppler flowmetry in the systematic review, these data were found to be based on studies with a high level of bias and serious shortfalls in study designs. The survey of specialists and GDP's showed that the use of pulp sensibility tests was relatively high amongst respondents while those of vitality tests were very low. Barriers and inconsistencies in the technique and recording of the results of sensibility tests were evident. The frequency and timing of using sensibility tests in line with international guidelines were stressed. The use of standardised techniques involving methods considered to improve reliability was highlighted. The results of the clinical studies showed that there was a high probability of false results when using LDF in assessing the pulp blood flow/pulp vitality. LDF 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 in the first clinical study. Within the limitations of the second clinical study, LDF showed better specificity than both EPT and ethyl chloride in predicting the outcome of the pulp at baseline but less at the end of follow-up. Due to the small sample size and relatively short follow-up period, the results of the second clinical study have been interpreted with caution.

Therefore, the published data on the accuracy of LDF can not be accepted as they are based on studies with unacceptable flaws in study design. Our

studies have shown that not only the use of LDF or even the experience of clinicians with its use is extremely low, but also its specificity and sensitivity were of a level which is unacceptable for recommending its meaningful clinical use.

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Table of Contents

List of Tables

List of Figures

Thesis layout

The thesis is presented in a chapter format. The first chapter presents an introduction and literature review. The following four chapters present the four studies of the thesis. Each chapter consists of materials and methods, results, discussion and conclusion. A general discussion and conclusion is presented at the end of the thesis.

Abbreviations

BSPD: British Society of Paediatric Dentistry

CBCT: Cone Beam Computed Tomography

CDH: Child Dental Health

CRD: Centre for Reviews and Dissemination

EF: Endo Frost

EPT: Electrical Pulp Test

GDC: General Dental Council

LDF: Laser Doppler Flowmetry

LPN: Local Professional Network

PO: Pulse Oximetry

StR: Speciality Registrar

SWM: Slow Wave Vasomotion

TDIs: Traumatic Dental Injuries

Chapter 1 Introduction and literature review

1.1 The prevalence of traumatic dental injuries

Traumatic dental injuries (TDIs) are common among children and have become a dental public health problem in childhood and adolescence. The oral region constitutes an area of 1% of the total body. However, TDIs represent 5% of all bodily injuries (Andersson, 2013). Therefore, dental traumatology research is a large component in paediatric dentistry.

The prevalence of TDIs is high throughout the world and varies within and between countries. The variation is due to different factors such as the lack of standardisation in study designs/methodologies used, dentition studied, socio-economic and behavioural differences between cultures and countries. In the permanent dentition, most studies report figures at approximately 20% of children and adolescents (Glendor, 2008).

In the UK, according to the 2013 Children's Dental Health (CDH) survey, around 12% of 12-year-olds and 10% of 15-year-olds were found to have evidence of TDIs to their incisors. It was also observed that the prevalence of trauma was higher in boys than in girls and the most affected teeth were the maxillary central incisors. Enamel fracture was the most common injury sustained (Pitts et al., 2015). These findings are quite similar in comparison to the 2003 CDH (Chadwick et al., 2006). However, there was a decline in TDIs in 15-year-old boys from 17% to 11% between 2003 and 2013.

When compared to 2003, there was an increase in the number of teeth with enamel fractures from 28.2 to 36 per thousand maxillary central incisors in 12-year-olds and a decrease from 28.2 to 20.9 in 15-year-olds. In addition, an increase in missing maxillary central incisors in both age groups was evident in 2013, from 0.5 to 2.1 and 0.1 to 1.1 maxillary central incisors per thousand in 12 and 15-year-olds, respectively (Pitts et al., 2015).

On an international level, reports from most countries have shown that around one-fourth of all school children had sustained TDIs (Glendor, 2008). Table 1.1 shows the prevalence of TDIs in the permanent teeth in different countries of the world (Lam, 2016; Glendor, 2008).

A valid study recruits a sample that represents a defined target population (Boyle, 1998). Some studies were carried out in both private and public schools (Livny et al., 2010; Nik-Hussein, 2001), which is more representative than collecting data from one domain of schools only (Al-Majed et al., 2011; Faus-Damia et al., 2011) or from a dental school service (Schatz et al., 2013). Moreover, studies that only include boys may overestimate the prevalence rates. For instance, a study that only included boys reported a high prevalence rate which may be explained by the fact that boys suffer more TDIs than girls (Petti, 2015; Al-Majed et al., 2011).

Furthermore, some studies limited their inclusion criteria to include only specific types of TDIs. For example, they included injuries to the supporting structures such as luxation injuries (Petti and Tarsitani, 1996; Taiwo and Jalo, 2011; Oldin et al., 2015) while other studies did not include luxation injuries (Nik-Hussein, 2001; Frujeri et al., 2014) or root fractures (Patel and Sujan, 2012). It should be

noted that many luxation injuries may be clinically missed at the time of examination due to the lack of radiographs at study settings and to the difficulty of obtaining ethical approvals to justify unnecessary additional radiographs. This could lead to underestimation of the prevalence of TDIs.

Using cross-sectional clinical examinations of children may also overlook many types of injuries especially if the injuries did not occur recently prior to the time of the examination such as luxation injuries (Bastone et al., 2000). Other studies are retrospective in nature (Petti and Tarsitani, 1996). The data collected from patient records at a particular point in time represent events that have occurred when treatment was only sought. The prevalence figures are an underestimation as there are no reliable means to quantify the number of patients not seeking professional care using this methodology (Lam, 2016).

A Swedish study adopted a 5 year longitudinal study using multiple ways to identify children with TDIs. A combination of yearly clinical examinations, retrospective interviews, retrospective dental records, prospective interviews, and prospective dental records were implemented in order to find and identify all children with TDIs in the study. The prevalence was reported to be high which may be due to the fact that having a bike is common among Swedish children. Also, there was a risk factor for children desired to ride advanced bikes at an early age (Oldin et al., 2015).

Table 1:1 International prevalence of TDIs to permanent teeth in different countries

Region	Authors	Age group	Sample size	Study setting	Prevalence %
Brazil	(Frujeri et al., 2014)	12	1118	Private and public schools	12.6
India	(Patel and Sujan, 2012)	8-13	3708	Private and public schools	8.8
Italy	(Petti and Tarsitani, 1996)	6-11	824	Two primary schools	20.3
Malaysia	(Nik-Hussein, 2001)	16	4085	Private and public schools	4.1
Nigeria	(Taiwo and Jalo, 2011)	12	719	Public schools	15.2
Palestine	(Livny et al., 2010)	11-12	804	Private and public schools	17.7
Saudi Arabia	(Al-Majed et al., 2011)	12-14	1216	Public schools	34
Spain	(Faus-Damia et al., 2011)	6-18	1325	Public schools	6.2
Sweden	(Oldin et al., 2015)	0-17	2363	Public Dental Service clinics	37.6
Thailand	(Malikaew et al., 2006)	11-13	2725	Public schools	35.0
USA	(Shulman and Peterson, 2004)	6-20	6558	Mobile examination centres	16.0
Switzerland	(Schatz et al., 2013)	6-13	1898	Dental school service	14.1

1.2 Aetiology and risk factors of TDIs in children

1.2.1 Aetiology

The aetiology of TDIs can be broadly classified into unintentional and intentional causes (child physical abuse/assault). The main causes of TDIs in young children include falls and collisions. Teenagers, on the other hand, are predominantly injured during sports activities and violence (fights, assaults) (Glendor, 2008).

Aetiological factors are influenced by population types, age groups, cultures, regions in the world and environments (Andersson, 2013). In a comparative study between the Sudan and Iraq, it was found that violence was the main aetiological factor of TDIs in 6– 12-year-old children (36% in Iraq and 71% in Sudan) (Baghdady et al., 1981). Engaging in sport activities was found to be the cause of TDIs in as low as 3 % in both countries. Sport has been reported as the main cause of TDIs among Japanese and UK teenagers (Uji and Teramoto, 1988; Blinkhorn, 2000). In Brazil, a similar occurrence of TDIs as a result of sport (19%) and violence (16%) was observed (Marcenes et al., 2000)

1.2.2 Risk factors

The following risk factors have been reported to influence TDIs:

- Increased overjet and lip incompetence are significant predisposing factors to TDIs. Two meta-analyses concluded that an overjet larger than 3 mm might double the risk of TDIs to children’s anterior teeth in comparison to those with an overjet less than 3 mm (Nguyen et al., 1999; Petti, 2015).

- Another major environmental risk factor for TDIs is material deprivation. It was reported that 34–44% of dental injuries in the UK have occurred in deprived areas (Hamilton et al., 1997; Marcenes and Murray, 2002). Studies concluded that the more deprived the area, the higher prevalence of dental injuries. Furthermore, overcrowded areas were found to have major influence on dental injuries (Marcenes and Murray, 2002; Marcenes and Murray, 2001) as such places are likely to have more crowded and unsafe playgrounds, sport facilities and schools.

- Emotionally stressful conditions, such as attention-deficit hyperactivity disorder (ADHD), are highly associated with dental trauma. A cross-sectional study was conducted in a private child psychiatric setting has shown a significant association between dental trauma and ADHD. The prevalence of TDIs, among a total of 475 children, was found to be 12.8 % (Sabuncuoglu et al., 2005). Furthermore, the risk of TDIs is more significant in these children especially before starting ADHD treatment (Sabuncuoglu, 2007).

- The presence of illness, physical limitations or learning difficulties are also associated with TDIs. Epileptic patients, for instance, are at a higher risk of sustaining TDIs as 40% of epileptic patients were found to have experienced TDIs (Costa et al., 2011). Moreover, TDIs occur at a higher frequency, 57%, among cerebral palsy patients. The uncontrolled head movement was the major risk factor increasing the risk for this cohort (Holan et al., 2005).

- Iatrogenic injuries, such as TDIs secondary to general anaesthesia intubation, have been found to vary from 0.04% to 12%. Most TDIs are accidentally caused by direct pressure during laryngoscopy and intubation, resulting in mainly crown fractures, luxation injuries or avulsions (Chadwick and Lindsay, 1996; Chadwick and Lindsay, 1998).

1.3 Classification of dental injuries

Currently, the accepted system is based on the World Health Organisation and modified by Andreasen et al. 2007 (Table 1:2). TDIs can result in different injury types involving:

- The hard dental tissues and the pulp.
- The periodontal tissues.
- The supporting bone.
- The gingiva and oral mucosa.

Table 1:2 Classification of dental injuries to the hard dental tissues and pulp, and to the periodontal tissues

Injuries to the hard dental tissues and the pulp	
Injury	**Criteria**
Enamel infraction	An incomplete fracture (crack) of the enamel without loss of tooth substance.
Enamel fracture	A fracture with loss of enamel only
Enamel-dentine fracture	A fracture with loss of enamel and dentine, but not involving the pulp
Complicated crown fracture	A fracture involving enamel, dentine, and exposing the pulp
Crown-root fracture	A fracture involving enamel, coronal and radicular dentine, and cementum
Root fracture	A fracture involving radicular dentine, cementum, and the pulp.
Injuries to the periodontal tissues	
Concussion	An injury to the tooth-supporting structures without abnormal loosening or displacement of the tooth.
Subluxation	An injury to the tooth supporting structures with abnormal loosening, but without displacement of the tooth.
Extrusive luxation	Partial displacement of the tooth out of its socket
Lateral luxation	Displacement of the tooth in a direction other than axially.
Intrusive luxation	Displacement of the tooth into the alveolar bone.
Avulsion	Complete displacement of the tooth out of its socket

1.4 The effect of trauma on the dental pulp and supporting structures

Various complications and consequences can result following TDIs. The type of complication and the likelihood of its development is dependent on several factors such as the type and severity of dental trauma. Therefore, accurate diagnosis and follow-up are important in managing acute and long-term complications. When a tooth suffers from a traumatic injury, variable degrees of damage occur to the periodontal structures and neurovascular bundle at the apex of the root (Trope, 2002; Bakland and Andreasen, 2004).

The most favourable outcome of TDIs is healing of the pulp and the surrounding tissues. Some injuries, such as enamel infraction and enamel fractures, have a very low risk to develop complications affecting the health and survival of traumatised teeth. However, others, such as intrusion and avulsion injuries, are often associated with complications of different types and severities such as crown discolouration, pulp necrosis, apical periodontitis, loss of marginal bone and root resorption followed by possible tooth loss. These healing complications can be predicted (Andreasen et al., 2006b) and consideration should be given to the fact that complications of dental trauma can occur several months or even years after the injury (Robertson, 1998; Robertson et al., 2000).

1.4.1 The effect on periodontal ligament (PDL)

The effect of TDIs on the PDL can be detected in situations where root resorption occurs (Andreasen and Andreasen, 1992; Trope, 2002). Complete

tissue regeneration occurs after minor injuries to the PDL which cause rupture of fibres. Recruitment of macrophages is stimulated when the injured tissues are removed in a more severe injury causing compression or crushing of the PDL (Andreasen, 1980).

When more severe injuries occur, such as lateral luxation and intrusive luxation, other bony structures are damaged. Root resorption frequently occurs following the recruitment of osteoclasts. The result could be surface resorption (repair-related resorption) or ankylosis-related resorption (replacement resorption), determined by the extent of the injury.

Repair-related resorption (also called surface resorption) is external, therefore, not progressive and shows repair with cementum. It is a transient process involving small areas on the root surface resulting from minor injuries such as subluxation. Also, it can be seen with avulsion injuries and root fracture injuries. It is self-limiting, and it shows spontaneous repair. Moreover, as long as there is no presence of bacteria in the root canal system, it is reversible (Andreasen and Andreasen, 1992).

On the other hand, extensive injured areas favour ankylosis-related (replacement) resorption over surface resorption. It is a relatively slower resorptive process. It is related to extensive damage to the PDL resulting in the lost vitality of the cells and damaged cementum (Lee et al., 2003; Nikoui et al., 2003). Lacking of the protective mechanism of PDL coverage, the cementum is exposed to osteoclasts that replace cementum and dentine with bone, resulting in a fusion of the tooth to bone (Andreasen et al., 1995).

Another aggressive type of resorption, related to root canal infection subsequent to trauma, is infection related resorption. Severe injuries such as intrusive luxation and avulsion injuries usually result in reduction or cutting of blood supply to the pulp. The resorption occurs when there is an untreated infection of the pulp canal as well as damage to the periodontal membrane and cementum. This damage to the cementum causes the pulp canal and dentinal tubules to become pathways for bacterial toxins within the canal capable of triggering osteoclastic activity (Andreasen and Andreasen, 1992; Trope, 2002). The delay or failure to eradicate bacteria and remove the necrotic pulp from the root canal system may lead to infection-related resorption at a rapid rate that may produce complete root resorption within a short term. Once detected, an intervention with root canal treatment can arrest the process.

1.4.2 The effect on the dental pulp

The degree of the damage to the pulpal blood and nerve supply depends on the severity of the injury (Andreasen and Pedersen, 1985). The damage may range from minor injuries such as local bleeding, stretching or compression of the nerve fibres and blood vessels as in concussion or subluxation injuries, to total severance of the blood and nerve supply in lateral luxation or intrusion injuries. If bacteria find access to an injured pulp, healing may be affected (Lauridsen et al., 2012a). The degree of recovery and repair of the dental pulp is related to the ability to maintain an intact vascular supply to the pulp following the injury. The response of the dental pulp is affected by the degree of injury to the neurovascular supply through the apical foramen, as well as exposed

dentinal tubules in cases of crown fracture injuries leading to bacterial access to the pulp (Love and Jenkinson, 2002; Andreasen et al., 2006a).

Pulpal outcomes, following dental injuries, include pulpal healing, pulp canal obliteration or pulpal necrosis. Pulpal healing usually occurs following minimal disruption of the neurovascular supply. The pulp may have the ability to continue functioning with reduced circulation until complete reconstruction or revascularisation is achieved.

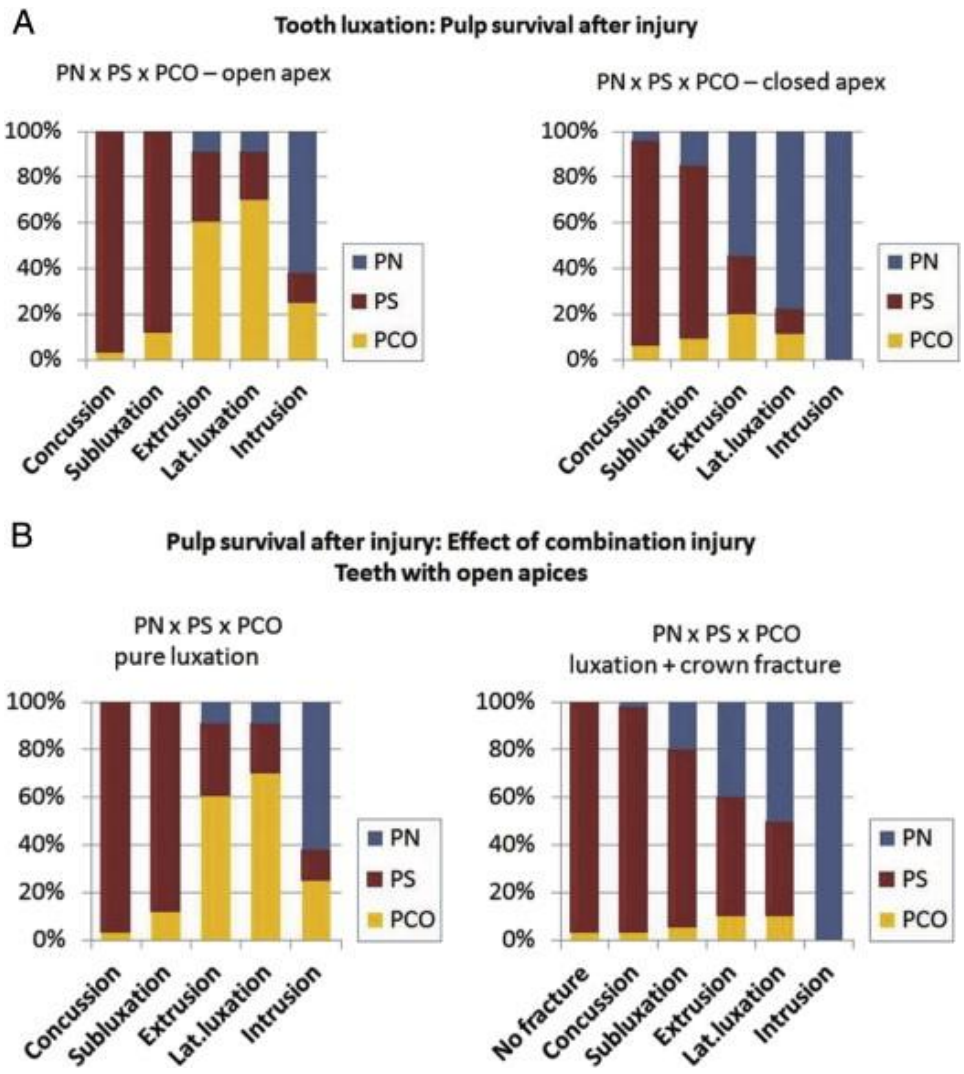
Varying degrees of pulp canal obliteration can occur affecting approximately 4–24% of traumatised teeth. Pulp canal obliteration is characterised by a radiographic narrowing of pulp canal space, and yellow discolouration of the crown clinically. Around 7–27% of such teeth may develop pulp necrosis (McCabe and Dummer, 2012).

Following TDIs, pulp necrosis can develop as a result of coronal bacterial invasion through exposed dentinal tubules, direct exposure of pulp tissues or rupture of the neurovascular supply to the pulp through the apical foramen associated with bacterial infection of the ischemic pulp preventing pulpal revascularisation (Andreasen et al., 2006b). Therefore, pulp necrosis following severance of the blood supply may occur through coagulation necrosis (sterile necrosis) to gangrenous necrosis (infection of infarcted tissue) as well in cases of coronal pulp exposure resulting in as liquefaction necrosis (Bakland and Andreasen, 2004).

Minor TDIs, such as subluxation and crown fracture without pulp exposure, are a low risk for pulp necrosis when they occur in isolation. However, if

combination injuries are involved in the same tooth, an increased risk of pulp necrosis is observed. Recent studies have shown a significant increase risk of pulp necrosis in teeth with concussion, subluxation, or lateral luxation with a concomitant crown fracture (Lauridsen et al., 2012a; Lauridsen et al., 2012b; Lauridsen et al., 2012c).

The diameter of the apical foramen and the stage of root development have a significant role and relationship with the development of pulp necrosis. These factors are associated with luxated teeth, avulsed replanted teeth and root fractures (Andreasen and Pedersen, 1985; Andreasen et al., 1995). It has been shown that a tooth with an apical diameter of 1.2 mm had a higher potential for recovery compared to one with an apical diameter of 0.7 mm (Andreasen and Kahler, 2015b). Immature teeth with incomplete root development have a higher pulpal vascular supply resulting in better prognosis, and a better chance of pulpal revascularisation and survival rate following dental injury (Figure 1:1).



*PN: Pulp necrosis, *PS: Pulp survival, *PCO: Pulp canal obliteration

Figure 1-1 Stacked bar charts showing:(A) Relationship between pulp survival and luxation injury in teeth with open and closed apices (Andreasen and Pedersen, 1985), (B) Pulp survival after injury: Effect of combination injury in teeth with open apices (Robertson et al., 2000).

1.5 The dental pulp

1.5.1 Pulp histology

The pulp is derived from neural crest cells of ectomesenchymal origin. It is a specialised soft connective tissue entirely enclosed by dentine in the pulp chamber and root canal(s) of the tooth. It is a richly vascularised and innervated tissue. Histologically, four distinct cell zones can be distinguished namely the odontoblastic zone, the cell-free zone, the cell-rich zone and the pulp core (Figure 1:2). The odontoblastic zone contains a pseudostratified layer of highly differentiated dentine-producing odontoblastic cells. The cell-free zone is a sub-odontoblastic zone in the coronal pulp measuring approximately 40 µm. This zone consists of branching cytoplasmic processes from cells situated in the adjacent cell-rich zone. This zone forms the main part of the sub-odontoblastic capillary plexus and contains the terminal branches of sensory and autonomic nerve fibres. Fibroblasts and undifferentiated cells are contained within the cell-rich zone. The undifferentiated cells have spindle-shaped nuclei that are arranged with their cytoplasmic processes perpendicular to the dentine in the coronal pulp or parallel to the dentine in the radicular pulp.

The pulp core consists of major vessels and nerves, and cells such as odontoblasts, fibroblasts, macrophages, other immunocompetent cells and undifferentiated cells. An extracellular matrix, rich in collagenous fibrils, elastin fibres, thin fibre bundles, large blood vessels and nerve trunks, is also contained within the pulp core. All of the pulpal constituents lie within a gel-like ground substance with high-water content, chondroitin sulfate, hyaluronic acid,

dermatan sulfate, proteoglycans and glycoproteins (Baume, 1980; Tziafas, 2007; Luukko et al., 2011).

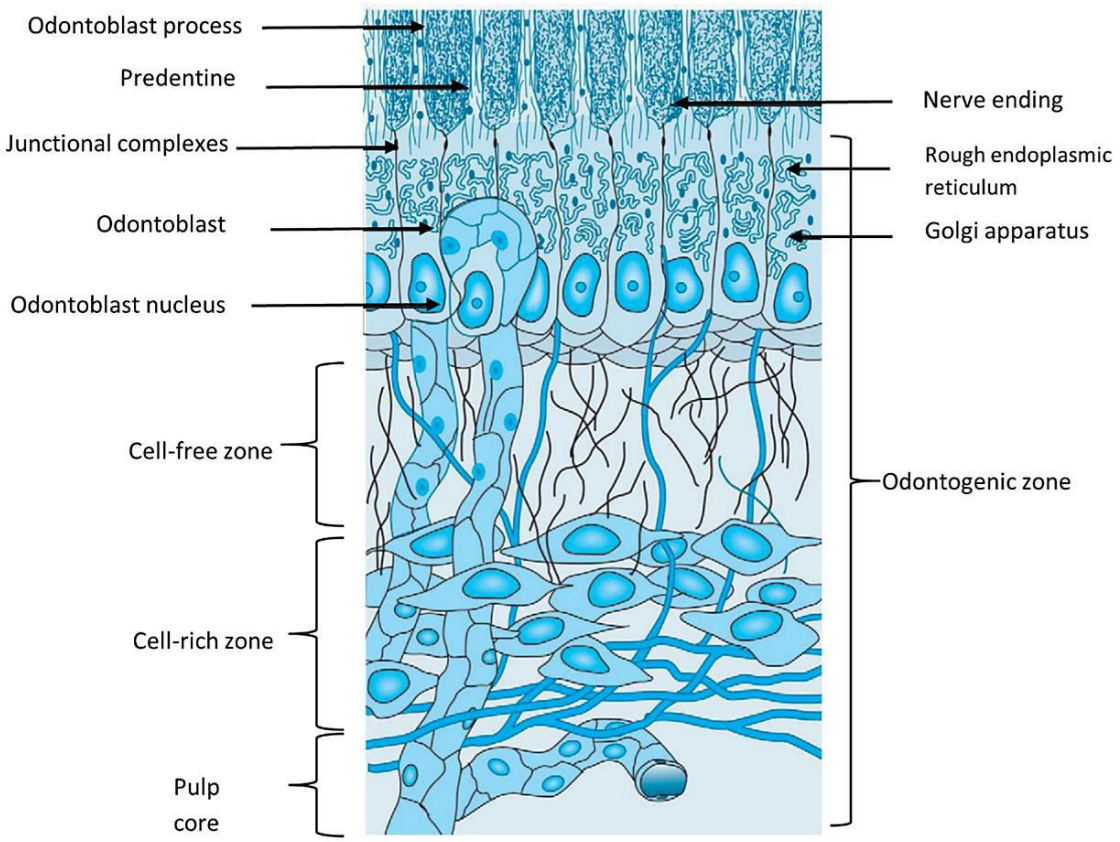


Figure 1-2 Schematic diagram showing cell zones of the dental pulp (Kumar, 2014)

1.5.2 Pulpal microcirculation

The pulpal microcirculation and blood vessels are supplied by branches of the maxillary artery, superior and inferior alveolar arteries in both maxillary and mandibular arches. These blood vessels enter the tooth through arterioles nourishing each dental pulp microvasculature (Yu and Abbott, 2007).

The vessels of the dental pulp are arranged in a hierarchy. The arterioles direct centrally and have branches to form a capillary network at the periphery of the pulp. This network provides the odontoblasts with a high source of nutrients. The dental pulp has rapid and relatively high blood flow. The blood flow is higher in the peripheral pulp than that of the central areas and in the coronal pulp than that of the radicular pulp. Around 90% of pulpal capillaries are found in the sub-odontoblastic zone (Yu and Abbott, 2007).

The dental pulp also contains numerous arteriovenous connections (shunts) that regulate blood flow, especially apically. These shunts are essential in the control of tissue pressure as well. The vessels can be arterio-venous anastomoses, venous-venous anastomoses or U-turn loops which provide direct communication between arterioles and venules. When the intra-pulpal pressure increases during pulpal inflammation, the shunt vessels open up to decrease the intra-pulpal pressure to allow the blood flow to be retained (Kim et al., 1983; Kim et al., 1984).

1.5.3 Pulpal revascularisation

Pulp ischemia as a result of partial or total disruption of the neurovascular supply can occur following TDIs. Pulp healing by revascularisation may occur as long as there is no bacterial infection and the size of the apical foramen is sufficient to allow neurovascular in-growth. Extension of the capillaries, through the apical foramen, to the ischemic pulp follows within a few days after the injury. The speed of this extension depends on the width of the pulp-periodontal interface. When the apical vascular supply is ruptured, end-to-end anastomosis occurs. The healing process starts apically and moves coronally.

Pulp survival, in some situations, with an intact odontoblastic layer may be caused by anastomoses to pre-existing microvasculature in the pulp. However, in other cases, pulp revascularisation appears to occur mostly by ingrowth of new vessels (Skoglund et al., 1978). Revascularisation may range from pulp regeneration, pulp repair with accelerated dentine formation (pulp canal obliteration), or pulp metaplasia (Andreasen, 2012). It has been shown in experimental replantation and auto-transplantation studies in dogs and humans that revascularisation of teeth with almost normal pulpal anatomy could be observed after replantation (Ohman, 1965; Skoglund and Tronstad, 1981).

1.5.4 Nerves of the pulp

The sensory nerves of the dental pulp are branches of the maxillary and mandibular divisions of the trigeminal nerve. The sensory nerves are involved in pulp pain perception and transduction. They follow the same route as that of the blood vessels progressing coronally and peripherally. The sensory nerves branch below the cell-rich zone to form the plexus of Raschkow. The plexus consists of myelinated A-beta and A-delta fibres (2–5μm in diameter) as well as smaller unmyelinated C-fibres (0.3–1.2μm) (Abd-Elmeguid and Yu, 2009).

A-fibres transmit pain to the thalamus producing a fast and sharp pain which is localised easily. The number of these myelinated fibres increases with tooth maturation. They do not fully develop or penetrate into the pulp until the tooth is completely formed. This mechanism can explain why immature teeth are less sensible than fully mature teeth. A-fibres respond to different stimuli. These stimuli such as probing or drilling, can cause rapid movement and flow of the dentinal fluid in the dentinal tubules through the hydrodynamic effect in

response to a stimulus which is likely to activate the pulpal nociceptors (Abd-Elmeguid and Yu, 2009).

A-fibres are classified according to the diameter and conduction velocities into A-beta and A-delta fibres. Approximately 90% of A-fibres are A-delta fibres. A-delta fibres have a smaller diameter than A-beta fibres. Thus, A-delta fibres have a slower conduction velocity than A-beta and C-fibres. Moreover, the A-delta fibres and C-fibres have a conduction velocity of 12 to 30 m/s and 0.5 to 2 m/s, respectively. Therefore, C-fibres have a higher excitability threshold.

A-fibres principally innervate the dentine. Most of the A-delta fibres are present in the coronal part of the pulp. The highest nerve density is located in the pulp horns. The free nerve endings infiltrate the dentinal tubules for a distance of 150 - 200 µm (Byers and Dong, 1983). The C-fibres, on the other hand, innervate the body of the pulp and are located in the pulp proper extending into the cell-rich zone. The C-fibres have a higher pain threshold. This pain is characterised as dull and aching pain. Thus, C-fibres need a stronger stimulus to be activated (Abd-Elmeguid and Yu, 2009).

In the existence of persistent pain, the threshold of the sensory neurons (nociceptors) may decrease. It occurs during inflammation of the pulp when A- and C-fibres respond differently. This explains the variable degree of pain in pulpitis. The C-fibres may survive and function in the presence of hypoxia which may explain the pain felt during root canal preparation of a necrotic pulp (Narhi et al., 1979; Olgart, 1985; Bender, 2000).

1.6 Diagnosis of pulp necrosis

One of the main problems in dental traumatology is the ability to diagnose pulpal health following dental trauma. The fundamental problem in diagnosing the pulpal status is that the dental pulp is enclosed within a calcified tissue; therefore, all existing diagnostic methods are of an indirect nature. For instance, conventional pulp tests measure only the conductivity of the pulpal nerve fibres, rather than pulpal vascular supply. Similarly, radiographs only provide information about the result of osteoclastic and osteoblastic activity in or around the root (Andreasen, 1989b).

Histological examination of the pulp is the most accurate method of evaluating the degree of inflammation or the presence of necrosis. Unfortunately, this is impossible in clinical scenarios. Therefore, an accurate assessment of the pulpal status is achieved through a combination of detailed patient history, clinical and radiographic findings suggestive of pulp necrosis (Sigurdsson, 2003).

1.6.1 Clinical findings associated with pulp necrosis

The following is a list of signs and symptoms that can help diagnose pulp necrosis:

- Tenderness to percussion: A positive correlation between tenderness to percussion and pulpal necrosis has been shown (Seltzer et al., 1963; Garfunkel et al., 1973; Dummer et al., 1980; Andreasen, 1989a). Careful examination should be performed to rule out other causes of tenderness to percussion such as periodontal trauma.

- Sinus tract: A discharging sinus in the alveolus is a definitive sign of infection which could be a consequence of pulp necrosis (Dummer et al., 1980). Careful examination is, however, required to rule out other sources of infection such as an associated periodontal abscess.

- Pulp testing: Sensibility of the pulp can be assessed using different tests. Conventional pulp testing methods involve the use of electric pulp testing (EPT) and thermal tests. These tests are not conclusive as there is no correlation between the sensibility threshold and the histological condition of the pulp (Seltzer et al., 1963; Mumford, 1967a; Jafarzadeh and Abbott, 2010a). The use of dental pulp tests will be discussed in depth in the next part of this chapter (Chapter 1, page 24).

- Crown discolouration: The crown colour of traumatised teeth should be noted, as colour changes may occur after dental trauma resulting in pink, yellow, brown, grey or a combination of these colour changes successive of pulpal changes. Pink or reddish discolouration may be seen 2-3 days after an injury which indicates intra-pulpal haemorrhage. This discolouration is usually reversible within a few weeks. However, the persistence of such colour or a grey colour development could indicate pulpal necrosis. Isolated colour change, however, is not a reliable indicator of pulpal necrosis (Jacobsen, 1980; Andreasen, 1989b).

1.6.2 Radiographic findings

Radiographic findings that may be suggestive of pulp necrosis are described as follows:

- Periapical radiolucency: Pulp necrosis or infection within the canal is reflected by a widened PDL space or an apical radiolucency. A periapical lesion is caused by the infection-induced release of a number of osteoclast activating factors. (Andreasen and Andreasen, 1992). These changes may develop 2-3 weeks post-trauma.

- Arrested root development: Continued apical development of an immature root can be a useful indicator of pulpal vitality, primarily when a vital contra-lateral tooth acts as a control (Andreasen et al., 2007). However, arrested apical development does not always indicate loss of pulpal vitality. Some teeth have shown to have hard tissue formation although they had arrested apical development. The hard tissue formation was in continuity with the periapical bone (Kling et al., 1986). Furthermore, apical root resorption may occur with orthodontic treatment due to pressure applied during tooth movement leading to a possible root shortening. Such teeth are usually asymptomatic with vital pulps. High and continuous orthodontic forces might cause disruption of the apical blood supply leading to loss of pulp vitality (Fuss and Trope, 1996).

Since no one test is able to accurately assess pulpal vitality, the diagnosis of pulp necrosis should be based on ‘’ two or more of the following signs: coronal discolouration, negative sensibility testing and periapical radiolucency’’ (Andreasen et al., 2007). Nevertheless, prophylactic root canal treatment may be indicated in some instances where the prognosis of pulpal healing is poor such as in severely intruded or replanted mature teeth with prolonged extra-alveolar and dry times (Albadri et al., 2010; Andersson et al., 2017).

In some cases, it has been noted that pulpal healing could occur even with more than two signs of loss of vitality. A lack of response to pulp tests is possible in otherwise vital teeth. Furthermore, transient apical breakdown and transient grey discolouration could develop in a small proportion of traumatised teeth. These findings may not necessarily indicate pulp necrosis as they may be related to pulpal repair and healing (Jacobsen, 1980; Andreasen, 1986; Andreasen, 1988). Thus, confirming pulpal status can sometimes be quite challenging. As a result, more accurate diagnostic techniques and tests for the evaluation of pulpal status are needed.

1.6.3 Cone Beam Computed Tomography (CBCT)

The advantage of using CBCT can overcome the challenges in image interpretation by creating three-dimensional images of the area to be examined. Therefore, CBCT can be a useful tool in endodontic diagnosis. Moreover, CBCT enables periapical radiolucencies to be detected before they would be apparent on conventional radiographs. Many clinical and laboratory studies have compared the diagnostic accuracy of CBCT with traditional radiography (Patel et al., 2015).

Clinical studies have shown that CBCT is significantly more likely to detect apical periodontitis compared to periapical radiography. One study compared the accuracy of CBCT and periapical radiographs from a consecutive sample of 888 imaging exams of patients with endodontic infection for the detection of apical periodontitis. They found the detected prevalence of apical periodontitis to be significantly higher with CBCT and that CBCT imaging detected 54.2% more lesions than intraoral radiography alone (Estrela et al., 2008). Similar

results were reported by another study where the preoperative assessment of the periapical condition of 37 premolars and 37 molars in the maxilla using periapical radiography was compared to CBCT. It was found that CBCT demonstrated significantly more lesions (34%) than conventional radiography (Low et al., 2008). Similar findings have been reported in other studies (Bornstein et al., 2011; Abella et al., 2012; Cheung et al., 2013)

The usefulness of the CBCT can not be disputed. As with any radiographic examination, the use of CBCT must be justified and the potential benefits should outweigh the exposure to ionizing radiation. This is especially relevant when assessing children who are more susceptible to the potential effects of ionizing radiation.

1.7 Dental pulp tests

Dental pulp tests are an essential component of the diagnostic process of the pulp status. There are a variety of dental pulp tests available. Basically, dental pulp tests are categorised into sensibility tests and vitality tests. Pulp sensibility tests assess the pulp‘s sensory response and the nerve supply of the pulp. In other words, sensibility is the ability to respond to a stimulus. Thermal tests (heat and cold), EPT and test cavity are examples of sensibility tests. On the other hand, vitality tests assess the pulp’s blood supply present within the pulp such as pulse oximeter and laser Doppler flowmetry (LDF) (Chen and Abbott, 2009). The ideal dental pulp test should *be ‘’non-invasive, painless, standardised, reproducible, reliable, inexpensive, easily completed, and objective’’* (Chambers, 1982).

1.7.1 Pulp sensibility tests

The most common pulp sensibility tests include thermal tests (cold and heat), EPT and test cavity. The clinically normal dental pulp should produce a mild to moderate response to a stimulus. When the stimulus is removed, the response diminishes after a few seconds (Pitt Ford and Patel, 2004). Most sensibility tests activate only the A-delta fibres as the degree of stimulus needed to activate A-delta afferent fibres is 25% of that needed to activate C fibres (Virtanen, 1985).

1.7.1.1 Thermal tests

Thermal tests stimulate the hydrodynamic movement of fluid in the dentinal tubules. This causes an expansion or a contraction of dentinal fluid within the tubules, causing a fast fluid movement. The movement of dentinal fluid causes stimulation of A-delta fibres in the pulp/dentine complex (Linsuwanont et al., 2007). In general, it has been reported that cold tests are more accurate than heat tests (Ehrmann, 1977).

1.7.1.1.1 Cold tests

Several methods have been used for cold testing such as ice sticks, refrigerant sprays, carbon dioxide snow (CO_2) and ethyl chloride. The major difference between all agents and methods is the temperature produced by each different test (Pitt Ford and Patel, 2004).

Ice sticks

The use of ice sticks is probably the simplest cold testing method. The temperature produced is 0°C. However, it is not accurate in adults, posterior teeth and in teeth with deposition of secondary or reparative dentine. Used sterilised local anaesthetic cartridges and the plastic covers of hypodermic needles can be used can be used as ice sticks. When using ice sticks, they should be placed in gauze to prevent melting the ice caused by warmth from the clinician's fingers (Ehrmann, 1977; Augsburger and Peters, 1981).

Refrigerant spray

The use of refrigerant spray is a common cold testing method. These agents produce higher thermal changes than ice sticks with a temperature decrease ranging between – 20 °C and - 50 °C, depending on the type of agent used (White and Cooley, 1977). Various refrigerant sprays are available. However, they are mainly based on dichlorodifluoromethane (DDM), tetrafluoroethane (TFE), or a propane-butane mixture (PBM) (Jafarzadeh and Abbott, 2010a).

DDM is commercially packaged as Endo-Ice (-50°C) (Colte`ne/Whaledent). TFE and PBM are commercially available as Green Endo-Ice (-26.2°C) (Colte`ne/Whaledent) and Endo-Frost (-50°C) (Colte`ne/Whaledent). One study has shown that PBM and TFE produced lower temperatures than TFE when assessed directly after application on a cotton swab. However, temperatures measured inside the pulp chamber were statistically similar in all agents (de Morais et al., 2008).

A carrier is needed, a cotton pellet, to apply the refrigerant spray. The cotton pellet should be saturated with the agent before the direct contact with tooth structure. Larger pellets have larger surface areas which allow for better thermal conduction. On the other hand, cotton buds and small cotton pellets have smaller surface areas, thus, less efficacious in thermal conduction (Jones, 1999). The cotton pellet is applied to the middle third of the of the crown. The cotton should be in contact with the tooth surface until the patient feels the stimulus or applied up to 5-8 seconds (White and Cooley, 1977).

Carbon dioxide snow (dry ice)

It is made from a pressurised liquid CO_2 cylinder with the dry ice collected in pencil sticks. Applying carbon dioxide snow to a temperature probe produced a temperature of –56 °C that is sufficient to provoke a response (Augsburger and Peters, 1981; Fuss et al., 1986). One study showed that CO_2 and a refrigerant spray (TFE) produced similar responses regardless of tooth type or restoration category. However, the refrigerant spray was faster in triggering a tooth response (Jones et al., 2002).

Ethyl chloride

Ethyl chloride (Chloroethane) is a colourless, flammable gas or refrigerated liquid. It has a faintly sweet odour with a temperature of -12.3 °C. It is available as a compressed spray and frequently used in medicine as a skin refrigerant (Jafarzadeh and Abbott, 2010a). Ethyl chloride is sprayed onto a cotton pellet to form a frost layer and then applied on to the surface of the tooth. It is important to test the contra-lateral tooth if sound, to allow the patient to understand the nature of the stimulus (Rowe and Pitt Ford, 1990).

One study has shown that ethyl chloride had the best prediction of the pulp vitality when compared to EPT (Moody et al., 1989). Another study showed that ethyl chloride was more accurate than EPT in identifying pulp necrosis (Petersson et al., 1999). Also, it was reported that the results using ethyl chloride were more accurate than hot gutta-percha. However, the results were not reproducible (Mumford, 1964). On the other hand, the use of ethyl chloride in pulp testing has been found to be less efficient than dry ice or DDM in premolars (Fuss et al., 1986).

1.7.1.1.2 Heat Tests

Heat causes the dentinal fluid to expand which stimulates A-delta fibres. However, C-fibres can be stimulated by pressure increase when heat is applied to an inflamed pulp producing long-term pain (Bender, 2000). Heated gutta-percha and instruments, electrical heat sources, and hot water baths can be a means of delivery of heat test. The accuracy of heat testing has been reported to be low when assessing pulp vitality. The absence of sensation to heat was not reliable to indicate pulp necrosis. Having a positive response to heat testing was more accurate in identifying vital teeth. However, cold testing and EPT are more reliable than heat testing (Petersson et al., 1999).

1.7.1.2 Electric pulp testing

Electric pulp testing provides a current to stimulate the A-delta fibres. The non-myelinated C- fibres do not respond to EPT as a significantly more powerful current is needed to stimulate these fibres (Narhi et al., 1979). The pulp is presumed to be vital or partially vital when the electrical current sensation is felt. This is done through a gradual increase in the level of the electrical current

conducted through the electrolyte of the tooth. A positive response is the result of an ionic shift in the dentinal fluid producing local depolarisation, and thus action potential is generated from the un-injured nerves (Pantera et al., 1993). In other words, a positive response suggests the presence of intact sensory fibres within the pulp. Patients would be feeling a brief sharp or tingling sensation from the tooth.

The EPT unit has a probe that is applied to the tested tooth. The tested tooth should be adequately dry to prevent the conduction of electrical current to the periodontium or other adjacent teeth (Pitt Ford and Patel, 2004). A suitable conducting medium should be used to coat the probe such as toothpaste or a special electrode gel (Mickel et al., 2006).

EPT is more reliable in assessing healthy vital teeth than assessing diseased non-vital teeth (Fuss et al., 1986; Peters et al., 1994; Petersson et al., 1999; Kamburoğlu and Paksoy, 2005; Gopikrishna et al., 2007; Weisleder et al., 2009; Saeed et al., 2011; Villa-Chavez et al., 2013). The opposite results were reported by Karayilmaz & Kirzioglu (2011) showing higher sensitivity than specificity when anterior teeth were only included in their study. The pooled sensitivity and specificity of EPT were determined to be 72% and 93 %, respectively (Mainkar and Kim, 2018).

1.7.1.3 Test cavity

In situations where pulpal status remains unidentified despite the use of a combination of the previously mentioned tests, the use of a test cavity can be justified. A test cavity involves a cut into dentine as this can provide a conclusive answer as long as the exposed dentinal tubules have direct

communication with the pulp. Patients respond when dentine is penetrated, if the pulp is vital, before the pulp chamber is reached. Patients should be adequately acquainted with what to expect and how to respond to the test cavity before conducting the test (Rowe and Pitt-Ford, 1990).

1.7.2 Limitations of sensibility tests

1.7.2.1 Correlation with the histological status of the dental pulp

No clear co-relation was found when studies have assessed the results of sensibility tests with the histological status of the pulp (Seltzer et al., 1963; Reynolds, 1966; Mumford, 1967a; Dummer et al., 1980). The nerve fibres are the last part of the pulp to undergo degeneration because they are somewhat resistant to necrosis. Therefore, a necrotic tooth can respond to stimulation (Fuss et al., 1986).

1.7.2.2 Dental trauma

Dental pulp tests have been shown to be unreliable soon after TDIs, as there may be no response to sensibility tests even though blood circulation may be restored (Ohman, 1965; Bhaskar and Rappaport, 1973). Following trauma, a lack of response to pulp testing may not be a true indication of the pulpal blood supply due to the state of shock the pulp is under with intra-myelin oedema, axonal swelling, and partial loss of myelin sheaths (Ozcelik et al., 2000). Never the less, the initial response to pulpal testing may serve as a baseline that can be compared with future results (Teitler et al., 1972). Changing the response from positive to negative during the follow-up period may suggest pulp

degeneration. Furthermore, the persistence of negative responses may indicate pulp necrosis. However, interpreting such results should be done with caution as such negative persistent responses might be transient (Andreasen and Kahler, 2015a).

After TDIs, a long period, which may range from 1–8 weeks, can occur before a response can be produced to pulp testing. Neural generation is slower than vascular regeneration in traumatised teeth and is sometimes even lacking. However, longer follow-up periods are required. Thus, a traumatised tooth that does not respond to sensibility tests is not necessary necrotic (Andreasen et al., 2007).

1.7.2.3 Subjectivity

Subjectivity is a major limitation of sensibility tests. Pulp sensibility tests rely on the patient's response to the stimulus. Therefore, false positive responses can occur in anxious or young patients (Cooley and Robison, 1980). Moreover, sensibility tests are sometimes difficult to apply, and the results are unreliable with children (Peters et al., 1994). Children, sometimes, are unable to define a subjective response to a stimulus. False responses can also occur when the clinician asks the child leading questions (Cohen and Hargreaves, 2006). Children have the ability to adjust their behaviour to avoid a painful unpleasant stimulus (Kennedy et al., 1987).

1.7.2.4 Stage of root development

The stage of root development has been shown to affect sensibility tests. The use of EPT is less reliable in teeth with immature apices because development

of the Raschkow's plexus does not entirely take place until the complete development of the roots (Fulling and Andreasen, 1976). Immature permanent teeth generally provide little or no response to EPT. A study has shown that only 11% of teeth in children with immature apices responded positively to EPT. Consequently, it has been suggested that CO_2 snow cold test could be more reliable than EPT for testing teeth with immature apices (Fulling and Andreasen, 1976; Klein, 1978).

1.8 Laser Doppler flowmetry (LDF)

1.8.1 Laser

Laser is an acronym which stands for ''Light Amplification by Stimulated Emission of Radiation''. The stimulated emission theory was first discussed by Einstein in 1916. It resulted in the development of the first working laser by Maiman in 1960. The first application of the laser was for the diagnosis and treatment of skin conditions. In dentistry, laser technology was introduced in the mid-1970's and its first application was for oral soft tissue surgery. The main characteristics of laser light are that it is delivered as waves, which are typically collimated, coherent and monochromatic, of a single wavelength (Nazemisalman et al., 2015).

1.8.2 Mechanism of LDF

The Doppler Effect was the principle used in developing LDF technology. In 1842, Christian Doppler, an Austrian physicist, suggested an explanation during observing the colours of stars for the frequency shift that takes place when the distance between a source of waves and an object changes with

time. The Doppler Effect is recognisable in everyday life with sound waves. For example, the change in pitch of the sirens that occurs in a passing emergency vehicle in the street when the vehicle moves toward and away from an observer (Toman, 1984).

Since the introduction of LDF in the medical field in 1972, the *in vitro* and *in vivo* effect of LDF on various tissues has been investigated (Riva et al., 1972; Stern, 1975). LDF was first presented in the dental literature in 1986 (Gazelius et al., 1986). The principle of using LDF is that the laser light is aimed and directed to the dental pulp through a fibre optic probe placed against the tested tooth. The laser light reaches the pulp through the dentinal tubules which act as a guide. The photons which interact with red blood cells will be Doppler–shifted according to the Doppler principle. The backscattered light from moving red blood cells will be frequency-shifted while the light from the static tissue stays un-shifted in frequency. 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. Thus, the signal is produced (Roeykens and De Moor, 2011).

This signal is a semi-quantitative measurement of blood flow, called the Flux signal, which is measured using arbitrary units. The Flux is defined as the number of moving red blood cells per second times their mean velocities. The flux result of the suspected non-vital tooth is usually compared to that of a healthy vital control tooth in order to assess the vitality of suspected teeth. The Flux signal from a tooth with a vital pulp should be higher than that of a tooth with a non-vital pulp (Roebuck et al., 2000).

Most of the moving objects within the pulp are red blood cells. As a result, measuring the Doppler-shifted backscattered light acts as an index of pulpal blood flow. LDF assesses the dynamic changes in blood flow by identifying cell movement in a limited volume of tissue (about 1 mm^3) (Oberg, 1990; Vongsavan and Matthews, 1993a; Vongsavan and Matthews, 1993b; Vongsavan and Matthews, 1996).

The original technique implemented by Gazeluis was conducted on five volunteers with vital and non-vital teeth. The LDF device used had one probe with three optical fibres placed close to the buccal tooth surface in the cervical region of the crown on a modified rubber dam clamp. A green rubber dam was used to avoid interference from the surrounding tissues.

The background level was first measured, by placing an aluminium film between the probe and the tooth to get a visual representation of the rhythm of the readings with no blood flow. This reading was compared to the measurements of vital and non-vital teeth obtained. Non-vital teeth resulted in lack of rhythmic pattern, and much lower output signal close to the background level when compared to normal vital contra-lateral teeth. Oscillations in non-vital teeth were absent, and the irregular fluctuations and spikes that occurred were related to movement artefacts (Gazelius et al., 1986). Also, regular oscillations were observed in recordings from normal vital teeth similar to an ECG recording. A light beam from a helium-neon (He–Ne) laser emitting at 632.8 nm was used in the original technique. Other studies have used other wavelengths of laser, 780–820 nm (Kimura et al., 2000).

1.8.3 Factors influencing the results of LDF

The use of LDF in the assessment of pulpal blood flow, as well as the results produced, are widely affected by some environmental and technique related factors.

1.8.3.1 Probe design

The Flux signal recorded from the dental pulp is affected by some variables such as the signal processing bandwidth filter (used to reduce signal noise), the wavelength of the laser beam, fibre diameter, fibre separation within the probe (the distance between fibres) and the probe position in relation to the gingival margin. Studies assessing these variables have been conducted.

1.8.3.1.1 Fibre diameter and separation

Ingolfsson et al. (1993) investigated the effect of probe design on the signals produced from vital teeth in 18 adult participants using 632.8 nm laser wavelength. Measurements were carried out on maxillary central incisors, mandibular central incisors and maxillary canines. Five different probes were used based on the diameter of the fibre and fibre separation. Each probe had three fibres arranged in a tringle. One fibre carried the light to the tooth, and the other two fibres carried the backscattered light to the LDF. The external diameter of all probes was 2.8 mm. In general, the probe design is described first by the diameter of the fibres used followed by the distance between the fibres such as 200/500 (both measured in μm).

The different fibre combinations used in this study were 200/1500, 200/1000, 200/800, 200/500 and 125/250. The results of the study showed that the probe with the largest separation of the fibres produced significantly higher values than other probes (Ingolfsson et al., 1993). Similar findings have been reported in another investigation (Odor et al., 1996a).

1.8.3.1.2 Wavelengths and bandwidths

Furthermore, other studies investigated the effect of wavelengths and bandwidths on LDF signals from vital and root filled teeth. The values for vital teeth were higher than those of root-filled teeth for the wavelengths used (633 nm and 810 nm). Recordings from 633 nm wavelength were of lower values than those of 810 nm light source. The effect of bandwidth was more inconstant as increasing bandwidth decreased the Flux readings with probe 100/125. However, with the other probes (200/500 and 200/375), the Flux output was the lowest using 14.9 kHz bandwidth, but similar using 3.1 kHz and 20 kHz bandwidth. Moreover, Flux values were increased with wider bandwidths when using 810 nm. On the other hand, when using 633 nm light source, the highest values were obtained from the narrowest bandwidth of 3.1 kHz in comparison to 14.9 kHz and 20 kHz bandwidths. (Odor et al., 1996a; Odor et al., 1996b).

Another study investigated the effect of different variables (wavelength, bandwidth filter, fibre separation and the distance of the probe from the gingival margin) on LDF recordings on vital and non-vital teeth. A total of 24 combinations were tested. The combination of the 633 nm laser beam with 3 kHz bandwidth using a probe with 500 μm fibre separation placed 2-3 mm from the gingival margin was shown to be the most reliable. It showed consistent

results of higher values of vital teeth. All combinations except the above combination had at least one recording that showed a Flux value of a non-vital tooth that was greater than a vital tooth (Roebuck et al., 2000).

1.8.3.2 Probe position

Studies have shown that the probe position affects the Flux results. Placing the probe close to the gingival area may increase the risk of including non-pulpal signals from the periodontal and surrounding tissues. An *in vivo* study found a significant difference and higher Flux values (+42%) when the LDF probe was placed in the cervical area compared with the incisal positioning of the probe (Hartmann et al., 1996).

Data obtained from another study indicated that the location of the probe on the labial surface of maxillary central incisors could affect pulpal blood flow measurements. This study was conducted on 13 participants aged 21 to 39 years and found a significant difference in Flux readings between incisal and cervical positions of the probe. The more incisal the probe was located, the lower values obtained (Ramsay et al., 1991). Moreover, another investigation of different positions of the probe in 10 vital teeth in 10 patients was carried out. It reported that Flux readings were significantly higher when placing the probe in gingival, mesial and distal positions than from the incisal positioning of the probe (Ingolfsson et al., 1994b).

1.8.3.3 Probe holder and stabilisation

To aid in stabilisation of the LDF probe, a holder may be constructed to fit over the teeth which also maintains an actual contact between the probe and tooth

structure. This also creates reproducible positioning of the probe which can be maintained for future readings.

Different LDF measurement methods and techniques have been used since the application of LDF in dentistry. The use of rigid splints constructed with different materials such as silicone (Gazelius et al., 1988; Olgart et al., 1988), green rubber base splint (Roeykens et al., 1999; Evans et al., 1999; Roebuck et al., 2000), plastic splint (Ramsay et al., 1991; Watson et al., 1992; Norer et al., 1999; Emshoff et al., 2004a), acrylic (Akpinar et al., 2004; Polat et al., 2004), self-curing resins (Ikawa et al., 2001; Sato et al., 2003; Soo-ampon et al., 2003), polyvinyl siloxane (Verdickt and Abbott, 2001; Setzer et al., 2013), polyurethane splint (Hartmann et al., 1996) or holding the probe manually (Wilder-Smith, 1988) have been used. However, the best LDF readings were achieved by dental splints constructed using dental putty impression material moulded over the teeth. The probe can then be placed by drilling a small hole in the splint (Jafarzadeh, 2009).

1.8.3.4 Non-pulpal signals

It has been shown that the signal produced from non-vital teeth is significantly lower than from vital teeth. It has been proposed that part of the signal registered for vital and non-vital teeth is derived from the blood flow of the surrounding tissues as otherwise non-vital teeth should show a Flux value of 0. In other words, signals obtained do not only represent pulpal blood flow but can be contaminated by signals from other tissues. In fact, laser light scatters broadly outside the tooth which may reach the periodontal tissue, resulting in contamination of the recording. As a result, studies have proposed the use of

isolation measures in order to reduce such contamination (Matthews and Vongsavan, 1993; Hartmann et al., 1996; Ikawa et al., 1999; Polat et al., 2005a). For example, Ikawa et al. (1999) evaluated scattered LDF light through human teeth *in vitro*. The results indicated that the light was scattered to a wide area outside the tooth with measurement of blood flow in surrounding tissues (Ikawa et al., 1999).

Furthermore, intra and extra coronal scattering of LDF's light was analysed *in vivo.* A study included 12 vital teeth from 12 adults (22– 29 years). A camera was used for imaging LDF's light during pulpal blood flow measurement. During the analysis of the photos, the laser beam was perceived to cause the tooth to shine like a lamp. Other tissues were also illuminated inside the mouth such as the tongue, lips, and other teeth. This study showed that certain precautions are mandatory during LDF measurements as the laser can scatter all around the tissues. Thus, crown and gingival isolation were recommended (Polat et al., 2005a).

A study that was conducted in order to determine the strength of signals originated from the pulp and those from other tissues in 26 vital teeth in 12 patients. The measurements taken after root canal treatment were 30% lower than the measurements taken before the procedure. The study results showed that a major part of the signal was not only originated from the dental pulp. Thus, LDF results may be inconsistent when a rubber dam is not used to aid in eliminating the unwanted scattered light (Polat et al., 2004).

Another study was carried out to determine what proportion of the signal obtained with or without the use of a black rubber dam in addition to self-curing

splints. Measurements were recorded from 22 healthy vital maxillary incisors in 14 adults (22-40) years. The black rubber dam significantly reduced the mean blood flow reading obtained from vital teeth by 73% (Soo-ampon et al., 2003). Furthermore, Hartmann et al. (1996) investigated vital teeth using a combination of polyurethane splints and a rubber dam. The use of rubber dam in addition to the polyurethane splint decreased the Flux values by 69% compared to the use of the splint alone (Hartmann et al., 1996).

Assessing two different wavelengths, Kijsamanmith (2011), compared the effect of rubber dam on Flux values using red (635 nm) and infrared (780 nm) light for recording pulpal blood flow in anterior teeth. Seven vital teeth in 5 patients using an acrylic splint were evaluated. It was found that the rubber dam decreased the Flux values by 82 % when infrared light (780 nm) was used and 56% when red light (635nm) was used. The study confirmed the importance of using an opaque rubber dam (Kijsamanmith et al., 2011a).

Akpinar et al. (2004) evaluated the effect of both labial and palatal gingivae on LDF measurements. Twenty vital upper central incisors from 20 volunteers were included in the study. First, data was collected from measurements without any gingival covering. Then, measurements were retaken after application of an opaque, no-eugenol based periodontal paste to cover the labial gingiva, the palatal gingiva, or both the labial and palatal gingivae (Peripac Paste, De Trey/Dentsply, Germany). The Flux values decreased by 46% when the opaque paste was applied on the labial gingiva, 10% on palatal gingiva only, and 63% labial and palatal gingivae (Akpinar et al., 2004).

In conclusion, non-pulpal signals, mainly from the periodontal blood flow, can considerably contaminate the Flux signal of the pulp. Thus, it is necessary to isolate the teeth under evaluation as the unwanted reflected light may contribute to the overall signal. Even with such precautions, a component of the signal may be due to the supporting structures. In other words, it is difficult to totally exclude the contamination from the surrounding tissues.

1.8.3.5 Penetration depth

The penetration depth of LDF light was determined for contact and non-contact probe tips in-vitro using fifty-one human teeth with singles roots only. A beam of a 780 nm wavelength was pointed cervically on the crown. A photograph was taken, using a digital camera which was fixed 10 cm away from the crown, in normal light conditions. After that, two photographs were taken in a night shot mode for infrared imaging when the LDF probe was in contact with the crown and when it was 1 mm away. The depth of the illumination in the root was measured as high and low density where the cemento-enamel junction was used as the buccal reference point. When the probe was placed in contact with the tooth, the mean depth of root illumination with high and low density was 4.28 $\pm$ 0.14 mm and 13.27 $\pm$ 0.27, respectively. When the non-contact probe was used, the mean depth of root illumination with high and low density was 4.36 $\pm$ 0.16 mm and 13.28 $\pm$ 0.30 mm, respectively (Polat et al., 2005b).

1.8.4 Clinical studies of LDF and traumatic dental injuries

A study has shown that LDF may be a useful tool to detect pulp revascularisation much earlier than the conventional dental pulp tests. LDF was

used to assess the pulpal blood flow values of avulsed permanent maxillary incisors in 17 patients aged 7-10 years using a wavelength of 632 nm. Four measurements were taken during the follow-up period; on the day of splint removal, at 12 weeks, 24 weeks and 36 weeks after splint removal. The authors concluded that LDF recordings correctly predicted the vitality in 100% of cases. The recordings for non-vital teeth correctly identified 80 % of the non-vital teeth (Strobl et al., 2003). This study, however, had a low sample size with no sample size calculation. Also, the follow-up period was short. Moreover, LDF cut-off threshold was not used.

The same group of researchers have published a series of similar studies (Emshoff et al., 2004a; Emshoff et al., 2004b; Emshoff et al., 2004c; Emshoff et al., 2004d). For example, Emshoff et al. (2004a) evaluated 80 patients undergoing dental trauma as a result of luxation injuries. The age range was 2–56 years where two LDF measurements were recorded for each tooth in two sessions, the day of splint removal and 12 weeks after splint removal. The cut-off values used were 2.9, 6.4 and 9.9 PU levels. Adverse outcomes were classified into different types according to clinical and radiographic signs such as type I (loss of sensitivity), type II (periapical radiolucency), type III (grey discolouration), type IV (loss of sensitivity and periapical radiolucency), and type V (loss of sensitivity, periapical radiolucency and grey discolouration of the crown). The cut-off value of PU ≤ 9.9 identified 100% of non-vital incisors. However, the specificity was very low. The age range and mix pf permenant and primary teeth was the major limitation in the study. Also, the follow-up period was short in addition to randomly selecting a range of cut-off values rather than a predetermined threshold.

Furthermore, another investigation included 404 permanent maxillary incisors that sustained luxation, avulsion, uncomplicated crown fractures or root fracture injuries. The study included 309 patients (5-56 years). The threshold levels used were (≤ 3.0 PU; > 3.0 and ≤ 6 PU; > 6.0 PU and ≤ 9.0 PU; > 9.0 PU and ≤ 12.0 PU; > 12.0 PU). Different sensitivity and specificity values were reported for the cut-off thresholds used in the study (Emshoff et al., 2008b).

In conclusion, the use of LDF in prospective studies has been reported only by the above group of researches in the literature. The studies lack clear aims and objectives. The age range in the studies was questionable as it included children with primary teeth as well. Different cut-off thresholds have been tested in each study to try to evaluate the accuracy of LDF with different statistical techniques. In addition, LDF has never been compared to other dental pulp tests in prospective studies.

1.8.5 Comparative studies of LDF with other dental pulp tests

The following studies will be described and discussed in further detail in the next chapter (Page 47).

Ingolfsson et al. (1994a) investigated if LDF and EPT could aid in distinguishing between vital and non-vital necrotic teeth. Eleven anterior teeth with clinically diagnosed necrotic pulps, where pulp necrosis was confirmed by root canal treatment after the test, were compared to the contralateral vital teeth. Ten other pairs of anterior teeth with vital pulps in ten patients were tested as well. The output signals originating from the teeth with necrotic pulps were significantly lower than those of vital teeth. On average, the signal was 42.7%

lower from the teeth with necrotic pulps than from the vital teeth. Four of 11 teeth with necrotic pulps gave a positive response to EPT. The sensitivity of LDF ranged between 81.8 % – 90 % and the specificity recorded for the probe 125/250 was 100%. With regards EPT, the sensitivity and specificity were 63.3% and 100%, respectively (Ingolfsson et al., 1994a).

Another cross-sectional study compared LDF with other methods of assessing pulpal vitality of traumatised anterior teeth including EPT and ethyl chloride. Measurements were recorded from 67 non-vital anterior teeth (55 patients), and the pulpal status was later confirmed by pulpectomy. Measurements were also recorded from 84 vital anterior teeth (84 patients). Analysis of the measurements allowed diagnostic criteria to be developed which resulted in sensitivity and specificity of 100 % for LDF. In comparison, the sensitivity and specificity for EPT were 87 % and 96%, and for ethyl chloride 92% and 89 %, respectively (Evans et al., 1999).

Karayilmaz and Kirzioglu (2011) evaluated and compared the reliability of LDF, pulse oximetry and EPT for assessing the pulpal status. Data were collected from 59 pairs of maxillary anterior teeth in 51 patients. The age range was 12–18 years. The study included non-vital teeth with completed endodontic treatment and healthy, contralateral teeth in the same patients. The difference between LDF values obtained from the vital and non-vital teeth was statistically significant. The findings of this study showed that LDF was found to be more reliable than pulse oximetry and EPT for assessing the pulpal status of human teeth. LDF could reliably differentiate between the vitality of teeth with a sensitivity and specificity of 100%. The calculated sensitivity was 91.5% for

EPT and 81.3% for pulse oximetry (PO). The specificity for EPT was 88.1% and for PO was 94.9%.

Chen and Abbott (2011) compared the clinical accuracy, reliability, and repeatability of LDF, EPT, and various thermal pulp sensibility tests including CO_2, Endo Frost and Ice. The study sample included 121 teeth in 20 subjects. The tested teeth included maxillary and mandibular incisors, canines, premolars and molars. Measurements were taken during 3 test sessions with a minimum of a 1-week interval. The accuracy of EPT (97.7%) was the highest. This was followed by CO_2 (97%), LDF (96.3%), Endo Frost (90.7%) and Ice (84.8%). Regarding repeatability, ice was the most repeatable test (ICC = 0.677). It was followed by LDF (ICC = 0.654), Endo Frost (ICC= 0.57), EPT (ICC = 0.434) and CO_2 (ICC = 0.432).

In conclusion, the use of the conventional dental pulp tests for the assessment of pulp sensibility in children's teeth relies on patients' cooperation, understanding and comprehension. The use of these tests can sometimes be challenging especially in the child population. LDF, on the other hand, is an objective method that may offer more reliable results when used with these patients. Thus, the aim of the thesis was to evaluate the use of LDF in the assessment of the pulp blood flow of permanent teeth through different types of studies.

The overall aims and objectives of the thesis were as follows:

- To systematically review and assess the available evidence for the use of LDF in evaluating and monitoring the pulp status of permanent teeth in comparison to other sensibility and/or vitality tests.

- To explore the methods and techniques used by UK GDPs and paediatric dental specialists in assessing pulp sensibility and vitality following dental trauma as well as to explore the limitations and barriers to the use of these tests.

- To conduct a cross sectional clinical study to assess the sensitivity, specificity, positive predictive value, negative predictive value as well as the repeatability of LDF, EPT and ethyl chloride. Also, to determine the most accurate LDF Flux threshold below which a tooth could be identified as non-vital.

- To clinically monitor pulp vitality and sensibility of traumatised teeth using LDF, EPT and ethyl chloride and to prospectively calculate the sensitivity, specificity and predictive values of each of the tests.

Chapter 2 Systematic review

The diagnostic accuracy of laser Doppler flowmetry in assessing pulpal blood flow in permanent teeth

Ghouth, N., et al. 2018. The diagnostic accuracy of laser Doppler flowmetry in assessing pulp blood flow in permanent teeth: A systematic review. *Dental Traumatology.* [Online]. 28 June 2018. [Accessed 3 Septemebr 2018]. Available from: https://doi.org/10.1111/edt.12424 (Appendix 1).

2.1 Abstract

Background/Aim: Pulp necrosis is a frequent complication following dental trauma. The diagnosis of the state of the dental pulp can be challenging as most commonly used diagnostic tools are subjective and rely on a response from the patient, potentially making their use unreliable, especially in the child population. The aim of the study was to systematically review the evidence on the use of laser Doppler flowmetry (LDF) in the assessment of the pulp status of permanent teeth compared to other sensibility and/or vitality tests.

Methods: A systematic literature search, using MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, www.clinicaltrials.gov and www.controlled-trials.com, in addition to citation and manual reference list searches, was conducted up to 15th January 2018. A risk of bias assessment was performed using the quality assessment for diagnostic accuracy studies tool (QUADAS-2) with all steps performed independently by two reviewers.

Results: Four studies with a high risk of bias were included in the final analysis. Laser Doppler flowmetry was reported to be more accurate in differentiating between teeth with normal pulps and pulp necrosis with a sensitivity of (81.8-100%) and specificity of 100 % in comparison to other vitality tests such as pulp oximetry (sensitivity = 81.3 %, specificity = 94.9 %) and sensibility tests such as electric pulp testing (EPT) (sensitivity = 63.3 – 91.5 %, specificity = 88 – 100 %).

Conclusion: Despite the higher reported sensitivity and specificity of laser Doppler flowmetry in assessing pulp blood flow, these data are based on studies with a high level of bias and serious shortfalls in study designs. More research is needed to study the effect of different laser Doppler flowmetry's parameters on its diagnostic accuracy and the true cut-off ratios over which a tooth could be diagnosed as having a normal pulp.

2.2 Background

Evidence-based medicine is ''*the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients''*. It ensures that decisions concerning patient care are not only based on experience, but also on scientific research (Rabb-Waytowich, 2009). Furthermore, it is the best available existing approach to suggest interventions that are scientific, safe, efficient and cost-effective (Kishore et al., 2014).

Systematic reviews have become increasingly important in aiding clinical decision making in dentistry. A systematic review can be defined as "a review of a clearly formulated question that attempts to minimise bias using systematic and explicit methods to identify, select, critically appraise and summarise relevant research'' (Needleman, 2002).

Systematic reviews are important because an evaluation followed by a summary of all the included studies of a specific research question is presented. Therefore, they make the available evidence more accessible to healthcare providers. Furthermore, conducting systematic reviews should follow a well defined clear protocol and design based on transparent, pre-

detailed and reproducible methods. When they are appropriately conducted, they provide reliable results. They can also determine whether a high level of evidence is lacking or not. Thus, this can guide future researchers (Oxman et al., 1994).

2.3 The aim of the review

The aim of this review was to systematically assess the evidence, from clinical studies, for the use of the laser Doppler flowmetry in assessing and monitoring the pulp status of permanent teeth in comparison to other sensibility and/or vitality tests.

2.4 Methods

The full research protocol was registered and published on PROSPERO, Centre for Reviews and Dissemination (CRD) at the University of York, UK (Registration details: CRD42016035457). This systematic review has been published in Dental Traumatology journal (Appendix 1).

2.4.1 Search strategy

A systematic electronic search, citation search and reference list screening were performed. The initial electronic databases search was performed on 2nd March 2016 and included MEDLINE (1946 to February week 3, 2016), EMBASE and EMBASE classic (1947 to 2nd March 2016) and Cochrane Central Register for Controlled Trials CENTRAL. In addition, a search for ongoing trials was conducted on two websites; www.clinicaltrials.gov and www.controlled-trials.com. Dissertation and thesis searches were performed

using ProQuest while conference abstracts and proceedings were searched using BIOSIS database. The electronic search strategy was formulated under the supervision of a specialist librarian (University of Leeds Library). The medical subject headings (MeSH) / keywords and the search strategy utilised for MEDLINE were as follows: (exp Dentistry OR Dent* OR exp tooth OR tooth* OR teeth* OR pulp* OR exp Dental pulp) AND (exp laser Doppler flowmetry OR Doppler* OR LDF*), with no limits used. The search strategy was adapted and applied to other databases. EndNote (X 7.4 Thomson Reuters) was used to manage references and remove duplicate records. The electronic search was repeated towards the end of the review process (15th January 2018). The electronic search was performed by one reviewer (Nahar Ghouth).

2.4.2 Inclusion criteria

The PICOS methodology was utilised in formulating the research question as follows:

Types of participants

- Participants over the age of six years.
- Studies of participants with vital / non-vital teeth
- Studies where tooth vitality/sensibility had been followed up for at least six months.

Types of interventions

Vitality testing of permanent teeth using laser Doppler flowmetry in comparison to other vitality and/or sensibility tests.

Types of comparators/ reference standard

- Studies where any type of vitality and/or sensibility tests were compared to LDF.

- Studies comparing vital to non-vital teeth with the following reference standards were included :

    - A known vital tooth with no clinical or radiographic signs or symptoms of loss of vitality, in addition to no history of trauma, no caries nor any dental anomalies (composite reference standard).

    - A known non-vital tooth (such as pulp extirpated / root canal treated teeth).

- Prognostic studies where LDF was used in assessing teeth with damaged and unknown pulp status such as traumatised teeth with the following reference standards (composite reference standard):

    - Signs of loss of vitality: clinical signs of loss of vitality such as abscess formation, sinus tract formation, tenderness to percussion/palpation, radiographic signs of periapical pathology, infection-related resorption and hyperaemic dental pulp upon root canal treatment.

    - Signs of vitality: Continuation of root formation on radiographic views in teeth with immature root formation and none of the signs stated below for loss of vitality.

Types of outcome measures

Outcome measures were defined in accordance to published criteria for such studies (Akobeng, 2007a)

The following primary outcome measures were identified:

- Sensitivity: Identifying non-vital teeth as non-vital
- Specificity: Identifying vital teeth as vital.

Additionally, the following secondary outcomes were identified:

- Positive predictive value*:* Patients having the disease when the test result was positive.
- Negative predictive value: Patients not having the disease when the test result was negative.
- Repeatability: As determined by the variation in repeat measurements made by the same measurement method, observer or rater on the same subject over a short period of time.
- Reproducibility: As determined by the variation in measurements made on a subject under changing conditions (different observes or rater).
- Reliability: As determined by the correlation between any two measurement methods made on the same subject.
- Flux ratio: The ratio between the vital and non-vital teeth under which a tooth could be considered non-vital.

Types of Studies included:

The following studies were included:

- Randomised controlled clinical studies.

- Controlled trials.
- Cross sectional studies including diagnostic cohort studies and diagnostic case-control studies.
- Prognostic or predictive studies with at least six months follow-up showing a clear reference standard to differentiate between vital and non-vital teeth.
- Studies presented in English language only.

2.4.3 Exclusion criteria

The following exclusion criteria were applied:

- Participants under the age of six years.
- Studies where primary outcomes of accuracy, sensitivity and specificity are not stated or not possible to calculate.
- Studies with a case series, case reports and *in vitro* design.
- Articles not written in English.
- Prognostic or predictive clinical studies with less than six months follow-up.

2.4.4 Study selection process

Electronic searching was performed by one reviewer (Nahar Ghouh) while two reviewers, Nahar Ghouth and Alaa BaniHani (Clinical lecturer and speciality registrar in paediatric dentistry), independently performed study selection, data collection and quality assessment. Any disagreement was resolved by consensus or consulting a third researcher, Hani Nazzal. Articles meeting the inclusion criteria were selected for full-text second screening. In situations in

which the decision could not be made based on title/abstract, the full articles were obtained. The authors were contacted for additional information when necessary.

2.4.5 Data extraction

A data extraction form (Appendix 2) was used as a framework to capture all the necessary information about the study characteristics and outcomes of the included studies. The form was based on the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care (CRD 2009) . The form was piloted by the two researchers independently using one of the included studies.

The first part of the data extraction sheet included information about the study such as author name, article title and date of the study. Then, the data recorded in the data extraction form included information about the aim/objectives of the study, study design, inclusion and exclusion criteria, randomisation and blinding.

Furthermore, the details of each study participants including sample size, age range and gender were then recorded. Details about the intervention were also reported which included the description of the diagnostic, control and reference tests. Further details about LDF such as the device used, rubber dam use, splint type used, the location of the LDF probe and the duration of LDF measurement were also recorded. The last part of the data extraction sheet included statistical techniques used, study outcomes and results.

2.4.6 Quality assessment

The quality assessment tool used to evaluate the included studies was the QUADAS-2 (Figure 2-1, Figure 2-2, Figure 2-3) which is recommended by the Cochrane collaboration, Agency for Healthcare Research and Quality, and the UK National Institute for Health and Clinical Excellence for use in systematic reviews of diagnostic accuracy studies. The QUADAS-2 tool assesses two aspects: risk of bias and applicability of concerns. These two aspects are assessed based on three domains: patient selection, index test and reference standard. In addition to these three domains, a fourth domain of flow and timing was also used for the assessment of the risk of bias. All domains should be rated as low risk of bias and low concerns regarding applicability in order for a particular study to be rated as having an overall low risk of bias and applicability concerns. Rating one domain as high would result in an overall judgment of high risk of bias/applicability concerns regardless if the other domains are rated as low (Whiting et al., 2011). Piloting of the quality assessment process on one of the included studies was performed in order to calibrate and train both assessors.

QUADAS-2

Phase 1: State the review question:

Patients (setting, intended use of index test, presentation, prior testing):
Index test(s):
Reference standard and target condition:

Phase 2: Draw a flow diagram for the primary study

Figure 2-1 Shows QUADAS-2 tool page 1 (Phase 1 and 2)

Phase 3: Risk of bias and applicability judgments

QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

DOMAIN 1: PATIENT SELECTION

A. Risk of Bias

Describe methods of patient selection:

- ❖ Was a consecutive or random sample of patients enrolled? Yes/No/Unclear
- ❖ Was a case-control design avoided? Yes/No/Unclear
- ❖ Did the study avoid inappropriate exclusions? Yes/No/Unclear

Could the selection of patients have introduced bias? **RISK: LOW/HIGH/UNCLEAR**

B. Concerns regarding applicability

Describe included patients (prior testing, presentation, intended use of index test and setting):

Is there concern that the included patients do not match the review question? **CONCERN: LOW/HIGH/UNCLEAR**

DOMAIN 2: INDEX TEST(S)

If more than one index test was used, please complete for each test.

A. Risk of Bias

Describe the index test and how it was conducted and interpreted:

- ❖ Were the index test results interpreted without knowledge of the results of the reference standard? Yes/No/Unclear
- ❖ If a threshold was used, was it pre-specified? Yes/No/Unclear

Could the conduct or interpretation of the index test have introduced bias? **RISK: LOW /HIGH/UNCLEAR**

B. Concerns regarding applicability

Is there concern that the index test, its conduct, or interpretation differ from the review question? **CONCERN: LOW /HIGH/UNCLEAR**

Figure 2-2 Shows QUADAS-2 tool page 2 (Domain 1 and 2)

DOMAIN 3: REFERENCE STANDARD

A. Risk of Bias

Describe the reference standard and how it was conducted and interpreted:

- ❖ Is the reference standard likely to correctly classify the target condition? Yes/No/Unclear
- ❖ Were the reference standard results interpreted without knowledge of the results of the index test? Yes/No/Unclear

Could the reference standard, its conduct, or its interpretation have introduced bias? **RISK: LOW /HIGH/UNCLEAR**

B. Concerns regarding applicability

Is there concern that the target condition as defined by the reference standard does not match the review question? **CONCERN: LOW /HIGH/UNCLEAR**

DOMAIN 4: FLOW AND TIMING

A. Risk of Bias

Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):

Describe the time interval and any interventions between index test(s) and reference standard:

- ❖ Was there an appropriate interval between index test(s) and reference standard? Yes/No/Unclear
- ❖ Did all patients receive a reference standard? Yes/No/Unclear
- ❖ Did patients receive the same reference standard? Yes/No/Unclear
- ❖ Were all patients included in the analysis? Yes/No/Unclear

Could the patient flow have introduced bias? **RISK: LOW /HIGH/UNCLEAR**

Figure 2-3 Shows QUADAS-2 tool page 3 (Domain 3 and 4)

2.5 Results

2.5.1 Search results

The total number of citations identified was 2890 (2569 at initial electronic search, 318 citations through final electronic search and 3 citations through reference list screening). After removal of duplicates (n = 784), 2106 potential eligible studies were identified. Following title and abstract screening, 2061 studies were excluded leaving 45 articles for full article assessment. One author was contacted for additional information about one particular study (Chandler, 1998) and the study was found to be a review paper. Forty-one studies were excluded resulting in four studies to be included in the final qualitative assessment (Figure 2-1) (Ingolfsson et al., 1994a; Evans et al., 1999; Chen and Abbott, 2011; Karayilmaz and Kirzioğlu, 2011).

Although the outcome measures were not specified in one of the included studies (Ingolfsson et al., 1994a), the study provided enough information to calculate the sensitivity and specificity of the tests, therefore allowing the reviewers to include it in the review. A summary of included studies' demographics and LDF machine used are presented in Tables 2.2 and 2.3.

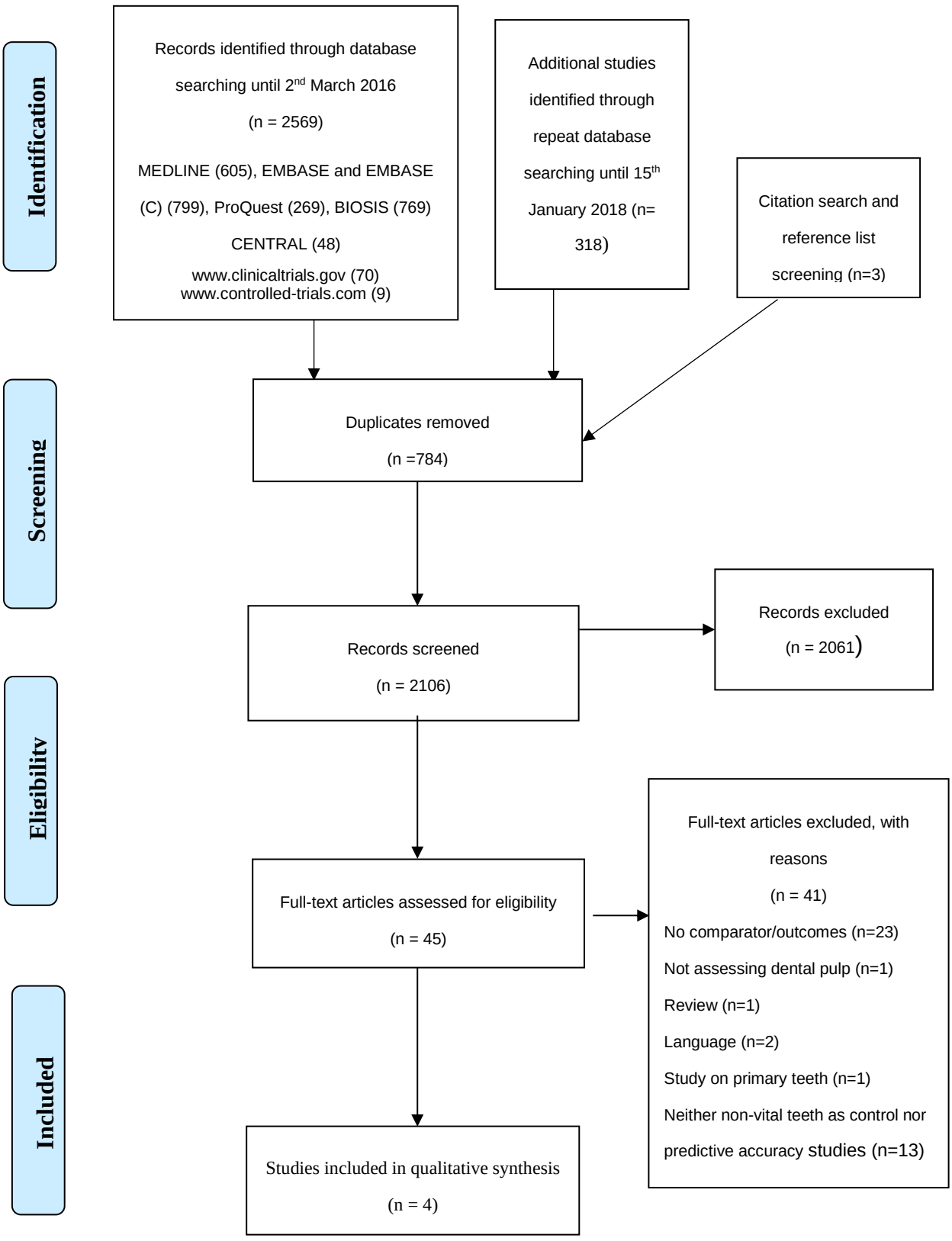


Figure 2-4 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2009) flowchart summarising the systematic review process in the identification of included studies.

Table 2:1 List of studies excluded following full article assessment showing exclusion reasons

Study authors	Reason for exclusion
(Gazelius et al., 1986; Olgart et al., 1988; Ramsay et al., 1991; Ingolfsson et al., 1993; Gazelius et al., 1993; Hartmann et al., 1996; Sasano et al., 1997; Musselwhite et al., 1997; Firestone et al., 1997; Roeykens et al., 1999; Roebuck et al., 2000; Ikawa et al., 2003; Soo-ampon et al., 2003; Emshoff et al., 2004a; Emshoff et al., 2004c; Strobl et al., 2004a; Strobl et al., 2004b; Polat et al., 2004; Emshoff et al., 2004b; Sasano et al., 2005; Strobl et al., 2005; Roy et al., 2008; Komatsu et al., 2009; Kijsamanmith et al., 2011b; Kijsamanmith et al., 2011a; Setzer et al., 2013; Ingolfsson et al., 1994b).	No outcome measures/ No direct comparison
(Odor et al., 1996a; Strobl et al., 2003; Emshoff et al., 2004c; Emshoff et al., 2004d; Emshoff et al., 2008a; Emshoff et al., 2010; Qu et al., 2014)	No direct comparison
(Amess et al., 1993)	Not assessing the dental pulp
(Chandler, 1998)	Review article (author contacted)
(Mirgazizov et al., 1999; Pypec, 2007)	Language

Table 2:2 A summary of the demographics and characteristics of included studies

Study	Study design	Sample size	Age	Teeth included
(Chen and Abbott, 2011)	Cross sectional	20 patients ; 121 teeth	18-74	Maxillary and mandibular incisors, canines, premolars and molars.
(Karayilmaz and Kirzioğlu, 2011)	Cross-sectional	51 patients; 59 pairs of anterior teeth	12-18	Maxillary central and lateral incisors
(Evans et al., 1999)	Cross-sectional	**Group 1**: 57 patients; 57 non-vital teeth and 53 vital control teeth. **Group 2:** 84 patients; 84 vital teeth	6.5-33.5	Maxillary and mandibular anterior teeth
(Ingolfsson et al., 1994a)	Cross-sectional	**Group 1:** 9 patients; 11 vital teeth and 11 non-vital teeth Group 2: 10 patients with 20 vital teeth	11-37	Maxillary and mandibular anterior teeth.
Study	**Disease characteristics**	**Comparators**	**Randomisation and blinding**	**Reference test**
(Chen and Abbott, 2011)	Teeth suspected or known to have pulp pathosis or provisionally diagnosed as having a healthy pulp.	▪ CO2 crystals ▪ Ice ▪ Endo Frost ▪ Electric pulp testing	No	Root canal treatment
(Karayilmaz and Kirzioğlu, 2011)	Endodontically treated teeth and healthy control teeth	EPT and Pulse oximetry	No	Clinical and radiographic examinations. Non-vital teeth had root canal treatment
(Evans et al., 1999)	Vital and non-vital teeth	EPT and ethyl chloride	No	No clinical/radiographic signs or symptoms of infection for the vital teeth. Bleeding on pulp extirpation for non-vital teeth.
(Ingolfsson et al., 1994a)	Vital and non-vital teeth	EPT	No	Pulp necrosis was confirmed by root canal treatment, while vital teeth tested positive to EPT, exhibited no discolouration and normal radiographic examination.

Table 2:3 A summary of LDF techniques used in the included studies

Study	LDF device used and wavelength	Splint used	Location of probe	Type of probe
(Chen and Abbott, 2011)	▪ MoorLAB/FloLAB; Moor Instruments Ltd, Axminster, UK. ▪ Wavelength: 780 nm	Polyvinyl	2–3mm above the gingival margin	▪ Double channel ▪ Two fibres with 500 µm fibre separation.
(Karayilmaz and Kirzioğlu, 2011)	▪ BLF21A Wavelength: 780 nm	Silicon-impression-based	2 mm above the gingival margin.	▪ Single channel ▪ Two fibres 200/500 µm.
(Evans et al., 1999)	▪ Perimed PF2b, Stockholm, Sweden. ▪ Wavelength: 632.8 nm	A two-stage green elastomeric splint.	Between 2 and 3 mm from the gingival margin.	▪ Single channel ▪ Two fibres with 500 µm fibre separation.
(Ingolfsson et al., 1994a)	▪ A Periflux PF3 laser, Perimed, Sweden. ▪ Wavelength: 632.8	Rubber base material	2-3 mm from the gingival margin.	▪ Double channel ▪ Three fibres arranged in a triangle ▪ Five probes used (fibre diameter/ fibre separation) µm ○ 200/1500 ○ 200/1000 ○ 200/800 ○ 200/500 ○ 125/250

Study	Rubber dam used	Duration of LDF measurement	LDF cut-off ratio used	Unit of measurement
(Chen and Abbott, 2011)	No	90 seconds	Diseased pulp flux/ known healthy pulp flux ratio is less than or equal to 0.6	Flux
(Karayilmaz and Kirzioğlu, 2011)	No	20 optimum seconds out of 45 seconds.	1/10 ratio between the pulpal blood flow values measured by LDF	PU*
(Evans et al., 1999)	No	3 min (where patient cooperation allowed	▪ Vital pulp: Flux ≥ 7.0 and SWV ≥ 1.6 P ▪ Non-vital pulp : Flux < 7.0 ▪ Intermediate vitality: : Flux ≥ 7.0 PU but amplitude SWV < 1.6	Flux and SWV** measured in PU.
(Ingolfsson et al., 1994a)	No	1.5 to 2 minutes	No cut off used, only significance difference between readings	Flux

* PU: Perfusion unit

** SWV: amplitude of slow wave vasomotion.

Table 2:4 A summary of the outcome measures reported for LDF in comparison to other sensibility and vitality tests.

Outcome measures	Tests	(Chen and Abbott, 2011)	(Karayilmaz and Kirzioğlu, 2011)	(Evans et al., 1999)	(Ingolfsson et al., 1994a)
Sensitivity (%)	LDF	---	100	100	81.8 – 90
	EPT	---	91.5	87	63.3
	PO	---	81.3	---	---
	EC*	---	---	92	---
Specificity (%)	LDF	---	100	100	100***
	EPT	---	88	96	100
	PO	---	94.9	---	---
	EC*	---	---	89	---
Positive predictive value (%)	LDF	---	---	---	100***
	EPT	---	88.5	---	100
	PO	---	94.1	---	---
Negative predictive value (%)	LDF	---	---	---	50***
	EPT	---	91.2	---	73
	PO	---	83.5	---	---
Accuracy (%)	LDF	96.3	---	---	---
	EPT	97.7	---	---	---
	CO2	97	---	---	---
	**EF	90.7	---	---	---
	ICE	84.8	---	---	---
Repeatability	LDF	0.65	---	---	---
	EPT	0.43	---	---	---
	CO2	0.43	---	---	---
	**EF	0.57	---	---	---
	ICE	0.67	---	---	---

* EC: Ethyl chloride ** EF: Endo Frost *** Probe 125/250

2.5.2 Study design and sample size

All included studies adopted a cross-sectional study design. The sample size varied between studies. Chen and Abbott (2011) included 20 patients with a total number of 121 teeth while Karayilmaz and Kirzioglu (2011) included 51 patients with 59 pairs of teeth. Furthermore, Evans et al. (1999) and Ingolfsson et al. (1994a) included 141 and 19 patients allocated between two groups in each study, respectively. Sample size calculation was not performed in all studies (Table 2:2).

2.5.3 Randomisation and blinding

Blinding and randomisation were not performed in any of the included studies (Table 2:2).

2.5.4 Participants' age

In three of the included studies (Ingolfsson et al., 1994a; Evans et al., 1999; Chen and Abbott, 2011), the participants' age ranges were very wide (11-37 years, 6.5-33.5 years and 18-74 years, respectively). The fourth study by Karayilmaz and Kirzioglu (2011) included a narrow age range (12-18 years) (Table 2:2).

2.5.5 Teeth evaluated in included studies

Two studies included both maxillary and mandibular anterior teeth (Ingolfsson et al., 1994a; Evans et al., 1999), Karayilmaz and Kirzioglu (2011) included only maxillary central and lateral incisors while Chen and Abbott (2011)

included maxillary and mandibular incisors, canines, premolars and molars (Table 2:2).

2.5.6 Disease characteristics and reference tests

Evans et al. (1999) and Ingolfsson et al. (1994a) included vital and non-vital teeth (non-endodontically treated at the time of LDF assessment). The pulp necrosis for non-vital teeth was confirmed by root canal treatment and no bleeding in both studies. The vital teeth exhibited no clinical or radiographical signs/symptoms of infection in both studies. On the other hand, Karayilmaz and Kirzioglu (2011) included teeth which had been already endodontically treated and healthy control teeth.

The fourth study included clinically normal pulps and teeth with diseased pulps. All teeth had temporary pulp diagnosis made by clinical and radiographic examinations before conducting the tests. Pulp necrosis was confirmed by subsequent endodontic treatment (Table 2:2) (Chen and Abbott, 2011).

2.5.7 Comparators

EPT was used in all four studies. Three of the studies compared LDF to sensibility testing alone, while Chen and Abbott (2011) compared LDF to pulse oximetry as a vitality test. EPT was the only comparator in one study (Ingolfsson et al., 1994a) while Evans et al., (1999) used EPT and ethyl chloride and Chen and Abbott (2011) used EPT, CO_2 crystals, Ice and refrigerant spray (Endo Frost). Karayilmaz and Kirzioglu (2011), on the other hand, compared the LDF to EPT and pulse oximetry (Table 2:2).

2.5.8 LDF device and technique characteristics

There was a variation in LDF devices and techniques used by the researchers in all included studies. For example, 780 nm laser wavelengths were used in the two studies (Chen and Abbott, 2011). On the other hand, 632.8 nm was used in the other two studies (Ingolfsson et al., 1994a; Evans et al., 1999; Karayilmaz and Kirzioğlu, 2011). Moreover, Chen and Abbott (2011) and Ingolfsson et al. (1994a) used a double channel LDF device, while Karayilmaz and Kirzioglu (2011) and Evans et al. (1999) used single-channel devices (Table 2:3)

The type of LDF probe used may be described in respect of the number of fibres, the diameter of each fibre and the distance between the fibres. With regards the type of probes used in the studies, Chen and Abbott (2011) and Evans et al. (1999) used a probe type with two fibres and 500 μm fibre separation (fibre diameter was not stated). Karayilmaz and Kirzioglu (2011) used a probe type with two fibres of 200/500 μm, while Ingolfsson et al., (1994a) used five different probes 200/1500, 200/1000, 200/800, 200/500 and 125/250 with three fibres arranged in a triangle (Table 2:3).

Regarding the LDF technique used, an isolation splint was used in all studies; however, a rubber dam was not used in any of the included studies. Furthermore, there were differences in the duration of LDF measurements between studies. Karayilmaz and Kirzioglu (2011) tested each tooth for 45 seconds with 20 optimum seconds included in the analysis. The other three studies (Chen and Abbott, 2011; Ingolfsson et al., 1994a; Evans et al., 1999)

tested each tooth for 1.5 minutes, 1.5 to 2 minutes and 3 minutes, respectively (Table 2:3).

Moreover, the cut-off threshold used showed inconsistency among studies. Chen and Abbott (2011) used a pre-determined cut-off ratio (Diseased pulp flux/ known healthy pulp flux ratio is less than or equal to 0.6). Another study used a post-analysis cut-off ratio of 0.1 ratio between the values measured for vital and non-vital teeth (Karayilmaz and Kirzioğlu, 2011).

Instead of a cut-off ratio, Evans et al. (1999) used a post-analysis Flux cut-off value and amplitude of slow wave vasomotion (SWV) to determine the vitality of teeth visually. The signal has movements in a regular rhythm and large amplitudes of high-frequency movements from the vital revealing of vasomotion. These high-frequency movements are low in amplitude and irregular in necrotic teeth. SWV was calculated as the mean amplitude of the largest three successive cycles. For vital teeth, the Flux and SWV cut-off values used were ≥ 7.0 and ≥ 1.6, respectively. For non-vital teeth, the cut-off value used was Flux < 7.0 PU. For intermediate vitality of the pulp (necrotic coronally but with increased probability of perfusion apically) *a* Flux ≥ 7.0 and SWV < 1.6 was used. On the other hand, Ingolfsson et al. (1994a), neither used a cut off ratio nor value in their study. Alternatively, a statistical difference was reported between vital and non-vital teeth (Table 2:3).

2.5.9 Outcome measures

LDF compared to sensibility and vitality tests

The LDF showed a sensitivity of 81.8-100 % and specificity of 100 % in three studies (Karayilmaz and Kirzioğlu, 2011; Evans et al., 1999; Ingolfsson et al., 1994a). LDF was compared to EPT in three studies with the EPT showing the sensitivity and specificity of 63.3% – 91.5% and 88-100%, respectively. LDF was compared to ethyl chloride in only one of the included studies, showing a sensitivity and specificity of 92 % and 89 %, respectively (Evans et al., 1999).

Accuracy and repeatability of LDF in comparison to four other dental pulp tests were reported in the fourth study with scores of 96.3% and 65%, respectively. The accuracy of EPT, CO_2 crystals, Endo Frost and Ice, in the fourth study, were 97.7%, 97%, 90.7% and 84.8%, respectively. The repeatability was 0.43 for EPT and CO_2 crystals. The repeatability was 0.57 and 0.67 for Endo Frost and Ice, respectively (Chen and Abbott, 2011). Pulse oximetry was compared to LDF in one study showing lower sensitivity (81.3%) and specificity (94.9 %) to that of LDF.

2.5.10 Quality analysis and level of evidence

Quality assessment of the included studies showed a high level of bias in all included studies. With regards to applicability concerns, one study exhibited high concerns regarding applicability (Chen and Abbott, 2011), while the other three studies exhibited low concerns (Karayilmaz and Kirzioğlu, 2011; Evans et al., 1999; Ingolfsson et al., 1994a) (Figure 2:5).

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Study 1	☹	☹	☹	☹	☹	☹	☺
Study 2	☹	☹	☹	☹	☺	☺	☺
Study 3	☹	☹	☹	☹	☺	☺	☺
Study 4	☹	☹	☹	☹	☺	☺	☺

☺Low Risk ☹High Risk

Figure 2-5 Suggested tabular Presentation for QUADAS-2 Results of included studies

2.6 Discussion

2.6.1 Diagnostic accuracy studies

Diagnostic accuracy studies assess how accurate a test can correctly identify the presence or absence of a disease for the purpose of developing treatment plans and treatment decisions (Schmidt and Factor, 2013). The diagnostic accuracy is generally represented by two measures, sensitivity and specificity (Akobeng, 2007a). The ideal dental pulp test should have a sensitivity and specificity of 100 %.

When bias occurs, the test accuracy consistently diverges from the real value leading to underestimation or overestimation of the true accuracy. In other words, poor estimation of accuracy can contribute to misdiagnosis and mistreatment. Therefore, it is critical for accuracy estimation to be reliable (Kohn et al., 2013).

Diagnostic accuracy studies are in general cross-sectional. Mainly, there are two methods to recruit subjects. One type of studies is sometimes called cohort type accuracy studies or single-gate. One set of inclusion criteria is used. The second type is sometimes called case-control type accuracy studies or Two-gate in which two different sets of inclusion/exclusion criteria are applied for those participants. The latter can be prone to bias. These terms, however, are not entirely applicable as diagnostic accuracy studies are not usually longitudinal (Rutjes et al., 2006).

When designing a diagnostic accuracy study, direct or head-to-head comparisons where the index test and the comparator are assessed in the same group of participants is a firm study design. All subjects receive all tests and the reference standard to evaluate the accuracy. Random allocation of study participants is considered the strongest design and offers the chance to avoid selection bias. This is followed by verification of the results by the reference standard (Knottnerus et al., 2002; Rutjes et al., 2005).

2.6.2 Reference standards

Among the principles considered during the quality assessment of the included studies was the use of reference standards. The reference standard may be explained as is the best currently available method to distinguish a condition against which the index test (LDF) is evaluated. Selection of the reference standard plays a very critical role with regards to the validity of a test accuracy study (Rutjes et al., 2006).

The reference standards used in the included studies, in order to identify a non-vital tooth as non-vital, were that non-vital teeth already had root canal

treatment in one study(Karayilmaz and Kirzioğlu, 2011), while the other three studies (Ingolfsson et al., 1994a; Evans et al., 1999; Chen and Abbott, 2011) used the presence of non-vital pulp or bleeding on pulpal extirpation and root canal treatment as reference standards. Bleeding following pulpal extirpation is a subjective sign of pulpal necrosis, therefore should not be used as a reference standard. The reference standard for vital teeth was based on the lack of clinical and radiographic signs/symptoms of infection which is appropriate for such studies. Incorrect initial classification of the vitality of the included teeth may result in over/underestimation of the accuracy of dental pulp tests used.

2.6.3 Test review bias (blinding)

Test review bias occurs when the results of the reference standard are known to the operator carrying out the diagnostic test while the test results are interpreted. Such an interpretation of the diagnostic tests is usually influenced by the knowledge of the other tests or the condition of the evaluated teeth. Thus, operator blinding to the condition of the teeth to be examined is mandatory in diagnostic accuracy studies (Schmidt and Factor, 2013). This, however, was lacking in all included studies.

The nature of the dental pulp tests makes them difficult to blind. One way to achieve this would be by utilising two assessors. One non-blinded assessor would assess case suitability, consent patients, randomise patients, and then apply isolation splints with a small window showing small areas of the teeth under assessment. The second blinded assessor would then assess the pulp

status of the teeth using the dental pulp tests. This technique requires an additional assessor, cost, time and effort.

2.6.4 Age range

With regards to the wide variation in the age range in three of the included studies (Ingolfsson et al., 1994a; Evans et al., 1999; Chen and Abbott, 2011), age-related pulpal changes could also contribute to changes in pulpal blood flow affecting Flux values and cut-off thresholds. Such changes include higher pulpal blood supply in immature teeth versus lower blood supply in calcified teeth or teeth with smaller pulp chambers due to secondary dentine formation (Ikawa et al., 2003). The authors recommend that more studies should include a younger age group, where the trauma occurs before root development is complete, as the assessment of pulp healing after trauma can be more challenging due to the child's anxiety often making routinely used sensibility tests less reliable.

Ageing affects the pulpal structure causing changes to the number of blood vessels, a decrease in the size and volume of the pulp, development of calcified tissue and arteriosclerotic changes. As a result, pulpal blood flow is significantly decreased with increased age of participants (Ikawa et al., 2003). These age-related changes have been associated with decreased pulpal sensibility in older patients. By having fewer nerve branches and increased mineralisation of the dental pulp nerves, those patients have weaker and delayed responses to thermal stimuli increasing the possibility of false-negative responses. Sclerosis of the dentinal tubules could also lead to a decreased flow

velocity of the dentinal fluid leading to further reduction in tooth sensibility (Carvalho and Lussi, 2017).

2.6.5 LDF device and technique characteristics

The included studies showed higher sensitivity and specificity of LDF in comparison to other sensitivity and vitality tests. However, the results of this systematic review highlighted the inconsistency and variability of the used LDF machine specifications (wavelength, probe specifications etc.) and application techniques (time of application, use of gingival isolation etc.) used in assessing pulpal vitality. Such variability and heterogeneity prevent comparison and quantitative synthesis of LDF's published results.

Factors such as the degree of LDF's laser penetration, gingival and periodontal signal contamination, the location of the LDF probe, the duration of the Flux measurement and the cut-off Flux threshold at which a tooth is considered non-vital should be taken into consideration when using LDF and when planning and executing any future studies.

2.6.5.1 Laser penetration and reflection

Laser penetration and reflection have been shown to be affected by tooth crown restorations (Chandler et al., 2014; Chandler et al., 2010). Therefore, the inclusion of heavily restored teeth in studies might affect the LDF accuracy. One of the studies included in this systematic review (Chen and Abbott, 2011) included heavily restored teeth and reported a high accuracy of LDF (96.3 %) in comparison to other dental pulp tests. Such an effect should have been

considered and reflected in the results of that study as Flux values might have been affected leading to misinterpretation and overestimation of the results.

2.6.5.2 Signal contamination

Studies have recommended the use of isolation measures to reduce such contamination from the surrounding tissues, as described in the previous chapter. An isolation splint was used in all included studies. However, none of the included studies used a rubber dam. The effect of signal contamination on the accuracy of LDF could have resulted in recording a proportion of unwanted blood flow in the final Flux outcome.

2.6.5.3 The location of LDF probe

The location of the probe on the crown surface of the assessed tooth has also been recognised as one of the factors affecting LDF measurements. It has been shown that the closer the probe to the gingiva, the higher the signal contamination, as discussed in the previous chapter. An isolation splint whereby the LDF probe was placed 2-3 mm away from the gingival margin has been used in all included studies. To avoid any technique errors, such as improper placement or movement of the splint, the probe tip should be placed at the level of the middle third of the crown, where possible.

2.6.5.4 Movement artefacts

There were inconsistencies between the studies with regards the duration of LDF measurements. It is well established that movement artefact, whether related to the patient or apparatus itself, affect LDF readings. Therefore,

allowing sufficient time for recording stable Flux recording is recommended (Jafarzadeh, 2009). Including unstable movement artefacts in the analysis may increase the Flux value leading to miss interpretation of the results. All studies lacked referencing whether movements artefacts were excluded from the analysis. Flux duration measurements ranged from 45 seconds(Karayilmaz and Kirzioğlu, 2011), to 3 minutes (Evans et al., 1999) in the included studies with no reference to allowing stable Flux readings except in one study where its authors did report that the optimum 20 seconds out of 45 seconds recorded were chosen in the data analysis (Karayilmaz and Kirzioğlu, 2011). However, using the term '*optimum''* to select measurement time is not entirely clear and was not properly defined.

2.6.5.5 Cut off threshold

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 diseased teeth. Ideally, a pre-specified threshold between a vital tooth and non-vital tooth must be established before conducting a clinical study (Whiting et al., 2011). It seems that there is no total agreement or consensus among studies with regards to a cut-off threshold when using LDF.

A pre-specified threshold was only mentioned in one of the studies included in this review (Chen and Abbott, 2011) with a cut off ratio of 0.6 used (a ratio ≥ 0.6 (diseased/healthy) indicated a healthy pulp). The authors based this ratio on the work of Ingolfsson et al. (1994a), included in this review and that of (Roebuck et al., 2000), not included in this review due to the lack of direct comparison with other sensibility/vitality tests. The study by Ingolfsson et al.

(1994a) showed that LDF results of 11 pairs of vital and non-vital teeth showing significantly lower Flux values for non-vital teeth in comparison to vital teeth using four different probes. The output signals for non-vital teeth were 39.2% lower when using probe 200/1500, 40.3% lower with probe 200/1000, 35.1 % lower with probe 200/800, 40.0% lower with probe 200/500, and 58.9% lower with probe 125/250. This study, however, showed spectrum bias, ‘’differences in disease severity’’, as four teeth were diagnosed with periapical radiolucencies, one tooth with submucosal abscess and one tooth with pulp canal obliteration. Teeth with such conditions should have been excluded as this could have caused inconsistencies in the accuracy estimates of dental pulp tests.

The other study which Chen and Abbott (2011) referred to when using the selected cut-off ratio evaluated the effect of bandwidth filter, laser wavelength, fibre separation and probe position on the vital/ non-vital ratios of Flux signals recorded from 11 vital and non-endodontically treated non-vital teeth. The combination of 633 nm with a 3 KHz bandwidth using a probe with a 500 μm placed 2-3 mm from the gingival margin was considered the most reliable combination. Moreover, this study resulted in a cut-off of vital teeth Flux/non-vital teeth Flux > 1.25 (a Flux ratio > 0.8 of diseased pulp / healthy pulp) (Roebuck et al., 2000).

2.7 Conclusion

Despite the higher reported sensitivity and specificity of LDF in assessing pulpal vitality, the data are based on studies with a high level of bias and serious shortfalls in study design. This systematic review highlights

inconsistencies in the evidence supporting the use of LDF in assessing the pupal vitality of permanent teeth. Further high quality diagnostic clinical studies are needed to determine LDF accurate cut-off ratios under which a tooth could be diagnosed as non-vital. More research is also needed to study the effect of LDF on its diagnostic accuracy before such a tool, which is relatively expensive, could be reliably recommended for routine clinical use in everyday practice.

Chapter 3 A cross-sectional survey

The use of dental pulp tests in children with dental trauma: a national survey of the British Society of Paediatric Dentistry's members

Ghouth N, Duggal MS, Nazzal H (2018) The use of dental pulp tests in children with dental trauma: a national survey of the British Society of Paediatric Dentistry's members. *British Dental Journal.* [In press].

3.1 Abstract

Background: Careful long-term monitoring of pulp vitality has been recommended by all dental trauma guidelines. It is essential to explore the methods and techniques used by UK dental practitioners in assessing pulp sensibility and vitality.

Aim: To study the use of dental pulp tests by paediatric dentists and general dental practitioners in children with dental trauma.

Design: A cross-sectional study utilising an 18-item questionnaire that was developed using the Bristol Online Survey (BOS) tool and circulated electronically to the members of the British Society of Paediatric Dentistry between June and August 2017.

Results: One hundred and forty-one respondents included in the analysis, paediatric dental specialists (56%) and GDPs (44%). Almost all specialists (93.7%) reported using sensibility tests routinely in comparison to 80.6% of GDPs. Child perception and cooperation were the most commonly reported barriers. GDPs mainly used cold testing, while specialists used cold and electric pulp tests equally. Inconsistencies in recording as well as documentation the results varied among respondents. Only a few specialists reported having some experience in using laser Doppler flowmetry.

Conclusions: The use of pulp sensibility tests was relatively high amongst respondents while those of vitality tests were very low. Barriers and inconsistencies in the technique and recording of the results of sensibility tests were evident. The frequency and timing of using sensibility tests in line with

international guidelines were stressed. The use of standardised techniques involving methods considered to improve reliability was highlighted.

3.2 Rationale of the study

Loss of pulp vitality is one of the sequelae of dental trauma and careful long-term monitoring of pulp vitality has been recommended in all dental trauma guidelines (Albadri et al., 2010; Diangelis et al., 2017). It is uncertain how dental practitioners follow the guidelines with the use of dental pulp tests.

In order to reduce and overcome the limitations of sensibility tests, some recommendations and techniques have been recommended in order to reduce the chance of such false results (Jafarzadeh and Abbott, 2010a; Jafarzadeh and Abbott, 2010b). Therefore, it was considered important to explore the methods and techniques used by UK general dental practitioners (GDPs) and paediatric dental specialists in assessing pulp sensibility and vitality following dental trauma, especially in the child population. This would also help understand limitations and barriers to the use of these tests.

3.3 Aims

This study aimed to investigate paediatric dentists' and GDPs' use of sensibility/vitality tests, in addition to the barriers to their routine use in assessing dental trauma in children.

3.4 Materials and methods

This was a cross-sectional study utilising a 18-item questionnaire, divided into four sections (Figure 3-1, Figure 3-2, Figure 3-3, Figure 3-4), that was

developed and piloted on a small group of 10 dentists (specialist paediatric dentists, speciality registrars in paediatric dentistry, postgraduate students in paediatric dentistry and GDPs) at Leeds Dental Institute for ease of understanding and reduction of the ambiguity of questions prior to administration. An electronic version was then developed using the Bristol Online Survey Tool (BOS), now known as online surveys.

Institutional ethical approval was obtained from the University of Leeds Research Ethics Committee prior to the commencement of the study (300317/NG/226) (Appendix 3). An invitation email explaining the aims of the survey questionnaire was circulated electronically to the members of the British Society of Paediatric Dentistry (BSPD) between 23^{ed} June and 15th August 2017 with a reminder email sent on 18th July 2017. Individual follow-up correspondence with non-respondents was not carried out due to the anonymity of the survey.

UK based paediatric dental specialists, paediatric dental trainees, GDPs working in the capacity of specialists in paediatric dentistry, such as non-specialist senior dental officers in paediatric dentistry, lecturers in paediatric dentistry or GDPs with advanced training in paediatric dentistry, and GDPs who were members of the British Society of Paediatric Dentistry were included in the study. Non-UK based practitioners and retired dentists/specialists were excluded. Information collected in the questionnaire included the following:

Part A: Demographic data including positions held and frequency of treating children with traumatised permanent teeth.

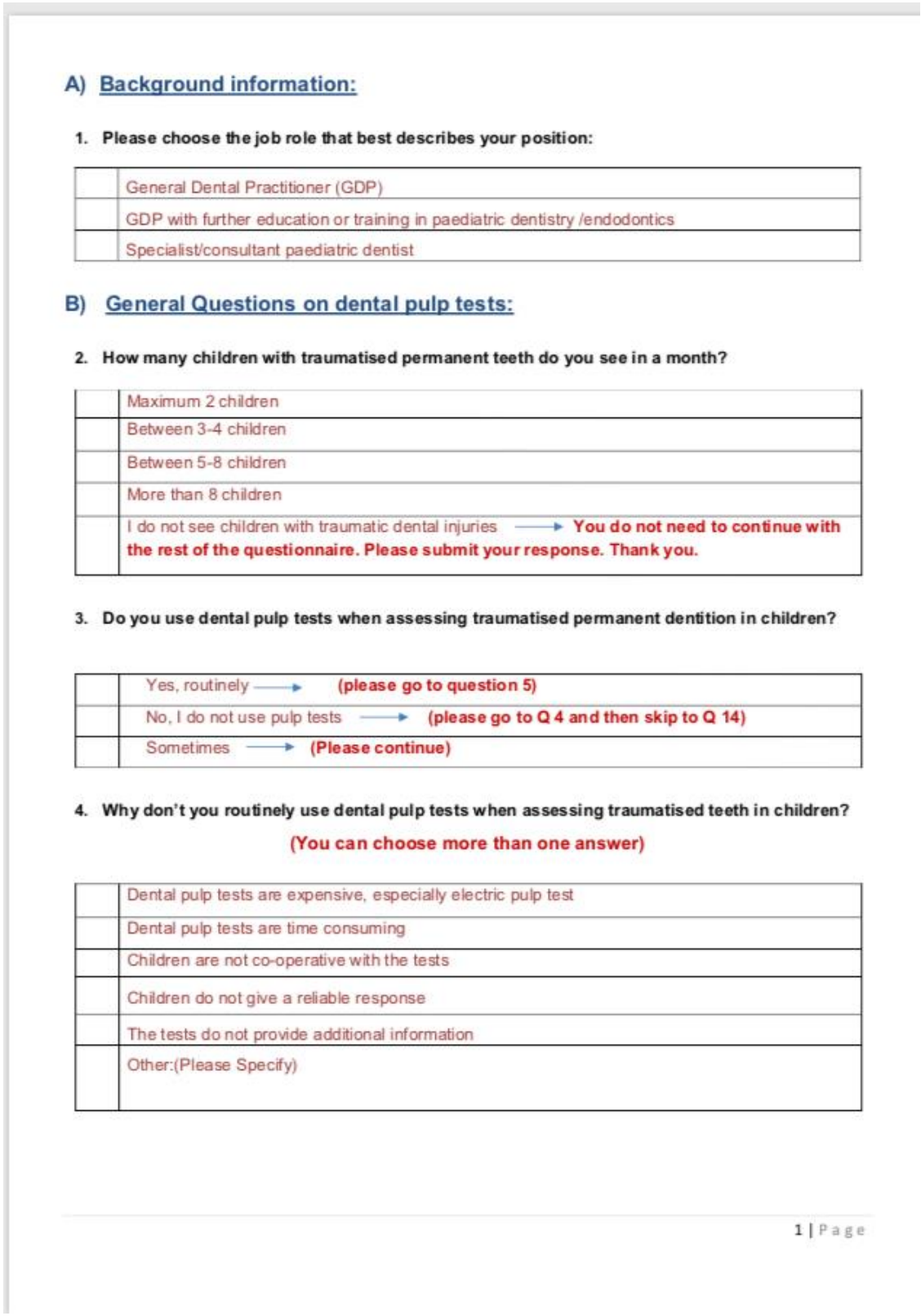
Part B: General questions on the clinical use of dental pulp tests.

Part C: Specific questions on the use of cold sensibility testing.

Part D: Specific questions on the use of EPT.

Part E: Specific questions on the use of LDF.

Data collected were entered into a statistics programme (IBM SPSS version 22). Descriptive statistics analysing participants' responses were computed.



A) Background information:

1. **Please choose the job role that best describes your position:**

	General Dental Practitioner (GDP)
	GDP with further education or training in paediatric dentistry /endodontics
	Specialist/consultant paediatric dentist

B) General Questions on dental pulp tests:

2. **How many children with traumatised permanent teeth do you see in a month?**

	Maximum 2 children
	Between 3-4 children
	Between 5-8 children
	More than 8 children
	I do not see children with traumatic dental injuries → **You do not need to continue with the rest of the questionnaire. Please submit your response. Thank you.**

3. **Do you use dental pulp tests when assessing traumatised permanent dentition in children?**

	Yes, routinely → **(please go to question 5)**
	No, I do not use pulp tests → **(please go to Q 4 and then skip to Q 14)**
	Sometimes → **(Please continue)**

4. **Why don't you routinely use dental pulp tests when assessing traumatised teeth in children?**

(You can choose more than one answer)

	Dental pulp tests are expensive, especially electric pulp test
	Dental pulp tests are time consuming
	Children are not co-operative with the tests
	Children do not give a reliable response
	The tests do not provide additional information
	Other:(Please Specify)

1 | Page

Figure 3-1 Page 1 of the questionnaire showing questions on background information and general questions on dental pulp tests

5. **When do you use dental pulp tests to assess traumatised permanent teeth in children? (Please choose only one answer)**

	Only Initially at the time of trauma
	On initial presentation and at specific intervals.
	Only When new symptoms arise
	Other: Please specify

6. **Which sensibility/vitality test(s) do you use in assessing traumatised permanent teeth in children? (You can choose more than one answer)**

	Cold testing
	Electric Pulp Testing
	Heat Testing
	Laser Doppler flowmetry
	Other(Please specify)

7. **Do you think sensibility tests are reliable when used with children in the permanent dentition?**

	Yes
	No
	Sometimes (please explain)

8. **Do you use any of the following methods in order to improve the reliability of the tests (You can choose more than one answer)**

	I apply a false positive reading such as (applying a dry cotton)
	I use a control tooth for the child to experience the desired sensation
	I repeat the test on each tooth
	I do not do anything in specific
	Other (Please specify)

Figure 3-2 Page 2 of the questionnaire showing additional general questions on dental pulp tests

(If you DO NOT use cold testing, please go to Q 12)

9. Which type of cold test(s) do you use? (You can choose more than one answer)

	Ice sticks
	Refrigerant sprays, such as Endo-Ice
	Carbon dioxide snow (dry ice)
	Ethyl chloride
	Other: (please Specify)

10. Do you apply the cold test for a specific period of time on each tooth?
(Please choose only one answer)

	I apply the cold test for a specific period of time on each tooth (time used is .....)
	I don't use it for specific time period/determined time

11. How do you record the results of cold tests? (Please choose only one answer)

	Positive and Negative
	Positive reliable/unreliable or Negative reliable/unreliable
	Other: (Please Specify)

12. (If you DO NOT use Electric Pulp Testing please go to Q 14)

When using EPT: (Please choose only one answer)

	I record the first reading
	I record the last reading
	I record all the readings
	I record the most reliable/consistent reading

Figure 3-3 Page 3 of the questionnaire showing questions on cold testing and EPT

13. How do you record the results of each tooth when using Electric Pulp Test?
(Please choose only one answer)

	Positive or negative only.
	Positive reliable/unreliable, or Negative reliable/unreliable
	I record the results as values only, such as 32,46.
	I record the results as values and degree of reliability such as 36 (reliable/unreliable)
	Other: Please Specify

14. Have you ever used laser Doppler flowmetry in assessing pulpal vitality in permanent teeth?

	Yes → **(please go to question 15)**
	No → **(please go to question 16)**

15. What are the reasons for using LDF as opposed to other sensibility tests?
(You can choose more than one answer)
(End of questionnaire. Thanks for completing this survey)

	It is more reliable than sensibility tests
	It allows assessment of tooth vitality (blood supply) rather than sensibility (nerve supply) to the tooth.
	It is objective (not dependent on child response)
	For research purposes
	Other: (Please Specify)

16. What do you think has prevented you from using it? **(Choose all that apply)**

	Never heard of laser Doppler flowmetry
	Lack of training
	Not enough research based on its use
	Technique difficulty
	Other:

Thank you for completing this survey

4 | Page

Figure 3-4 Page 4 of the questionnaire showing additional questions on EPT and LDF

3.5 Results

The results are presented as absolute frequencies as well as percentages.

3.5.1 Participants

The email invitation was sent to all BSPD members (732 members) with approximately 192 UK registered specialists. The membership includes both UK registered paediatric dentistry specialists and GDPs with an interest in children's dentistry. A total of 149 respondents completed the survey, of which eight respondents were excluded (two retired dentists, two special care dentists and four dentists who did not treat patients with dental trauma). The remaining 141 respondents were split into paediatric dental specialist (79, 56%) and GDP groups (62, 44%). The paediatric dental specialist group included 68 registered paediatric dental specialists, eight paediatric dental trainees and three speciality dentists. Consequently, a specialist response rate of 35% (68 BSPD registered specialists out of 192 BSPD registered specialists) was achieved in this survey and an overall response rate for all the members of 20.3% (149 out of 732).

3.5.2 Dental trauma experience

More than half of the specialists (45/79, 57%) reported seeing more than eight patients a month, while the majority of GDPs (42/62, 67.7%) reported seeing a maximum of two children with a history of dental trauma in a month (Figure 3-5).

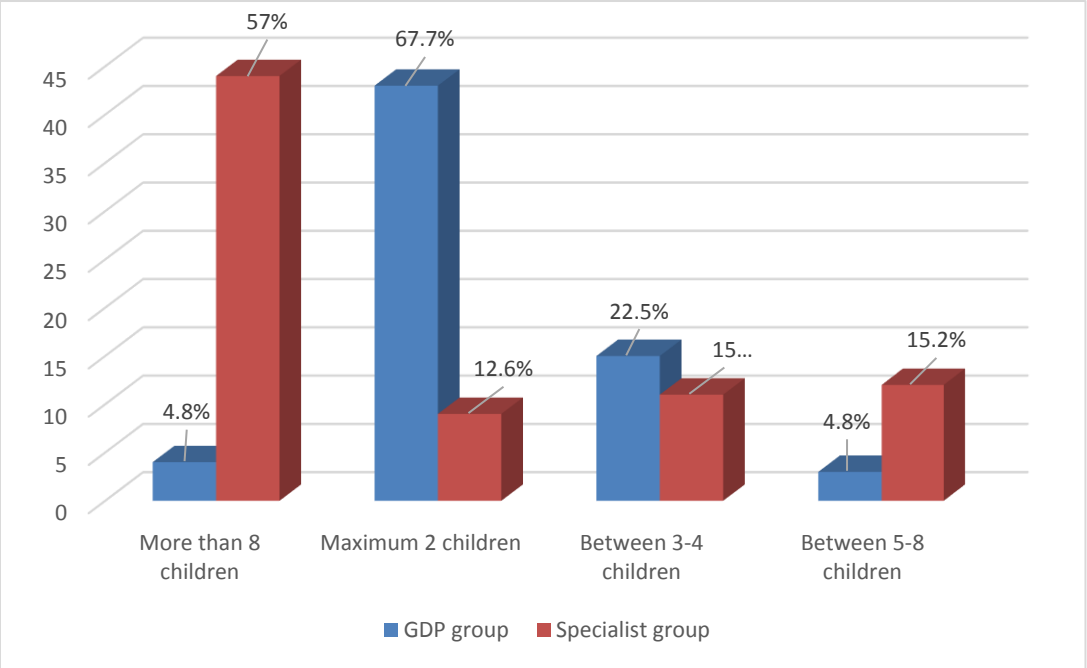


Figure 3-5 Bar chart showing the number of children with traumatised permanent teeth, per group of respondents, seen in a month

3.5.3 General use of dental pulp tests

The majority of the respondents (124/141, 87.9%), with almost all specialists (74/79, 93.7%) reported using sensibility pulp tests routinely in the management of traumatised teeth in children in comparison to (50/62, 80.6%) of GDPs (Figure 3:6).

Furthermore, most of the respondents reported using dental pulp tests at an initial presentation following dental trauma and then at specific intervals (128/141, 90.8%). Almost all of the specialists (78/79, 98.7%) reported using dental pulp tests on initial presentation and then at specific intervals, in comparison to 83.9% of GDPs (52/62) (Figure 3:7).

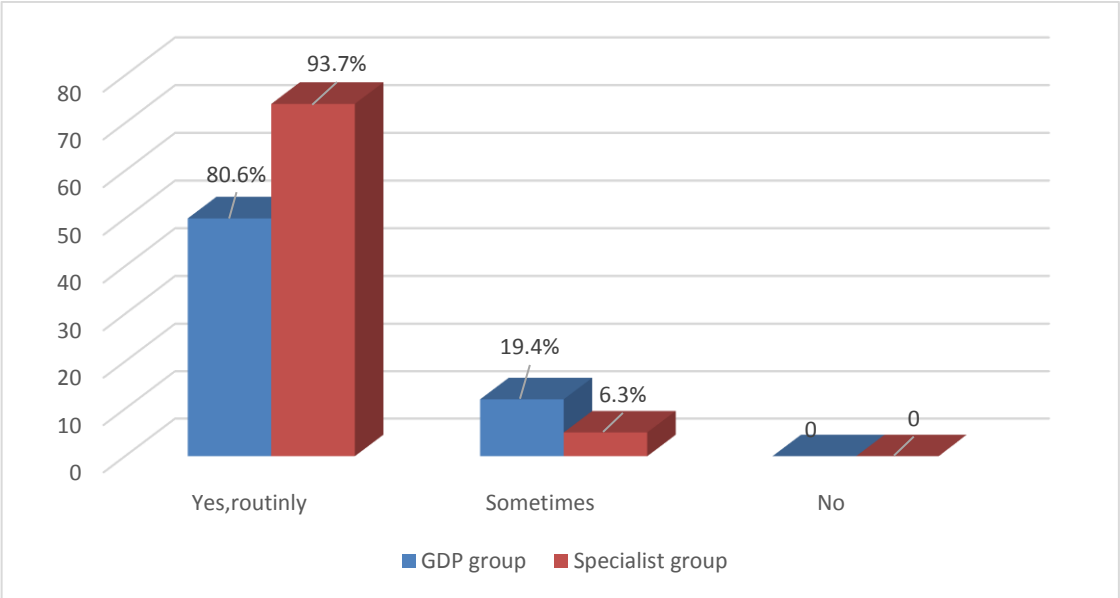


Figure 3-6 Bar chart showing the overall frequency of using dental pulp tests among participants in the management of traumatised teeth in children

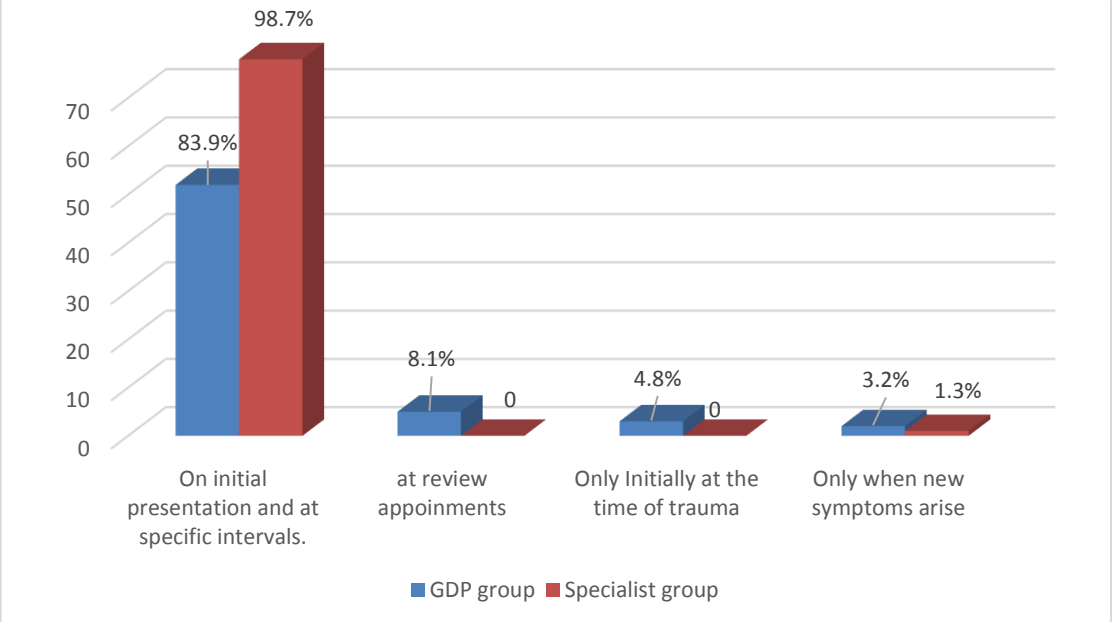


Figure 3-7 Bar chart showing the timing of using dental pulp tests following traumatic dental injuries among the two groups

Different barriers to the use of sensibility testing among those who reported not using the tests routinely were reported with child perception and cooperation being the mostly reported barriers among both groups. Other barriers were also reported including the cost of the tests, time requirements, and lack of extra information provided by these tests (Figure 3-8).

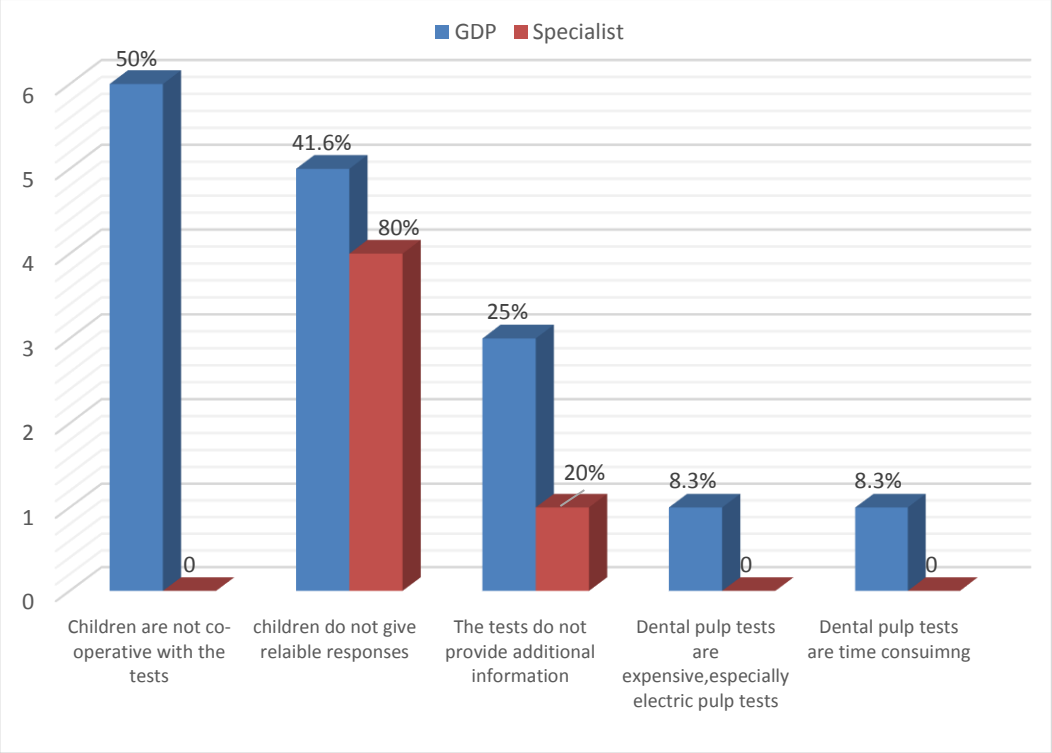


Figure 3-8 Reported barriers for routine use of dental pulp sensibility tests per group

3.5.4 Type of sensibility/vitality tests used

The most common type of sensibility/vitality tests used by all respondents was cold testing (137/141, 97.2%) followed by EPT (94/141, 66.7%). None of the respondents reported using LDF. Six respondents (4.2%) reported the use of heat testing.

GDPs mainly used cold testing 60/62 (96.8%) rather than other tests such as EPT (28/62, 45.2%), while specialists used cold and EPT tests equally (77/79,

97.5%) and (76/79, 96.2%), respectively (Figure 3:9). One participant provided additional information stating that '*"if other tests are inconclusive, then test cavity without local anaesthetic".*

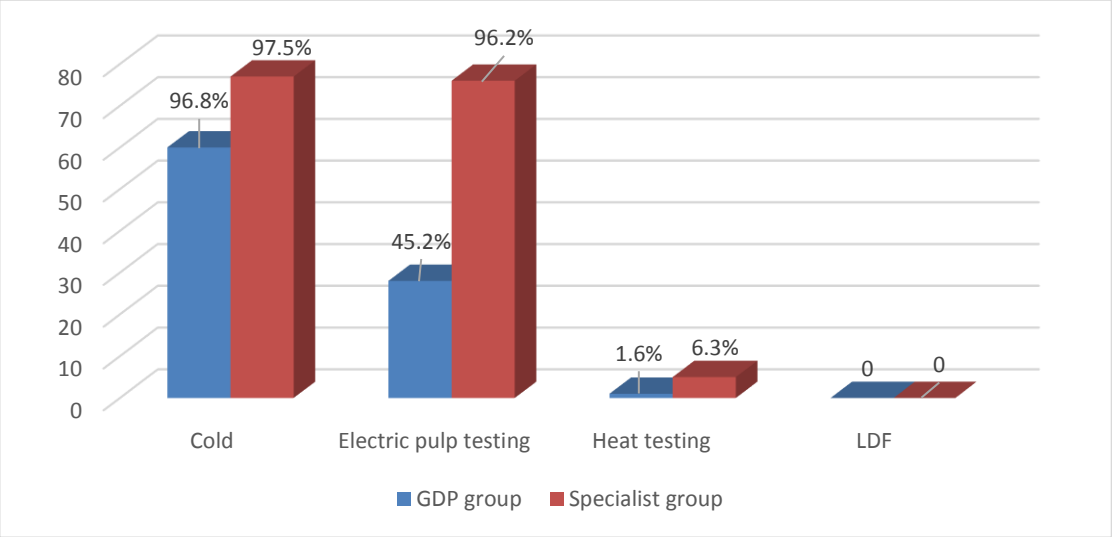


Figure 3-9 Bar chart showing types of sensibility/vitality tests used by respondents per group

3.5.5 Reliability of sensibility tests

The reliability of dental pulp tests was considered inconsistent with almost half the number of GDPs (32/62, 51.6%) and almost two-thirds of the specialist group (50/79, 63.3%) considering these tests to be sometimes reliable. Participants were asked if whether sensibility tests were reliable or not (Figure 3:10).

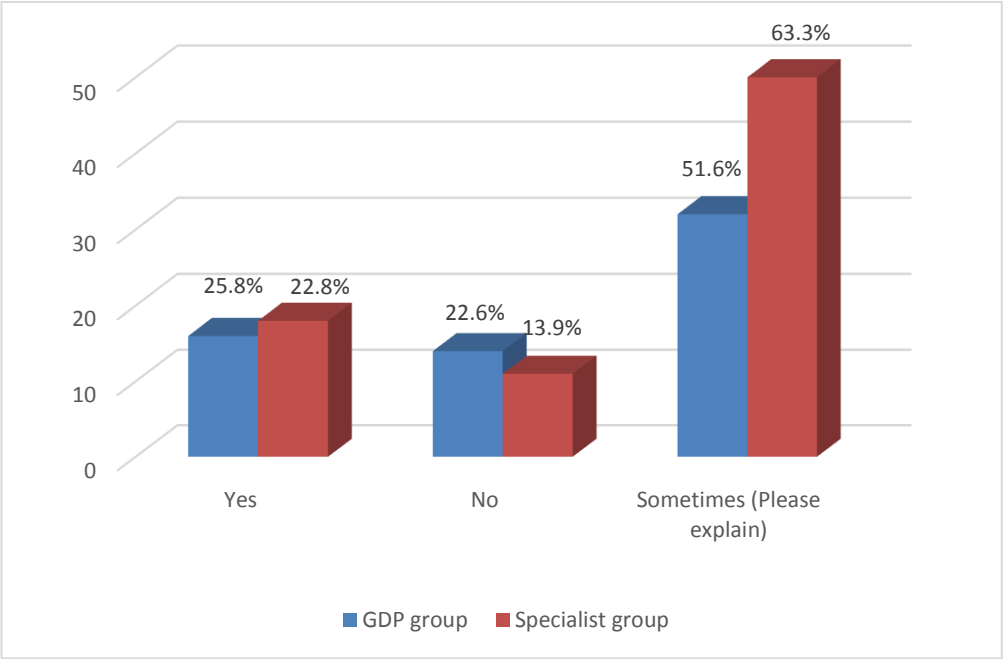


Figure 3-10 Bar chart showing the perception of the reliability of sensibility tests by respond

Different reasons for inconsistency of reliability were reported including children's understanding and cooperation, anxiety and stress, age, root formation, lack of test reliability in the early stage of trauma and issues with sensitivity and specificity of the tests.

Techniques used to improve test reliability in children are shown in Figure 3:11. The most commonly used method by both the GDP group and specialist group was the use of a control tooth, while the least commonly used method was applying a false positive reading.

In order to improve the reliability of dental pulp tests, 15/62 (24.2%) of GDPs apply a false positive reading such as (applying a dry cotton pledget), use a control tooth for the child to experience the desired sensation, and they repeat the test on each tooth. This is in comparison to 28/79 (35.4%) in the specialist group who used these three methods in combination.

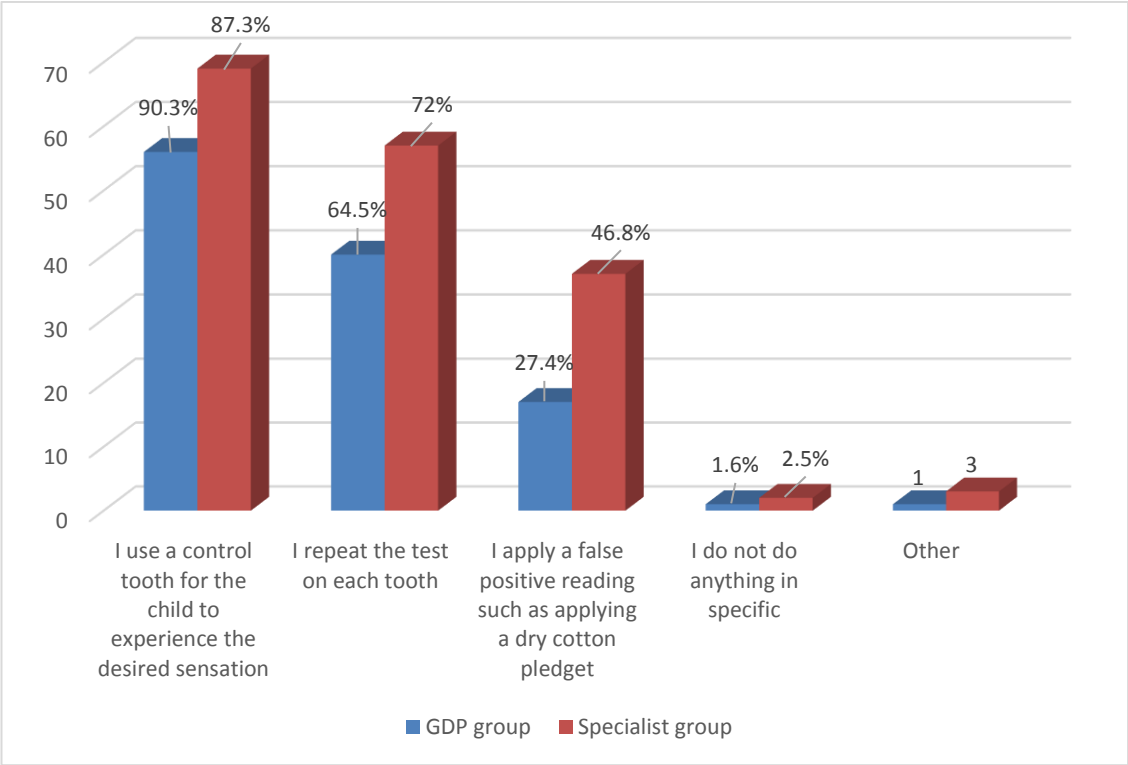


Figure 3-11 Bar chart showing practical techniques performed by respondents in improving the reliability of sensibility tests

Furthermore, 20/62 (32.2%) GDPs and 20/79 (25.3%) in the specialist group use a control tooth and repeat the test without applying a false positive reading. Almost one-third of GDPs 20/62 (35.4%) and 12/79 (15.2%) of the specialist group use a control tooth for a child to experience the desired sensation but neither apply a false positive reading nor repeat the test.

The following were responses from respondents showing other techniques:

- *''Let them feel the tingle on their hand''.*
- *''If I have a child who I suspect to be giving unreliable results and I am highly suspicious that an incisor is non-vital, I will significantly increase the rate of increase on the electric pulp tester. Before applying the EPT I create a false positive by making the child think the EPT is touching the tooth by*

touching it with a metal instrument, and after ~15 second I touch the EPT to the metal instrument to complete the circuit. Generally, there is either an immediate response from the child, or the EPT quickly reaches its maximum value without the child reacting".

3.5.6 Cold test

Participants who answered ''Yes'' to using cold testing were required to complete this section. Otherwise, participants had to skip to the next part, part D, of the questionnaire.

Almost all respondents reported using cold tests (139/141, 98.6%) with only two respondents (one GDP and one specialist) reported not using this type of sensibility testing. Ethyl chloride was reported as the most commonly used cold testing agent with comparable use between the two groups. The second most used cold test reported was refrigerant sprays such as Endo ice 52/141 (36.8%), of which GDPs 24/64 (37.5 %) and similarly by specialists in 28/75 (37.3%). Only one GDP 1/64 (1.5%) reported using ice sticks. Three specialists 3/75 (4 %) reported the use of carbon dioxide snow (dry ice) (Figure 3:12).

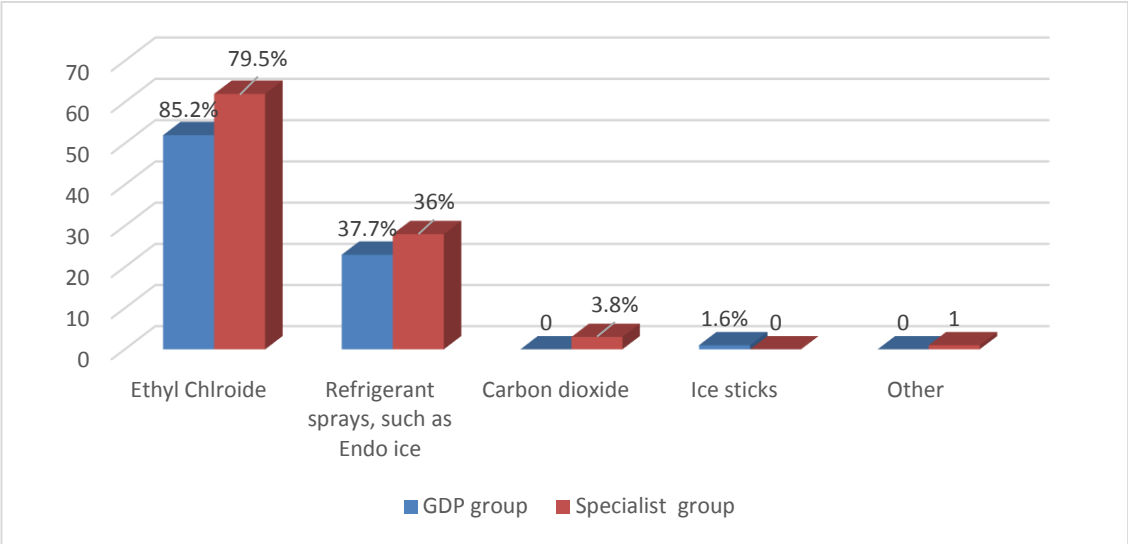


Figure 3-12 Bar chart showing types of cold tests used per group

Three-quarters of all respondents (106/139, 76.2 %), of which 80.3% (49/61) and 73% (57/78) were GDPs and specialists, respectively, did not apply the cold test for a specific period on each tooth. Those who did, however, used a range of time between 1 and 20 seconds per tooth.

Inconsistencies in recording the results of the cold test were also observed with the majority of GDPs (43/61, 70.5%) and specialists (55/78, 70.5%) recording the results as positive and negative with no record of reliability of results. Nearly quarter of the respondents, in addition to recording positive or negative also specify the degree of reliability of the cold tests by stating whether the results were reliable or unreliable, 18/61 (29.5%) GDPs and 23/78 (29.5%) specialists.

3.5.7 EPT use among respondents

Participants who answered ‘’Yes’’ to using EPT were required to complete this section. Otherwise, participants had to skip to the next part, part E, of the questionnaire. Almost half of the GDPs (30/62, 48.4%) and the majority of the specialists (67/79, 85%) reported using EPT when treating traumatised permanent teeth in children. Thus, 97 participants completed this section.

More than half of all participants 52/97 (53.6%) record the most reliable/consistent reading of EPT, of which GDPs 22/30 (73.3%) and specialists 30/67 (44.8%). Furthermore, 19/67 (28.3%) of the specialists only record the first reading and 14/67 (21%) record all the readings of EPT applied. In comparison, 9/30 of GDPs (30%) record all the readings and none record only the first reading. One participant in the specialist group, a speciality registrar (StR) in paediatric dentistry, 1/67 (1.5%) reported recording the average of all readings (Figure 3:13).

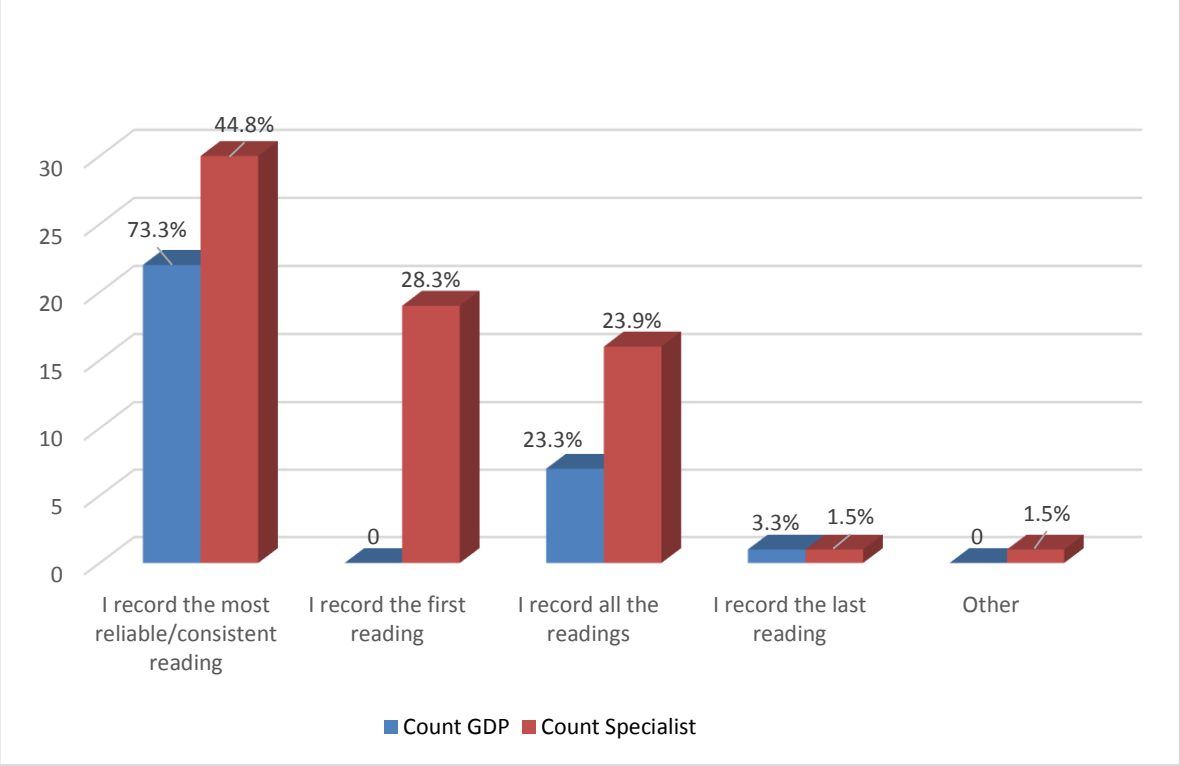


Figure 3-13 Bar chart showing EPT recording per group

Documentation of the results of the EPT varied among respondents with most specialists (48/67, 71.6%) and just over half of GDPs (17/30, 56.6%) documenting the numerical values of the EPT rather than whether the results were reliable or unreliable (Figure 3:14).

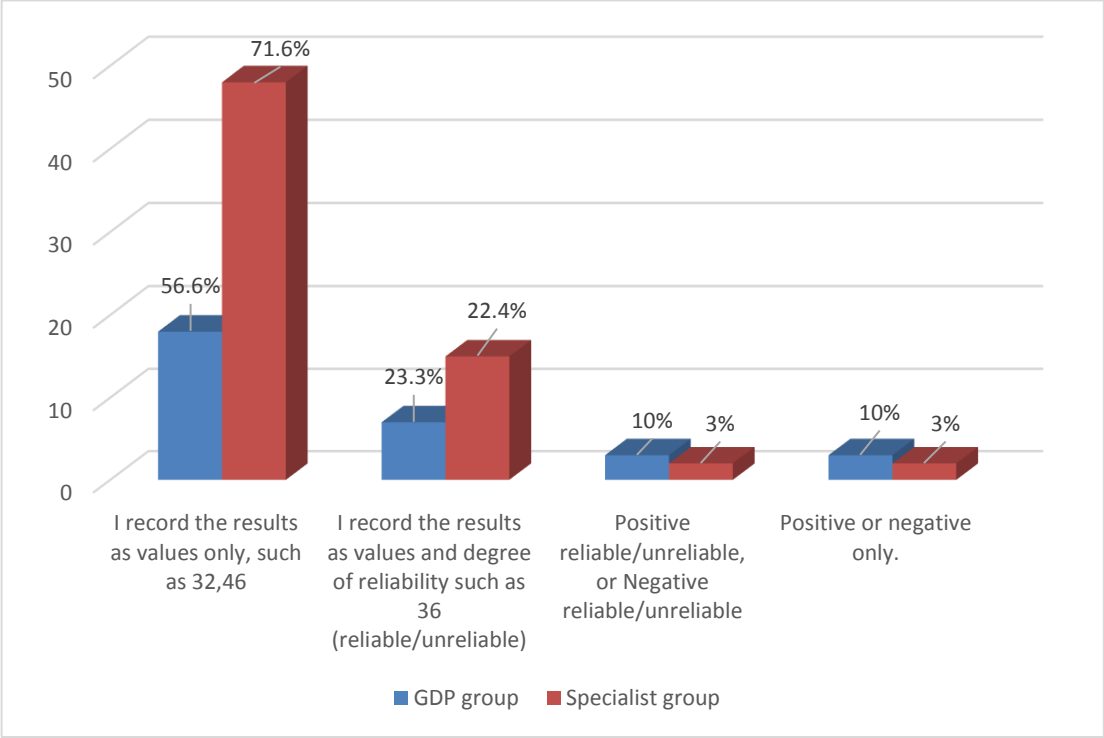


Figure 3-14 Bar chart showing different methods used in documenting the results of the EPT per group

3.5.8 LDF use among respondents

Only 9/141 (6.4%) respondents reported having some experience in using LDF, of which all were specialists. The main reason reported for using the LDF was the need for a test able to assess tooth vitality (blood flow) rather than sensibility (nerve supply). One specialist used it for research purposes, and another used it as a "*Trial use prior to considering purchase of equipment".* The

respondent further mentioned that he/she found the machine *''not reliable enough to be of more use than EPT''.*

When participants were asked about the barriers in using LDF in dental trauma, the main barriers for GDPs were that they never heard of LDF 29/62 (47.1%) in comparison to lack of training as reported by the specialist group (33/70, 45%) (Figure 3:15).

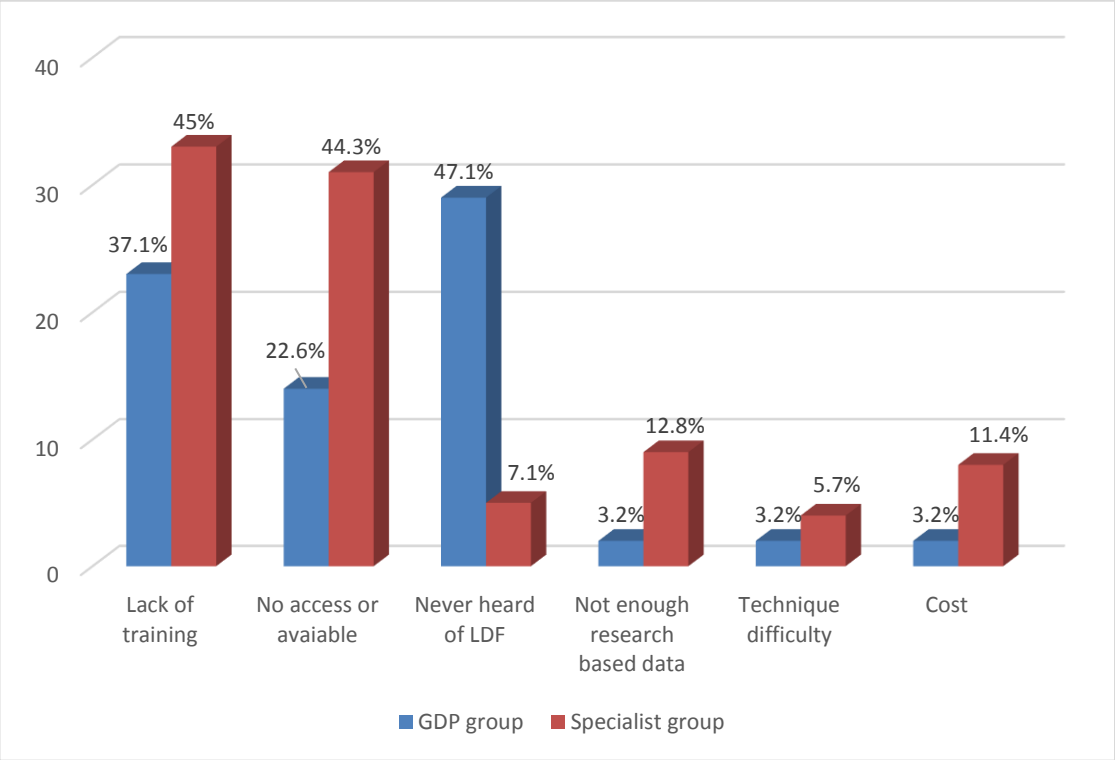


Figure 3-15 Bar chart showing the reasons/barriers in using LDF in dental trauma

3.6 Discussion

Survey research is a method of collecting information about a specific sample through responses to specific questions. The term survey is relatively broad and may include questions used in personal or 'face-to-face' interviews,

telephone interview, a focus group or questions on a self-administered survey. Questionnaires are important for gathering data about ideas or concepts such as opinions, attitudes, knowledge, experiences and beliefs (Ponto, 2015). Surveys may be circulated electronically or by mail. The chosen technique depends on the amount and type of information desired, the target sample size, investigator time and financial limitations (Burns et al., 2008).

The potential use of the internet as a research tool is growing making electronic survey methodology increasingly popular. The main advantages of using electronic surveys are ease of execution, and the possibility of conducting extensive surveys while reducing the costs (Braithwaite et al., 2003). However, researchers have found that obtaining satisfactorily high response rates is difficult compared to a mail method of conducting a survey. Two meta-analyses have shown that e-mail surveys generally have significantly lower response rates, between 11 and 20% on average, than mail survey methodology (Manfreda et al., 2008; Shih and Fan, 2009).

An online rather than a postal method of delivery was used for this survey because of inability to obtain a list of specialists and GDPs addresses due to data protection. Initially, an attempt was made to get the practitioners (GDPs and paediatric dentistry specialists) contact details in order to send postal surveys by contacting the GDC. Unfortunately, due to a recent change in the GDC's published members' information, such information was no longer available online. In addition, the GDC was neither able to share their members' addresses or willing to forward electronic surveys to their members.

An attempt was then made to distribute the survey to all practitioners in the Yorkshire and Humber region through contacting the Local Professional Network (LPN). Initial approval from the LPN's chairperson was obtained in order to distribute the survey electronically. Therefore, a change in the survey mode was made, and an online questionnaire was developed. Unfortunately, our email invite was never forwarded by the LPN's chairperson to the region's practitioners, as agreed, despite attempts to remind them through emails and in person.

The BSPD kindly agreed to assist us in circulating the electronic survey to all their members. The BSPD was not able to share their members' contact details, but agreed to forward an electronic survey to all their registered members. As a result, the survey was distributed through the BSPD mailing list.

The authors acknowledge that a few UK based specialists might not be members of the BSPD. That being said, the results included the participation of a large number of UK based specialists and practitioners working in the capacity of paediatric dental specialists, with a reasonably good representation of paediatric dental specialists across the country. The cohort of GDPs might not fully represent UK GDPs as those BSPD GDP members are likely to be more interested in managing children than the average GDP population.

A number of reasons may explain the response rate achieved in this survey. The BSPD membership does not only include the UK registered practitioners such as paediatric dental specialists, trainees in paediatric dentistry and GDPs who have an interest in children's dentistry, but also include other members not targeted in this survey such as special care dentists, nurses, therapists, retired dentists, and non-UK based dentists/specialists. Unfortunately, the BSPD does

not hold a detailed list in order to exclude those members and therefore the response rate of those eligible to complete the survey is actually higher than reported.

The survey response rate could have also been affected by lack of response from those BSPD members who might have felt that they had nothing to contribute to the survey as they infrequently treat children with dental trauma. Moreover, there may have been a matter of research fatigue that has affected some BSPD members that consequently affected the response rate. BSPD members who have received the invite e-mail may have felt that they had already completed research questionnaires in the past and felt that this questionnaire was not necessary to complete.

In general, there are some additional possible reasons for the low response rate of electronic surveys including technical errors with the server that could lead to loss of responses, and the e-mails may be easily deleted or forgotten (Braithwaite et al., 2003). Some other factors could have influenced the response rate in this study including a lack of interest in completing the survey or in the subject matter.

There are some ways that could be adopted in order to increase response rates. Systematic reviews have found that reminders, such as that used in our survey, and telephone contact have a positive influence on response rates. For postal surveys, some researchers have suggested the use of 3 follow-up reminders as each reminder is expected to increase the response rate. Reminders may be sent after one week, three weeks and seven weeks following the initial mailing of the questionnaire. Electronic reminders sent to

healthcare providers been shown to be an effective way to increase response rates substantially (Burns et al., 2008). However, some other researchers suggest that frequent reminders may possibly irritate the survey respondents leading to no response (Nulty, 2008). In this study, one reminder was sent to all respondents after nearly four weeks from sending the original invitation. The number of respondents, just before sending the reminder, was 106 which increased to 149 after sending the reminder.

Shorter versus longer questionnaires, to a lesser extent, have been shown to influence response rates (Edwards et al., 2002; Nakash et al., 2006). Piloting the questionnaire was undertaken in order to identify any need for question clarification, rephrasing and to consider any other comments provided. It was done by participants from different clinical experience levels. Piloting the questionnaire led to the identification and removal of two leading questions which further reduced the time required to complete the questionnaire to a maximum of 5 minutes.

Anonymity and confidentiality of questionnaires is another technique shown to improve responses (Nulty, 2008). We utilised this in our survey as we have not asked respondents for any identifiable information. This prevented us from sending specific reminders and the authors believe that this made it easier for respondents to take part in the survey.

Extending the duration of a survey's availability could improve response rates as that increases the chance of survey completion. This was utilised in our survey as a total time period of 8 weeks was allowed for participation in the survey. Similarly, incentives can increase the response rate and can be offered

electronically (online vouchers, drawing for a prize) (Nulty, 2008). Incentives were not used in our survey questionnaire and these might have improved the response rate.

Long-term monitoring of pulp vitality has been recommended by all dental trauma guidelines in order to avoid unwanted complications (Albadri et al., 2010; Andersson et al., 2017; Diangelis et al., 2017). According to the International Association of Dental Traumatology (IADT) guidelines, dental pulp tests should be performed as part of the clinical examination at the time of trauma and at the specific review intervals depending on the type of the dental trauma (Diangelis et al., 2017). False negative results may occur up to weeks or even months following dental injuries due to the loss of sensory function transiently or permanently, despite an intact vascular supply. However, providing no response initially followed by obtaining a response at subsequent visits may be indicative of a recovering pulp. On the other hand, a transition from obtaining a response to no response may be indicative of a pulp that is possibly undergoing degeneration (Andreasen and Kahler, 2015a).

Despite their limitations, sensibility tests are extremely useful tools in assessing/monitoring pulp health. Lauridsen et al. (2012) showed the importance of using EPT at initial trauma in identifying teeth at increased risk of pulp necrosis (Lauridsen et al., 2012a; Lauridsen et al., 2012b; Lauridsen et al., 2012c). Therefore, the routine use of sensibility tests by most respondents especially at initial trauma was in line with published guidelines. More exposure of specialists to children with dental trauma could explain the discrepancy in the routine use of sensibility tests by the two groups with more specialists than GDPs using these tests routinely.

Around 1.3% of specialists reported using sensibility tests only when symptoms arise, and around 5% of GDPs reported using sensibility tests only at initial trauma. It is recommended to assess pulpal sensibility at the initial visit as a positive response at the first visit after trauma has been shown to be a good prognostic sign and is hence recommended (Andreasen et al., 2007). However, the lack of pulp assessment at follow-up visits is not in line with current dental traumatology guidelines and could increase identifying early potential complications of pulp necrosis such as pain, infection and bone/root resorption secondary to delayed pulp assessment.

The overwhelming use of cold testing and EPT among all respondents could be attributed to the availability, ease of use of these tests, cost-effectiveness, and high accuracy reported for these tests (Alghaithy and Qualtrough, 2017). Cold dental pulp tests were used by most respondents, and ethyl chloride was the most commonly used agent. This survey, however, highlighted the lack of standardisation in the type and technique used with this test among respondents which is likely to affect the results obtained.

Most respondents used ethyl chloride over other cold tests. This could be due to the availability of ethyl chloride. One study compared the reliability ethyl chloride and refrigerant spray (DDM) in premolars and found that the refrigerant spray provided more reliable positive responses than ethyl chloride (Fuss et al., 1986). Different studies evaluating the use of cold tests have shown that the sensitivity of Endo-ice ranged from 81%-100% and for ethyl chloride ranged from 43% to 92 %. The specificity for Endo-ice and ethyl chloride was (76%-100%) and (89% to 100%), respectively. However, there is insufficient high-

quality evidence to appropriately assess the accuracy of cold tests (Mejare et al., 2012).

The correct use of cold tests is important in improving accuracy, reliability and reproducibility of these tests. Since cold tests are subjective, a clear understanding by the patients of the exact nature and feeling of the applied cold stimulus as well as how to respond are important in reducing false results. Applying the cold stimulus to unaffected teeth prior to using these tests on affected teeth (with questionable pulp status), so that patients are aware of the cold stimulus sensation, is important in reducing false results. This was performed by the majority of respondents in both groups in this survey. Since applying EPT with the current switched off to test the reliability of the test has been recommended (Jafarzadeh and Abbott, 2010b). Likewise, the use of dry cotton pellets to test patient compliance and understanding of the cold test is also recommended. Unfortunately, this was only reported by almost a 1/3 of GDPs and less than half of specialists responding to the survey.

The reliability and consistency of ethyl chloride and refrigerant sprays are important during their clinical application. The application of the cotton pellet to the middle third of the labial/buccal surface of the crown for 5-8 seconds is recommended (Dachi et al., 1967; White and Cooley, 1977). This is sufficient to determine a tooth's sensibility (Dachi et al., 1967; White and Cooley, 1977). Adjacent or contra lateral teeth should be tested first in order for the patient to feel the normal sensation and to establish a baseline response. Avoiding contact with the gingival tissues is also important in order to reduce false positive results.

The use of the EPT was also inconsistent among respondents and therefore likely to affect the results obtained. When using EPT, a positive response results from an ionic shift in the dentinal fluid within the tubules initiating local depolarisation and thus the generation of action potential from undamaged nerves (Pantera et al., 1993). A positive response suggests the existence and presence of intact sensory fibres in the pulp with the ability to respond to the stimulus. However, necrotic pulp tissue can have electrolytes in the pulp space. The electrolytes have the ability to conduct the electrical current to sensory fibres down the pulp canal, mimicking a positive response from the pulp (Apfel and Gerstein, 1973).

Different techniques are recommended in order to reduce false positive and false negative results associated with the use of the EPT. Applying the EPT on unaffected teeth prior to use in order to enhance patient understanding is needed. If possible, a contralateral tooth may be tested first to establish a baseline response. This was performed by the majority of respondents in both groups as shown in this survey. Drying the tooth is essential in preventing false positive results due to electrical conduction to the adjacent teeth, or periodontium (Pitt Ford and Patel, 2004). Teeth may be tested at least two times to confirm the responses and ensure consistency (Bender et al., 1989). Around 64% of GDPs and 72% of specialists in the survey reported repeating the test on each tooth. This reflects that they understood the importance of repeating the tests at least twice on each tooth. Moreover, it is important to change the order of the teeth assessed as this has also been reported to increase the reliability of EPT (Jafarzadeh and Abbott, 2010b). The electric

current can be switched off by removing the conducting medium, and to repeat the test (Jafarzadeh and Abbott, 2010b).

Another method is to change the speed of the current applied so that a faster current is applied. The numerical values of EPT have significance only if there is a high difference between the traumatised tooth and the vital control teeth (Andreasen and Kahler, 2015a). Therefore, the numerical value of the responses should be recorded for each tooth. Most of the respondents in the present survey record the numerical values of EPT, However, around 20% of GDPs and 6 % of specialists reported recording EPT as positive and negative without recording the values.

Patients need to fully understand what type of feeling to expect with EPT and what to do in response to that. Patients usually report a sharp sensation or a tingling sensation. The threshold may vary between patients and teeth (Mumford, 1967b). The value of sensibility tests is highly dependent on a number of factors including patient understanding, compliance and cooperation and the degree of root development. Therefore, this can limit their use in some children, patients with learning disabilities or with limited communication. This was reported by respondents showing good understanding and appreciation of these limitations. Therefore, recording the results of such techniques with a comment on the reliability of the results and/or any limiting factors should be encouraged.

The ability of LDF in measuring pulp blood flow rather than innervation lead to its use to test pulp vitality. However, the cost of the equipment is considered to be high when compared to other pulp tests. Moreover, it is technique sensitive

and careful interpretation of the results should also be considered (Jafarzadeh, 2009).

This survey aimed at assessing the knowledge, experience and barriers to the use of LDF amongst respondents. Interestingly, none of the GDPs had any experience with the use of LDF with almost half of the GDPs reporting lack of awareness of such a test in the first place. In comparison, most specialists were aware of such a test, although, very few reported some experience in using this test.

Lack of training and unavailability of the device were the most commonly chosen barriers by both groups in addition to almost half of the GDPs having never heard of LDF. Interestingly, cost was selected by a few participants as one of the barriers to using this technique. This might be associated with the high proportion of the respondents who were not aware of LDF. In addition, technique difficulty and the lack of well-conducted studies in assessing the sensitivity and specificity of LDF were other barriers chosen by participants.

The systematic review conducted in Chapter 2 of this thesis has shown, based on low-quality evidence, that LDF had better accuracy than the traditional pulp sensibility tests while this survey questionnaire has highlighted the need for high accuracy objective tests able to assess pulp vitality of teeth with minimal dependency on patient’s cooperation and understanding. Therefore, conducting a well-designed clinical study to assess the accuracy of LDF was of great importance prior to recommending the use of such an expensive technique in practice.

Although the use of pulp sensibility tests was relatively high when assessing traumatised teeth in children, dental practitioners should:

1) Routinely use sensibility tests with all traumatised teeth mainly at baseline and key review appointments as per IADT guidelines.
2) Use a standardised technique able to reduce false results as described above and in order to be accurately compared with future pulp test results.
3) Record the reliability of the results depending on their assessment of patient understanding, cooperation and response to contralateral healthy teeth and repeated measurements.
4) Interpret the results of the sensibility tests within the overall clinical assessment due to the inherent limitations of these tests.

3.7 Conclusion

This survey highlighted the relatively high use of pulp sensibility tests among GDPs and specialists with inconsistency in the use of the techniques and recording of results. Several barriers usually associated with the child patient, including cooperation, understanding and age were identified. The knowledge and use of vitality tests such as LDF, was extremely low amongst GDPs and specialists. Conducting high-quality accuracy studies assessing LDF is deemed necessary before this could be recommended for use by GDPs and/or specialists in assessing pulp vitality.

Chapter 4 Clinical study 1

The diagnostic accuracy of laser Doppler flowmetry in the assessment of pulp status in paediatric patients

This study has been submitted for publication to the Journal of Endodontics

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.

Methodology: A cross-sectional cohort diagnostic accuracy study with randomisation was carried out in children. Participants had one maxillary central or lateral incisor with either a completed root canal treatment or pulp extirpation and a contra-lateral tooth with vital pulp. The outcome measures included the cut-off threshold for LDF and the sensitivity, specificity and predictive values as well as the repeatability of each test. The Receiver Operating Characteristic (ROC) curve and the contingency 2X2 table were used for analysis. 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 aged 8-16 years. There was a significant difference between the Flux values for teeth with vital and non-vital pulps. 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: The results of this study showed that there was a high probability of false results when using LDF in assessing the pulp blood flow/pulp vitality. Therefore, LDF 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.

4.1 Rationale of the study

The use of electrical and thermal pulp tests for the assessment of pulp sensibility in children's teeth relies on patients' cooperation, understanding and comprehension. The use of these tests can sometimes be challenging, especially in the child population. Relying on children's responses to stimuli can sometimes be unreliable. LDF, on the other hand, is an objective method that may offer more reliable results when used with these patients.

Although there are several studies on the use of LDF, the evidence is weak and often derived from studies with compromised designs, methodologies and high levels of bias (Ghouth et al., 2018). In addition, there is a huge variation in the flux threshold used by these studies in determining pulp status (Flux threshold below which teeth are considered non-vital). Therefore, assessing the LDF's accuracy and Flux threshold using a well-designed study was deemed important.

4.2 Research Aim, objectives, and hypotheses

4.2.1 The aim of the study

To assess whether LDF is more accurate than the conventional pulp sensibility tests (EPT and ethyl chloride) in assessing the pulp vitality status of permanent anterior teeth in paediatric patients.

4.2.2 Study objectives

- To assess the sensitivity, specificity, positive and negative predictive values of LDF, EPT and ethyl chloride.
- To assess the repeatability of each method.
- To determine the most accurate flux threshold below which a tooth could be identified as non-vital when using LDF.

4.2.3 Hypotheses

4.2.3.1 Null hypothesis

LDF is as accurate as the conventional methods (EPT and ethyl chloride pulp tests) in assessing pulp status of permanent anterior teeth in paediatric patients.

4.2.3.2 Alternative hypothesis

LDF is more accurate than the conventional method in assessing pulp status of permanent anterior teeth in paediatric patients.

4.3 Materials and methods

The study protocol was registered at the International Standard Randomised Controlled Trial Number (ISRCTN) registry (ISRCTN12547356). The following section describes the materials used and methods applied for LDF, EPT and ethyl chloride.

4.4 Materials

This section describes the details of the tools and materials used in the study.

4.4.1 LDF

The device used in the study had the following specifications:

- MoorVMS-LDF2, Laser Doppler Monitor LDF, dual channel (Moor Instruments, Axminster, UK) (Figure 4:1).
- Laser Safety Classification: Class 1 per IEC 60825-1:2007, Class 1 per 21 CFR 1040.10 and 1040.11.
- Output power 2.5 mW max.
- Wavelength 785 nm ± 10 nm
- Frequency filter 15 KHz probe.
- Probe diameter 1.5 mm.
- Two fibres with fibre separation of 500 μm.
- Fibre diameter 200 μm.

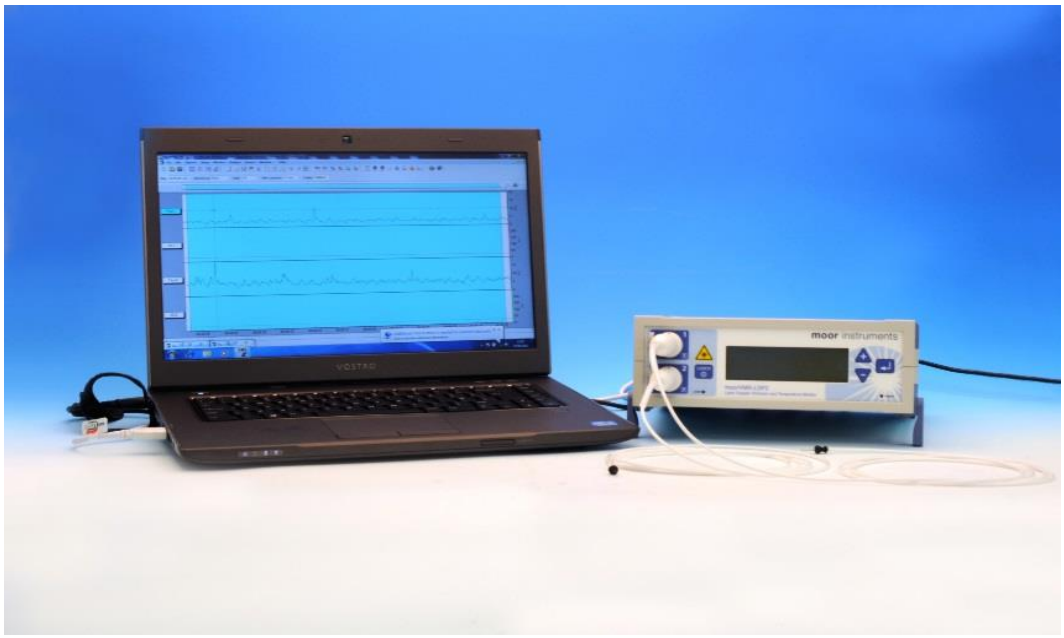


Figure 4-1: LDF device connected to a laptop

4.4.2 EPT

Vitality scanner 2006 (Sybron Endo, Sybron Dental Specialties, Glendora, California, USA) (Figure 4:2).

Figure 4-2: Electric pulp tester

4.4.3 Ethyl chloride

Ethyl Chloride (Axongesic; BTC Invest, Praha, Czech Republic) (Figure 4:3).

4.4.4 Other materials used in the study

- Rubber dental dam, latex free, light blue squares 6'' x 6'' (UnoDent, Essex, England).
- Aquagel medium (Fabricado por, ECOLAB, Leeds, UK)
- IMPREP *ac,* Additional-cured Hydrophilic Vinyl Polysiloxane Impression material (UnoDent, Essex, England).

Figure 4-3 Ethyl chloride

4.5 Methods

4.5.1 Study design

A cross-sectional, cohort diagnostic accuracy study using a randomised controlled study design was used.

4.5.2 Ethical approval

Ethical approval was obtained from RES North West - Greater Manchester East (Ref # 15/NW/0583) (Appendix 4). NHS permission was then obtained at The Leeds Teaching Hospital NHS Trust (LTHT) (Ref # DT15/307) (Appendix 5).

The study documents included the following (Appendix 6):

- Assent
- Consent
- Information sheet for the person with parental responsibilities
- Patient information sheet for children 8-12 years of age.
- Patient information sheet for children 12-16 years of age.
- Invitation letter

4.5.3 Recruitment

Dental records of patients who attended the trauma clinic at the Leeds Dental Institute were assessed for possible suitability for inclusion in this study prior to their forthcoming trauma clinic appointments. Information leaflets which included an invitation letter, a letter to the person with parental responsibility and an age-specific letter for children to read (either 8-12 years old or 12-16 years old) were posted to potentially suitable patients two weeks prior to their forthcoming trauma clinic appointment.

On the day of the appointment and following the child's examination/treatment session, the chief investigator approached patients, assessed each potential participant clinically and further explained the study to the parent/person with

parental responsibility. Informed consent was then obtained from participants fitting the inclusion criteria. Participants were offered the choice of either having the measurements made at that session or during any of the subsequent appointments.

4.5.3.1 Inclusion Criteria

Children were recruited into the study when they fulfilled the following inclusion criteria:

- Aged between 8-16 years.
- Medically fit and well (ASA I, II).
- Understood English language and able to understand instructions.
- Showed an acceptable level of cooperation.
- Had one non-vital maxillary central or lateral incisor with root canal treatment or pulp extirpation and, when possible, a contra-lateral vital tooth with no history of dental trauma, no tenderness to percussion, no periapical radiolucency nor associated sinus tract.
- Had minimal restoration covering less than half the labial crown surface of all teeth assessed (non-vital and vital).

4.5.3.2 Exclusion Criteria

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

- Medically compromised children.
- With learning disabilities.

- With a history of moderate and significant behaviour management problems
- With communication barriers such as not understanding or speaking English language.
- With heavily restored teeth (restorations covering more than half the labial surface) and when you placing the LDF probe against enamel due to restoration was not possible,
- On routine analgesics, antidepressants or antihypertensive drugs.
- With non-vital teeth treated with regenerative endodontic techniques.
- With teeth showing abnormal crown colour.
- With vital teeth showing pulp canal obliteration.
- With contra-lateral vital teeth 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 signs of loss of vitality.

4.5.4 Sample size/power calculation

The sample size was calculated based on a pilot study that was conducted in the Department of Paediatric Dentistry, Leeds Dental Institute (Nazzal H., et al 2014). The study consisted of 15 patients and aimed to assess the accuracy of LDF, EPT and ethyl chloride in assessing the vitality and pulpal regeneration of non-vital immature permanent incisors. The sensitivity of LDF, ethyl chloride and EPT were estimated to be 87.5%, 88.7%, and 62.5% respectively. As a result, the number of patients required to achieve the power of 80%, at 95%

significance difference with effect size as 25% using a one-sided test was calculated using online software (http://www.stat.ubc.ca/~rollin/stats/ssize/) and calculated to be 37 participants per group.

4.5.5 Randomisation

Following consent and assent, on the day of conducting the recordings, participants were randomly assigned to either group (Test or Control) using a computer-generated random list that was made by an independent person. The independent person concealed the allocation sequence in sequentially numbered, opaque, and sealed envelopes. Each patient chose one envelope prior to commencing the chosen test(s).

4.5.6 Pulp assessment

4.5.6.1 Test group

The included non-vital and vital teeth were assessed twice with LDF. The assessment was performed as follows:

- The chief investigator prepared and calibrated the LDF device prior to use. Calibration was performed as per manufacturer's instructions using the recommended Brownian motion of polystyrene microspheres in water (Figure 4:4).

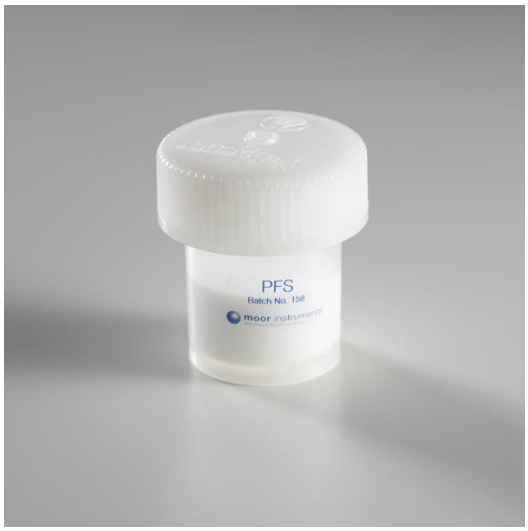


Figure 4-4 Probe Flux standard (10ml)

- Pre and post-decontamination of the probes were carried out using a 3-part decontamination system for non-laminated medical devices following the manufacturer's instructions (Tristel, Trio 50, Tristel Solutions limited, Cambridgeshire, UK). The use of the wipes was documented in the Trio Wipes System Audit Trail-Record Book and checked by the infection control head nurse (Figure 4:5).

Figure 4-5 Trio Wipes System and Audit Trail-Record Book (http://www.tristel.com)

- An impression was taken for each patient for splint construction using IMPREP *ac,* Additional-cured Hydrophilic Vinyl Polysiloxane Impression material (UnoDent, Essex, England). Small holes were drilled in the splint labially at the level of the middle third of all teeth to be assessed using a tungsten carbide round bur with a slow speed handpiece in order to accommodate and stabilise the LDF probes (Figure 4:6, Figure 4:7).

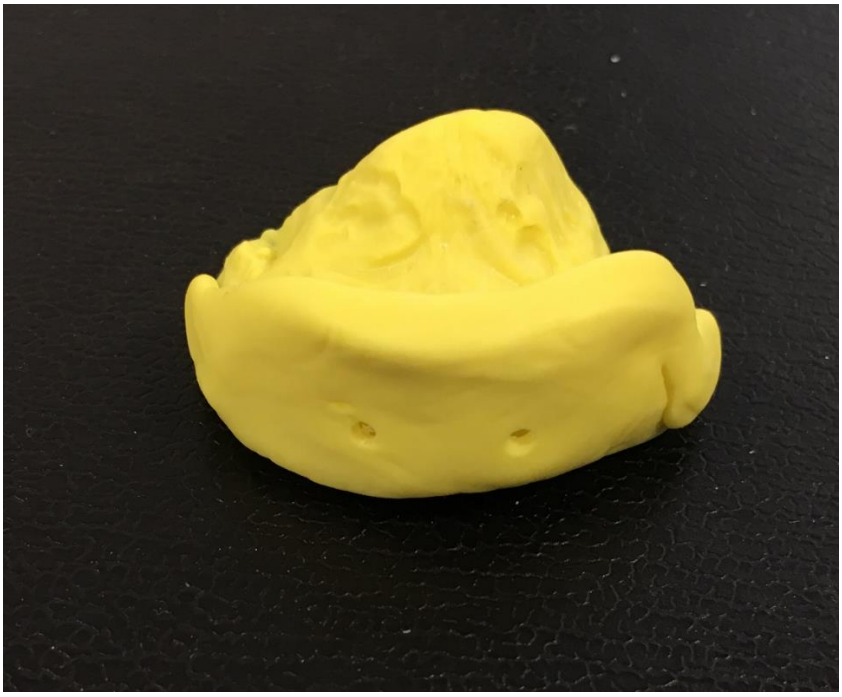


Figure 4-6 A frontal view of the splint showing the drilled holes used to guide and stabilise the LDF probes.

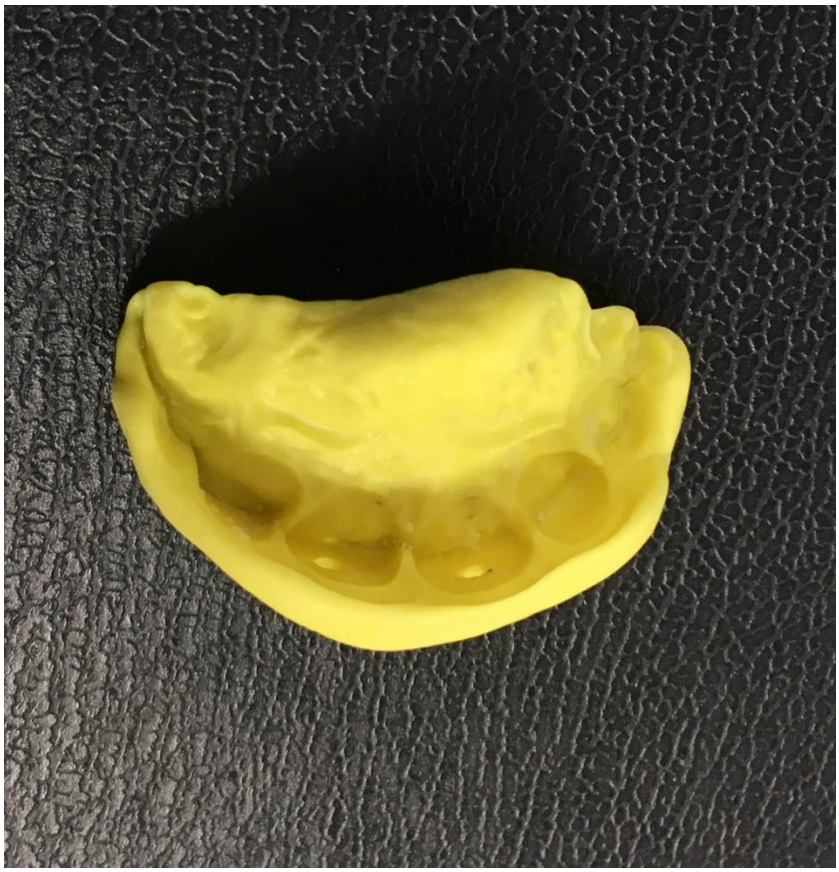


Figure 4-7 A top view of the splint showing the drilled holes used to guide and stabilise the LDF probes.

- Participants were asked to rest for a few minutes while the splints were prepared for intra-oral use before the start of LDF signal recording.
- Teeth were isolated using a small piece of rubber dam (Figure 4:8).

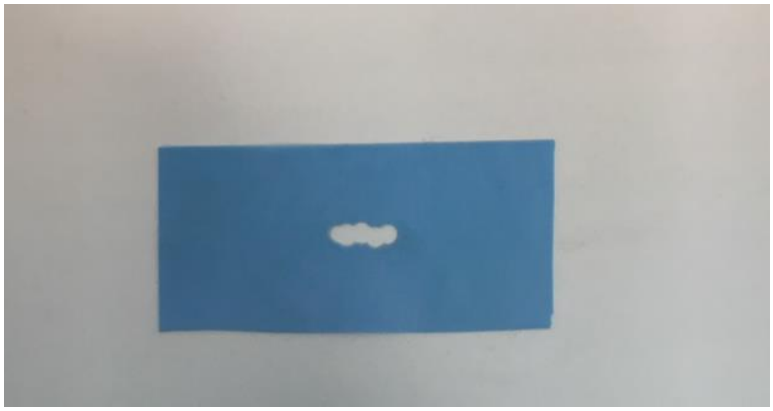


Figure 4-8 Rubber dam used to isolate the teeth tested by LDF.

- The splint was then fitted over the rubber dam and care was taken not to displace the rubber dam and expose the gingival tissues.
- Once the splint was stable, the LDF's probes were passed through the labial holes, made into the splint, against the labial surfaces of the teeth assessed with one probe placed against the vital tooth and the second against the non-vital tooth allowing simultaneous recordings with the participant sitting in a semi-supine position.
- Movement of the participant or the probes were avoided as much as possible.
- A 30-second interval of stable LDF flux was recorded (Figure 4:9, Figure 4:10). A stable recording was achieved when there were no movement artefacts.
- Two measurements per tooth were obtained to assess repeatability.
- All data were stored on an encrypted laptop and backed up on the University of Leeds secured server. The laptop was kept in a locked cabinet in a password protected office when not in use.

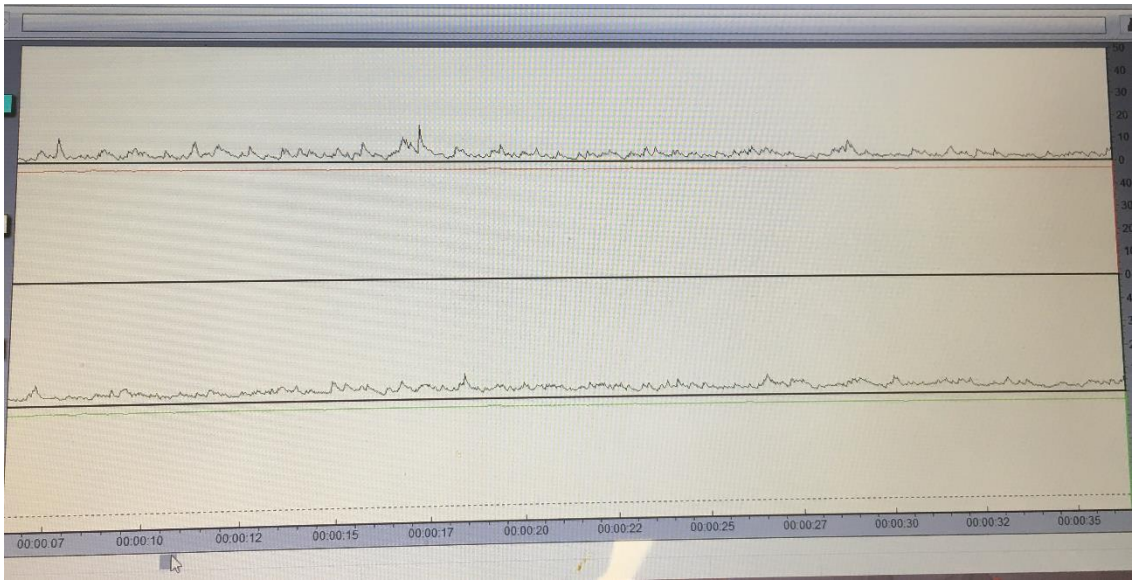


Figure 4-9 An example of a stable LDF recording

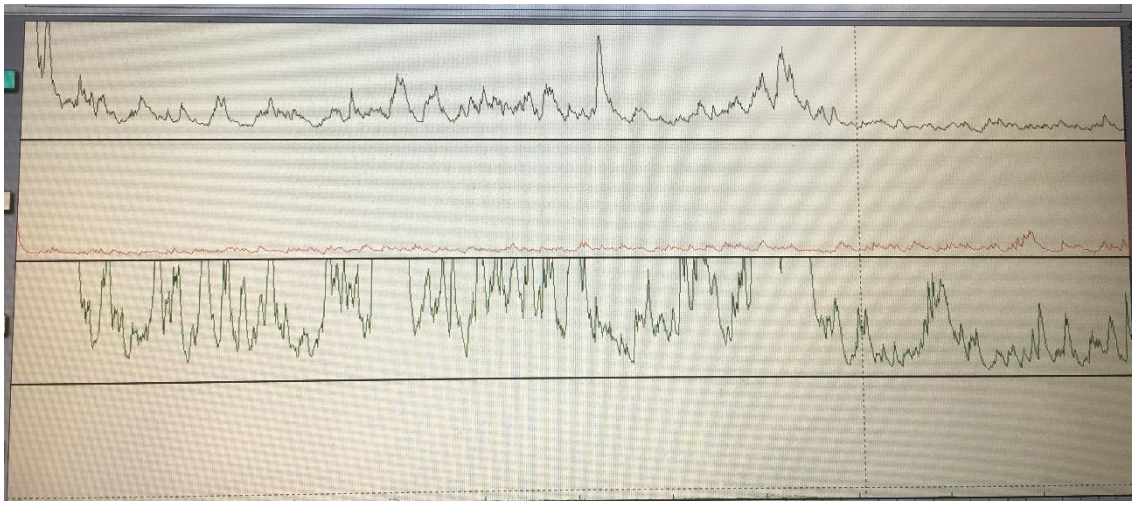


Figure 4-10 An example of unstable LDF recording.

4.5.6.2 Control group

Pulp sensibility was assessed using EPT followed by ethyl chloride as described under 4.5.6.2.1 and 4.5.6.2.2. 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 patient in order to improve participants' understanding and compliance as experiencing the normal feeling of both tests prior to the assessment could improve the accuracy of the tests.

4.5.6.2.1 EPT

EPT was conducted as follows:

- The maxillary anterior teeth were isolated with cotton rolls. Then, the teeth were dried with cotton rolls and dried using air spray.

- Each participant was asked to hold the metal end of the EPT's probe. Once a tingling sensation was felt, participants were asked to let go of the probe. EPT was performed with a conducting medium (Aquagel medium, Fabricado por, ECOLAB, Leeds, UK).

- Two recordings were carried out for each of the vital and non-vital teeth. During the first recording of both teeth, the rate of voltage change was set to 5 for the first recording. The voltage at which the patient felt a sensation was then recorded.

- The second recording of both teeth was carried out after increasing the rate of voltage change to 8.

- Any sensation felt by participants at any time before EPT reached the maximum voltage of 80 on the scale was considered positive.

- A negative result was recorded if the participant did not feel any sensation up to a voltage of 80.

- For an overall positive, reliable and consistent response to be recorded, a positive response in both measurements should have been recorded.

- For an overall negative, reliable and consistent response, a negative response in both measurements should have been recorded.

- An unreliable EPT measurement was recorded when different responses were obtained (one measurement is positive while the other is negative).

4.5.6.2.2 Ethyl chloride

Three applications were performed for each tooth using ethyl chloride. The first application was performed with a cold sprayed cotton pledget. A dry un-sprayed cotton pledget was used in the second application. The third application was performed again with a cold sprayed cotton pledget.

Following EPT recordings, all teeth were re-isolated and re-dried as described during EPT measurement. Ethyl chloride cold testing was performed as follows:

- Each participant was asked to raise their left hand when he/she felt any cold sensation.

- During the first application, a cotton pledget was sprayed with ethyl chloride until it was saturated. After removal of the excess by shaking the cotton pledget, it was applied to the first tooth for 5 to 8 seconds. This method was applied to one tooth followed by the second tooth. The response for each tooth was recorded as positive or negative.

- The second application for both teeth was carried out using a dry cotton pledget and followed by recording the response of both teeth.

- Finally, 2 minutes following the first application, the third application was performed again as described in the first application taking. The response of each tooth was recorded as positive or negative.

- A positive response was recorded when patients raised their hands indicating sensation within 5-8 seconds of application.

- A negative response was recorded when patients did not raise their hands indicating lack of sensation within 5-8 seconds of application.

- An overall positive, reliable and consistent response was recorded when positive responses in the first and third applications and a negative response in the second application were recorded.

- An overall negative, reliable and consistent response was recorded when negative responses in the first and third applications and a negative response in the second application were recorded.

- An overall unreliable response was recorded when disagreement in measurement between the first and third applications was recorded (one positive and one negative) and/or a positive response to the second application was recorded.

4.5.7 Data collection

A data collection sheet was used to collect the demographic and clinical data of each participant and its corresponding conducted test results. The data collection sheet included information such as age, sex, type of trauma, stage of root development as well as the results of the tests (Appendix 7).

4.5.8 Statistical analysis

The data obtained from the study was analysed using IBM SPSS (Statistical Package for Social Science) statistics version 23.

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 teeth vital and non-vital teeth.

4.5.8.1 Test group

4.5.8.1.1 Determining the LDF's cut-off threshold

Using the Receiver Operating Characteristic curve (ROC curve), two methods were used to calculate LDF's cut-off threshold:

1) Assessment of LDF's cut-off threshold based on the Flux values obtained for vital and non-vital teeth.

2) Assessment of LDF's cut-off threshold based on the Flux ratios obtained for each participant (Flux non-vital tooth/ Flux vital tooth).

Ideally, a ROC curve should be as close as possible to the upper left corner indicating perfect sensitivity and specificity. In other words, the closer the curve to the upper left corner, the better the sensitivity and specificity are. Coordinates of the ROC curves will be presented in tables which show the full range of cut-off values and ratios that can be obtained from the data and their corresponding sensitivity and 1-specificity.

4.5.8.1.2 Determining the sensitivity, specificity and predictive values

Using the tables obtained from the ROC curve, the sensitivity and specificity of the cut-off values and cut-off ratios were determined. The positive and negative predictive values table were calculated using the traditional 2X2 (Akobeng, 2007a) (Table 4:1).

Table 4:1 The traditional 2x2 table

	Tooth status		Total
	Vital	Non-vital	
Test positive (non-vital)	a	b	a+b
Test negative (Vital)	c	d	c+d
Total			

a: True positive, b: False positive, c: False negative, d: True negative

4.5.8.2 Control group

The sensitivity, specificity, positive and negative predictive values were calculated using the traditional 2X2 (Akobeng, 2007a) (Table 4:1). Sensitivity analysis was used to assess these outcomes when study participants provided unreliable results as each unreliable response was firstly excluded then was considered as positive or negative.

The 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)”.*

4.5.8.3 Assessment of the repeatability of LDF, EPT and ethyl chloride

Repeatability 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).*

Kappa scores were used to assess the repeatability of EPT and ethyl chloride while inter-class correlation was used to measure the repeatability of the LDF. The following levels of agreement were considered appropriate for the extent of agreement (Landis and Koch, 1977):

- Poor if $k < 0.00$
- Slight if $0.00 \leq k \leq 0.20$
- Fair if $0.21\ k \leq 0.40$
- Moderate if $0.41 \leq k \leq 0.60$
- Substantial if $0.61 \leq k \leq 0.80$
- Almost perfect if $k > 0.80$

4.6 Results

4.6.1 Baseline demographics and clinical characteristics

4.6.1.1 Age, gender and the type of dental trauma

The study included 74 participants with an overall mean age of 12.4 years (SD=2), with an age range of 8-16 years. The mean age for the test group (LDF) was 12.1 (SD=2) years (range: 8-15 years) and for the control group was 12.7 (SD=2) years (range: 9-16 years). There was no significant difference between the two groups in terms of participants' age (P= 0.25) (Table 4:2, Table 4:3).

The study included more male participants (n= 46, 62.2%) than female participants (n= 28, 37.8%). However, there was no significant difference between the test and control groups in terms of gender distribution (P= 0.47) (Table 4:2).

Recruited participants had sustained different types of dental trauma including crown fractures, luxation injuries, avulsion, root fractures and concomitant injuries. The most frequent type of trauma sustained was enamel-dentine fractures with a percentage of 39.2%. There was no significant difference between the groups in the distribution of the types of dental trauma (p= 0.18) (Tabe 4:2). Moreover, Table 4:4 summarises the distribution of the type of dental trauma among gender in both groups.

Table 4:2 Study demographics

Variable		Test group n (%)	Control group n (%)	Total	P-value
Age (years)					
	Mean (SD)	12.1 (2)	12.7 (2)	--	0.25
Gender					
	Male	21 (56.7)	25 (67.6)	46 (62.2)	
	Female	16 (43.3)	12 (32.4)	28 (37.8)	
	Total	37	37	74	0.47
Type of traumatic dental injury					
Enamel-dentine fracture		14 (38)	15 (40.5)	29 (39.2)	
Complicated crown fracture		--	6 (16.2)	6 (16.2)	
Concussion		1 (2.7)	--	1 (2.7)	
Subluxation		3 (8.1)	1 (2.7)	4 (10.8)	
Extrusive luxation		1 (2.7)	3 (8.1)	4 (10.8)	
Intrusive luxation		1 (2.7)	--	1 (2.7)	
Avulsion		13 (35)	7 (19)	20 (27)	
Lateral Luxation		3 (8.1)	3 (8.1)	6 (16.2)	
Enamel-dentine fracture with lateral luxation		1 (2.7)	--	1 (2.7)	
Mid root fracture		--	1 (2.7)	1 (2.7)	
Enamel fracture with subluxation		--	1 (2.7)	1 (2.7)	
Total		37	37	74	0.18

Table 4:3 Age distribution among the two groups

Age	Group		Total
	Test	Control	
8	1	0	1
9	5	2	7
10	3	3	6
11	4	6	10
12	6	8	14
13	7	4	11
14	6	4	10
15	5	9	14
16	0	1	1
Total	37	37	74

Table 4:4 Distribution of the type of dental trauma among males and females in both groups

Type of dental trauma	Gender		Total
	Male	Female	
Enamel-dentine fracture	19	10	29
Complicated crown fracture	6	0	6
Concussion	1	0	1
Subluxation	1	3	4
Extrusive luxation	1	3	4
Intrusive luxation	1	0	1
Avulsion	11	9	20
Lateral Luxation	4	2	6
Enamel-dentine fracture with lateral luxation	0	1	1
Mid root fracture	1	0	1
Enamel fracture with subluxation	1	0	1
Total	46	28	74

4.6.1.2 Stage of root development and tooth type

The stage of root development of all tested teeth was classified according to the following classification (Jonsson and Sigurdsson, 2004):

- Stage 1: One quarter to half root length
- Stage 2: Half to three-quarters of root length
- Stage 3: three-quarters to full root length
- Stage 4:full root length and wide open foramen (diameter > 2mm)
- Stage 5: full root length and half open apical foramen (diameter 1-2 mm)
- Stage 6: full root length and closed apical foramen

The most frequent degree of the stage of root development was full root length with closed apical foramen (76.4% of all tested teeth) (Table 4:5).. There was no significant difference between the groups in the stage for root development for vital and non-vital teeth, $P > 0.05$ (Table 4:6).

The vast majority of the teeth included in the study were central incisors (72.2%). In the test group, more than half of the vital teeth were central incisors (62.2%) and most of which had full root length and closed apical foramen (82.6%). The majority of non-vital teeth in the LDF group were also central incisors (89.2%).

In the control group, there was an equal distribution of vital teeth between central incisors and lateral incisors with full root length and closed apical foramen. Most of the non-vital teeth were central incisors (86.5%). There was

no significant difference between both groups in relation to the tooth type for vital and non-vital teeth, 0.31 and 1.0, respectively (Table 4:6).

Table 4:5 Frequency of the stage of root development in all tested teeth (Jonsson and Sigurdsson, 2004)

Stage of root development	Frequency (%)
Stage 1: One quarter to half root length	0
Stage 2: Half to three-quarters of root length	0
Stage 3: Three-quarters to full root length	0
Stage 4: Full root length and wide open foramen (diameter > 2mm).	7 (4.7)
Stage 5: Full root length and half open apical foramen.	28 (18.9)
Stage 6: Full root length and closed apical foramen.	113 (76.4)
Total	**148 (100)**

Table 4:6 Comparison between the control group and test group in relation to the stage of root development and tooth type for non-vital teeth

Group	Stage of root development	P value	Tooth type		Total	P value
			Central incisor n (%)	Lateral incisor n (%)	n (%)	
Test (Vital teeth)	Full root length and half open apical foramen	0.15	4 (17.4)	1 (7)	5 (13.5)	0.48
	Full root length and closed apical foramen		19 (82.6)	13 (93)	32 (86.5)	
			23 (62.2)	14 (37.8)	37	
Control (Vital teeth)	Full root length and wide open foramen (diameter > 2mm)		0	1 (5.5)	1 (2.8)	
	Full root length and half open apical foramen		0	1 (5.5)	1 (2.8)	
	Full root length and closed apical foramen		19 (100)	16 (89)	35 (94.4)	
			19 (51.3)	18 (48.7)	37	
Test (Non-Vital teeth)	Full root length and wide open foramen (diameter > 2mm)	0.22	4 (12)	1 (25)	5 (13.5)	0.72
	Full root length and half open apical foramen		10 (30.3)	1 (25)	11 (29.7)	
	Full root length and closed apical foramen		19 (57.7)	2 (50)	21 (56.8)	
			33 (89.2)	4 (10.8)	37	
Control (Non-Vital teeth)	Full root length and wide open foramen (diameter > 2mm)		1 (3)	0	1 (2.7)	
	Full root length and half open apical foramen		10 (31)	1 (20)	11 (30)	
	Full root length and closed apical foramen		21 (66)	4 (80)	25 (67.3)	
			32 (86.5)	5 (13.5)	37	

4.6.2 Test group (LDF)

Prior to calculating the sensitivity, specificity and predictive values, it was important to identify Flux threshold whereby teeth would be considered vital/ non-vital. Therefore, the Receiver operating characteristic (ROC) curve was used to analyse the Flux values obtained from the two recordings for the vital and non-vital teeth.

As explained in the statistical analysis section, (4.5.8.1.1), two methods were used to calculate LDF's cut-off threshold. Analysis of the values and ratios (non-vital teeth Flux/vital teeth Flux) have been carried out to determine the best sensitivity and specificity that can be achieved from the data.

4.6.2.1 Descriptive analysis of LDF recordings

The mean Flux values for vital teeth were higher than those of non-vital teeth for both recordings (Table 4:7). There was a significant difference between the average Flux values of the two recordings for vital and non-vital teeth ($P < 0.05$) (Table 4:8). The full dataset for the two recordings of vital and non-vital teeth and their averages are presented in Table 4:9. Also, frequency tables showing the Flux values for vital teeth in the two recordings and the Flux values for non-vital teeth in the two recordings will be presented for further description of the data (Appendix 8, 9).

Table 4:7 Descriptive analysis of LDF recordings for vital and non-vital teeth

	Recording 1 (vital teeth)	Recording 1 (Non-vital teeth)	Recording 2 (vital teeth)	Recording 2 (Non-vital teeth)
Mean Flux	9.87	6.36	10.61	7.40
SD	5.16	5.11	6.47	6.10
Minimum	3.40	1.70	3.50	2.0
Maximum	28.90	27.60	34.90	27.80

Table 4:8 Paired t-test comparing the mean Flux for vital and non-vital teeth

Status	Mean Flux	SD	95% Confidence Interval		Sig (2-tailed)
			Upper	lower	
Non-vital	6.88	5.46	-6.75	-9.37	.00
Vital	10.24	5.63			

Table 4:9 The Flux values for vital and non-vital teeth per study participant

Participant	Vital 1	Vital 2	Average	Non-vital 1	Non-vital 2	Average
1	9.6	6.8	8.2	5.1	3.1	4.1
2	8.4	14.3	11.3	5.3	9.3	7.3
3	9.9	9.5	9.7	4.9	4.7	4.8
4	9.4	9.9	9.6	4.1	4.2	4.1
5	4.2	7	5.6	3.3	2.8	3.0
6	5	3.9	4.4	3.2	2.6	2.9
7	7.9	8.8	8.3	1.7	2.3	2
8	9.3	3.7	6.5	1.9	3.1	2.5
9	5.5	6.8	6.1	3.3	4.5	3.9
10	3.4	5.9	4.6	2	3.3	2.6
11	9.6	8.8	9.2	2.9	4.6	3.7
12	10.6	7.6	9.1	4.2	4.3	4.2
13	7	4.5	5.7	5	5.6	5.3
14	7.4	10.7	9.0	5.3	4.9	5.1
15	19.3	17.2	18.2	9	8.1	8.5
16	15.8	27	21.4	11.9	25.2	18.5
17	9	12.2	10.6	7.9	9.6	8.7
18	6.6	7.2	6.9	8.2	8.5	8.3
19	20.1	24.2	22.1	18.3	22	20.1
20	28.9	34.9	31.9	27.6	27.8	27.7
21	4.4	4.9	4.6	3.1	3.5	3.3
22	7.5	10.4	8.9	4.5	4.4	4.4
23	10.1	9.7	9.9	8.2	8.1	8.1
24	12.4	11.4	11.9	2.8	4.9	3.8
25	15.1	11.1	13.1	13.8	12.7	13.2
26	7.6	11.9	9.75	6.1	8.6	7.3
27	6.6	8.2	7.4	5.6	7.4	6.5
28	3.4	5.8	4.6	2.8	3.4	3.1
29	15.2	15.5	15.3	4	5.9	4.9
30	12.5	12.8	12.6	6.8	10.4	8.6
31	6	3.5	4.75	3.7	2.8	3.2
32	9.1	7	8.05	7.9	5.4	6.6
33	6.3	8	7.15	13	12.3	12.6
34	7.3	8.2	7.75	5.8	7.4	6.6
35	9.1	9.7	9.4	2.7	2	2.3
36	12.7	9.8	11.2	2.8	2.9	2.8
37	13.2	13.9	13.5	6.7	11.5	9.1

4.6.2.2 Assessment of LDF's cut-off values

The ROC analysis of the values obtained from each LDF recording will be presented separately followed by their average.

4.6.2.2.1 LDF recording 1

ROC curve for recording 1 (vital and non-vital teeth) shows that there was no ideal value as the cut-off for both high sensitivity and specificity (Figure 4:11). Furthermore, the area under the curve was very small, 0.23. When observing Table 4: 10, a reverse relationship between sensitivity and specificity is evident. Flux values for vital teeth and non-vital teeth are presented in scatter charts (Figure 4:12, Figure 4:13).

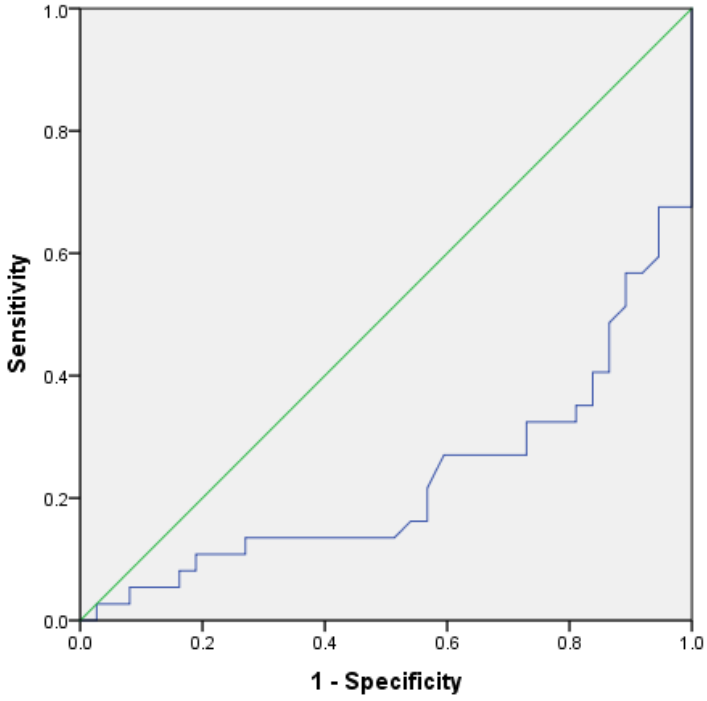


Figure 4-11 ROC curve for the values of recording 1 of vital and non-vital teeth

Table 4:10 Coordinates of the ROC curve for the values of recording 1, showing the possible cut-off values obtained with their corresponding sensitivity and specificity

Flux value	Sensitivity	1 - Specificity
.700	1.000	0.0
1.800	.97	0.0
1.950	.94	0.0
2.350	.91	0.0
2.750	.89	0.0
2.850	.81	0.0
3.000	.78	0.0
3.150	.75	0.0
3.250	.73	0.0
3.350	.67	0.0
3.550	.67	.05
3.850	.64	.05
4.050	.62	.05
4.150	.59	.05
4.300	.56	.08
4.450	.56	.10
4.700	.54	.10
4.950	.51	.10
5.050	.48	.13
5.200	.45	.13
5.400	.40	.13
5.550	.40	.16
5.700	.37	.16
5.900	.35	.16
6.050	.35	.19
6.200	.32	.19
6.450	.32	.21
6.650	.32	.27
6.750	.29	.27
6.900	.27	.27
7.150	.27	.30
7.350	.27	.32
7.450	.27	.35
7.550	.27	.38
7.750	.27	.40
8.050	.21	.43
8.300	.16	.43
8.700	.16	.46
9.050	.13	.48
9.200	.13	.54
9.350	.13	.57
9.500	.13	.59
9.750	.13	.65
10.000	.13	.67
10.350	.13	.70
11.250	.13	.73
12.150	.10	.73
12.450	.10	.76
12.600	.10	.78
12.850	.10	.81
13.100	.08	.81
13.500	.08	.84
14.450	.05	.84
15.150	.05	.86
15.500	.05	.89
17.050	.05	.92
18.800	.02	.92
19.700	.02	.94
23.850	.02	.97
28.250	.00	.97
29.900	.00	1.0

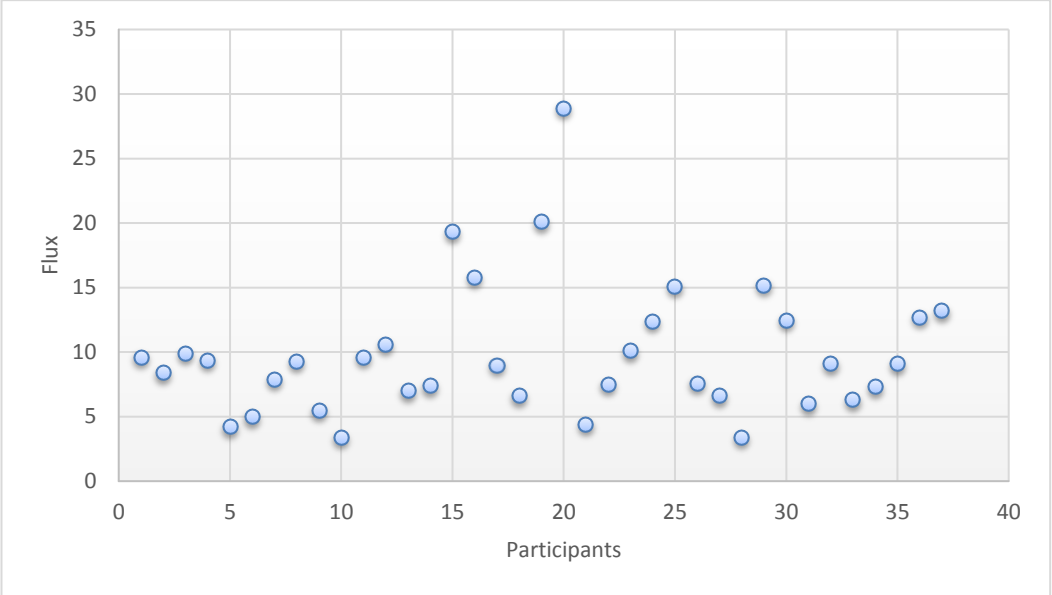


Figure 4-12 Scatter chart showing Flux values for vital teeth in recording 1

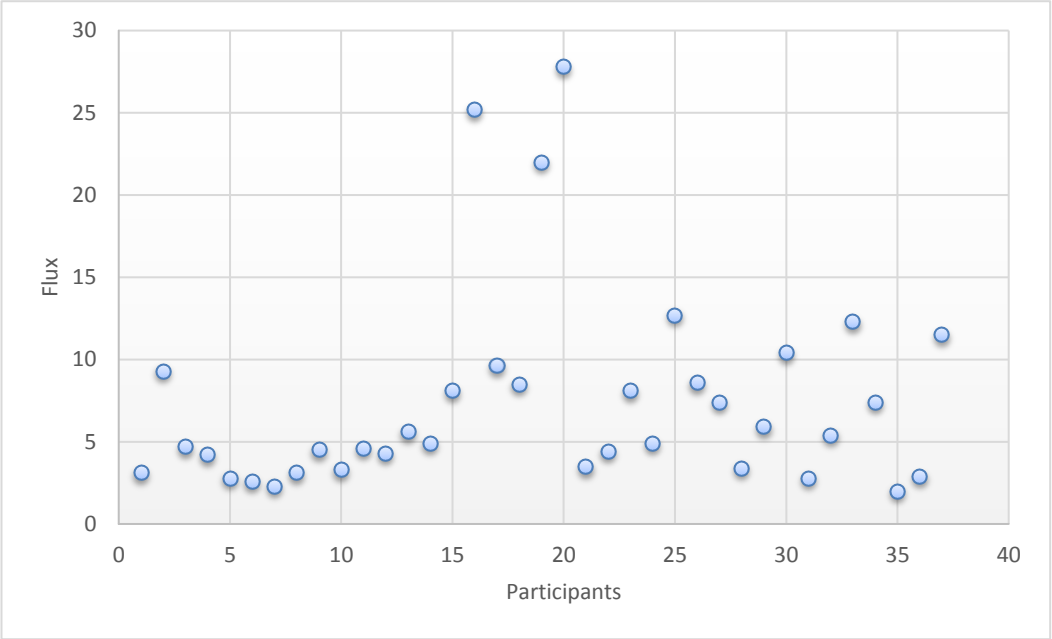


Figure 4-13 Scatter chart showing the Flux values for non-vital teeth in recording 1

4.6.2.2.2 LDF recording 2

ROC curve for recording 2 (vital and non-vital teeth) shows again that there was no ideal value as a cut-off for both high sensitivity and specificity. The area under the curve = 0.28 (Figure 4:14). When observing Table 4:11, a reverse relationship between sensitivity and specificity is evident. Flux values for vital teeth and non-vital teeth are presented in scatter charts (Figure 4:15, Figure 4:16).

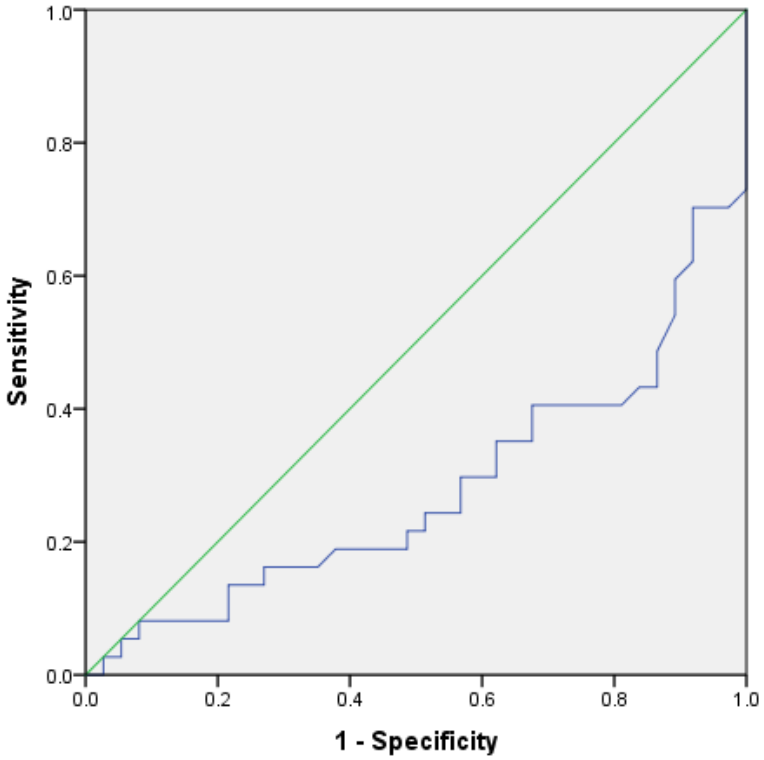


Figure 4-14 ROC curve for the values of recording 2 of vital and on-vital teeth

Table 4:11 Coordinates of the ROC curve for the values of recording 2 showing the possible cut-off values obtained with their corresponding sensitivity and specificity

Flux values	Sensitivity	Specificity
1.000	1.000	.00
2.150	.973	.00
2.450	.946	.00
2.700	.919	.00
2.850	.865	.00
3.000	.838	.00
3.200	.784	.00
3.350	.757	.00
3.450	.730	.00
3.600	.703	.02
3.800	.703	.05
4.050	.703	.08
4.250	.676	.08
4.350	.649	.08
4.450	.622	.08
4.550	.595	.10
4.650	.568	.10
4.800	.541	.10
5.150	.486	.14
5.500	.459	.14
5.700	.432	.14
5.850	.432	.16
6.350	.405	.19
6.900	.405	.24
7.100	.405	.30
7.300	.405	.32
7.500	.351	.32
7.800	.351	.35
8.050	.351	.38
8.150	.297	.38
8.350	.297	.43
8.550	.270	.43
8.700	.243	.43
9.050	.243	.48
9.400	.216	.48
9.550	.216	.51
9.650	.189	.51
9.750	.189	.57
9.850	.189	.60
10.150	.189	.62
10.550	.162	.65
10.900	.162	.67
11.250	.162	.70
11.450	.162	.73
11.700	.135	.73
12.050	.135	.76
12.250	.135	.78
12.500	.108	.78
12.750	.081	.78
13.350	.081	.81
14.100	.081	.84
14.900	.081	.86
16.350	.081	.89
19.600	.081	.92
23.100	.054	.92
24.700	.054	.94
26.100	.027	.94
27.400	.027	.97
31.350	.000	.97
35.900	.000	1.0

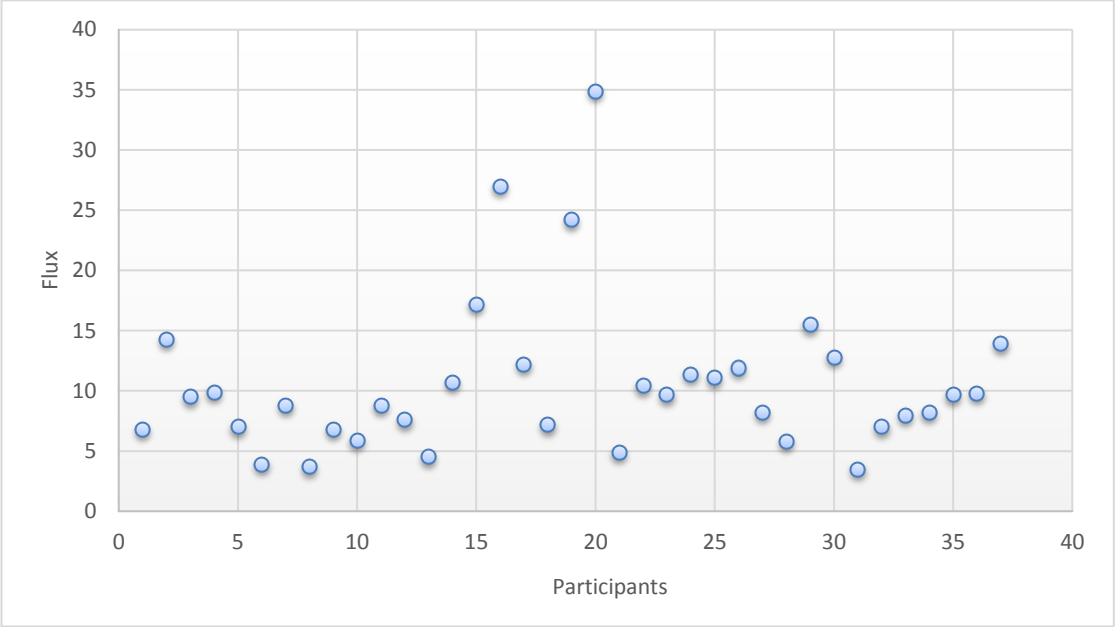


Figure 4-15 Scatter chart showing Flux values for vital teeth in recording 2

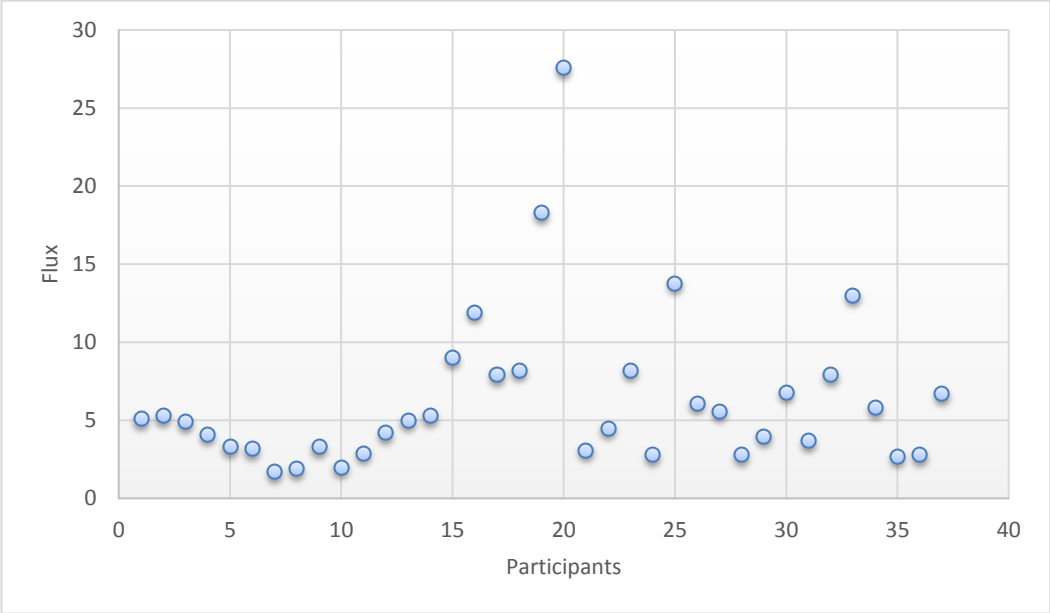


Figure 4-16 Scatter chart showing the Flux values for non-vital teeth in recording 2

4.6.2.2.3 The average of the two LDF recordings

ROC curve shows that there was no ideal value as a cut-off for high sensitivity and specificity. The area under the curve was equal to 0.24 indicating a very small value which was less than 0.5, indicating that the test does worse than chance (Figure 4:17). When observing Table 4:12, a reverse relationship between sensitivity and specificity is evident. Thus, it was not possible to get an ideal cut-off from the values obtained from the recordings. The average Flux values of the two recordings for vital teeth and non-vital teeth are presented in scatter charts (Figure 4-18, Figure 4-19). The best cut-off value identified was 6.3 Flux with a sensitivity of 43.2%, a specificity of 21% a positive predictive value of 35.5% and negative predictive value of 16 %.

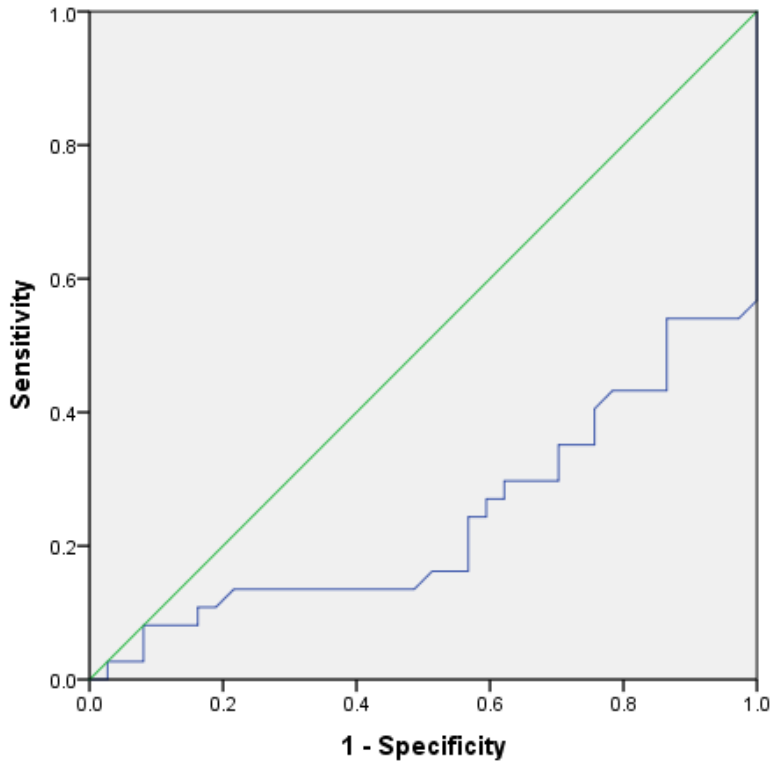


Figure 4-17 ROC curve for the average values of the two recordings of LDF (vital and non-vital teeth)

Table 4:12 Coordinates of the ROC curve for the average Flux values of the two recordings showing the possible cut-off values obtained with their corresponding sensitivity and specificity

Flux values	Sensitivity	Specificity
1.0000	1.000	00
2.1750	.973	00
2.4250	.946	00
2.5750	.919	00
2.7500	.892	00
2.8750	.865	.00
2.9750	.838	.00
3.0750	.811	.00
3.1750	.784	.00
3.2750	.757	.00
3.5250	.730	.00
3.8000	.703	.00
3.8750	.676	.00
4.0000	.649	.00
4.1250	.622	.00
4.2000	.595	.00
4.3500	.568	.00
4.5250	.541	.02
4.6250	.541	..05
4.7000	.541	.10
4.7750	.541	.13
4.8750	.514	.13
5.0250	.486	.13
5.2000	.459	.13
5.4500	.432	.13
5.6750	.432	.16
5.9500	.432	.19
6.3250	.432	.21
6.5500	.405	.24
6.6250	.378	.24
6.7750	.351	.24
7.0250	.351	.27
7.2250	.351	.30
7.3250	.324	.30
7.3750	.297	.30
7.5750	.297	.32
7.9000	.297	.35
8.1000	.297	.38
8.1750	.270	.38
8.2750	.270	.40
8.3500	.243	.40
8.4500	.243	.43
8.5750	.216	.43
8.6750	.189	.43
8.8500	.162	.43
9.0000	.162	.46
9.0750	.162	.48
9.1500	.135	.51
9.3000	.135	.54
9.5250	.135	.57
9.6750	.135	.59
9.7250	.135	.62
9.8250	.135	.65
10.2500	.135	.67
10.9250	.135	.72
11.3000	.135	.73
11.6250	.135	.75
12.2750	.135	.78
12.8750	.108	.811
13.1750	.108	.84
13.4000	.081	.84
14.4500	.081	.86
16.8000	.081	.89
18.4000	.081	.92
19.3500	.054	.92
20.7750	.027	.92
21.7750	.027	.94
24.9250	.027	.97
29.8000	.000	.97
32.9000	.000	1.0

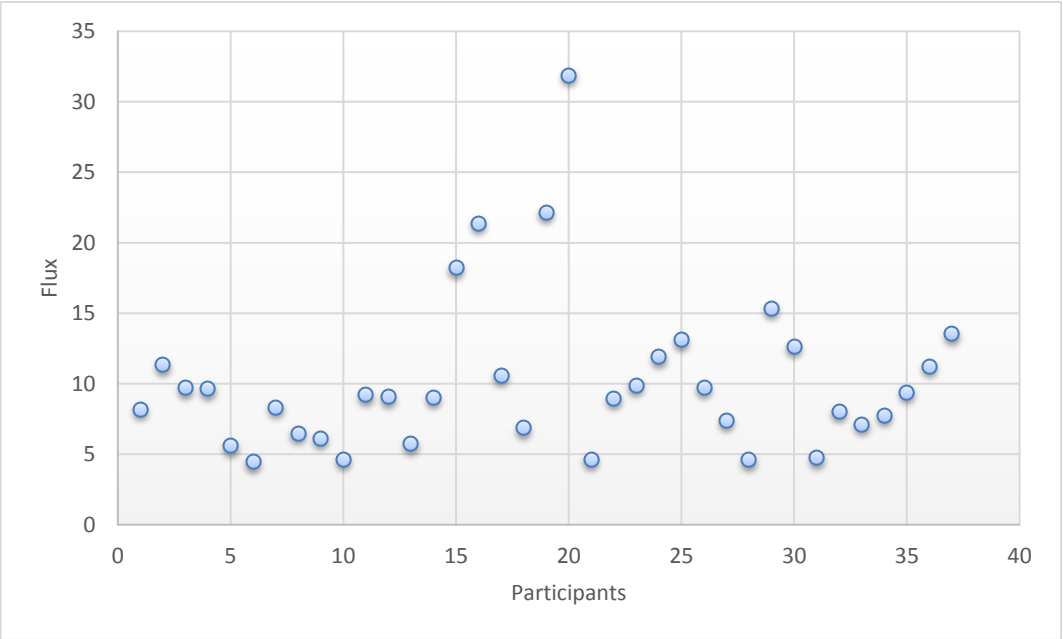


Figure 4-18 Scatter chart showing the average Flux values for vital teeth in recording 1 and 2

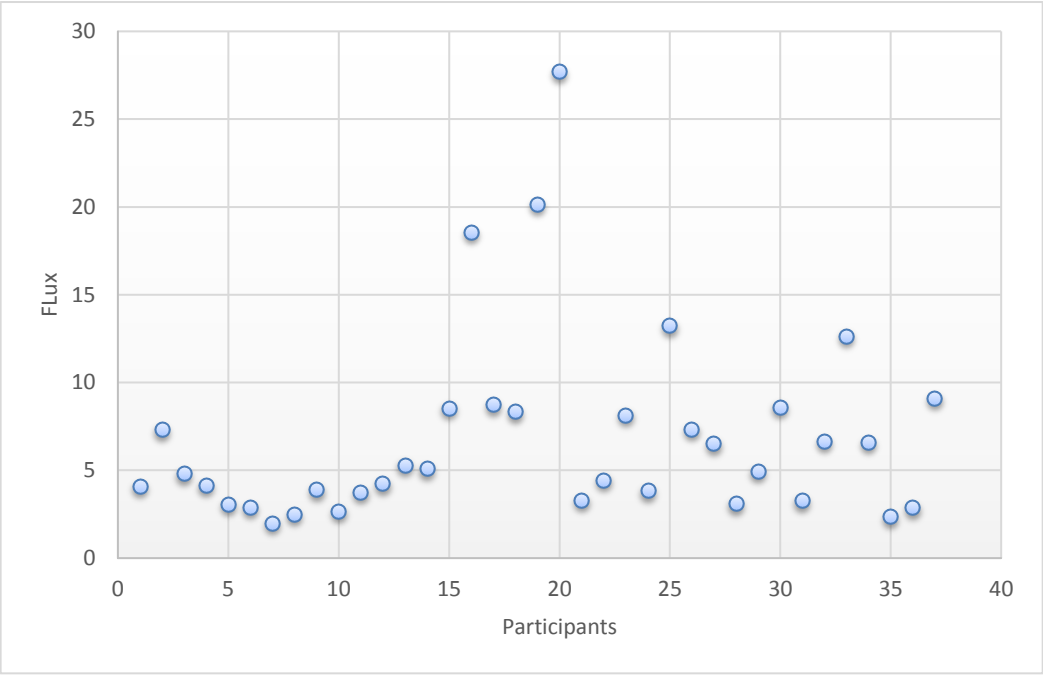


Figure 4-19 Scatter chart showing the average Flux values for non-vital teeth in recording 1 and 2

4.6.2.3 Assessment of LDF's using cut-off ratios rather than values

Based on the Flux ratios obtained for each participant (Flux non-vital tooth/ Flux vital tooth). The ROC analysis of the ratios obtained from each LDF recording will be presented for recording 1, recording 2 and followed by their average. The ratios between the Flux values of vital and non-vital teeth for all study participants are presented in Table 4:13.

Table 4:13 The ratios of the two recordings and their average per patient

Participant	Ratio recording 1	Ratio recording 2	Average
1	0.53	0.46	0.50
2	0.63	0.65	0.64
3	0.49	0.49	0.49
4	0.44	0.42	0.43
5	0.79	0.40	0.54
6	0.64	0.67	0.65
7	0.22	0.26	0.24
8	0.20	0.84	0.38
9	0.60	0.66	0.63
10	0.59	0.56	0.57
11	0.30	0.52	0.41
12	0.40	0.57	0.47
13	0.71	1.24	0.92
14	0.72	0.46	0.56
15	0.47	0.47	0.47
16	0.75	0.93	0.87
17	0.88	0.79	0.83
18	1.24	1.18	0.83
19	0.91	0.91	0.91
20	0.96	0.80	0.87
21	0.70	0.71	0.71
22	0.60	0.42	0.50
23	0.81	0.84	0.82
24	0.23	0.43	0.32
25	0.91	1.14	0.99
26	0.80	0.72	0.75
27	0.85	0.90	0.88
28	0.82	0.59	0.67
29	0.26	0.38	0.32
30	0.54	0.81	0.68
31	0.62	0.80	0.68
32	0.87	0.77	0.83
33	2.06	1.54	0.57
34	0.79	0.90	0.85
35	0.30	0.21	0.25
36	0.22	0.30	0.25
37	0.51	0.83	0.67

4.6.2.3.1 LDF recording 1

ROC curve (Figure 4:20) shows little improvement as the curve has shifted upwards and to the left resulting in a higher point representing the sensitivity and specificity. However, the area under the curve is still small = 0.45. The best cut – off ratio that can be obtained is 0.5 yielding a sensitivity of 0.68 and a specificity of 0.50 for recording 1 (Tble 4:14).. Flux ratios for vital teeth and non-vital teeth in recording 1 are presented in a scatter chart for further demonstration of the data (Figure 4:21).

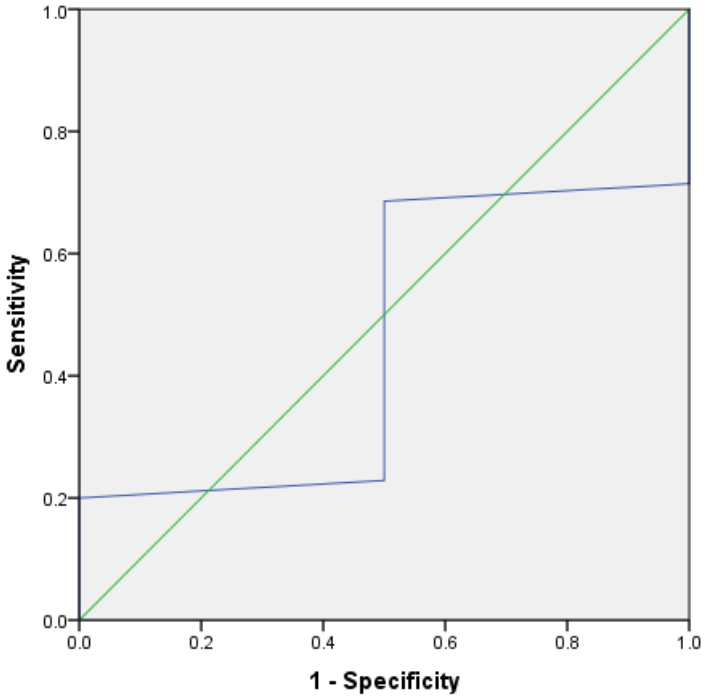


Figure 4-20 ROC curve for the ratio of recording 1

Table 4:14 Coordinates of the ROC curve for the ratios of recording 1

Ratio	Sensitivity	Specificity
.0000	1.000	0.0
.2100	.971	0.0
.2250	.914	0.0
.2450	.886	0.0
.2800	.857	0.0
.3500	.800	0.0
.4200	.771	0.0
.4550	.743	0.0
.4800	.714	0.0
.5000	.686	.50
.5200	.657	.50
.5350	.629	.50
.5650	.600	.50
.5950	.571	.50
.6100	.514	.50
.6250	.486	.50
.6350	.457	.50
.6700	.429	.50
.7050	.400	.50
.7150	.371	.50
.7350	.343	.50
.7700	.314	.50
.7950	.257	.50
.8050	.229	.50
.8150	.200	1.0
.8350	.171	1.0
.8600	.143	1.0
.8750	.114	1.0
.8950	.086	1.0
.9350	.029	1.0
1.0000	.000	1.0

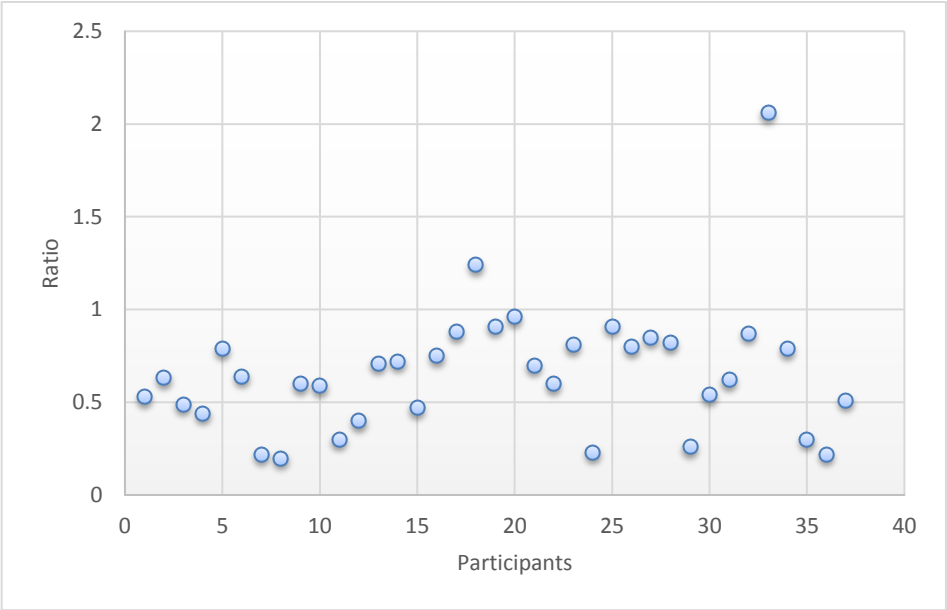


Figure 4-21 Flux ratios between vital and non-vital teeth in recording 1

4.6.2.3.2 LDF recording 2

ROC curve (Figure 4:22) shows a decrease in the ROC curve as the area under the curve = 0.26. The best cut–off ratio that can be obtained is 0.65 yielding a sensitivity of 0.50 and a specificity of 0.33 (Table 4:15). Flux ratios for vital teeth and non-vital teeth in recording 2 are presented in a scatter chart for further demonstration of the data (Figure 4:23).

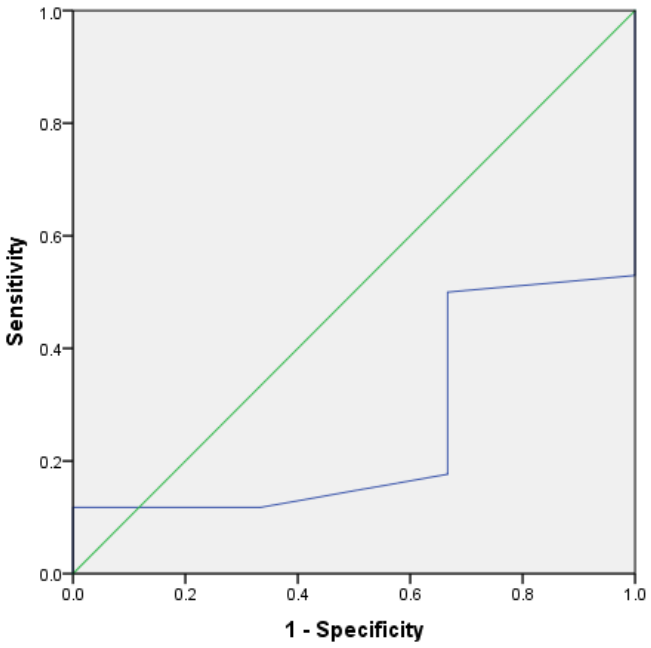


Figure 4-22 ROC curve for the ratio of recording 2

Table 4:15 Coordinates of the ROC curve for the ratio of recording 2

Ratio	Sensitivity	Specificity
.0000	1.000	.00
.2350	.971	.00
.2800	.941	.00
.3400	.912	.00
.3900	.882	.00
.4100	.853	.00
.4250	.794	.00
.4450	.765	.00
.4650	.706	.00
.4800	.676	.00
.5050	.647	.00
.5400	.618	.00
.5650	.588	.00
.5800	.559	.00
.6200	.529	.00
.6550	.500	.33
.6650	.471	.33
.6900	.441	.33
.7150	.412	.33
.7450	.382	.33
.7800	.353	.33
.7950	.324	.33
.8050	.235	.33
.8200	.206	.33
.8350	.176	.33
.8600	.118	.66
.8900	.118	1.0
.9050	.059	1.0
.9200	.029	1.0
1.0000	.000	1.0

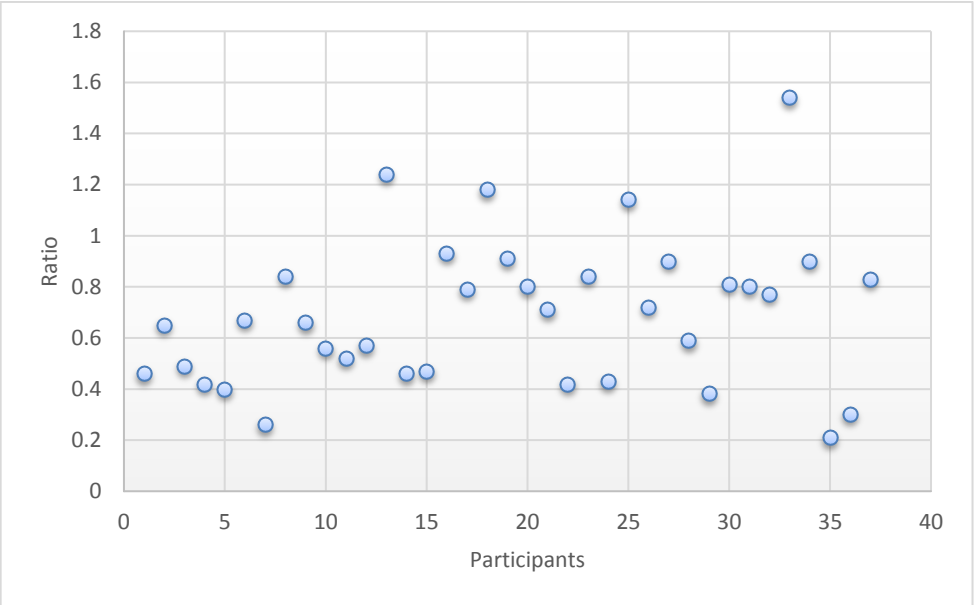


Figure 4-23 Flux ratios between vital and non-vital teeth in recording 2

4.6.2.3.3 The average of the two LDF recordings

When calculating the ratios based on the two recordings, the following ROC curve was obtained (Figure 4-24). The area under the curve = 0.25. The average Flux ratios of the two recordings for vital teeth and non-vital teeth are presented in a scatter chart for further demonstration of the data (Figure 4:25).

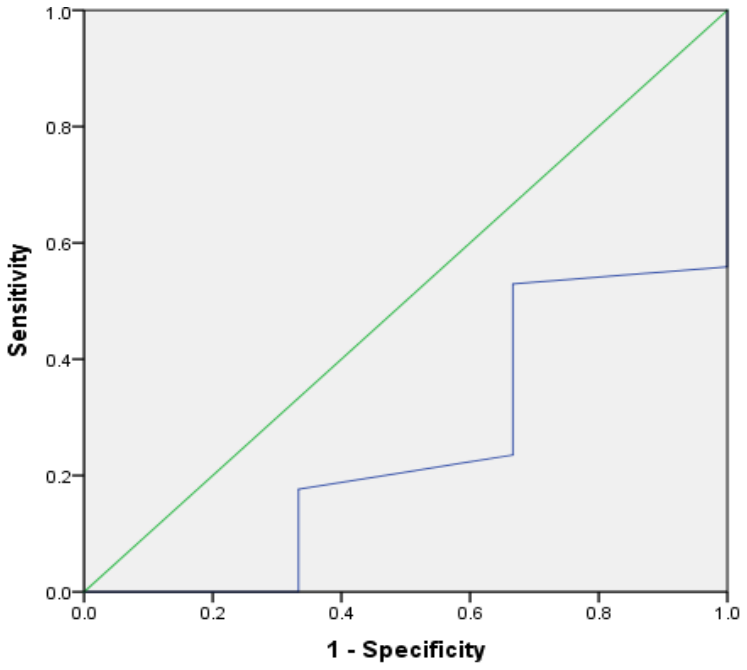


Figure 4-24 ROC curve for the ratio of the average

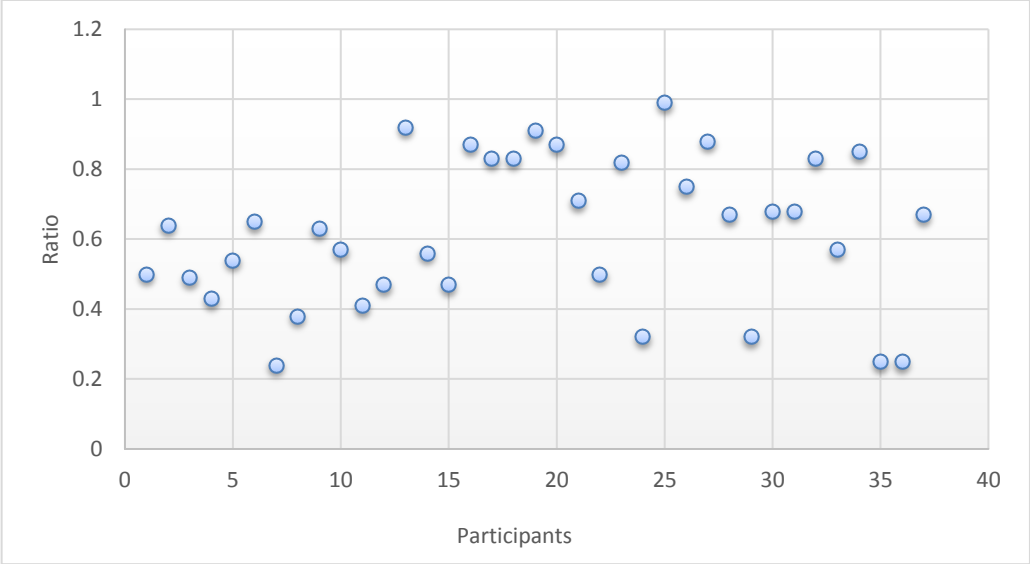


Figure 4-25 The average ratios of vital and non-vital teeth

The best cut–off ratio that can be obtained was 0.6 yielding a sensitivity of 53 % and a specificity of 33% (Table 4:16). Thus, when using the 2X2 table (Table 4:17), positive and negative predictive values are approximately as follows:

- Positive predictive value = a / (a+b) = 44.4%
- Negative predictive value = d/ (c+d) = 41.2 %

Table 4:16 Coordinates of the ROC curve for the average ratios of recordings 1 and 2

Ratio	Sensitivity	Specificity
.0000	1.000	.00
.2450	.971	.00
.2850	.912	.00
.3500	.853	.00
.3950	.824	.00
.4200	.794	.00
.4500	.765	.00
.4800	.706	.00
.4950	.676	.00
.5200	.618	.00
.5500	.588	.00
.5650	.559	.00
.6000	.529	.33
.6350	.500	.33
.6450	.471	.33
.6600	.441	.33
.6750	.382	.33
.6950	.324	.33
.7300	.294	.33
.7850	.265	.33
.8250	.235	.33
.8400	.176	.66
.8600	.147	.66
.8750	.088	.66
.8950	.059	.66
.9150	.029	.66
.9550	.000	.66
1.0000	.000	1.0

Table 4:17 2x2 table for LDF based on the cut-off ratio of 0.6

	Tooth status		Total
	Non-vital	vital	
Test dead	20	25	45
Test alive	17	12	29
Total	37	37	74

4.6.2.4 Outliers

By looking at the original values for LDF, there are a few values that may be considered outliers. To define outliers, we used three standard deviations to identify outliers. Thus, the following was applied:

4.6.2.4.1 Vital teeth

- **Recording 1 for vital teeth**

The mean Flux value was 9.87 (SD = 5.16). Any outlier existing above the following value would be considered an outlier:

Mean + (3 X SD) = (9.87+ (3 x 5.16)) = 25.35

Thus only one value existed above this estimate which is 28.9.

- **Recording 2 for vital teeth**

The mean Flux value was 10.61(SD = 6.47). Any outlier existing above the following value would be considered an outlier:

Mean + (3 X SD) = (10.61 + (3 x 6.47)) = 30.02

Thus only one value fell into this estimate which is 34.9.

4.6.2.4.2 Non-vital teeth

- **Recording 1 for non-vital teeth**

The mean Flux was 6.36 (SD = 5.11). Any outlier existing above the following value would be considered an outlier:

Mean + (3 X SD) = (6.36 + (3 x 5.11)) = 21.69.

Thus only one value existed above this estimate which is 27.6.

- **Recording 2 for non-vital teeth**

The mean Flux was 7.40 (SD = 6.10). Any outlier existing above the following value would be considered an outlier:

Mean + (3 X SD) = (7.40 + (3 x 6.10)) = 25.7

Thus only one value existed above this estimate which is 27.8.

Thus, the outliers obtained from the above calculations belong to one participant only. Re-calculating the ROC curves for the average values and ratios after removing the outliers showed no difference in the outcomes. The area under the curve for the values and ratios were 0.22 and 0.24, respectively.

4.6.3 The control group (EPT and ethyl chloride)

4.6.3.1 EPT

The sensitivity, specificity, positive predictive value and negative predictive value for EPT were calculated based on the 2x2 table calculation presented in Table 4:18.

Table 4:18 showing 2x2 calculation of sensitivity, specificity and predictive values for EPT

		Tooth status		Total
		Non-vital	Vital	
	Test non-vital	31[(a)]	1[(b)]	32
	Test vital	2[(c)]	33[(d)]	35
	unreliable	4	3	7
Total		37	37	74

(a) True Positive (b) False positive (c) False negative (d) True negative

The data showed, for vital teeth, that 33 participants responded positively to EPT, while one participant responded negatively and three participants provided unreliable responses (Table 4:19). Furthermore, for non-vital teeth, 31 participants responded negatively to EPT, while two participants responded positively and four provided unreliable results (Table 4:20).

Table 4:19 The response to EPT for all participants (vital teeth)

Participant	Recording 1	Recording 2	Result
1	Positive	Negative	Unreliable
2	Positive	Positive	Positive
3	Positive	Positive	Positive
4	Negative	Positive	Unreliable
5	Positive	Positive	Positive
6	Positive	Positive	Positive
7	Positive	Positive	Positive
8	Positive	Positive	Positive
9	Positive	Positive	Positive
10	Positive	Positive	Positive
11	Positive	Positive	Positive
12	Positive	Positive	Positive
13	Positive	Positive	Positive
14	Positive	Positive	Positive
15	Positive	Positive	Positive
16	Positive	Positive	Positive
17	Positive	Positive	Positive
18	Positive	Positive	Positive
19	Positive	Positive	Positive
20	Positive	Positive	Positive
21	Positive	Positive	Positive
22	Positive	Positive	Positive
23	Positive	Positive	Positive
24	Positive	Positive	Positive
25	Negative	Negative	Negative
26	Positive	Positive	Positive
27	Positive	Positive	Positive
28	Positive	Negative	Unreliable
29	Positive	Positive	Positive
30	Positive	Positive	Positive
31	Positive	Positive	Positive
32	Positive	Positive	Positive
33	Positive	Positive	Positive
34	Positive	Positive	Positive
35	Positive	Positive	Positive
36	Positive	Positive	Positive
37	Positive	Positive	Positive

Table 4:20 The response to EPT for all participants (non-vital teeth)

Participant	Recording 1	Recording2	Result
1	Negative	Negative	Negative
2	Negative	Positive	Unreliable
3	Negative	Negative	Negative
4	Negative	Negative	Negative
5	Negative	Negative	Negative
6	Negative	Negative	Negative
7	Positive	Negative	Unreliable
8	Positive	Positive	Positive
9	Negative	Negative	Negative
10	Negative	Negative	Negative
11	Negative	Negative	Negative
12	Negative	Negative	Negative
13	Negative	Negative	Negative
14	Negative	Negative	Negative
15	Negative	Negative	Negative
16	Negative	Negative	Negative
17	Negative	Negative	Negative
18	Negative	Negative	Negative
19	Negative	Negative	Negative
20	Positive	Positive	Positive
21	Negative	Negative	Negative
22	Negative	Negative	Negative
23	Negative	Negative	Negative
24	Negative	Negative	Negative
25	Negative	Negative	Negative
26	Negative	Negative	Negative
27	Positive	Positive	Unreliable
28	Negative	Positive	Unreliable
29	Negative	Negative	Negative
30	Negative	Negative	Negative
31	Negative	Negative	Negative
32	Negative	Negative	Negative
33	Negative	Negative	Negative
34	Negative	Negative	Negative
35	Negative	Negative	Negative
36	Negative	Negative	Negative
37	Negative	Negative	Negative

Sensitivity analysis was used to assess the outcomes when study participants provided unreliable results as each unreliable response was first excluded then was considered as positive and negative.

When all unreliable responses were excluded, then the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 31 / 33 = 94 %
- Specificity = d / (b+d) = 33 / 34 = 97.6 %
- Positive predictive value= a / (a+b) = 96.9 %
- Negative predictive value = d / (c+d) = 94.3 %

When the unreliable results were calculated as positive responses (Table 4:21), the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 83.8 %
- Specificity = d / (b+d) = 97.3 %
- Positive predictive value = a/ (a+b) = 96.9 %
- Negative predictive value = d/ (c+d) = 85.7 %

Table 4:21 2x2 table for EPT when unreliable responses are calculated as positive responses

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	31	1	32
Test vital	6	36	42
Total	37	37	74

When the unreliable results were calculated as negative responses (Table 4:22), the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 94.6 %
- Specificity = d / (b+d) = 89.2 %
- Positive predictive value = a / (a+b) = 89.7 %
- Negative predictive value = d / (c+d) = 94.3 %

Table 4:22 2x2 table for EPT when unreliable responses are calculated as negative responses

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	35	4	39
Test vital	2	33	35
Total	37	37	74

Therefore, the range of the above outcomes for EPT are as follows:

- Sensitivity = 83.8 – 94.6 %
- Specificity = 89.2 – 97.6 %
- Positive predictive value = 89.7 – 96.9 %
- Negative predictive value = 85.7 - 94.3 %

4.6.3.2 Ethyl chloride

The sensitivity, specificity, positive predictive value and negative predictive value were also calculated based on the original 2x2 table (Table 4:23).

Table 4:23 2x2 table for ethyl chloride

	Tooth status		Total
	Non-vital	vital	
Test non-vital	30	7	37
Test vital	3	27	30
Unreliable	4	3	7
Total	37	37	74

The data showed that 27 participants responded positively to ethyl chloride when vital teeth were assessed, while 7 participants responded negatively and 3 participants responded unreliably (Table 4:24). Moreover, 30 participants responded negatively to ethyl chloride when non-vital teeth were assessed, while three responded positively and four responded unreliably (Table 4:25).

Table 4:24 The response to ethyl chloride for all patients (vital teeth)

Participant	Recording 1	Recording 2	Recording 3	Result
1	Positive	Negative	Positive	Positive
2	Positive	Negative	Positive	Positive
3	Positive	Negative	Positive	Positive
4	Positive	Negative	Positive	Positive
5	Negative	Negative	Negative	Negative
6	Positive	Negative	Positive	Positive
7	Negative	Negative	Negative	Negative
8	Positive	Negative	Positive	Positive
9	Positive	Negative	Positive	Positive
10	Negative	Positive	Positive	Unreliable
11	Positive	Negative	Negative	Unreliable
12	Positive	Negative	Positive	Positive
13	Negative	Positive	Positive	Unreliable
14	Positive	Negative	Positive	Positive
15	Positive	Negative	Positive	Positive
16	Positive	Negative	Positive	Positive
17	Negative	Negative	Negative	Negative
18	Negative	Negative	Negative	Negative
19	Positive	Negative	Positive	Positive
20	Positive	Negative	Positive	Positive
21	Negative	Negative	Negative	Negative
22	Positive	Negative	Positive	Positive
23	Negative	Negative	Negative	Negative
24	Positive	Negative	Positive	Positive
25	Positive	Negative	Positive	Positive
26	Positive	Negative	Positive	Positive
27	Positive	Negative	Positive	Positive
28	Negative	Negative	Negative	Negative
29	Positive	Negative	Positive	Positive
30	Positive	Negative	Positive	Positive
31	Positive	Negative	Positive	Positive
32	Positive	Negative	Positive	Positive
33	Positive	Negative	Positive	Positive
34	Positive	Negative	Positive	Positive
35	Positive	Negative	Positive	Positive
36	Positive	Negative	Positive	Positive
37	Positive	Negative	Positive	Positive

Table 4:25 The response of ethyl chloride for all patients (non-vital teeth)

Participant	Recording 1	Recording 2	Recording 3	Result
1	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative
6	Negative	Negative	Negative	Negative
7	Negative	Positive	Positive	Unreliable
8	Positive	Negative	Positive	Positive
9	Negative	Negative	Negative	Negative
10	Negative	Negative	Negative	Negative
11	Negative	Negative	Negative	Negative
12	Negative	Negative	Negative	Negative
13	Negative	Negative	Negative	Negative
14	Positive	Positive	Negative	Unreliable
15	Negative	Negative	Negative	Negative
16	Negative	Negative	Negative	Negative
17	Negative	Positive	Negative	Negative
18	Negative	Negative	Negative	Negative
19	Negative	Negative	Negative	Negative
20	Negative	Negative	Negative	Negative
21	Positive	Negative	Positive	Positive
22	Negative	Negative	Negative	Negative
23	Negative	Negative	Negative	Negative
24	Negative	Positive	Negative	Unreliable
25	Negative	Negative	Negative	Negative
26	Negative	Negative	Negative	Negative
27	Negative	Positive	Negative	Negative
28	Positive	Positive	Positive	Positive
29	Negative	Positive	Negative	Negative
30	Negative	Negative	Negative	Negative
31	Negative	Negative	Negative	Negative
32	Negative	Negative	Negative	Negative
33	Negative	Negative	Negative	Negative
34	Negative	Positive	Positive	Unreliable
35	Negative	Negative	Negative	Negative
36	Negative	Negative	Negative	Negative
37	Negative	Negative	Negative	Negative

The sensitivity analysis was also applied to the results of ethyl chloride. When all unreliable responses were excluded, then the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 90.9 %
- Specificity = d/ (b+d) = 79.4 %
- Positive predictive value = a / (a+b) = 81.1 %
- Negative predictive value = d/ (c+d) = 90 %

When the unreliable results were calculated as positive responses (Table 4:26), then the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 81.1 %
- Specificity = d / (b+d) = 81 .1 %
- Positive predictive value = a / (a+b) = 81.1 %
- Negative predictive value = d / (c+d) = 81.1 %

Table 4:26 2x2 table for ethyl chloride when unreliable responses are calculated as positive responses

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	30	7	37
Test vital	7	30	37
Total	37	37	74

When the unreliable results were calculated as negative responses (Table 4:27), then the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 91.9 %
- Specificity = d / (b+d) = 73 %
- Positive predictive value = a / (a+b) = 77.3 %
- Negative predictive value = d / (c+d) = 90 %

Table 4:27 2x2 table for EPT when unreliable responses are calculated as negative responses

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	34	10	44
Test vital	3	27	30
Total	37	37	74

Therefore, the range of the above outcomes for ethyl chloride are as follows:

- Sensitivity = 81.1 – 91.9 %
- Specificity = 73 – 81.1 %
- Positive predictive value = 77.3 – 81.1 %
- Negative predictive value = 81.1 – 90 %

4.6.4 Repeatability

This section presents the repeatability of LDF, EPT and ethyl chloride. The repeatability will be presented in three methods:

- The repeatability of vital teeth only
- The repeatability of non-vital teeth only

- The repeatability when analysing vital and non-vital teeth recordings together.

4.6.4.1 Test group (LDF)

- Interclass Correlation for the repeatability of vital teeth = 0.891
- Interclass Correlation for the repeatability of non-vital teeth = 0.880
- When analysing all the two recordings for both vital and non-vital teeth together, Interclass Correlation = 0.851.
    - Lower bound = 0.729
    - Upper bound = 0.920

4.6.4.2 Control group

4.6.4.2.1 EPT

- Kappa score for the repeatability of vital teeth = 0.36
- Kappa score for the repeatability of non-vital teeth = 0.80
- When analysing all the two recordings of EPT for both vital and non-vital teeth together, Kappa score = 0.86
- Asymptotic Standardised Error = 0.06
    - Upper bound = 0.86 + (2 x 0.06) = 0.98
    - Lower bound = 0.86 – (2 x 0.06) = 0.74

4.6.4.2.2 Ethyl chloride

- Kappa score for the repeatability of vital teeth = 0.77

- Kappa score for the repeatability of non-vital teeth = 0.54

- When analysing all the two recordings for both vital and non-vital teeth together, Kappa score = 0.81

- Asymptotic Standardised Error = 0.07
    - Upper bound = 0.81 +(2 X 0.07) = 0.95
    - Lower bound = 0.81 - (2 X.07) = 0.67

4.7 Discussion

4.7.1 Study design and sample calculation

This study adopted a cross-sectional study design with a random allocation of study participants to the study group (LDF) or a control group. A simple randomisation technique, to avoid selection bias, using a computer-generated random list by an independent person was used. The independent person also concealed the allocation sequence in sequentially numbered, opaque, and sealed envelopes. Concealing the allocation sequence from the principal investigator who assigned participants to the groups until assignment also helped to prevent selection bias. Thus, it prevented the principal investigator from being able to predict which intervention will be allocated next. This study design has been recommended for diagnostic accuracy studies (Knottnerus et al., 2002; Rutjes et al., 2005). None of the published accuracy studies had

randomised study participants (Ingolfsson et al., 1994a; Evans et al., 1999; Chen and Abbott, 2011).

The sample size was calculated based on a pilot study that was conducted in the Department of Paediatric Dentistry to assess the vitality and pulpal regeneration of non-vital immature permanent incisors (Nazzal., et al 2014). Even though the aim of that pilot study was different from the aim of our study, it used the same LDF device and technique we used in our study. Also, the same EPT and ethyl chloride have been used making it a reliable and valid study to use in sample size determination. The sample size was based on a power of 80%. A power of 90% resulted in a significantly larger sample size.

4.7.2 Patient characteristics

The participants in our study were similar to those in the study by Karayilmaz and Kirzioglu, (2011), the only study that compared LDF to other dental pulp tests in children or young adults, in terms of the reference standard used for vital and non-vital teeth. In both studies, the non-vital teeth had root canal treatment, and the vital teeth were based on clinical and radiographical examinations. Also, maxillary central and lateral incisors were only included in both studies. According to the Child Dental Health Survey 2013, maxillary central incisors were the most likely teeth to be affected by TDIs. Karayilmaz and Kirzioglu (2011) included teenagers and young adults aged 12-18 years old, while in our study, we included a younger age group (8-16 years) in order to assess the accuracy of the dental pulp tests used in the child population.

Cooperation and understanding the English language were two of the important criteria for inclusion in our study. The EPT and ethyl chloride tests are subjective. Therefore, it was important that the study participants should fully understand and comprehend the instructions given by the investigator. In addition, the LDF is very sensitive to movement and requires that study participants to stay still during the time of recordings.

4.7.3 Bias

The purpose of diagnostic accuracy studies is to study how well the results of a specific test under assessment agree with the reference standard. Choosing an ideal reference standard is fundamental. It is the best available method to establish the presence or absence of a disease. In other words, the test accuracy is calculated based on the consideration that the reference standard is flawless with 100% sensitivity and specificity. The use of an inappropriate reference standard can cause an error in diagnoses (classification bias) and can result in underestimation of the performance of the test. Errors due to defective reference standards can possibly bias the assessment of the diagnostic accuracy of the index test (Rutjes et al., 2006).

The present study included a composite reference standard for vital teeth in our study. Since TDIs usually affect more than one tooth, patients with an original extensive dental trauma involving multiple teeth were not included in the study. Our study included teeth that had no evidence of being traumatised as reported by the treating dentist. Also, vital teeth should have exhibited all the signs and symptoms of healthy normal pulps. All attempts were made not to include any vital tooth showing any signs of having compromised vitality. A control tooth

from the opposing arch could have been chosen. However, this requires an additional splint made and it will add to the technique difficulty of LDF as allowing simultaneous recording of vital and non-vital teeth from both arches would not be feasible.

There are different types of bias that can affect diagnostic accuracy studies such as test review bias, partial verification bias, spectrum bias, incorporation bias, and classification bias. Test review bias involves the knowledge of the results of the reference standard while interpreting the results of the test. The degree at which the results can be biased is determined by the degree of subjectivity involved in interpreting the test. Operators are more likely be influenced by the results of the reference standard when a specific test depends on the subjective element during the evaluation of the test. Since the results of LDF are objective, test review bias did not significantly affect our study even though the study lacked operator blinding. Even though the use of EPT and ethyl chloride are subjective in general, they were used in a standardised method throughout the study to reduce any subjectivity that may affect the results. Ideally, the operator conducting the tests should be, ideally, blinded to either the pulp tests or the condition of the tested teeth. An attempt was made to include a blinded investigator to carry out the pulp tests, but that was not possible.

Furthermore, all participants in a diagnostic accuracy study should receive verification by the reference test. In other words, the status of vital and non-vital teeth should be verified by the same method for all teeth prior to the tests. Failure to do that can cause bias to the accuracy, and it is identified as partial verification bias (Schmidt and Factor, 2013). All study participants in our study

received verification by the reference test in which the non-vital teeth had either pulp extirpation or a full root canal treatment. Polat et al. (2004) showed no significant difference in LDF recordings between empty and filled root canals. All the vital teeth also received verification by the reference standard used in the study both radiographically and clinically. Partial verification bias can be eliminated by verifying all participants.

Spectrum bias which is the difference in disease severity was avoided in this study as all participants had a vital and non-vital tooth based on clear inclusion, exclusion criteria and reference standards. Therefore, this type of bias was avoided in this study.

Incorporation bias occurs when the result of the index test is used as a criterion for the reference test and aids to establish the final diagnosis. Clinically, LDF was not part of the reference standard used in this study. However, the comparators (EPT and ethyl chloride) were used as part of the reference tests for the vital control teeth as the included teeth should have responded normally to sensibility tests before including the teeth in the study. Ideally, the diagnosis of both vital and non-vital teeth should be made blindly before conducting the tests. This is what occurred in our study where all participants had a final diagnosis by consultants who were not involved in the study (Worster and Carpenter, 2008).

4.7.4 Laser penetration and reflection

Laser penetration and reflection for LDF have been shown to be affected by crown restorations (Chandler et al., 2010; Chandler et al., 2014). Therefore, the

inclusion of heavily restored teeth was avoided during the recruitment of study participants. Teeth with more than half of the crown restored or crown discolouration were not included in the study. For standardisation purposes, all teeth were not discoloured and had less than half of the crown restored in order to allow LDF and EPT probes as well as ethyl chloride cotton pledget placement on the middle third of the crown in contact with sound tooth structure. Teeth were not included when the LDF probe could not be placed against enamel due to restoration.

Since the use of LDF depends on the transmission of light and the detection of backscattered light from the pulpal tissue, blood pigments presented in the dentinal tubules following injury can hinder the transmission of light which has been shown in a case report. It was also confirmed in the same investigation that LDF was unable to measure blood flow beneath a bruised fingernail which affirmed that blood pigment was an effective absorbent of the laser light even where the underlying tissue had a high blood flow. The results of the investigation showed that LDF can not be used in assessing pulpal blood flow in a tooth that has crown discolouration, but can be used to monitor blood flow once the discoloration has resolved (Heithersay and Hirsch, 1993).

One of the studies included in our systematic review (Chen and Abbott, 2011) included heavily restored teeth and reported a high accuracy of LDF (96.3 %) in comparison to other dental pulp tests. Such an effect should have been considered and reflected in the results of that study as Flux values might have been affected leading to misinterpretation and overestimation of the results.

4.7.5 Signal contamination

Studies have proposed the use of isolation measures in order to reduce non-pulpal contamination by the surrounding tissues. A splint using polyvinylsiloxane in addition to rubber dam isolation were used in this study to decrease the impact of non-pulpal blood flow. The use of rubber dam in addition to the splint is supported by studies in the literature. The use of rubber dam has shown to reduce the mean blood flow by 56-82 % (Hartmann et al., 1996; Soo-ampon et al., 2003; Kijsamanmith et al., 2011a). All the comparative studies included in the systematic review used an isolation splint only without the additional use of rubber dam.

4.7.6 Movement artefacts

The literature shows inconsistency with regards the optimum duration of LDF recording as it ranged from 20 seconds to three minutes of acquisition time. Furthermore, it is well established that movement artefact, whether related to the patient or apparatus itself, affects LDF readings. Therefore, allowing sufficient time for recording stable Flux recording is recommended (Jafarzadeh, 2009). Valid and correct acquisition require 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. Thus, three minutes of LDF recording represents a long period of time, especially for children. A stable 30-second interval, as free as possible from movement artefacts, based on the advice from the LDF manufacturer, was used to calculate the mean Flux values for each patient. A study has shown that there was no statistically significant difference among six 30-second time intervals on stable results measured

(Miron et al., 2010). Thus, a stable 30-second interval adopted in the present study was sufficient to assess the pulp blood flow. To achieve a stable 30 second-interval in children required two to three minutes due to the difficulty for children to avoid movements as the LDF probes are very sensitive and any slight movements can affect the results.

4.7.7 LDF values

In theory, pulp extirpated teeth should provide very low Flux values because there is no pulpal blood flow. In our experimental conditions, the average Flux values for vital teeth was higher than those of non-vital teeth for both recordings. There was a significant difference between the average Flux values for vital and non-vital teeth. However, the Flux values of non-vital teeth were higher than those of vital teeth in a few recordings. This finding is similar to Roebuck et al. (2000) where they evaluated the effect of wavelength, bandwidth, and probe design and position on assessing the vitality of anterior teeth when using the LDF. Most of the combinations used resulted in at least one recording where a Flux value of a non-vital tooth was higher than the vital tooth. This may be an additional limitation of LDF, and therefore adds to the difficulty in interpreting the results.

Fluctuations and heterogeniety of Flux values were observed in our data. This finding was similar to another study where LDF results of vital and non-vital teeth showed non-interpretable Flux values and LDF values for vital and non-vital teeth were not significantly different (Roy et al., 2008).

4.7.8 LDF's cut-off threshold

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 diseased teeth. Ideally, a pre-specified threshold between vital and non-vital teeth must be established before conducting a clinical study (Whiting et al., 2011). Unfortunately, there is currently no consensus as to the LDF cut-off threshold except for a few suggestions which are based on low-quality research.

A pre-specified threshold has only been mentioned in one study in the literature (Chen and Abbott, 2011). The cut-off ratio used to indicate a healthy pulp was a ratio of diseased/healthy ≥ 0.6. The ROC analysis used to analyse the results of the current study showed a cut-off ratio of diseased/healthy ≥ 0.6 yielded the best possible combination of sensitivity and specificity. These values were much lower than those shown by Chen and Abbott (2011). The sensitivity and specificity of the cut-off ratio, in the current study, were 53 and 33% respectively, in comparison to an accuracy of 97% in Chen and Abbott's (2011) study. The major difference between the results is the fact that the definition of the outcomes and study design are different.

Chen and Abbott (2011) evaluated 121 teeth, nine teeth of which were non-vital, in 20 participants with a wide age range (18-74). They included maxillary and mandibular incisors, canines, premolars and molars, 28 of which had moderate or extensive restorations. These inconsistencies and the lack of standardised inclusion criteria made the comparison among teeth unreliable. Furthermore, they defined accuracy as a measure of test efficacy explored by

RMANOVA and pairwise comparisons examining between-test accuracy differences.

Another study used a very low ratio of 1 ⁄ 10 between the pulp blood flow values measured by LDF. The ratio was calculated after data analysis. The basis of this ratio was not clear and the authors did not perform power calculation or randomisation (Karayilmaz and Kirzioğlu, 2011). Applying this ratio to the data in the current study showed 100% sensitivity and 0% specificity.

The study by Evans et al. (1999) used a cut-off value rather than a ratio. The cut-off value used was 7.00 PU. This value resulted in 100% sensitivity and specificity. It was unclear how the authors decided such a value, as they only indicated that analysis of the data allowed the diagnostic criteria to be devolved. In addition, no power calculation or randomisation was performed. Applying this value to the data in the present study showed poor sensitivity and specificity of 35% and 27 %, respectively. Applying a random cut-off value would result in overestimation of the true accuracy. ROC analysis avoids the possibility of random selection of cut-off threshold and eliminates the subjectivity in interpreting the results.

4.7.9 ROC analysis

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 determination of the cut-off threshold which results in the best sensitivity and specificity that may be attained. The curve is achieved by calculating the

sensitivity and specificity of the test at every possible cut-off point, then plotting sensitivity (on the y-axis) against 1-specificity (on the x-axis) for different threshold values.

The 45° diagonal line on the graph connecting the points (0, 0) to (1, 1) is the ROC curve correlative to a random chance. This line represents a reference line which also represents the characteristics of a test that are completely useless in distinguishing between diseased and non-diseased subjects. The point on the ROC curve at the upper left-hand corner (0, 1) represents the perfect test (100% sensitivity and 100% specificity).Thus, the closer the ROC curve gets to 0 and 1 the better the test is at differentiating between diseased and non-diseased teeth.

In addition, the area under the curve is a reflection of how good the test is and provides a summary measure that basically averages diagnostic accuracy across the range of the values. The area under the curve provides a global summary of the accuracy of the test. The larger the area under the curve, the better the test. A perfect test would have an area under the curve value of 1.0, while a completely useless test would have a value of 0.5. When the estimated area under the curve is less than 0.5, it indicates that the test outcomes are worse than chance (Zou et al., 2007; Akobeng, 2007b).

The ROC curves in our study resulted in very small values for the area under the curve for both LDF Flux values and ratios calculated, which were less than 0.5, indicating that the test outocmes are worse than chance. This means that the results of LDF can be misleading in clinical practice showing a high chance of false positive and false negative results. In other words, LDF can incorrectly

classify subjects with the disease as negative, free of disease, and subjects with no disease as positive, having the disease.

4.7.10 Sensitivity and specificity

Even though our data showed a significant difference between LDF values of vital and non-vital teeth, the sensitivity and specificity of LDF in our study have been shown to be less than the reported values in the literature. This could be contributed to the method of analysing the data as no LDF accuracy study has been reported using ROC curve analysis. The published LDF studies that used the same definition of sensitivity and specificity used in our study reported 100% sensitivity and specificity of LDF (Evans et al., 1999; Karayilmaz and Kirzioğlu, 2011).

EPT, on the other hand, has shown to have an average sensitivity and specificity of 83.8 – 94.6 % and 89.2 – 97.6 %, respectively. This is in agreement with the literature as EPT is more reliable in assessing healthy vital teeth than diseased non-vital teeth (Fuss et al., 1986; Peters et al., 1994; Petersson et al., 1999; Kamburoğlu and Paksoy, 2005; Gopikrishna et al., 2007; Weisleder et al., 2009; Saeed et al., 2011; Villa-Chavez et al., 2013). Karayilmaz and Kirzioglu, (2011), on the other hand, reported opposite results as the sensitivity was higher than the specificity when only anterior teeth were included in their study.

It has been suggested that EPT is affected by age and is unreliable in assessing teeth with immature apices because of the lack of the development of the plexus of Raschkow at the pulp-dentinal junction. The stage of root development had no effect on EPT results in our study which showed high sensitivity and specificity values. This could be explained by the fact that the mean age of the control group was 12.7 years old and approximately 94% of the vital teeth tested by EPT had full root length with closed apical foramina. Also the children had higher understanding and cooperation for the test than a younger group of participants.

The results of the current study showed a sensitivity and specificity of 81.1 – 91.9 % and 73 – 81.1 % for ethyl chloride, respectively. The sensitivity is comparable to those reported by Petersson et al. (1999), (83%), and Evans et al. 1999 (92%) (Petersson et al., 1999; Evans et al., 1999). Other researchers have reported lower sensitivity Dummer et al. (1980) (68%), Garfunkel et al. (1973), (75%), Johnson et al. (1970) (35%) and Fuss et al. 1986 (53%) (Dummer et al., 1980; Fuss et al., 1986; Garfunkel et al., 1973; Johnson et al., 1970).

The specificity in the present study was higher than those reported by Dummer et al. (1980) (70%), Johnson et al. (1970) (49%) and Garfunkel et al. 1973 (57%) (Dummer et al., 1980; Garfunkel et al., 1973; Johnson et al., 1970), and lower than the studies by Evans et al., 1999 (89%), Petersson et al. (1999) (90%) and Fuss et al. 1986 (100%) (Evans et al., 1999; Petersson et al., 1999; Fuss et al., 1986). The differences in sensitivity and specificity among different studies may be due to the variations in study design, study populations, techniques implemented in the studies and interpretation of the results.

4.7.11 EPT and ethyl chloride techniques

Electrical and cold stimulation of the dental pulp have two different mechanisms of action according to the hydrodynamic theory. This theory implies that cold stimuli induce neurons to act as mechanoreceptors that react to the movement from the thermal contraction of the dentinal fluid. The electrical stimulus causes depolarization of nerve membranes. Consequently, the application of cold testing appears to have no effect on electrical stimulation of 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). Five to eight-second application of cold tests is sufficient to determine the responsiveness of the teeth in the majority of the cases (White and Cooley, 1977).

4.8 Conclusion

LDF has been considered more accurate and reliable than other dental pulp tests with most studies in the literature advocating its use for the objective assessment of the pulp. However, all existing LDF studies were assessed as having high levels of bias and being based on compromised statistical and study designs. The design of the current study was in line with the recommended research design for diagnostic accuracy studies. The results of this study show a high probability of false positive and false negative results when using LDF to assess dental pulpal blood flow. Therefore, within the limitations of this study, the results of the study suggest that LDF is unable to

reliably differentiate between vital and non-vital teeth in children between the ages of 8-16 years, with an acceptable level of confidence.

Further technical development will be needed to allow the more convenient use of LDF especially in the child population before it can be recommended for routine clinical use for the assessment of the dental pulp. Further development of this technique assessing different wavelengths and probe diameter is recommended.

Chapter 5 Clinical study 2

A prospective study to assess the diagnostic accuracy of laser Doppler flowmetry in predicting pulp vitality of traumatised teeth in paediatric patients

5.1 Abstract

Aim/objectives: To monitor pulp sensibility/vitality of traumatised teeth using LDF, EPT and ethyl chloride and to prospectively investigate the sensitivity, specificity and predictive values of each of the tests.

Methods: Children who sustained dental trauma to an anterior tooth with uncertain pulp vitality requiring monitoring for a minimum of 12 months were included in the study. Recordings of dental pulp tests were carried out at baseline and at the end of follow-up period. The number of participants required to achieve a power of 90%, with a 95% level of confidence was 26.

Results: The study included a convenience sample size of 15 participants with a mean age of 10.7 years (SD=1.66), age range 8-14 years. The mean follow-up period was 7.29 months (SD 1.9) with a range of (6-12 months). All traumatised teeth remained vital at the end of follow-up except one tooth. The specificity of LDF at baseline was 80% compared to 66.6% and 60-73.3% for EPT and ethyl chloride, respectively. At the end of the follow-up period, LDF showed lower specificity (71.4 %) than EPT (78.5 – 85.7 %) and ethyl chloride (71.4 – 78.5 %).

Conclusion: Within the limitations of this study, LDF has shown better specificity than the EPT and ethyl chloride in predicting the outcome of the pulp at baseline but less at the end of follow-up. Due to the small sample size and relatively short follow-up period, these results should be interpreted with caution.

5.2 Research Aim, objectives, and hypotheses

5.2.1 The aim of the study

To prospectively assess the accuracy of LDF in determining pulp vitality of traumatised anterior teeth when compared to EPT and ethyl chloride.

5.2.2 Study objectives

- To monitor pulp vitality and sensibility of traumatised teeth using LDF, EPT and ethyl chloride.
- To prospectively calculate the sensitivity, specificity and predictive values of each of the tests.

5.2.3 Hypotheses

5.2.3.1 Null hypothesis

There is no difference between the ability of LDF, EPT and ethyl chloride in assessing pulp vitality of traumatised anterior teeth in children.

5.2.3.2 Alternative hypothesis

LDF is more accurate in assessing pulp vitality of traumatised teeth than the EPT and ethyl chloride.

5.3 Materials and methods

The The study protocol was registered online at ClinicalTrails.gov (NCT03005197). The following section describes the materials and methods used in this study.

5.3.1 Materials

The materials used in this study were the same materials used in the previous study as described in section 4.4.

5.3.2 Methods

5.3.2.1 Study design

This was a prospective diagnostic accuracy cohort study.

5.3.2.2 Ethical approval

Ethical approval was obtained from RES Yorkshire & The Humber - Leeds East Research Ethics Committee (Ref # 17/YH/025) (Appendix 10). NHS permission was then obtained at The Leeds Teaching Hospital NHS Trust (LTHT) (Ref # DT16/232) (Appendix 11)

The study documents included the following (Appendix 12):

- Assent

- Consent
- Information sheet for the person with parental responsibilities
- Patient information sheet for children 6-12 years of age.
- Patient information sheet for children 12-16 years of age
- Invitation letter

5.3.2.3 Recruitment

Children referred to the paediatric dentistry trauma clinic at Leeds Dental Institute were assessed for inclusion in this study. Whenever possible, information leaflets were posted to any patient deemed suitable for inclusion in the study two weeks prior to their forthcoming appointment. Information leaflets included an invitation letter, a letter to the person with parental responsibility and an age-specific letter for children to read (either 8-12 years old or 12-16 years old).

On the day of the appointment and following the child's examination/treatment session, the chief investigator approached patient's parent/person with parental responsibility, assessed each potential participant clinically and further explained the study to the parent/ person with parental responsibility. Informed consent and assent were then obtained from participants fitting the inclusion criteria (listed below). Patients were offered to have the assessment required for the purposes of the research done on the day or at a future follow-up appointment.

Children attending our emergency clinics with dental trauma were also, when possible, approached for inclusion in our study. The study was explained to the

children and their parents/ person with parental responsibility and information leaflets were provided. Children and their parents/ person with parental responsibility were offered to have the assessment done on the day or at future follow-up appointments.

5.3.2.4 Inclusion Criteria

Children were recruited into the study when they fulfilled the following inclusion criteria:

- Children aged 6-16 years.
- Medically fit (ASA I, II) children.
- Children who understood English language and were able to understand instructions.
- Children with an acceptable level of cooperation.
- Children with a traumatised anterior tooth, regardless of the type of trauma, with a restoration covering less than half the labial crown surface and uncertain pulp vitality requiring monitoring for a minimum of 12 months.
- Children with a non-traumatised anterior tooth with a restoration covering less than half the labial crown surface, for use as a control tooth.

5.3.2.5 Exclusion Criteria

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

- Medically compromised children.
- Children with learning disabilities.

- Children with a history of moderate and significant behaviour management problems
- Children with a communication barrier such as not understanding or speaking English language.
- Children with either traumatised or control tooth with a restoration covering more than half the labial crown surface.
- Children on routine analgesics, antidepressants or antihypertensive drugs.
- Children with traumatised teeth where pulp extirpation was deemed necessary.
- Children with the non-traumatised tooth 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 signs of loss of vitality
    - pulp canal obliteration
    - Extensive caries
    - Developmental anomalies
    - Symptomatic teeth

5.3.2.6 Sample size/power calculation

The The sample size was calculated following a consultation with a statistician and was based on the results of the previous study of this thesis (Chapter 4). The sample size calculation was based on LDF, EPT and ethyl chloride sensitivities of 53%, 89.2% and 86.5 % respectively. As a result, the number of

patients required to achieve a power of 90%, 95% level of confidence with an effect size of 35% using one-sided test was 26 subjects as calculated using an online software (http://www.stat.ubc.ca/~rollin/stats/ssize/). Fifteen per cent dropout rate was used therefore an extra four patients were added resulting in a total number of 30 patients.

5.3.2.7 Pulp assessment

Pulp assessment of traumatised and control teeth was carried out using LDF, EPT and ethyl chloride. The methods used to conduct the three tests were the same methods described in the previous chapter (section 4.5.6). Recordings of all tests were performed by a single operator and were carried out at baseline and at the end of the follow-up.

5.3.2.8 Clinical assessment

For each included participant, the following clinical data were recorded:

- Type of injury sustained by the participant.
- Tenderness to percussion test: Positive or negative.
- Tenderness to palpation: Positive or negative.
- Colour: Normal, yellow, grey.
- Mobility: Miller Index (0-3) (Laster et al., 1975):
    - 0: No movement when force applied
    - 1: Tooth can be moved less than 1 mm in the buccolingual or mesiodistal direction.

- 2: Tooth can be moved 1 mm or more in the buccolingual or mesiodistal direction. No mobility in the occlusoapical direction (vertical mobility)
- 3: Tooth can be moved 1 mm or more in the buccolingual or mesiodistal direction. Mobility in the occlusoapical direction is also present.

5.3.2.9 Radiographic assessment

Radiographic assessment was carried out using the baseline radiograph and a second radiograph at the end of follow-up or when clinically indicated.

Using the baseline radiographs, the stage of root development was recorded according to the following classification (Jonsson and Sigurdsson, 2004):

- Stage 1: One quarter to half root length
- Stage 2: Half to three-quarters of root length
- Stage 3: three-quarters to full root length
- Stage 4:full root length and wide open foramen (diameter > 2mm)
- Stage 5: full root length and half open apical foramen (diameter 1-2 mm)
- Stage 6: full root length and closed apical foramen

The following radiographic signs of loss of pulp vitality were assessed:

- Cessation of root development in comparison to other non-traumatised contralateral teeth.

- Evidence of pathological resorption.
- Evidence of periapical radiolucency.

The pulp status was determined by the consultant/specialist overseeing the care of the participant utilising all clinical and radiographic evidence available excluding the results of LDF. Where a tooth was deemed non-vital, pulp extirpation was carried out and no further follow-up as part of the study was provided. Those participants normally continued attending the clinics for further treatment/review depending on their future treatment needs. The end of follow-up or the development of pulp necrosis were considered as endpoints for study participants.

5.3.2.10 Data collection

A data collection sheet was used to collect the demographic and clinical data. The data collection sheet included information such as age, sex, type of trauma, stage of root development as well as the results of the tests (Appendix 13).

5.3.2.11 Statistical analysis

The data obtained from the study was analysed using IBM SPSS (Statistical Package for Social Science) statistics version 23. Descriptive statistics were used to describe the participants' demographics and characteristics as well as the ability of the tests to predict the out come of each participant at baseline and end of follow-up.

Traumatised teeth showing evidence of loss of vitality will be referred to as non-vital teeth while those not showing any signs of loss of vitality will be referred to as vital teeth.

5.3.2.11.1 LDF

The sensitivity, specificity and predictive values were calculated using the traditional 2X2 (Table 4.1) explained in Chapter 4 (4.5.8.1.2) and based on the cut-off ratio of 0.6 (Flux value of traumatised tooth /Flux value of control tooth) obtained from the previous study in this thesis (Chapter 4).

5.3.2.11.2 EPT and ethyl chloride

The sensitivity, specificity, positive and negative predictive values were also calculated using the traditional 2x2 table for the traumatised teeth. Sensitivity analysis was used to assess the outcomes when study participants provided unreliable results as each unreliable response was firstly excluded then was considered as positive or negative.

The 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.*

5.4 Results

5.4.1 Participants’ demographics and clinical characteristics

The study included a convenience sample size of 15 participants who were recruited for this study between March 2017 and January 2018. The mean age was 10.7 years (SD=1.66), and the age range was between 8-14 years (Figure 5:1).The mean follow-up period was 7.29 months (SD 1.9), median (6.0) and range (6-12 months) (Figure 5-2). The study included more male participants (n= 9, 60%) than female participants (n= 6, 40%).

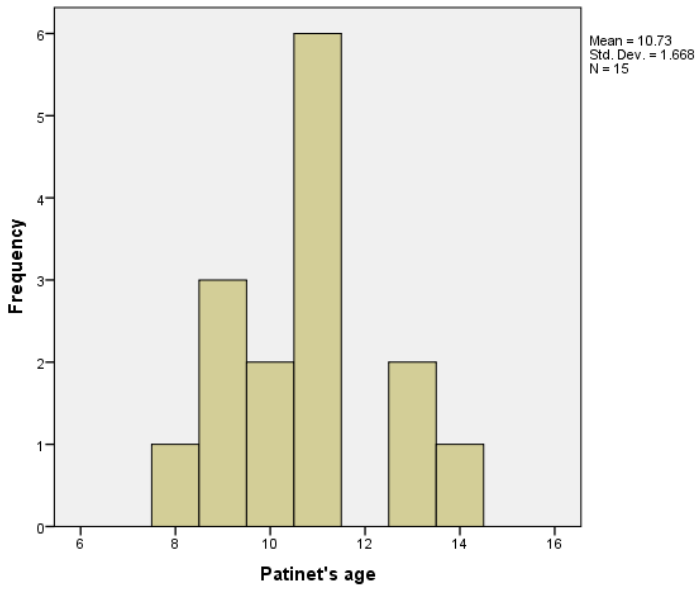


Figure 5-1 Histogram showing age distribution

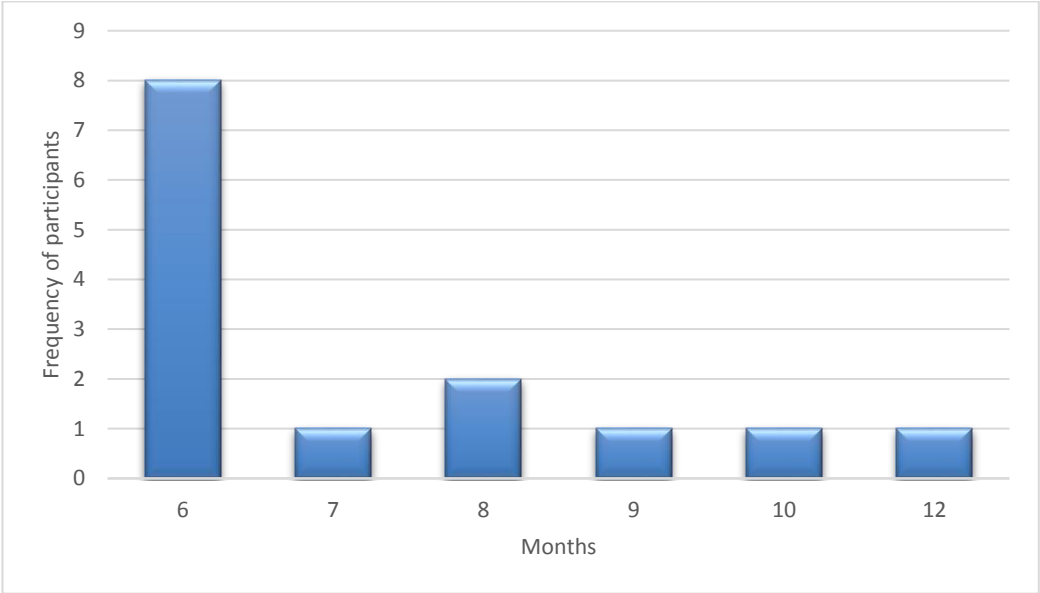


Figure 5-2 Bar chart showing the follow-up period of the participants in months

Recruited participants had sustained different types of dental trauma including crown fractures, luxation injuries, avulsion and root fractures. The most frequent type of trauma sustained was enamel-dentine fractures followed by

root fractures. Table 5:1 summarises the distribution of the types of dental trauma among gender. Most of the traumatised teeth were central incisors (87%), and most of the vital control teeth were lateral incisors (80%) (Table 5:2).

Table 5:1 The distribution of the type of dental trauma among gender

		Gender		Total
		male	female	
Type of trauma	Enamel Dentine fracture	2	3	5
	Subluxation	2	1	3
	Extrusion	1	1	2
	Avulsion	1	0	1
	Mid root fracture	2	1	3
	Apical root fracture	1	0	1
Total		9	6	15

Table 5:2 The stage of root development and tooth type in relation to the included teeth

Tooth	Stage of root development	Central incisor	Lateral incisor	Total
Traumatised tooth	Full root length and wide open foramen (diameter > 2mm)	1	0	1
	Full root length and half open apical foramen	6	1	7
	Full root length and closed apical foramen	6	1	7
		13	2	15
Vital control tooth	Full root length and wide open foramen (diameter > 2mm)	1	2	3
	Full root length and half open apical foramen	2	3	5
	Full root length and closed apical foramen	0	7	7
		3	12	15

At base line, the traumatised teeth showed no mobility, had normal colour and no tenderness to percussion and palpation. Only one traumatised tooth exhibited grade I mobility, grey discolouration and tenderness to percussion (Table 5:3). This tooth remained vital throughout the review period. At the end of follow-up, 14 teeth showed no clinical or radiographical signs/symptoms of loss of vitality (Table 5:4).

One participant developed pulp necrosis one month after the baseline recordings and showed tenderness to palpation with an intra-oral swelling. The patient was seen at an emergency appointment where one of our colleagues assessed the tooth clinically and radiographically. Sensibility testing using EPT and ethyl chloride were negative for the traumatised tooth.

Table 5:3 Clinical parameters of all teeth at baseline and follow-up per study participant

n	Tooth	Baseline				End of follow-up			
		Mobility	Colour	Percussion	Palpation	Mobility	Colour	Percussion	Palpation
1	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
2	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
3	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
4	T	0	Normal	(-)	(-)	--	--	--	--
	C	0	Normal	(-)	(-)	--	--	--	--
5	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
6	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
7	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
8	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
9	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
10	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
11	T	1	Grey	(+)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
12	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
13	T	1	Normal	(-)	(-)	1	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
14	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)
15	T	0	Normal	(-)	(-)	0	Normal	(-)	(-)
	C	0	Normal	(-)	(-)	0	Normal	(-)	(-)

Table 5:4 The outcome of each traumatised tooth in relation to participants' demographics and other factors

n	Age	Gender	Type of trauma	Trauma occurred	Length of follow-up (months)	Outcome
1	11	Female	Mid root fracture	2 months	10	Vital tooth-connective tissue healing
2	14	Male	Subluxation	3 weeks	6	Vital
3	9	Male	Avulsion	2 weeks	8	Vital
4	10	Female	Enamel dentine fracture	3 months	1	Non-vital: tooth developed a swelling one month after baseline
5	13	Male	Mid root fracture	4 months	7	Pulp canal obliteration-hard tissue healing
6	11	Female	Enamel dentine fracture	5 months	6	Vital
7	11	Male	Extrusion	4 months	9	Vital-blunting of apex
8	9	Male	Enamel dentine fracture	4 months	12	Vital
9	9	Male	Mid root fracture	2 months	6	Vital-connective tissue healing
10	8	Female	Extrusion	2 months	8	Vital-evidence of continuing root development
11	11	Female	Subluxation	2 months	6	Vital
12	11	Male	Enamel dentine fracture	2 weeks	6	Vital
13	10	Male	Subluxation	4 months	6	Vital
14	11	Female	Enamel dentine fracture	6 months	6	Vital
15	13	Male	Apical root fracture	1 month	6	Vital

5.4.2 LDF

Baseline and end of follow-up recordings are presented in Table 5:5, showing the ratios (Flux traumatised / Flux control) and the Flux values. Based on a cut-off ratio of 0.6, LDF was able to accurately predict the outcome of the pulp in 12/15 teeth (80%) at baseline recordings and 10/14 teeth (71%) at the follow-up visit representing the specificity at base line and at the follow-up visit. The

2x2 tables for LDF at baseline and follow-up are presented in (Table 5:6 and Table 5:7).

Table 5:5 LDF recordings for the two visits

n	Tooth	Baseline recording				Follow-up recording			
		R1	R2	Mean	Ratio	R1	R2	Mean	Ratio
1	T	14.4	17.2	15.8	0.76	7.2	8.9	8.0	0.71
	C	18.4	23.2	20.8		10.6	12	11.3	
2	T	8.3	8.6	8.4	0.53	14.9	16.8	15.8	1.02
	C	12.7	19.1	15.9		15.3	15.7	15.5	
3	T	2	1.7	1.8	0.92	8.8	13.1	10.9	1.70
	C	1.8	2.2	2		5.5	7.3	6.4	
4	T	7.7	6.5	7.1	0.79	--	--	--	--
	C	9.4	8.5	8.9		--	--	--	
5	T	8.4	11.8	10.1	0.88	5.5	5.2	5.3	0.48
	C	10.5	12.3	11.4		11	11.2	11.1	
6	T	6.1	5.4	5.7	0.45	9	6.9	7.9	0.94
	C	12.8	12.7	12.7		6.9	10	8.4	
7	T	3	4.8	3.9	0.48	3.9	4.2	4.0	0.59
	C	4.6	11.5	8.0		8.1	5.6	6.8	
8	T	10	14.6	12.3	1.2	8.5	9.3	8.9	0.59
	C	9.1	10.3	9.7		13.1	16.6	14.8	
9	T	5.9	6.1	6	0.72	3.9	2.7	3.3	0.71
	C	8.5	8	8.2		4.3	5	4.6	
10	T	2.2	2.2	2.2	1.63	15.2	15.6	15.4	0.88
	C	1.6	1.1	1.3		12.8	21.9	17.3	
11	T	13	14.7	13.8	0.92	6.8	11.5	9.15	0.8
	C	14.1	16	15.0		10	12.8	11.4	
12	T	2.8	2.9	2.8	1.05	3.9	4.5	4.2	0.62
	C	2.3	3.1	2.7		6.3	7.1	6.7	
13	T	12.2	16.4	14.3	1.16	6.9	8.4	7.6	0.81
	C	10.5	14.1	12.3		7.4	11.3	9.3	
14	T	5.3	7.1	6.2	1.28	7.4	8	7.7	1.33
	C	4.3	5.4	4.8		5.2	6.3	5.7	
15	T	6	5.9	5.9	0.92	2	1.9	1.9	0.44
	C	7.1	5.8	6.4		3.5	5.3	4.4	

T: Traumatised tooth C: Control tooth R: Recording

The following values were calculated when using the below 2x2 table (Table 5:6) to calculate the possible accuracy values at baseline:

- Sensitivity = a / (a+c) = (0+0/0) = 0%
- Specificity = d / (b+d) = 12 / 15 = 80%
- Positive predictive value = a / (a+b) = 0 / (0+4) = 0%
- Negative predictive value = d / (c+d) = 12/ (0+12) = 100 %

Table 5:6 2x2 table for LDF at baseline

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	0[(a)]	3[(b)]	3
Test vital	0[(c)]	12[(d)]	12
Total	0	15	15

(a) True Positive (b) False positive (c) False negative (d) True negative

The following values were also calculated when using the below 2x2 table (Table 5:7) to calculate the possible accuracy values at the end of follow-up:

- Sensitivity = a / (a+c) = 0 / (0+0)= 0%
- Specificity = d / (b+d) = 10 / (4+10) = 71.4 %
- Positive predictive value= a / (a+b) = 0 / (0+4)= 0%
- Negative predictive value = d / (c+d) = 10 / (0+10)= 100%

Table 5:7 2x2 table for LDF at Follow-up visit

	Tooth status		Total
	Non-vital	Vital	
Test Non-vital	0[a]	4[b]	4
Test alive	0[c]	10[d]	10
Total	0	14	14

(a) True Positive (b) False positive (c) False negative (d) True negative

5.4.3 EPT

EPT was able to detect the sensibility of traumatised teeth in 10/15 participants (66.6%) at base line (Table 5:8 2x2 table for EPT at baseline and 11/14 (78.5%) at the follow-up visit (Table 5:9). All EPT recordings are presented in Table 5:10 EPT recordings at baseline and follow-up. One tooth showed evidence of pulp canal obliteration, and the EPT result was unreliable at the follow-up visit

Table 5:8 2x2 table for EPT at baseline

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	0[a]	5[b]	5
Test vital	0[c]	10[d]	10
Total	0	15	15

(a) True Positive (b) False positive (c) False negative (d) True negative

At base line, the following values were calculated when using the 2x2 table (Table 5:8) to calculate the possible accuracy values of EPT:

- Sensitivity = a / (a+c) = 0 / (0+0) = 0%
- Specificity = d / (b+d) = 10 / (10+5) = 66.6%
- Positive predictive value= a / (a+b) = 0 / (0+5)= 0%
- Negative predictive value = d / (c+d) = 10 / (0+10)= 100%

Table 5:9 2x2 table for EPT at Follow-up visit

	Tooth status		Total
	Non-vital	vital	
Test non-vital	0[(a)]	2[(b)]	2
Test vital	0[(c)]	11[(d)]	11
Unreliable	0	1	1
Total	0	14	14

(a) True Positive (b) False positive (c) False negative (d) True negative

At follow-up, when the unreliable responses were excluded, then the outcomes were calculated as follows (Table 5:9):

- Sensitivity = a / (a+c) = 0 / 0 = 0%
- Specificity = d / (b+d) = 11 / 13 = 84.6%
- Positive predictive value= a / (a+b) = 0/2 = 0%
- Negative predictive value = d / (c+d) = 11/11 = 100%

When the unreliable results were calculated as positive responses the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0 / 0 = 0%
- Specificity = d / (b+d) = 12 / 14 = 85.7%
- Positive predictive value = a / (a+b) = 0 / 2= 0
- Negative predictive value = d / (c+d) = 12 / 12=100

When the unreliable results were calculated as negative responses, the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0 / 0 = 0%
- Specificity = d / (b+d) = 11 / 14= 78.5 %
- Positive predictive value = a / (a+b) = 0 / 3 = 0%
- Negative predictive value = d / (c+d) = 11 / 11 = 100 %

Therefore, the final outcomes for EPT were as follows:

- Sensitivity = 0 %
- Specificity = 78.5 – 85.7 %
- Positive predictive value = 0%
- Negative predictive value = 100 %

Table 5:10 EPT recordings at baseline and follow-up

n	Tooth	Baseline			End of follow-up		
		Recording 1	Recording 2	Result	Recording 1	Recording 2	Result
1	T	Negative	Negative	Negative	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
2	T	Negative	Negative	Negative	Negative	Negative	Negative
	C	Positive	Positive	Positive	Positive	Positive	Positive
3	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
4	T	Positive	Positive	Positive	--	--	--
	C	Positive	Positive	Positive	--	--	--
5	T	Positive	Positive	Positive	Negative	Positive	Unreliable
	C	Positive	Positive	Positive	Positive	Positive	Positive
6	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
7	T	Negative	Negative	Negative	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
8	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
9	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
10	T	Negative	Negative	Negative	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
11	T	Negative	Negative	Negative	Negative	Negative	Negative
	C	Positive	Positive	Positive	Positive	Positive	Positive
12	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
13	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
14	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive
15	T	Positive	Positive	Positive	Positive	Positive	Positive
	C	Positive	Positive	Positive	Positive	Positive	Positive

5.4.4 Ethyl chloride

Ethyl chloride was able to reliably detect the sensibility of traumatised teeth in 9/15 participants (60%) at base line and 10/14 at the follow-up visit (71.4%). Two participants showed unreliable responses at baseline. One of which showed unreliable response at the follow-up visit as the tooth exhibited pulp canal obliteration. The 2x2 tables to calculate the possible outcomes are presented in Table 5:11 and Table 5:12. All recordings are presented in Table5:13.

Table 5:11 2x2 table for ethyl chloride at baseline

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	0[(a)]	4[(b)]	4
Test vital	0[(c)]	9[(d)]	9
Unreliable	0	2	2
Total	0	15	15

(a) True Positive (b) False positive (c) False negative (d) True negative

Table 5:12 2x2 table to ethyl chloride at the follow-up visit

	Tooth status		Total
	Non-vital	Vital	
Test non-vital	0[(a)]	3[(b)]	3
Test vital	0[(c)]	10[(d)]	10
Unreliable	0	1	1
Total	0	14	14

(a) True Positive (b) False positive (c) False negative (d) True negative

At baseline, when the unreliable responses were excluded, the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0 / 0 = 0 %
- Specificity = d / (b+d) = 9 / 13 = 69.2 %
- Positive predictive value= a / (a+b) = 0 / 4 = 0 %
- Negative predictive value = d / (c+d) = 9 / 9 = 100 %

When the unreliable results were calculated as positive responses the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0 / 0 = 0 %
- Specificity = d / (b+d) = 11 / 15 = 73.3 %
- Positive predictive value = a/ (a+b) = 0 / 4 = 0 %
- Negative predictive value = d/ (c+d) = 11 / 11 = 100

When the unreliable results were calculated as negative responses, the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0 / 0 = 0 %
- Specificity = d / (b+d) = 9 / 15 = 60%
- Positive predictive value = a / (a+b) = 0 / 6 = 0
- Negative predictive value = d / (c+d) = 9 / 9 = 100

Therefore, the overall outcomes for ethyl chloride are at baseline as follows:

- Sensitivity = 0%
- Specificity = 60-73.3%
- Positive predictive value = 0
- Negative predictive value = 100%

At follow-up, when the unreliable responses were excluded, then the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0/3=0
- Specificity = d / (b+d) = 10/13= 77%
- Positive predictive value= a / (a+b) =0/3=0
- Negative predictive value = d / (c+d) = 10/10=100%

When the unreliable results were calculated as positive responses the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0
- Specificity = d / (b+d) = 11/14=78.5%
- Positive predictive value = a/ (a+b) = 0
- Negative predictive value = d/ (c+d) = 11/11=100

When the unreliable results were calculated as negative responses, the outcomes were calculated as follows:

- Sensitivity = a / (a+c) = 0/0=0
- Specificity = d / (b+d) = 10/14= 71.4%
- Positive predictive value = a / (a+b) =0
- Negative predictive value = d / (c+d) = 10/10= 100%

Therefore, the range of the above outcomes for ethyl chloride are as follows:

- Sensitivity = 0
- Specificity = 71.4 – 78.5 %
- Positive predictive value = 0
- Negative predictive value = 100%

A summary of LDF, EPT and ethyl chloride in relation to the outcome and type of trauma for all study participants are presented in Table 5:14.

Table 5:13 Ethyl chloride recordings at baseline and follow-up

n		Baseline				Endo of follow-up			
		Rec 1	Rec 2	Rec 3	Result	Rec 1	Rec 2	Rec 3	Result
1	T	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
2	T	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
3	T	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
	C	Positive	Positive	Positive	Unreliable	Positive	Negative	Positive	Positive
4	T	Positive	Negative	Positive	Positive	--	--	--	--
	C	Positive	Negative	Positive	Positive	--	--	--	--
5	T	Positive	Negative	Positive	Positive	Positive	Negative	Negative	Unreliable
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
6	T	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
7	T	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
8	T	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
9	T	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
10	T	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
	C	Positive	Positive	Positive	Unreliable	Positive	Negative	Positive	Positive
11	T	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Negative
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
12	T	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Unreliable
13	T	Positive	Negative	Negative	Unreliable	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
14	T	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive
	C	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
15	T	Positive	Negative	Negative	Unreliable	Positive	Negative	Positive	Positive
	C	Positive	Positive	Positive	Unreliable	Positive	Negative	Positive	Positive

Table 5:14 A summary of LDF, EPT and ethyl chloride in relation to the outcome and type of trauma for all study participants

n	Baseline					End of follow-up				Final outcome
	Type of trauma	**Flux ratio**	**LDF result**	**EPT**	**EC***	**Flux ratio**	**LDF result**	**EPT**	**EC***	
1	Mid root fracture	0.76	Vital	(-)	(-)	0.71	Vital	(+)	(-)	Vital tooth-connective tissue healing
2	Subluxation	0.53	Non-vital	(-)	(-)	1.02	Vital	(-)	(+)	Vital
3	Avulsion	0.92	Vital	(+)	(+)	1.7	Vital	(+)	(+)	Vital
4	Enamel-dentine fracture	0.79	Vital	(+)	(+)	N/a	N/a	N/a	N/a	Non-vital: tooth developed swelling one month after baseline
5	Mid root fracture	0.88	Vital	(+)	(+)	0.48	Non-vital	Unreliable	Unreliable	Pulp canal obliteration-hard tissue healing
6	Enamel-dentine fracture	0.45	Non-vital	(+)	(+)	0.94	Vital	(+)	(+)	Vital
7	Extrusion	0.48	Non-vital	(-)	(-)	0.59	Non-vital	(+)	(-)	Vital-blunting of apex
8	Enamel-dentine fracture	1.2	Vital	(+)	(+)	0.59	Non-vital	(+)	(+)	Vital
9	Mid root fracture	0.72	Vital	(+)	(+)	0.71	Vital	(+)	(+)	Vital-connective tissue healing
10	Extrusion	1.63	Vital	(-)	(+)	0.88	Vital	(+)	(+)	Vital-evidence of continuing root development
11	Subluxation	0.92	Vital	(-)	(+)	0.8	Vital	(-)	(-)	Vital
12	Enamel-dentine fracture	1.05	Vital	(+)	(+)	0.62	Vital	(+)	(+)	Vital
13	Subluxation	1.16	Vital	(+)	Unreliable	0.81	Vital	(+)	(+)	Vital
14	Enamel-dentine fracture	1.28	Vital	(+)	(-)	1.33	Vital	(+)	(+)	Vital
15	Apical root fracture	0.92	Vital	(+)	Unreliable	0.44	Non-vital	(+)	(+)	Vital

EC* Ethyl chloride

The specificity of LDF, on the other hand, at baseline was 80% compared to 66.6% and 60-73.3% for EPT and ethyl chloride, respectively. At the end of the follow-up period, the LDF showed lower specificity (71.4 %) than EPT (78.5 – 85.7 %) and ethyl chloride (71.4 – 78.5 %).

5.5 Discussion

5.5.1 Study design and sample size

This study adopted a prospective design to assess the ability of LDF to detect the vitality and changes in the pulp blood flow of traumatised teeth. A standardised approach was used regarding the implementation of all tests with one investigator carrying out all the recordings at base line and follow-up visits. All traumatised teeth were tested using the three dental pulp tests selected for the study. The use of a parallel group study design would have allowed randomisation however would also require a larger sample size, therefore was not adopted.

At the start of this study, we aimed to recruit 30 patients and review those patients over a period of 1 year. Unfortunately, due to staff changes at our department, reduction in the number of trauma clinics, reduction in the number of patients per trauma clinic, and reduction in the numbers of new patients seen at each clinic we were unable to recruit the required number of participants in this study. In addition, a number of patients attending our department refused or were hesitant to participate in the study further affecting the final number of patients recruited.

5.5.2 Patients' characteristics

The study included participants with dental trauma regardless of the type of the injury. Children with at least one traumatised and one non-traumatised control tooth for comparison were included. This was mainly for LDF recordings as two teeth were needed for Flux comparison. This was one of the limitations of the study as the control tooth should have been ideally non-traumatised based on clinical history. Clinical and radiographic examination was used to confirm the non-traumatised tooth diagnosis, in order for the child to be included in the study. However, when dental trauma occurs, it is quite difficult sometimes for the clinician to be completely certain that the teeth other than the obviously injured were not injured despite normal clinical and radiographic findings. Including teeth as control from patients who had suffered trauma could have caused an impact on the LDF results, although all efforts were made to ensure that the teeth included as controls were non-traumatised with vital pulps.

Children may be apprehensive during the first few visits after trauma which could have had an impact on study recruitment and conducting the baseline recording. Also, children with heavily restored teeth were not considered for inclusion in the study. In addition, children with multiple traumatised teeth were not considered for inclusion as it would be challenging to get a suitable control tooth. These criteria emphasise the limitation of LDF for its routine use as the results depend on the presence of a suitable non-traumatised tooth which is not always possible due to the extensive nature of traumatic dental injuries children may sustain. These variable factors allowed

recruitment of only 15 patients over a period of ten months and a follow up period of a minimum of six months for those recruited towards the end of that period in order to finalise the study within the available time.

5.5.3 The cut-off threshold

The cut-off threshold used in the present study was the cut-off ratio obtained from the previous study in the thesis (Chapter 4) (Flux vital tooth / Flux non-vital tooth ≥ 0.6) which unfortunately showed low sensitivity and specificity. However, using this threshold in the present study showed acceptable specificity of LDF.

5.5.4 Study results

We were unable to calculate the sensitivity of any of the tests used as none of the teeth were considered non-vital at baseline and, unfortunately, the only tooth that lost vitality during the recall period was assessed during an emergency visit whereby the principle investigator was not contacted. Therefore comparison between the tests mainly relied on specificityand negative predictive values.

The study results show that interpretation of the LDF values alone could falsely indicate pulp necrosis. Placing reliance on the LDF ratio on its own to determine pulp vitality is not a reliable method because the results showed inconsistency between the changes in Flux ratios and the pulp status after six months.

A longer observation period could have revealed more reliable and useful results as a change in the pulpal status of some of teeth could have developed. Loss of pulp vitality following dental trauma has been shown to develop with a fairly large time variation as it may develop early or months after an injury (Andreasen and Pedersen, 1985; Andreasen, 1989a).

The specificity of LDF decreased at the follow-up visit from 88% to 71.4% indicating the possibility of some vital teeth undergoing pulp necrosis in the follow-up period. The specificity of LDF in the current study is lower than that reported by Strobl et al. (2003), where the specificity was shown to reach 100%. These authors evaluated the LDF in diagnosing revascularisation of replanted avulsed permanent maxillary central incisors whereby a group of children (7-10 years of age) were examined using LDF 4 times over nine months of follow-up. However, this study included a small sample size (17 children) with no power calculation. Moreover, a cut-off ratio was not used as the final pulp status was based on the difference between the values of the traumatised teeth and the control teeth. If the traumatised tooth had similar values to those of the control tooth or the values had shown a continuous increase, the traumatised tooth was considered vital. Such technique of identifying pulp vitality was not supported by any research.

On the other hand, the specificity of both EPT and ethyl chloride in the present study increased at the follow-up visit. This may be explained by neuronal regeneration as a period of several weeks can occur before a normal response can be elicited. However, interpreting such results should be done with caution as negative persistent response might be transient (Andreasen et al., 2007; Andreasen and Kahler, 2015b).

Studies assessing the accuracy of sensibility tests after TDIs are extremely rare. A prospective study evaluating the accuracy of using EPT and Endo-Frost after TDIs was carried out in 78 patients ranging from 6 to 22 years of age for a mean follow-up period of 20 months (Bastos et al., 2014). However, this study involved no sample size calculation. These authors showed the specificity of EPT and Endo-Frost to be lower than the values obtained in our study, at baseline. The specificity of EPT and Endo-Frost were 56.3% and 52.9 %, respectively (Bastos et al., 2014). This could be explained by the fact that having only children in our study could have overestimated the specificity due to the subjectivity of the tests. At the end of follow-up, the specificity of EPT and Endo-Frost were increased to 88.5% and 76 % indicating neuronal regeneration.

Furthermore, the method used to record the results in the study, (Bastos et al., 2014), was unreliable as for a positive/negative response to be recorded for a tooth, two consecutive positive/negative results were required. Thus, an element of bias may have been incorporated into the evaluation as several attempts could have been carried out in order to achieve two consecutive positive or negative results which could have influenced the reliability of the patients' responses.

In the present study, one participant developed pulp necrosis one month after the baseline recordings and showed tenderness to palpation with an intra-oral swelling. The patient was seen at an emergency appointment where one of our colleagues assessed the tooth clinically and radiographically. Sensibility testing using EPT and ethyl chloride were negative for the traumatised tooth. A sensitivity analysis whereby an

assumption that the LDF measurement of the non-vital tooth at follow up has shown a vital pulp result would not affect the LDF's sensitivity, specificity or positive predictive values, while, the negative predictive value would be decreased from 100 % to 90.9%.

5.6 Conclusion

Within the limitations of this study, LDF has shown better specificity than the EPT and ethyl chloride in predicting the outcome of the pulp at baseline but less at the end of follow-up. Due to the small sample size and relatively short follow up period, these results should be interpreted with caution.

Chapter 6 General discussion and conclusion

The aim of the thesis was to evaluate the use of LDF in the assessment of the pulp blood flow of permanent teeth through different types of studies. In order to assess the accuracy of the LDF, four different studies were conducted.

The first part of this thesis involved conducting literature review and systematic review in order to assess the available evidence for the use of LDF in assessing and monitoring the pulp status of permanent teeth in comparison to other sensibility and/or vitality tests. This systematic review highlighted the lack of high-quality studies assessing LDF's accuracy over traditional sensibility tests and the need for further studies with improved study designs. Furthermore, this review also highlighted the lack of a scientifically determined LDF cut-off threshold based on well-designed

studies with adequate sample size, randomisation, blinding, and sound participants' selection. The systematic review was the first to specifically focus on the use of LDF and it has been published in the journal of 'Dental Traumatology' (Ghouth et al., 2018).

The second part involved exploring the methods and techniques used by UK GDPs and paediatric dental specialists in assessing pulp sensibility and vitality following dental trauma, especially in the child population. This survey also explored the limitations and barriers to the use of these tests. The survey conducted highlighted very limited knowledge and experience amongst the survey respondents with regards to the use of LDF. Some GDPs had never heard of LDF while the percentage of specialists who had used LDF was negligible. The LDF's equipment cost, technique sensitivity and insufficient evidence were some of the limitations identified for the use of such device in assessing pulp vitality. The survey was the first dental survey exploring the use, knowledge and techniques of dental pulp tests by a group of GDPs and paediatric dental specialists. This contributes greatly to the understanding of how dental pulp tests are used in children's dentistry in the UK. The survey has been accepted for publication in the British Dental Journal (Appendix 14).

The results of the first two studies showed the need for further assessment of the LDF's accuracy, in children who sustain dental trauma, using sound diagnostic accuracy methodology and study design. Therefore, the following two studies were designed and executed.

The first clinical study involved a cross-sectional, cohort, diagnostic accuracy clinical study with randomisation that aimed at assessing whether LDF was more accurate than conventional pulp sensibility tests (EPT and ethyl chloride) in assessing the pulp vitality status of permanent anterior teeth in paediatric patients. Not only is this study the first to report low accuracy measurements of LDF in comparison to other sensibility tests, but also that the accuracy measurements were lower than those acceptable for clinical use. Almost certainly, the higher figures for accuracy reported in previous studies were due to a poor study design in which there was a lack of sample size calculation, randomisation, blinding, reference standards and the use of inappropriate statistical analysis methods. The manuscript of this study has been submitted for publication in the international Endodontic Journal (Appendix 15).

Finally, a prospective clinical study to assess the diagnostic accuracy of LDF in predicting pulp vitality of traumatised teeth in paediatric patients was also carried out. This was the first study to prospectively assess LDF using evidence-based pre-determined cut-off ratio which based on the results obtained from study 3 in this thesis. Unfortunately, due to departmental changes, the targeted sample size and follow up period were not achieved. Despite these limitations, the study showed that the accuracy of LDF in predicting the vitality of traumatised teeth was more acceptable than the outcomes obtained from the first clinical study. These results should however be interpreted with caution.

Within the limitations of the clinical studies conducted, the LDF, with the specifications used in this study, may be used with extreme caution in

interpreting the results indicating that the use of LDF to assess the pulp is questionable. The results showed a high probability of false results when using LDF suggesting that LDF was unable to differentiate between vital and non-vital teeth with acceptable accuracy in children between the ages of 8-16 years. 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 overcome its major limitations to allow more convenient use of the device especially in the child population before it can be recommended for routine clinical use for the assessment of the dental pulp.

In summary, the following is a list to answer the overall aims of the thesis:

- There was a lack of high-quality studies assessing LDF's accuracy over other dental pulp tests and the need for further studies with improved study designs.
- There was a lack of a scientifically determined LDF cut-off threshold based on well-designed studies with adequate sample size, randomisation, blinding, and sound participants' selection.
- The survey conducted highlighted that the use of pulp sensibility tests was relatively high amongst respondents while the use of vitality tests was very low.
- Barriers and inconsistencies in the technique and recording of the results of sensibility tests were evident.
- Several barriers usually associated with the child patient, including cooperation, understanding and age were identified.

- The LDF's equipment cost, technique sensitivity and insufficient evidence were some of the limitations identified for the use of LDF in assessing pulp vitality.
- LDF was less accurate than conventional pulp sensibility tests (EPT and ethyl chloride) in assessing the pulp vitality status of permanent anterior teeth in paediatric patients.
- The repeatability of LDF, EPT and ethyl chloride were comparable.
- LDF showed better specificity than EPT and ethyl chloride in predicting the outcome of the pulp in traumatised teeth at baseline but less at the end of follow-up.

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Appendices

Appendix 1: The systematic review (accepted manuscript)

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Article type : Comprehensive Review

Title: The diagnostic accuracy of laser Doppler flowmetry in assessing pulp blood flow in permanent teeth: A systematic review.

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Running Title: Laser Doppler flowmetry in assessing pulp vitality.

Key Words: Diagnostic accuracy, Laser Doppler flowmetry, Pulp blood flow, Pulp vitality.

Conflict of interest statement

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Abstract:

Background/Aim: Pulp necrosis is a frequent complication following dental trauma. The diagnosis of the state of the dental pulp can be challenging as most commonly used diagnostic tools are subjective and rely on a response from the patient, potentially making their use unreliable, especially in the child population. The aim of the study was to systematically review the evidence on the use of laser Doppler flowmetry in the assessment of the pulp status of permanent teeth compared to other sensibility and/or vitality tests.

Methods: A systematic literature search, using MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, www.clinicaltrials.gov and www.controlled-trials.com, in addition to citation and manual reference list searches, was conducted up to 15th January 2018. A risk of bias assessment was performed using the quality assessment for diagnostic accuracy studies tool (QUADAS-2) with all steps performed independently by two reviewers.

Results: Four studies with a high risk of bias were included in the final analysis. Laser Doppler flowmetry was reported to be more accurate in differentiating between teeth with normal pulps and pulp necrosis with a sensitivity of (81.8-100%) and specificity of 100 % in comparison to other vitality tests such as pulp oximetry (sensitivity = 81.3 %, specificity = 94.9 %) and sensibility tests such as electric pulp testing (sensitivity = 63.3 – 91.5 %, specificity = 88 – 100 %). *Conclusion*: Despite the higher reported sensitivity and specificity of laser Doppler flowmetry in assessing pulp blood flow, these data are based on studies with a high level of bias and serious shortfalls in study designs. More research is needed to study the effect of different laser Doppler flowmetry's parameters on its diagnostic accuracy and the true cut-off ratios over which a tooth could be diagnosed as having a normal pulp.

Introduction

The prevalence of traumatic dental injuries is reported to be approximately 20% in children and adolescents with higher percentages reported in adults.[1] Pulp necrosis is one of the sequelae of traumatic dental injuries, which if not managed appropriately could lead to pain, and infection.[2] Therefore, accurate diagnosis of the pulp status of traumatised permanent teeth is an essential component in the management of dental injuries and long term survival of traumatised teeth.[3] Accurate pulp diagnosis is achieved through a combination of the patient history, clinical and radiographic assessments including the use of sensibility and/or vitality tests which are an integral part of the diagnostic process.[4]

Several diverse sensibility and vitality pulp tests are available. Sensibility is defined as the ability to respond to a stimulus. Sensibility tests offer an assessment of pulp health through the stimulation of pulp nerve fibres, therefore, relying on the patient's understanding and cooperation. On the other hand, vitality indicates the presence of blood supply within the

tissues. Thus, vitality testing involves assessing the pulp's blood supply offering an objective approach to assessing pulp blood flow that is not reliant on the patient's understanding and response to stimuli.[5] Vitality tests include laser Doppler flowmetry (LDF), pulse oximetry and more recently the use of ultrasound Doppler flowmetry.[6,7]

LDF was first described in the dental literature in 1986.[8] The primary technique utilised a light beam originating from a helium–neon (He–Ne) laser emitting with a wavelength of 632.8 nm. Other laser wavelengths have since been used such as 780–820 nm. The laser light reaches the dental pulp from a fibre optic probe positioned against the tooth being assessed. When entering the tissues, the laser light is absorbed and scattered by the moving and circulating red blood cells. The photons that interact with red blood cells are Doppler–shifted according to the Doppler principle. The backscattered and returned light is then detected and registered by a photodetector leading to a signal production. The unit used to record the scattered signals or "the concentration and velocity of cells " is termed Flux and assigned an arbitrary unit termed the perfusion unit (PU).[6]

The objectivity, non-reliance on patient's understanding and response, non-invasiveness and ability to test blood supply rather than sensation offers excellent advantages over pulp sensibility tests. The results of LDF, however, should be carefully interpreted due to the inability of the device to measure blood flow in absolute units, in addition to the non-linear relation between the signal output and blood flow rate.[9] Other drawbacks include signal contamination by gingival or periodontal blood supply, high equipment cost in comparison to other pulp tests and the need for patient cooperation as any movement of the apparatus or patient could affect the results.[10,11] Th aim of this review was to systematically assess the evidence from clinical studies on the use of LDF in assessing and monitoring the pulp status of permanent teeth compared to other sensibility and/or vitality tests.

Materials and Methods

The full research protocol was registered and published on PROSPERO, Centre for Reviews and Dissemination (CRD) at the University of York, UK (Registration details: CRD42016035457). A systematic electronic search, citation search and reference list screening were performed. The initial electronic databases search was performed on 2nd March 2016 and included MEDLINE (1946 to February week 3, 2016), EMBASE and EMBASE classic (1947 to 2nd March 2016) and Cochrane Central Register for Controlled Trials CENTRAL. In addition, a search for ongoing trials was conducted on two websites; www.clinicaltrials.gov and www.controlled-trials.com. Dissertation and thesis searches were performed using ProQuest while conference abstracts and proceedings were searched using BIOSIS database. The electronic search strategy was formulated under the supervision of a specialist librarian (University of Leeds Library). The medical subject headings (MeSH) / keywords and the search strategy utilised for MEDLINE were as follows: (exp Dentistry OR Dent* OR exp tooth OR tooth* OR teeth* OR pulp* OR exp Dental pulp) AND (exp laser Doppler flowmetry OR Doppler* OR LDF*), with no limits used. The search strategy was adapted and applied to other databases. EndNote (X 7.4 Thomson Reuters) was used to manage references and remove duplicate records. The electronic search was repeated towards the end of the review process (15th January 2018).

The PICOS methodology was utilised in formulating the research question. The types of participants included were over the age of six years, participants with normal and necrotic pulps and studies where tooth vitality/sensibility had been followed up for at least six months. Types of intervention and comparators included vitality testing of permanent teeth using LDF compared to any type of vitality and/or sensibility tests.

Studies comparing healthy and necrotic pulps were included with the reference standards included a tooth with a pulp known to be normal with no clinical or radiographic signs or symptoms of loss of blood supply, in addition to no history of trauma, no caries nor any dental anomalies (composite reference standard). Moreover, a tooth known to have no pulp (such as pulp extirpated / root canal treated teeth).

Prognostic studies where LDF was used in assessing teeth with damaged and unknown pulp status such as traumatised teeth were also included. The reference standards for this type of studies were a composite reference standard which included signs of loss of blood supply including clinical signs of loss of blood supply and presence of infection in the root canal system such as abscess formation, sinus tract formation, tenderness to percussion / palpation, radiographic signs of periapical pathology, infection related resorption and hyperaemic dental pulp upon root canal treatment. Signs of a normal pulp included continuation of root formation on radiographic views in teeth with immature root formation and none of the signs stated above for loss of blood supply.

Outcome measures were defined in accordance to published criteria for such studies.[12,13] The primary outcome measures included sensitivity, identifying necrotic and infected teeth as having a necrotic and infected pulp, and specificity, identifying normal teeth as having a normal pulp. Additionally, the secondary outcomes included positive predictive value, negative predictive value, repeatability, reproducibility, reliability and Flux ratio.

This systematic review included randomised controlled clinical studies, controlled trials, cross sectional studies including diagnostic cohort studies and diagnostic case-control studies. Prognostic or predictive studies were also included. Studies presented in English language only were included.

The exclusion criteria were participants under the age of six years, studies where primary outcomes of accuracy, sensitivity and specificity are not stated or not possible to calculate. Case series, case reports, reviews and in vitro studies were also excluded. Prognostic or predictive clinical studies with less than six months follow up were as well excluded.

Electronic searching was performed by one reviewer (N.G) while two reviewers (N.G and A.B) performed study selection, data collection and quality assessment. Any disagreement was resolved by consensus or consulting a third researcher (H.N). Articles meeting the inclusion criteria were selected for full text screening. The authors were contacted for additional information when necessary. A data extraction form was based on the Centre for Review and Dissemination guidance for undertaking reviews in health care. The form was piloted using one of the included studies.

The quality assessment tool used to evaluate the included studies was the QUADAS-2, which is recommended by the Cochrane collaboration, Agency for Healthcare Research and Quality, and the UK National Institute for Health and Clinical Excellence for use in systematic reviews of diagnostic accuracy studies. The QUADAS-2 tool assesses two aspects: risk of bias and applicability of concerns. These two aspects are assessed based on three domains: patient selection, index test and reference standard. In addition to these three domains, a fourth domain of flow and timing was also used for the assessment of risk of bias. All domains should be rated as low risk of bias and low concerns regarding applicably in order for a particular study to be rated as having a low risk of bias and applicability concerns.[14]. Piloting of the quality assessment process on one of the included studies was performed in order to calibrate and train both assessors.

Results

The total number of citations identified was 2890 (2569 at initial electronic search, 318 citations through final electronic search and 3 citations through reference list screening (Figure 1). After removal of duplicates (n = 784), 2106 potential eligible studies were identified. Following title and abstract screening, 2061 studies were excluded leaving 45 articles for full article assessment. Forty one studies were excluded leaving four studies to be included in the final qualitative assessment (Figure 1). [15-18] Although the outcome measures were not specified in one of the included studies, the study provided enough information to calculate the sensitivity and specificity of the tests, therefore, allowing it to be included.[18]

All included studies adopted a cross sectional diagnostic cohort design. Blinding and randomisation were not performed in any of the included studies. The participant's age range (Table 1) was very wide in three of the included studies (6.5-74 years),[15,17,18] while the fourth study included a narrow age range (12-18 years).[16]

There were large variations in LDF devices and techniques used in all included studies (Table 2). In terms of LDF device characteristics, there were variations in the laser wavelength used (780 nm was used in two studies, [15,16] while 632.8 nm was used in the other two studies [17,18]) and the probe characteristics (number of probes, fibre diameter and fibre separation) (Table 2).

In terms of LDF technique used, there were also differences in the duration of LDF measurements (20 seconds - 3 minutes) and the cut-off ratio used in identifying tooth vitality in all included studies (Table 2). An isolation splint was used in all studies; however, a rubber dam was not used in any study.

LDF showed a sensitivity of 81.8-100 % and specificity of 100 % in three studies.[16-18] LDF was compared to electric pulp testing (EPT) in three studies with EPT showing sensitivity and specificity of 63.3% – 91.5% and 88-100%, respectively.[16-18] LDF was compared to ethyl chloride in only one of the included studies, showing sensitivity and specificity of 92 % and 89 %, respectively.[17] Accuracy and repeatability of LDF in comparison to four other dental pulp tests were reported in the fourth study with a score of 96.3% and 65%, respectively.[15] Pulse oximetry was compared to LDF in one study showing lower sensitivity (81.3%) and specificity (94.9 %) to that of LDF (Table 3).[16]

The quality assessment showed a high level of bias in all included studies in terms of patient selection, index testing, reference standards, as well as flow and timing as shown in Figure 2.[15-18] With regards to applicability concerns, one study exhibited high concerns regarding applicability,[15] while three studies exhibited low concerns (Figure 2).[16-18]

Discussion

This systematic review focused on assessing the accuracy of LDF compared to all other sensibility and vitality tests in assessing the pulp status of permanent teeth. Four studies with high levels of bias were identified.[15-18]

Some of the principles or criteria assessed during quality assessment of the included studies were the use of reference standards and blinding. The reference standard is the best currently available tool in identifying a condition against which the index test (LDF) is evaluated. Selection of the reference standard plays a very critical role with regards the validity of a test accuracy study.[19] The reference standards used in the included studies, in order to identify a tooth with pulp necrosis as truly having a necrotic pulp, was root canal treatment in one study,[16] the presence of necrotic pulp or bleeding on pulp extirpation and root canal treatment

in the other studies.[15,17,18] Bleeding following pulp extirpation is a subjective sign of pulp necrosis, therefore, should not be used as a reference standard. The reference standard for teeth with normal pulps was based on the lack of clinical and radiographic signs/symptoms of infection which is appropriate for such studies. Incorrect initial classification of the pulp status of the included teeth may result in over/under estimation of the dental pulp tests used.

Test review bias (blinding) occurs when results of the reference standard are known to the operator carrying out the diagnostic test while the test results are interpreted. The nature of the tests makes it hard to blind the examiner. However, the use of isolation splints with small windows showing teeth under assessment could allow blinding of the examiner to the pulp status of the assessed teeth while using different sensibility/vitality tests. Interpretation of the diagnostic tests is usually influenced by the knowledge of the other tests or the condition of the teeth to be tested. Therefore, operator blinding of the examined tooth condition is mandatory in diagnostic accuracy studies.[20] This, however, was lacking in all included studies.

The studies included showed higher sensitivity and specificity of LDF compared to other sensitivity and vitality tests. However, the results of this systematic review highlight the inconsistencies and variabilities of the LDF machine's specifications (wavelength, probe specifications etc.) and application techniques (time of application, use of gingival shields etc.) used in assessing pulp blood flow. Such variability prevents comparison and synthesis of the LDF's published results. Factors such as the degree of LDF's laser penetration, gingival and periodontal signal contamination, the location of LDF's probe, the duration of the Flux measurement and the cut-off Flux value/threshold to which a tooth is considered to have no blood supply should be taken into consideration when using LDF and when planning and executing any future trial.

Laser penetration has been shown to be affected by crown restorations and crown colour change.[21,22] Therefore, inclusion of heavily restored teeth in studies might affect the LDF's accuracy. One of the studies included in this systematic review included heavily restored teeth and reported a high accuracy of LDF (96.3 %) in comparison to other dental pulp tests.[15] Such an effect should have been considered and reflected in the results of that study as Flux values might have been affected leading to misinterpretation of the results.

There were inconsistencies between the studies with regards to the duration of LDF measurement. It is well established that movement artefacts, whether related to the patient or apparatus itself, affect LDF readings. Therefore, allowing sufficient time for recording stable Flux recording is recommended.[6] Including unstable movements' artefacts in the analysis may increase the Flux value leading to mis-interpretation of the results. Flux duration measurement ranged from 45 seconds,[16] to 3 minutes [17] in the included studies, with no reference to allowing stable Flux readings.

Another crucial factor in diagnostic accuracy studies is the use of a cut-off ratio/threshold (diseased pulp Flux/ known healthy pulp Flux) to aid the diagnosis. Ideally, a pre-specified threshold between a healthy tooth and a tooth with pulp necrosis must be established.[14] A pre-specified threshold was only mentioned in one of the studies included in this review with a cut-off ratio of 0.6 used (a ratio $\geq$ 0.6 (diseased/healthy) indicated a healthy pulp).[15] The authors based this ratio on the work of Ingolfsson et al,[18] which included in this review, and that of Roebuck et al, [23] which is not included in this review due to the lack of direct comparison with other sensibility/vitality tests.

LDF results of 11 pairs of healthy and necrotic pulps showing a significant lower Flux values for necrotic pulps in comparison to healthy pulps using four different probes have been reported in the study of Ingolfsson et al.[18] That study, however, showed spectrum bias,

differences in disease severity, as four teeth were diagnosed with periapical radiolucencies, one tooth with submucosal abscess and one tooth with pulp canal obliteration. Teeth with such conditions should have been excluded as this could have caused inconsistencies in the accuracy estimates of the tests.

Roebuck et al assessed the effect of bandwidth filter, laser wavelength, fibre separation and probe position on the healthy/necrotic pulp ratios of Flux signals recorded from 11 healthy and non-endodontically treated teeth with pulp necrosis have been reported.[23] The combination of 633 nm with a 3 KHz bandwidth using a probe with a 500 μm placed 2-3 mm from the gingival margin was considered the most reliable combination. Moreover, a cut-off ratio, used in determining pulp necrosis, was recommended if healthy pulps Flux / necrotic pulps Flux > 1.25 (a Flux ratio > 0.8 diseased/healthy) compared to the 0.6 reported.

Despite the limitations of these two studies, and indeed this systematic review, these studies highlighted the need for better quality diagnostic accuracy studies assessing the effect of different combinations of LDF parameters (such as wavelengths, probes used) on the cut-off ratio used in diagnosing pulp status before LDF could be recommended for clinical use.

Age related pulp changes could also contribute to changes in pulp blood flow, thus affecting Flux and Flux cut-off values. Such changes include higher pulp blood supply in immature teeth versus lower blood supply in calcified teeth or teeth with smaller pulp chambers due to secondary dentine formation.[24] There was a wide variation in age range in three included studies with the ages of the subjects ranging from 6.5-74 years.[15,17,18] More studies are recommended which should include a younger age group, where trauma occurs before root development is complete, as the assessment of pulp healing after trauma can be more challenging due to the child's anxiety often making routinely used sensibility tests less reliable.

Conclusion

Despite the higher reported sensitivity and specificity of LDF in assessing pulp vitality, these data are based on studies with high level of bias and serious shortfalls in study designs. This systematic review highlights inconsistencies in the evidence supporting the use of the LDF in assessing pulp vitality of permanent teeth. Further high quality diagnostic clinical trials are needed to determine LDF's true cut-off ratios over which a pulp could be diagnosed as necrotic. More research is also needed to study the effect of different LDF parameters on its diagnostic accuracy before such a tool, which is relatively expensive, could be reliably recommended for routine use in everyday practice.

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Legends to Tables

Table 1: A summary of the demographics and characteristics of the four included studies.

Table 2: A summary of LDF techniques used in the four included studies.

Table 3: A summary of the outcome measures reported for LDF in comparison to other sensibility and vitality tests as reported in the included studies.

Legends to Figures

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart summarising the systematic review process in the identification of included studies.

Figure 2: A tabular presentation of the results of the QUADAS-2 quality assessment of the studies included.

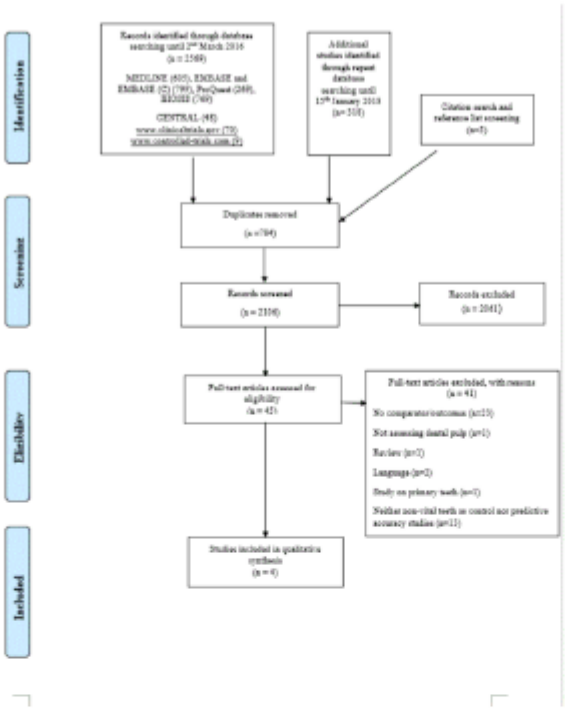
Study/Year	Study design	Sample size	Age	Teeth included	Disease characteristics	Comparators	Randomisation	Blinding	Reference test
Chen 2011 (13)	Cross sectional	20 patients ; 121 teeth	18-74	Maxillary and mandibular incisors, canines, premolars and molars.	Teeth suspected or known to have pulp pathosis or previously diagnosed as having a healthy pulp.	• CO_2 (carbon dioxide crystals) • Ice • Refrigerant spray (Endo Frost) • Electric pulp testing	No	No	Root canal treatment
Kamyabinaz 2011 (14)	Cross sectional	51 patients ; 50 pairs of anterior teeth	12-18	Maxillary central and lateral incisors	Endodontically treated teeth and healthy control teeth	Electric pulp testing and Pulse oximetry	No	No	Clinical and radiographic examinations. Palpless teeth had root canal treatment
Evans 1989 (15)	Cross sectional	Group 1: 57 patients ; 57 teeth with necrotic pulps and 53 healthy control teeth Group 2: 84 patients ; 84 vital teeth	6.5-33.5	Maxillary and mandibular anterior teeth	Teeth with healthy and necrotic pulps	Electric pulp testing and ethyl chloride	No	No	No clinical / radiographic signs or symptoms of infection for the healthy teeth No bleeding on pulp extirpation for non-vital teeth
Ingolfsson 1994 (16)	Cross sectional	Group 1: 9 patients; 11 healthy teeth and 11 teeth with necrotic pulps. Group 2: 10 patients with 20 healthy teeth	11-37	Maxillary and mandibular anterior teeth.	Teeth with healthy and necrotic pulps	Electric pulp testing	No	No	Pulp necrosis was confirmed during root canal treatment Healthy teeth tested positive to EPT , exhibited no discolouration and normal radiographic examination.

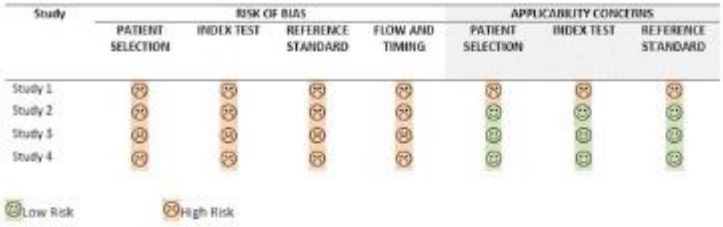
Study	Rubber dam used	Splint used	Location of probe	LDF device used and wavelength	Type of probe	Duration of LDF measurement	LDF cut-off ratio used	Unit of measurement
Chen 2011 (13)	No	Polyvinyl	2-3mm above the gingival margin	• MoorLAB/PeLAB ; Moor Instruments Ltd, Axminster, UK • Wavelength: 780 nm	• Double channel • 0.5mm fibre separation.	90 seconds	Discussed pulp flux/ known healthy pulp flux ratio is less than or equal to 0.6	Flux
Kanzyilmaz 2010 (14)	No	Silicon-impression-based	2 mm above the gingival margin.	• BLF21A • Wavelength: 780 nm	• Single channel • Diameter: 1.5 mm, two fibres in 0.2 mm diameter central 0.5 mm apart.	20 optimum seconds out of 45 seconds.	1 / 10 ratio between the pulp blood flow values measured by LDF	PU
Evans 1999 (15)	No	A two-stage green elastomeric splint.	Between 2 and 3 mm from the gingival margin.	• Perimed PF2b, Stockholm, Sweden. • Wavelength: 632.8 nm	• Single channel • Probe with 0.5 mm fibre separation.	5 min (where patient cooperation allowed)	*LDF healthy pulp*: flux equal or more than 7.0 PU and amplitude SWV equal or more than 1.6 PU *LDF severely pulp* : flux < 7.0 PU (determined post calculation and analysis, not before) *LDF Intermediate healthy pulp*: flux equal or more than 7.0 PU but amplitude SWV < 1.6 PU	Two LDF signal variables were measured virtually; Flux and SWV. Both measured in PU.
Ingolfsson 1994 (16)	No	Rubber base material)	2-3 mm from the gingival margin.	• A Periflux PF3 laser,Perimed, Sweeden. • Wavelength: 632.8	• Double channel • Five probes used (fibre diameter/ fibre separation) mm o 0.2/1.5 o 0.2/1.0 o 0.2 / 0.8 o 0.2 / 0.5 o 0.125 / 0.25	1.5 to 2 minutes	No set off used, only significance difference between readings.	Flux

PU: Perfusion unit PBF: Pulp blood flow SWV: amplitude of slow wave vasomotion.

Outcome measures	Tests	Chen 2011 (13)	Kanzyilmaz 2010 (14)	Evans 1999 (15)	Ingolfsson 1994 (16)
Sensitivity (%)	LDF	---	100	100	81.8 – 90
	RPT	---	91.3	87	68.3
	PO	---	91.3	---	---
	EC	---	---	92	---
Specificity (%)	LDF	---	100	100	100
	RPT	---	88	96	100
	PO	---	94.8	---	---
	EC	---	---	89	---
Positive predictive value (%)	LDF	---	---	---	100 (probe 125/250)
	RPT	---	88.5	---	100
	PO	---	94.1	---	---
Negative predictive value (%)	LDF	---	---	---	50 (probe 125/250)
	RPT	---	91.2	---	75
	PO	---	88.5	---	---
Accuracy (%)	LDF	96.3	---	---	---
	RPT	87.7	---	---	---
	COL	87	---	---	---
	EF	90.5	---	---	---
	ICE	84.8	---	---	---
Repeatability	LDF	0.65	---	---	---
	RPT	0.43	---	---	---
	COL	0.43	---	---	---
	EF	0.57	---	---	---
	ICE	0.67	---	---	---

EC: Ethyl chloride EF: Endo Frost


Identification
Screening
Eligibility
Included
Records identified through database searching until 2nd March 2016 (n = 2568)
MEDLINE (605), EMBASE and EMBASE (C) (799), PreQuest (268), BIOSIS (749)
CENTRAL (48)
www.clinicaltrials.gov (75)
www.controlled-trials.com (27)
Additional studies identified through repeat database searching until 15th January 2018 (n= 310)
Citation search and reference list screening (n=5)
Duplicates removed (n = 784)
Records screened (n = 2194)
Records excluded (n = 2041)
Full-text articles assessed for eligibility (n = 42)
Full-text articles excluded, with reasons (n = 41)
No comparator outcomes (n=23)
Not assessing dental pulp (n=1)
Review (n=2)
Language (n=2)
Study on primary teeth (n=1)
Neither non-vital teeth or control nor predictive accuracy studies (n=13)
Studies included in qualitative synthesis (n = 0)



Appendix 2: Data extraction form (Systematic review)

Date Version:

Data extraction proforma

- Name of Review:

Date of Data extraction:	
Researcher performing data extraction	
Author	
Article title	
Type of publication (e.g. journal article, conference abstract)	
Country of origin	
Space for additional notes	

Study characteristics	
Aim/objectives of the study	
Study design	
Study inclusion and exclusion criteria	
Recruitment procedures used (e.g. if applicable details of randomisation, blinding)	

Participant characteristics	
Age:	Gender:
Disease characteristics:	
Number of participants (in each group if present) eligible, enrolled, or randomised that is reported in the study:	

Date Version:

Intervention and setting	
Setting in which the intervention was delivered	
Description of the diagnostic test and other tests used(index test)	
Description of the diagnostic test and other tests used(index test)	
Reference test	
Rubber dam used	
Splint used	
Intervention details (sufficient replication of feasible):	

Outcome data/results:	
Outcomes and time points (i) collected; (ii) reported	
➢ **For prospective studies:** Number of participants enrolled Number of participants included in analysis Number of withdrawals, exclusions, lost to follow-up	
For each outcome of interest:	
Sensitivty,specificity and other outcomes	
Outcome definition	
Unit of measurement (if relevant)	
Method used during LDF measurement	
LDF ratio used	
Length of follow-up, number and/or times of follow-up measurements	
Statistical techniques used	
Any subgroup analysis	

Appendix 3: Ethical approval for the survey

Message sent to Outlook message list

DREC ref: 300317/NG/226

Julie McDermott

Mon 15/05/2017 11:27

To:Nahar Ghouth <dnnng@leeds.ac.uk>;

Cc:Julia Csikar <J.I.Csikar@leeds.ac.uk>; Hani Nazzal <H.M.Nazzal@leeds.ac.uk>; Jinous Tahmassebi <J.Tahmassebi@leeds.ac.uk>;

Dear Nahar

DREC ref: 300317/NG/226
Title: The use of sensitivity/vitality tests in the management of dental trauma in children amongst paediatric dentists and general dental practitioners

Thank you for submitting the above application to the Dental Research Ethics Committee. Your application has been reviewed and I am pleased to inform you that it has been accepted.

Documents reviewed

Document name	Version number and date
Ethics application form	Dated 15/05/2017
Study protocol	Version 5 30/03/2017
Online questionnaire	Version 1 30/03/2017

With best wishes for the success of your project.

Please note: You are expected to keep a record of all your approved documentation and other documents relating to the study such as sample consent forms, signed consent forms, participant information sheets and risk assessments. These documents should be kept in your study file and may be subject to an audit inspection. If your project is to be audited, you will be given at least 2 weeks' notice.

It is our policy to remind everyone that it is your responsibility to comply with Health and Safety, Data Protection and any other legal and/or professional guidelines there may be.

Kind regards,

For and on behalf of
Dr Julia Csikar
DREC Chair

Appendix 4: Ethical approval (RES) for clinical study 1

NHS
Health Research Authority
National Research Ethics Service

RES Committee North West - Greater Manchester East
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7816
Fax: 0161 625 7299

08 September 2015

Mr Nahar Ghouth
The Worsley Building
Clarendon Way
Leeds, West Yorkshire
LS2 9LU

Dear Mr Ghouth

Study title:	**The Diagnostic Accuracy of Laser Doppler Flowmetry for the Assessment of Pulp Survival Following Dental Trauma in Paediatric Patients.**
REC reference:	**15/NW/0583**
IRAS project ID:	**173671**

Thank you for your letter of 18 August 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Rachel Heron, nrescommittee.northwest-gmeast@nhs.net Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of

Document	*Version*	*Date*
Covering letter on headed paper [Cover letter]		08 August 2015
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)	1	17 September 2014
GP/consultant information sheets or letters	3	20 May 2015
GP/consultant information sheets or letters [GDP letter]	4	30 July 2015
Letters of invitation to participant	3	20 May 2015
Letters of invitation to participant [Invitation letter]	4	30 July 2015
Other	1	20 May 2015
Other	1	31 January 2015
Other	1	30 January 2015
Other	1	28 January 2015
Participant consent form	3	20 May 2015
Participant consent form [assent form]	3	20 May 2015
Participant consent form [Consent]	4	30 July 2015
Participant information sheet (PIS) [8 - 12 year olds]	3	20 May 2015
Participant information sheet (PIS) [12 - 16 year olds]	3	20 May 2015
Participant information sheet (PIS) [Parents]	3	20 May 2015
Participant information sheet (PIS) [PIS 8-12 years]	4	30 July 2015
Participant information sheet (PIS) [PIS 12-16 years]	4	30 July 2015
Participant information sheet (PIS) [PIS parents]	4	30 July 2015
Participant information sheet (PIS) [Information sheet 12-16 years]	5	18 August 2015
REC Application Form [REC_Form_29062015]		29 June 2015
Research protocol or project proposal	2	15 June 2015
Summary CV for Chief Investigator (CI)	1	20 May 2015
Summary CV for supervisor (student research)		

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/NW/0583	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

On behalf of
Professor Janet Marsden
Vice-Chair

Email: nrescommittee.northwest-gmeast@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Faculty ethics administrator*
Anne Gowing, Leeds Teaching Hospitals NHS Trust

Appendix 5 : NHS permission at The Leeds Teaching Hospital NHS Trust (LTHT)

The Leeds Teaching Hospitals NHS
NHS Trust

Mobeen Fazal

02/10/2015

Mr Nahar Ghouth
The Worsley Building
Clarendon Way
Leeds, West Yorkshire
LS2 9LU

Research & Innovation
Leeds Teaching Hospitals NHS Trust
34 Hyde Terrace
Leeds
LS2 9LN

Tel: 0113 392 0162
Fax: 0113 392 0146

r&d@leedsth.nhs.uk
www.leedsth.nhs.uk

Dear Mr Nahar Ghouth

Re: NHS Permission at LTHT for: The Diagnostic Accuracy of Laser Doppler Flowmetry for the Assessment of Pulp Survival Following Dental Trauma in Paediatric Patients.
LTHT R&I Number: DT15/307
REC: 15-NW-0583

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&I Department.

The study must be conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable), the terms of the Research Ethics Committee favourable opinion (if applicable) and NHS Trust policies and procedures (see http://www.leedsth.nhs.uk/research/) including the requirements for research governance and clinical trials performance management listed in appendix 1 and 2 . NHS permission may be withdrawn if the above criteria are not met including the requirements for clinical trials performance

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority ""Clinical Negligence Scheme for NHS Trusts"" for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity with NHS Permission

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm. Should there be any changes to the research team please ensure that you inform the R&I Department and that s/he obtains an appropriate contract, or letter of access, with the Trust if required.

Yours sincerely

Anne Gowing
Research Governance Manager

Appendix 6: Study documents for clinical study 1

Version number 4 Date 30.07.2015 Centre Number: 1

Leeds School of Dentistry- The Centre for Oral Health Sciences
Department of Paediatric Dentistry
A Centre for Children with Special Needs
Level 6, Worsley building
Clarendon way
Leeds LS2 9LU
T (Direct Line) +44 (0)1133436177
T (Enquiries) +44 (0)1133436138
F +44 (0) 1133436140
E m.s.duggal@leeds.ac.uk

UNIVERSITY OF LEEDS

Consent Form

Title of project: The diagnostic accuracy of laser Doppler flowmetry for the assessment of pulp survival following dental trauma in paediatric patients.

(A study to assess the best way to tell if a tooth is alive or dead?)

Name of Researcher: Nahar Ghouth

Please initial Box

1. I confirm I have read and understood the information sheet dated 30.07.2015 (Version 4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my child's participation is voluntary and that my child is free to withdraw at any time without giving any reason and without my child's medical care or legal rights being affected. ☐
3. I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the school of dentistry, from regulatory authorities or from the NHS Trust, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child's records. ☐
4. I agree to my GDP being informed of my child's participation in the study. ☐
5. I agree for my child to take part in the above study. ☐

----------	----------	----------
Name of Patient	Date	Signature
----------	----------	----------
Name of Person taking assent	Date	Signature

Monty S Duggal
BDS MDSc FDS (Leeds) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

Version number 3 Date 20.05.2015 Centre Number: 1

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Department of Paediatric Dentistry
A Centre for Children with Special Needs
Level 6, Worsley building
Clarendon way
Leeds LS2 9LU
T (Direct Line) +44 (0)1133436177
T (Enquiries) +44 (0)1133436138
F +44 (0) 1133436140
E m.s.duggal@leeds.ac.uk

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Patient Identification Number for this study:

ASSENT FORM

Title of project: A study to assess the best way to tell if a tooth is alive or dead?

Name of Researcher: Nahar Ghouth

Please initial Box

1. I am happy with the explanation given to me by the dentist. ☐
2. I know that I don't have to take part in the study and I can ask the dentist to stop at any time. ☐
3. I agree to take part in the study. ☐

----------	----------	----------
Name of Patient	Date	Signature
----------	----------	----------
Name of Person taking assent	Date	Signature

Monty S Duggal
BDS MDSc FDS (Peads) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

Version number 4 Date 30.07.2015

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Title of study: The diagnostic accuracy of laser Doppler flowmetry for the assessment of pulp survival following dental trauma in paediatric patients.

"A study to assess the best way to tell if a tooth is alive or dead?''

Information for children 8-12 years of age

Why has the dentist asked me to read this paper?

You broke your tooth and the nerve inside your tooth is now damaged. We have a new laser machine that can tell us how damaged your tooth is, but we need first to test if it is working. By doing this, you can help other children when they break their teeth.

Your tooth might feel cold or you might feel slight tingling sensation when we use our machines. The new laser machine has a tiny camera and a magic light which we will shine on your tooth. We will show you and explain everything before we use it.

Will anything hurt?
Nothing will hurt at all. If you feel anything you don't like, just tell us and we will stop.

Do I have to take part?
No

How long will it take?
It should not take long, just around half an hour (30-40 minutes)

Can I change my mind?
Of course you can change your mind anytime. You do not even need to tell us the reason you changed your mind.

Will you still fix my tooth if I choose not to take part in the study?

Yes, of course.

Thank you for reading this information leaflet.

Monty S Duggal
BDS MDSc FDS (Leeds) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

Double-click to hide white space

Version number 4 Date 30.07.2015

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Information for the person with parental responsibility

Title of study: The diagnostic accuracy of laser Doppler flowmetry for the assessment of pulp survival following dental trauma in paediatric patients.

"A study to assess the best way to tell if a tooth is alive or dead?"

Please take time to read the following information carefully. If you have any questions please ask us on your next visit.

Why have I been given this leaflet?

We would like to invite your child to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being carried out and what it will involve.

What is the purpose of this study?

Your child sustained an injury to one or more of his/her teeth causing the nerve inside the tooth to die. As a result, a root canal treatment was/will be carried out to treat the injured tooth. It is difficult to determine if the nerve in the tooth is dead or alive with the current techniques. We would like to assess a new technique that we think could help us to make this decision in the future. To do this we use a laser machine which is safe, painless, and has been routinely used for almost 30 years in dentistry for both adults and children. This machine measures the blood supply inside the tooth and so we can find out if the tooth is alive. Your child's participation will help us compare the new machine to the conventional techniques (the electrical test and cold test) and help children with injured teeth in the future.

We will test your child's teeth using either the conventional methods or the laser machine. We anticipate that your child's participation in our study would only take a maximum of 30 to 40 minutes and during your appointment if possible. A small number of patients returning to our department in the future for further treatment or review would be approached to have their teeth tested again.

Would my child be disadvantaged by using either of the two techniques?

No, your child will not be affected at all. The reason we are asking your child to help with the study is that we already know he/she has one alive and one dead tooth. This will help us check if either of the two techniques is good enough in differentiating between already known live

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and dead teeth. Your participation, however, would help us better use the laser machine which could help other children in the future.

Who is doing the study?

This study will be conducted at the children's department at the Leeds Dental Institute by one of the paediatric dentistry trainees (Nahar Ghouth), under the supervision of (Dr Hani Nazzal) a Clinical Lectrurer and Speciality Registrar, and the head of the Paediatric Dentistry department (Professor Monty Duggal).

Do I have to take part?

No

What will happen if I take part in this study?

During your next appointment, you would be able to ask Nahar Ghouth, any questions you might have about the study. If you require further time to consider the information and make a decision, we will arrange to see you again at your next appointment or arrange a separate appointment should that be more convenient for you.

If you decide to participate, the following will occur:

1) You will be asked to sign a document (Consent) stating that you are happy for your child to take part in the study and understand what is involved.

2) We will assess the status of the injured tooth and the alive vital tooth with one of the previously mentioned methods.

3) Some participants will be asked to undergo a second test during one of the follow up appointments.

Are there any side effects?

There are no side effects using all methods. The laser technique is a safe and painless technique that has been used for almost 30 years with both adults and children.

Can I withdraw from the study at any time?

You can withdraw from the study at any time without giving any reasons. Your child will still receive the appropriate treatment and review depending on the treatment he/she received.

Monty S Duggal
BDS MDSc FDS (Paed) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

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Will the information I give be kept confidential?

All information regarding your child's treatment is confidential and will be stored in your child's NHS records, which are only accessible by NHS staff. Any data we collect will be stored using non-identifiable information on password-protected computers.

What will happen to the results of the study?

The results of the study will be discussed with other colleagues in the department, presented at conferences and in scientific journals. Your child's identifiable information will not be used in these settings.

Should you want to know the results of the study, please let one of the research team know and we will inform you of the results after we obtain them.

Who reviewed this study?

All research carried out by the NHS is reviewed by an independent group of people, called research ethics committee, to protect your safety, rights, wellbeing and dignity prior to starting the research.

What if I need further information or need to complain?

Should you need any other information or like to complain please contact one of the research team using the following contact details. Alternatively, you could contact Patient Advice and Liaison Service on (0113 2067168) or patient relations department on Telephone (01132066261).

Chief Investigator	**Primary supervisor**	**CO-Supervisor:**
Nahar Ghouth	Hani Nazzal	Prof Monty Duggal
Postgraduate student	NIHR clinical lecturer and speciality registrar	Head of the Paediatric Dentistry department
Leeds Dental Institute	Leeds Dental Institute	Leeds Dental Institute
Clarendon Way	Clarendon Way	Clarendon Way
LS2 9LU	LS2 9LU	LS2 9LU
Tel.: 01133438139	Tel.: 01133438139	Tel.: 01133436177
Email:dnng@leeds.ac.uk	Email: denha@leeds.ac.uk	Email: M.S.Duggal@leeds.ac.uk

Thank you for taking the time to read this information sheet and for considering this study.

Monty S Duggal
BDS MDSc FDS (Peads) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

Version number 5 Date 18.08.2015

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Title of study: The diagnostic accuracy of laser Doppler flowmetry for the assessment of pulp survival following dental trauma in paediatric patients.

"A study to assess the best way to tell if a tooth is alive or dead?"

Information for children 12-16 years of age

Why have I been given this leaflet?

We would really like your help with our study. This letter will help you decide whether you want to help us or not.

Why me?

You had an injury to your tooth and the nerve inside the tooth died. As a result, your dentist did a treatment called root canal treatment on that tooth. We have a new laser machine that can help us to test if the nerve inside your tooth is alive or dead, and we need to test if it is working well.

We will compare the laser machine to the current methods we use these days. So, we might test your tooth with either technique. The laser machine has a tiny camera and a light which we will shine on your tooth. We will explain every step to you before we do it and we will stop if you raise your hand.

Why are we doing this study?

The current technique used to test the nerve inside the tooth is not very good. We hope this new machine would help other children when they damage their teeth. By helping us with this study, you are helping other children.

Do I have to take part?
No

Will anything hurt?
Nothing will hurt at all. If you feel anything you don't like, just tell us and we will stop.

How long will it take?
It should not take long, just around half an hour (30 -40 minutes).

Can I change my mind?
Of course you can change your mind anytime. You do not need to tell us the reason you changed your mind.

Will you still fix my tooth if I do not do take part in the study?
Yes, of course.

Thank you for reading this information leaflet.

Monty S Duggal
BDS MDSc FDS (Peads) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

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Date 30.07.2015

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Parental Invitation to Participation letter

Name of Researcher : Nahar N. Ghouth

Dear Parent/Guardian:

We would like to invite you to take part in our study Titled: The diagnostic accuracy of laser Doppler flowmetry for the assessment of pulp survival following dental trauma in paediatric patients.

"A study to assess the best way to tell if a tooth is alive or dead?"

I am conducting a research project comparing two different methods testing whether the tooth is alive or not after dental injuries. Your child has one dead tooth and therefore would be suitable for our study.

These methods are well used in dentistry, in addition to being used for many years on both adults and children. The methods are very simple, non invasive and your child will not feel any pain. One of the methods involves the use of a laser machine which uses very low powered laser. This machine has been safely used previously all over the world with no side effects reported. This machine has also been used in our department on children for the past 2 years.

During your next visit, you will be able to ask any questions and hopefully be in a position to make a decision regarding your child's participation.

Please be advised that should you decide not to take part in the study, we would still provide your child with the treatment needed at that appointment

Thank you for taking the time to read this letter and the attached information leaflets. Should you have any questions please do not hesitate to either contact me prior to your appointment or ask me during the appointment session.

Many thanks,

Nahar Ghouth
Postgraduate student in Paediatric dentistry
Under the supervision of Dr Hani Nazzal and Prof Monty Duggal

Monty S Duggal
BDS MDSc FDS (Peads) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
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Version 4 30/07/2015

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GDP's Address

Date:
Dear (name of GDP):
Reg.: (patient's name)
D. O. Birth: (patient's date of birth)
Patient's address

Thank you for referring this patient to the Leeds Dental Institute for the management of his/her dental trauma.

I would like to inform you that (patient's name) and their legal guardian has agreed to take part in our study titled: The diagnostic accuracy of laser Doppler flowmetry for the assessment of pulp survival following dental trauma in paediatric patients

The patients will be randomly assigned into one of the following two groups:

Group 1: Assessment of pulp vitality using laser Doppler flowmetry.
Group 2: Assessment of pulp sensibility using electrical pulp tester and ethyl chloride.

(Patient's name) is recruited in this study as they have a non vital tooth. (Patient's name) will receive his/her treatment by one of my colleagues depending on his/her treatment needs regardless of their contribution in this study.

We shall keep you informed of the patient's progress and please do not hesitate to contact me should you need any further information.

Kind Regards,

Nahar Ghouth
Postgraduate student in Paediatric Dentistry
Under the supervision of Prof Duggal
Consultant and Head of Paediatric Dentistry Department

Monty S Duggal
BDS MDSc FDS (Paeds) RCS (Eng) PhD
Head of Department of Paediatric Dentistry

The Leeds Teaching Hospitals NHS
NHS Trust

Appendix 7: Data collection sheets for LDF, EPT and ethyl chloride

LDF

Patient # Age: sex:

Type of trauma:

Vital tooth (central incisor-lateral incisor) Stage of root development:

Non-vital tooth (central incisor –lateral incisor) Stage of root development:

Measurement 1

Vital tooth	Non vital tooth
FLUX	FLUX

Measurement 2

Vital tooth	Non vital tooth
FLUX	FLUX

EPT

Patient # Age: sex:

Type of trauma:

Vital tooth (central incisor-lateral incisor) Stage of root development:

Non-vital tooth (central incisor –lateral incisor) Stage of root development:

Vital tooth

Measurement 1:

Value:

Measurement 2:

Value:

Non vital tooth

Measurement 1:

Value:

Measurement 2:

Value:

Cold

Vital Tooth	Non vital tooth
Measurement 1: Positive or negative.	Measurement 1: Positive or negative
Measurement 2: Positive or negative	Measurement 2: Positive or negative
Measurement 3: positive or negative	Measurement 3: positive or negative

Appendix 8: Frequency table showing Flux values for vital teeth in recording 1 and 2

Flux recording 1	Frequency	Flux recording 2	Frequency
3.4	2	3.5	1
4.2	1	3.7	1
4.4	1	3.9	1
5	1	4.5	1
5.5	1	4.9	1
6	1	5.8	1
6.3	1	5.9	1
6.6	2	6.8	2
7	1	7	2
7.3	1	7.2	1
7.4	1	7.6	1
7.5	1	8	1
7.6	1	8.2	2
7.9	1	8.8	2
8.4	1	9.5	1
9	1	9.7	2
9.1	2	9.8	1
9.3	1	9.9	1
9.4	1	10.4	1
9.6	2	10.7	1
9.9	1	11.1	1
10.1	1	11.4	1
10.6	1	11.9	1
12.4	1	12.2	1
12.5	1	12.8	1
12.7	1	13.9	1
13.2	1	14.3	1
15.1	1	15.5	1
15.2	1	17.2	1
15.8	1	24.2	1
19.3	1	27	1
20.1	1	34.9	1
28.9	1	--	--

Appendix 9: Frequency table showing Flux values for non-vital teeth in recording 1 and 2

Flux recording 1	Frequency	Flux value for recording 2	Frequency
1.7	1	2	1
1.9	1	2.3	1
2	1	2.6	1
2.7	1	2.8	2
2.8	3	2.9	1
2.9	1	3.1	2
3.1	1	3.3	1
3.2	1	3.4	1
3.3	2	3.5	1
3.7	1	4.2	1
4	1	4.3	1
4.1	1	4.4	1
4.2	1	4.5	1
4.5	1	4.6	1
4.9	1	4.7	1
5	1	4.9	2
5.1	1	5.4	1
5.3	2	5.6	1
5.6	1	5.9	1
5.8	1	7.4	2
6.1	1	8.1	2
6.7	1	8.5	1
6.8	1	8.6	1
7.9	2	9.3	1
8.2	2	9.6	1
9	1	10.4	1
11.9	1	11.5	1
13	1	12.3	1
13.8	1	12.7	1
18.3	1	22	1
27.6	1	25.2	1
--	--	27.8	1

Appendix 10: Ethical approval (RES) for clinical study 2

NHS
Health Research Authority
Yorkshire & The Humber - Leeds East Research Ethics Committee
Jarrow Business Centre
Rolling Mill Road
Jarrow
NE32 3DT

Telephone: 0207 104 8081

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

20 February 2017

Mr Nahar Ghouth
The Worsley Building
Clarendon Way
LS2 9LU

Dear Mr Ghouth

Study title:	**A prospective study to assess the diagnostic accuracy of laser Doppler flowmetry in assessing pulp vitality of traumatised teeth in Paediatric patients**
REC reference:	**17/YH/0025**
IRAS project ID:	**217461**

Thank you for your letter of 20th February, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper [Covering Letter]	1	20 February 2017
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Sponsor letter]		08 September 2016
GP/consultant information sheets or letters [General Dental Practitioner]	1	10 October 2016
IRAS Application Form [IRAS_Form_12012017]		12 January 2017
IRAS Application Form XML file [IRAS_Form_12012017]		12 January 2017
IRAS Checklist XML [Checklist_20022017]		20 February 2017
Letter from sponsor [Sponsor letter]		08 September 2016
Letters of invitation to participant [Invitation Letter]	2	15 February 2017
Participant consent form [Consent]	3	15 February 2017
Participant consent form [Assent]	2	15 February 2017
Participant information sheet (PIS) [Parents information sheet]	3	15 February 2017
Participant information sheet (PIS) [PIS children 6-12]	3	15 February 2017
Participant information sheet (PIS) [PIS 12-16 years old]	2	15 February 2017
Research protocol or project proposal [Protocol]	8	14 December 2016
Summary CV for Chief Investigator (CI) [Chief Investigator CV]	1	20 May 2015
Summary CV for supervisor (student research) [Supervisor CV]	1	22 June 2015

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

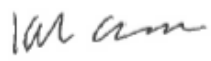
We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

17/YH/0025	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

pp



Dr Rhona Bratt
Chair

Email:nrescommittee.yorkandhumber-leedseast@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Anne Gowing, Research & Innovation Department Leeds Teaching Hospitals Trust*

Health Research Authority

Mr Nahar Ghouth
The Worsley Building
Clarendon Way
LS2 9LU

Email: hra.approval@nhs.net

02 March 2017

Dear Mr Ghouth

Letter of HRA Approval

Study title:	**A prospective study to assess the diagnostic accuracy of laser Doppler flowmetry in assessing pulp vitality of traumatised teeth in Paediatric patients**
IRAS project ID:	**217461**
REC reference:	**17/YH/0025**
Sponsor	**University of Leeds**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details

RE: IRAS 217461 Laser Doppler flowmetry in assessing the vitality of traumatised teeth Outcome of Application for HRA Approval.

Please find attached a letter informing you of the outcome of your application for HRA Approval.

Please read the attached documents with care.

You may now commence your study at those participating NHS organisations in England that have confirmed their capacity and capability to undertake their role in your study (where applicable). Detail on what form this confirmation should take, including when it may be assumed, is given in Appendix B of the HRA Approval letter.

If you have any queries please do not hesitate to contact me.

Kind regards

Alison

Alison Thorpe | Senior Assessor
Health Research Authority
Nottingham HRA Centre, The Old Chapel,
Royal Standard Court, Nottingham NG1 6FS
E: alison.thorpe1@nhs.net | T: 020 7104 8064
| www.hra.nhs.uk

Would you like to receive the latest updates on HRA work? Sign up here

For more information on the HRA Approval process Click here

Please note my working days are Tuesday – Friday

Appendix 11: R& I e-mail approval

Dear Nahar,

Re. A prospective study to assess the diagnostic accuracy of laser Doppler flowmetry, R&I No: DT16/232

This email confirms that the Leeds Teaching Hospitals NHS Trust has the capacity and capability to deliver the above research study, based upon Protocol version 8.0 dated 14/12/2016. You may now begin the study at this organisation.

It is the responsibility of the principal investigator to ensure that the study is conducted in accordance with the terms of the Health Research Authority approval and Leeds Teaching Hospitals NHS Trust policies and procedures including the requirements for research governance and clinical trials performance management. These are available at http://www.leedsth.nhs.uk/assets/Uploads/PI-responsibilities-v1.3-210716.docx

If you have any queries please do not hesitate to contact the R&I team at leedsth-tr.lthtresearch@nhs.net.

Best wishes,

Anne Gowing
Research Governance Manager, Research & Innovation Department

Appendix 12: Study documents for clinical study 2

IRAS Number 217461

Leeds School of Dentistry- The Centre for Oral Health Sciences
Department of Paediatric Dentistry
A Centre for Children with Special Needs
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Information for the person with parental responsibility

"A study to assess the best way to tell if a tooth is alive or dead?''

Please take time to read the following information carefully. If you have any questions please ask us on your next visit.

Why have I been given this leaflet?

We would like to invite your child to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being carried out and what it will involve.

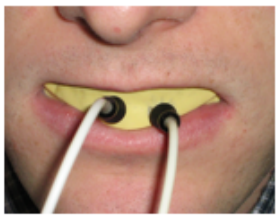
What is the purpose of this study?

Your child sustained an injury to one or more of his/her teeth causing the nerve inside the tooth to be damaged and there is possibility that the tooth will be dead. As a result, we will monitor the injured teeth with the conventional tools as well as a new tool using a laser device. It is difficult to determine if the nerve in the tooth is dead or alive with the current techniques. So we are introducing a new technique that we think could help us to make this decision.

To do this we use a laser machine which is safe, painless, and has been routinely used for almost 30 years in dentistry for both adults and children. This machine measures the blood supply inside the tooth and so we can find out if the tooth is alive or dead. Your child's participation will help us compare the new machine to the conventional techniques (the electrical test and cold test) and help children with injured teeth in the future.

We will test your child's teeth using all the conventional methods and the laser machine. We anticipate that your child's participation in our study would take approximately 15 minutes and during your appointment.

You do not need to come for specific visits for the research. It will be done during the normal follow up visits as part of your child's normal care which will be every 3 months and will take only 15 minutes in addition to your normal appointment.



The Leeds Teaching Hospitals NHS
NHS Trust

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What is the benefit to my child taking part in this research?

The laser machine is an additional diagnostic tool to the conventional tools we currently have. It will help us in the decision making regarding whether the tooth is alive or dead hopefully to avoid the complications of an unknown dead tooth which include pain, infection and swelling.

Would my child be disadvantaged by using either of the two techniques?

No, your child will not be affected at all. The reason we are asking your child to help with the study is that we already know he/she has an injured tooth and we are not sure whether this tooth alive or dead. This will help us check if this new technique is good enough in predicating the condition of the injured tooth. Your child participation, however, would help us better use the laser machine which could help other children in the future. We will inform your child's General Dental Practitioner about the research study.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Leeds or Leeds Teaching Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.'

Who is doing the study?

This study will be conducted at the children's department at the Leeds Dental Institute by one of the paediatric dentistry trainees (Nahar Ghouth), under the supervision of (Dr.Hani Nazzal) a Clinical Lecturer and Consultant Paedaitric Dentist, and an Honorary Consultant Paediatric Dentist (Professor Monty Duggal).

Does your child have to take part?

No.

What will happen if your child takes part in this study?

During your child's next appointment, you would be able to ask Nahar Ghouth, any questions you might have about the study. If you require further time to consider the information and make a decision, we will arrange to see you again at your next appointment or arrange a separate appointment should that be more convenient for you.

If you decide that your child will participate, the following will occur:

1) You will be asked to sign a document (Consent) stating that you are happy for your child to take part in the study and understand what is involved.

2) We will assess the status of the injured tooth/teeth and the alive teeth for your child with the previously mentioned methods.

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Are there any side effects?

There are no side effects using all methods. The laser technique is a safe and painless technique that has been used for almost 30 years with both adults and children.

Taking x-rays is a part of your routine care. If your child takes part in this study he/she will not undergo any additional *x-rays*.

X-rays use ionising radiation to form images of your child's body and/or provide treatment and/or provide your doctor with other clinical information. Ionising radiation can cause cell damage that may, after many years or decades, turn cancerous. The chances of this happening to your child are the same whether your child takes part in this study or not."

Can your child withdraw from the study at any time?

Your child can withdraw from the study at any time without giving any reasons. Your child will still receive the appropriate treatment and review depending on the treatment he/she received.

Will the information I give be kept confidential?

All information regarding your child's treatment is confidential and will be stored in your child's NHS records, which are only accessible by NHS staff. Any data we collect will be stored using non-identifiable information on password-protected computers. All research documents with patients' information will be stored in a locked filing cabinet at Leeds Dental Institute that will only be accessible to the chief investigator. For audit purposes, the data will be stored for 3 years.

What will happen to the results of the study?

The results of the study will be discussed with other colleagues in the department, presented at conferences and in scientific journals. Your child's identifiable information will not be used in these settings.
Should you want to know the results of the study, please let one of the research team know and we will inform you of the results after we obtain them.

Who reviewed this study?

All research carried out by the NHS is reviewed by an independent group of people, called research ethics committee, to protect your safety, rights, wellbeing and dignity prior to starting the research.

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What if I need further information or need to complain?

Should you need any other information or like to complain please contact one of the research team using the following contact details. Alternatively, you could contact Patient Advice and Liaison Service on (0113 2067168) or patient relations department on Telephone (01132066261).

Chief Investigator	**Primary supervisor**
Nahar Ghouth	Hani Nazzal
Postgraduate student	NIHR clinical lecturer and speciality registrar
Leeds Dental Institute	Leeds Dental Institute
Clarendon Way	Clarendon Way
LS2 9LU	LS2 9LU
Tel.: 01133438139	Tel.: 01133438139
Email:dnnng@leeds.ac.uk	Email: denha@leeds.ac.uk

Thank you for taking the time to read this information sheet and for considering this study.

IRAS Number 217461

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Consent Form

Title of project: A prospective study to assess the diagnostic accuracy of laser Doppler flowmetry in assessing pulp vitality of traumatised teeth in paediatrcic patients.

(A study to assess the best way to tell if a tooth is alive or dead?)

Name of Researcher: Nahar Ghouth

Please initial Box

1. I confirm I have read and understood the information sheet dated 27/02/2017 (Version 4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my child's participation is voluntary and that my child is free to withdraw at any time without giving any reason and without my child's medical care or legal rights being affected. ☐
3. I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the school of dentistry, from regulatory authorities or from the NHS Trust, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child's records. ☐
4. I agree to my General Dental Practitioner being informed of my child's participation in the study. ☐
5. I agree for my child to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Parent	Date	Signature
Name of Person taking assent	Date	Signature

Version number 2 Date 15.02.2017 Centre Number: 1
IRAS Number 217461

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Patient Identification Number for this study:

ASSENT FORM

Title of project: A study to assess the best way to tell if a tooth is alive or dead?

Name of Researcher: Nahar Ghouth

Please tick Box

1. I am happy with the explanation given to me by the dentist. ☐
2. I know that I don't have to take part in the study and I can ask the dentist to stop at any time. ☐
3. I agree to take part in the study. ☐

----------	----------	----------
Name of Patient	Date	Signature
----------	----------	----------
Name of Parent	Date	Signature
----------	----------	----------
Name of Person taking assent	Date	Signature

The Leeds Teaching Hospitals NHS
NHS Trust

Version number 2 Date 15.02.2017

IRAS Number 217461

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Parental Invitation to Participation letter

Name of Researcher : Nahar N . Ghouth

Dear Parent/Guardian:

We would like to invite you to take part in our study Titled:

"A study to assess the best way to tell if a tooth is alive or dead?''

I am conducting a research project comparing two different methods testing whether the tooth is alive or not after dental injuries. Your child has an injured tooth/teeth and therefore would be suitable for our study.

These methods are well used in dentistry, in addition to being used for many years on both adults and children. The methods are very simple, non invasive and your child will not feel any pain. One of the methods involves the use of a laser machine which uses very low powered laser. This machine has been safely used previously all over the world with no side effects reported. This machine has also been used in our department on children for the past few years. The other methods involve the use of a cold test where your child might feel a cold sensation and an electrical test where your child might feel a tingling sensation.This is done to assess if the tooth is responsive or not.

During your next visit, you will be able to ask any questions and hopefully be in a position to make a decision regarding your child's participation.

You do not need to come for specific visits for the research. It will be done during the normal follow up visits as part of your child's care which will be every 3 months and will take only 15 minutes in addition to your normal appointment.

Please be advised that should you decide not to take part in the study, we would still provide your child with the treatment needed at that appointment

Thank you for taking the time to read this letter and the attached information leaflets. Should you have any questions please do not hesitate to either contact me prior to your appointment or ask me during the appointment session.

Many thanks,

Nahar Ghouth
Postgraduate student in Paediatric dentistry
Under the supervision of Dr Hani Nazzal and Prof.Monty Duggal

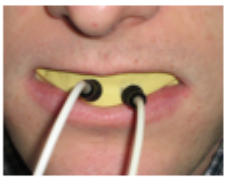
"A study to assess the best way to tell if a tooth is alive or dead?''

Information for children 6-12 years of age

Why has the dentist asked me to read this paper?

We need your help in our experiment. You broke your tooth and the nerve inside your tooth is now damaged. We have a new laser machine that can tell us how damaged your tooth is. By doing this, you can help other children in the future when they break their teeth.

Your tooth might feel cold or you might feel a slight tingling sensation when we use our machines. The new laser machine has a tiny camera and a magic light which we will shine on your tooth. We will show you and explain everything before we use it.



Will anything hurt?

Nothing will hurt at all. If you feel anything you don't like, just tell us and we will stop.

Do I have to take part?

No

How long will it take?

It should not take long, just around thirty minutes (30 minutes). You do not need to come for specific visits for the research. It will be done during the normal follow up visits as part of your care which will be every 3 months and will take only 15 minutes in addition to your normal appointment.

Can I change my mind?

Of course you can change your mind anytime. You do not even need to tell us the reason you changed your mind.

Will you still fix my tooth if I choose not to take part in the study?

Yes, of course.

Thank you for reading this information leaflet.

Version number 2 Date 15.02.2017

IRAS Number 217461

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''A study to assess the best way to tell if a tooth is alive or dead?''

Information for young people 12-16 years of age

Why have I been given this leaflet?

We would really like your help with our study. This letter will help you decide whether you want to help us or not.

Why me?

You had an injury to your tooth and the nerve inside the tooth is injured. As a result, we need to monitor the injured teeth as the nerve might die. It is difficult to say when the nerve in the tooth is dead or not with the current techniques. So we have a new technique that we think could help us to make this decision. We have a new laser machine that can help us to test if the nerve inside your tooth is alive or dead, and we need to test if it can predict the condition of your tooth.

We will compare the laser machine to the current methods we use these days. So, we will test your tooth with both techniques. The laser machine has a tiny camera and a light which we will shine on your tooth. We will explain every step to you before we do it and we will stop if you raise your hand.

Why are we doing this study?

The current technique used to test the nerve inside the tooth is not very good. We hope this new machine would help other children when they damage their teeth. By helping us with this study you may be helping other children in the future.

Do I have to take part?

No

Version number 2 Date 15.02.2017
IRAS Number 217461

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Will anything hurt?

Nothing will hurt at all. If you feel anything you don't like, just tell us and we will stop.

Taking x-rays is a part of your routine care. If you take part in this study you will not undergo any additional x-rays. X-rays use ionising radiation to form images of your body and/or provide treatment and/or provide your doctor with other clinical information. Ionising radiation can cause cell damage that may, after many years or decades, turn cancerous. The chances of this happening to you are the same whether you take part in this study or not."

How long will it take?

It should not take long, just around fifteen minutes (15 minutes).

You do not need to come for specific visits for the research. It will be done during the normal follow up visits as part of your care which will be every 3 months and will take only 15 minutes in addition to your normal appointment.

Can I change my mind?

Of course you can change your mind anytime. You do not need to tell us the reason you changed your mind.

Will you still fix my tooth if I do not do take part in the study?

Yes, of course.

Thank you for reading this information leaflet

Appendix 13: Data collection sheet for clinical study 2

Patient: Age: Sex: 1-Male 2-Female

Type of injury:

Tooth	TTP	Tenderness to palpation	Mobility	Colour	EC	EPT	Root formation
	Positive or negative	Positive or negative	0, 1, 2, 3	Normal,yellow, grey			
	Positive or negative	Positive or negative	0, 1, 2, 3	Normal,yellow, grey			

Tooth	TTP	Tenderness to palpation	Mobility	Colour	EC	EPT	Root formation
	Positive or negative	Positive or negative	0, 1, 2, 3	Normal,yellow, grey			
	Positive or negative	Positive or negative	0, 1, 2, 3	Normal,yellow, grey			

Tooth	TTP	Tenderness to palpation	Mobility	Colour	EC	EPT	Root formation
	Positive or negative	Positive or negative	0, 1, 2, 3	Normal,yellow, grey			
	Positive or negative	Positive or negative	0, 1, 2, 3	Normal,yellow, grey			

Appendix 14 : Accepted manuscript in the British Dental Journal

The use of dental pulp tests in children with dental trauma: a national survey of the British Society of Paediatric Dentistry's members

Abstract:

Background: Careful long-term monitoring of pulp vitality has been recommended by all dental trauma guidelines. It is essential to explore the methods and techniques used by UK dental practitioners in assessing pulp sensibility and vitality.

Aim: To study the use of dental pulp tests by paediatric dentists and general dental practitioners in children with dental trauma.

Design: A cross-sectional study utilising an 18-item questionnaire that was developed using the Bristol Online Survey (BOS) tool and circulated electronically to the members of the British Society of Paediatric Dentistry between June and August 2017.

Results: One hundred and forty-one respondents included in the analysis, paediatric dental specialists (56%) and GDPs (44%). Almost all specialists (93.7%) reported using sensibility tests routinely in comparison to 80.6% of GDPs. Child perception and cooperation were the most commonly reported barriers. GDPs mainly used cold testing, while specialists used cold and electric pulp tests equally. Inconsistencies in recording as well as documentation the results varied among respondents. Only a few specialists reported having some experience in using laser Doppler flowmetry.

Conclusions:

The use of pulp sensibility tests was relatively high amongst respondents while those of vitality tests were very low. Barriers and inconsistencies in the technique and recording of the results of sensibility tests were evident. The frequency and timing of using sensibility tests in line with international guidelines were stressed. The use of standardised techniques involving methods considered to improve reliability was highlighted.

Introduction:

Dental trauma, affecting incisors, has been shown to affect 12% and 10% of the UK's 12 and 15-year-old children, respectively.[1] Complications such as loss of pulp vitality and root resorption could develop as a consequence of such injuries leading to long-term irreversible damage or even tooth loss.[2] The risk of pulp necrosis after crown fractures ranges between 0.2 and 6%, increasing with concomitant luxation injuries.[3,4] Pulp necrosis after luxation injuries ranges between 15 and 59% with the highest frequency associated with intrusive luxation. The least occurrence of pulpal necrosis, on the other hand, is following concussion and subluxation injuries.[5,6] Consequently, accurate diagnosis and monitoring of the pulp status and periodontal tissues of traumatised teeth are essential.

The use of dental pulp sensibility/vitality tests is an integral part of the pulp assessment process following dental trauma.[7] An ideal pulp test should provide a ''*simple, objective, standardised, reproducible, non-painful, non-injurious, accurate and inexpensive*'' way of assessing the condition of the pulp tissue.[8] Several diverse sensibility and vitality pulp diagnostic tests are available.

Sensibility tests offer an assessment of pulp health through the stimulation of pulp nerve fibres. Vitality testing, on the other hand, involves assessing the tooth's blood supply offering an objective approach to assessing pulp vitality that is not reliant on patients' understanding and response to stimuli. Among vitality tests, laser Doppler flowmetry (LDF) has been developed for the assessment of pulp blood flow. Studies suggest that LDF is able to determine pulp vitality (blood supply) offering a better pulp evaluation of traumatised teeth in comparison to other dental pulp tests.[9]

Thermal and electric pulp testing (EPT) are the most commonly used pulp sensibility tests. The use of these conventional pulp tests in assessing pulp sensibility of children's' teeth is subjective and relies on patient's understanding and cooperation which can be challenging especially in the child population. Thus, false positive/negative results are often associated with the use of sensibility tests which can sometimes be misleading in clinical situations. [10]

There are recommendations and techniques to overcome some of the limitations of sensibility tests. [10,11] Therefore, it was considered important to explore the methods and techniques used by UK general dental practitioners (GDPs) and paediatric dental specialists in assessing pulp sensibility and vitality, especially in the child population following dental trauma. This would also help understand compliance, limitations and barriers to the use of the tests in complying with current guidelines. This survey aimed to investigate paediatric dentists' and GDPs' use of sensibility/vitality tests and the barriers to routinely using such tests in assessing dental trauma in children.

Methods

This was a cross-sectional study utilising an 18-item questionnaire aiming to investigate the use of sensibility and vitality tests in the management of dental trauma in children amongst UK paediatric dentists and GDPs. Institutional ethical approval was obtained from the University of Leeds Research Ethics Committee prior to the commencement of the study (300317/NG/226). The questionnaire was developed using the Bristol Online Survey (BOS) tool, now known as online surveys, and piloted on a small group of 10 dentists (Specialist paediatric dentists, specialist registrars in paediatric dentistry and GDPs) for ease of understanding and reduction of the ambiguity of questions prior to administration. An invitation email explaining the aims of the survey questionnaire was circulated electronically to the members of the British Society of Paediatric Dentistry (BSPD) between 23ed June and 15th August 2017 with a reminder email sent on 18th July 2017. Individual follow-up correspondence with non-respondents was not carried out due to the anonymity of the survey. The UK based paediatric dental specialists, paediatric dental trainees, GDPs working in the capacity of specialists in paediatric dentistry, such as non-specialist senior dental officers in paediatric dentistry, lecturers in paediatric dentistry or GDPs with advanced training in paediatric dentistry, and GDPs were included in the study. Non-UK based practitioners and retired dentists/specialists were excluded. Information collected in the questionnaire included the following:

Part A: Demographic data including positions held and frequency of treating children with traumatised permanent teeth.

Part B: General questions on the clinical use of dental pulp tests.

Part C: Specific questions on the use of cold sensibility testing.

Part D: Specific questions on the use of EPT.

Part E: Specific questions on the use of the LDF.

Data collected were entered into a statistics program (IBM SPSS version 22). Descriptive statistics analysing participants' responses were computed.

Results

- **Participants:**

The email invite was sent to all BSPD members (732 members), the membership of which included both UK registered paediatric dentistry specialists and GDPs who have an interest in children's dentistry. A total of 149 respondents completed the survey; of which 8 respondents were excluded (2 retired dentists, 2 special care dentists and 4 dentists who did not treat children with dental trauma).

The remaining 141 respondents were split into a paediatric dental specialist (79, 56%) and GDP groups (62, 44%). The paediatric dental specialist group included 68 registered paediatric dental specialists, eight paediatric dental trainees and three speciality dentists. Consequently, a specialist response rate of 35% (68 BSPD registered specialists out of 192 BSPD registered specialists) was achieved in this survey and an overall response rate of 20.3% (149 out of 732). The GDP group included 10 Community Dental Practitioners and 52 GDPs. A GDP response rate could not be calculated as the BSPD does not hold an overall number of GDP members.

- **Dental trauma experience:**

More than half of the specialists (45/79, 57%) reported seeing more than 8 patients a month, while, the majority of GDPs (42/62, 67.7%) reported seeing a maximum of 2 children with a history of dental trauma a month (Fig. 1).

- **General use of dental pulp tests:**

The majority of the respondents (124/141, 87.9%), with almost all specialists (74/79, 93.7%) reported using sensibility pulp tests routinely in the management of traumatised teeth in children in comparison to (50/62, 80.6%) of GDPs (Table 1).

Different barriers to the use of sensibility testing among those who reported not using the tests routinely were reported with child perception and cooperation being the mostly reported barriers among both groups. Other barriers were also reported including the cost of the tests, the tests are time-consuming, and they do not provide additional information.

On average, most of the respondents reported using dental pulp testing at initial presentation and then at specific intervals (128/141, 90.8%). Almost all of the specialists (78/79, 98.7%) reported using dental pulp tests on initial presentation and specific intervals, in comparison to 83.9% of GDPs (52/62) (Table 1).

Type of sensibility/vitality tests used

The most common type of sensibility/vitality tests used by all respondents was cold testing (137/141, 97.2%) followed by EPT (94/141, 66.7%). None reported using LDF. Six respondents (4.2%) reported the use of heat testing.

GDPs mainly used cold testing 60/62 (96.8%) rather than other tests such as EPT (28/62, 45.2%), while specialists used cold and EPT tests equally (77/79, 97.5%) and (76/79, 96.2%), respectively (Fig. 2).

Reliability of sensibility tests

The reliability of dental pulp tests was considered inconsistent with almost half the number of GDPs (32/62, 51.6%) and almost two-thirds of the specialist group (50/79, 63.3%) considering these tests to be sometimes reliable (Table 2). Different reasons for such inconsistency of reliability were reported including children's understanding and cooperation, anxiety and stress, age, root formation, tests are not reliable in the early stage of trauma and issues with sensitivity and specificity of the tests. Techniques used in improving test reliability in children are shown in Table 2.

Different techniques have been used by the respondents in order to improve reliability of dental pulp tests such as using a control tooth for the child to experience the desired sensation, repeating the test on each tooth, and applying a false positive reading such as applying a dry cotton pledget (Table 2). The most commonly used single method by both the GDP and specialist groups was the use of a control tooth, while the least commonly used method was applying a false positive reading.

- **Cold test use among respondents:**

Almost all respondents reported using cold tests (139/141, 98.6%) with ethyl chloride being reported as the most commonly used cold testing agent with comparable use between the two groups (Fig. 3). Three-quarters of all respondents (106/139, 76.2 %), of which 80.3% (49/61) and 73% (57/78) were GDPs and specialists, respectively, did not apply the cold test for a specific period on each tooth. Those who did, however, used a range of time between 1 and 20 seconds per tooth.

Inconsistencies in recording the results of the cold test were also observed with the majority of GDPs (43/61, 70.5%) and specialists (55/78, 70.5%) recording the results as positive and negative with no record of reliability of results.

- **EPT use among respondents:**

Almost half of the GDPs (30/62, 48.4%) and the majority of the specialists (67/79, 85%) reported using EPT when treating traumatised permanent teeth in children.

Documentation of the results of the EPT varied among respondents with most specialists (48/67, 71.6%) and just over half of GDPs (17/30, 56.6%) documented the numerical values of the EPT rather than whether the results were reliable or unreliable. Approximately, 20% of both groups equally reported recording whether the results were reliable or not (Fig. 4.a and b). There were differences in the recording of sensibility test results as detailed in Figure 4b with more than half of all participants 52/97 (53.6%) recording only the most reliable/consistent EPT reading, of which 22/30 (73.3%) GDPs and 30/67 (44.8%) specialists.

- **LDF use among respondents**

Only 9/141 (6.4%) respondents reported having some experience in using LDF, of which all were specialists. The main reason reported for using the LDF was the need for a test able to assess tooth vitality (blood flow) rather than sensibility (nerve supply).

Different barriers to the use of LDF by GDPs were reported as the lack of knowledge of such technique was the mostly reported barrier for GDPs, (29/62, 46.7 %), compared to the lack of training as reported by the specialist group (33/70, 47%) (Fig. 5).

Discussion

Loss of tooth vitality is one of the sequalae of dental trauma, and careful long-term monitoring of pulp vitality has been recommended by all dental trauma guidelines in order to avoid unwanted complications.[12,13,14] Different pulp sensibility and vitality tests are available, however, to date no one test has been shown, based on high-quality evidence, to be more superior in terms of sensitivity and specificity.[15] It has been argued that the use of LDF, whereby pulp blood flow is measured, is more appropriate and accurate in assessing pulp vitality rather than sensibility, therefore, reducing false negative and false positive results.[9]

The authors acknowledge that few UK based specialists might not be members of the BSPD. That being said, the results included the participation of a large number of UK based specialists and practitioners working in the capacity of paediatric dental specialists, with a reasonably good representation of paediatric dental specialists across the country. Also, such a cohort of GDPs might not fully represent UK GDPs as those BSPD GDP members are likely to be more interested in managing children with dental trauma than the average GDP population.

An attempt was made initially to get a wider sample of GDPs and paediatric dentistry specialists by contacting the GDC. Unfortunately, due to a recent change in the GDC's published member's information, such information was no longer available online. In addition, the GDC was neither able to share their members' addresses nor willing to forward electronic surveys to their members. Furthermore, attempting to distribute the survey to all practitioners in the Yorkshire and Humber region through contacting the Local Professional Network (LPN), was also unsuccessful. The BSPD was not able to share their member's contact details, but

agreed to forward an electronic survey to all their registered members. The survey was distributed through the mailing list.

The results of this survey showed a reasonable exposure of both specialists and GDPs to children with traumatised permanent teeth with the specialists expectedly reporting more exposure than GDPs. Such difference in exposure to this group of children is understandable since UK GDPs refer most trauma cases, especially severe traumatic injuries, to paediatric dental specialists for management.[16] It is essential that general dental practitioners have a sound knowledge about managing dental trauma, especially the initial treatment and management. [17]

Despite their limitations, sensibility tests are extremely useful tools in assessing/monitoring pulp status and should be used as part of clinical examination at initial trauma time and review appointments as recommended by the IADT. Lauridsen et al.[18,19,20] showed the importance of using EPT at initial trauma in identify teeth at increased risk of pulp necrosis. Therefore, the routine use of sensibility tests by most respondents especially at initial trauma was in line with published guidelines. More exposure of specialists to children with dental trauma could explain the discrepancy in the routine use of sensibility tests by the two groups with more specialists than GDPs using these tests routinely. Around 1.3% of specialists reported using sensibility tests only when symptoms arise, and around 5% of GDPs reported using sensibility tests only at initial trauma.

The overwhelming use of cold and EPT among all respondents could be attributed to the availability, ease of use of these tests, the cost-effectiveness, and high accuracy reported of these tests.[21] The lack of use of vitality tests such as LDF among respondents could be attributed to the higher cost, and lack of high-quality evidence supporting the superiority of this technique over other sensibility tests.[15] In addition, very few specialists have reported having a previous experience using LDF mainly in research.

Ethyl chloride and refrigerant sprays cold agents have been used by most respondents. Ethyl chloride has a temperature of -12.3 °C while the temperature produced by different refrigerant sprays such as Endo-Ice, Green Endo-Ice and Endo-Frost varies and ranges from -20 °C to -50 °C.[10] The sensitivity of ethyl chloride has been reported to range between 53 and 92 % while that of Endo Ice refrigerant spray ranges between 81 to 100%. Specificity, on the

other hand, ranged between 89-100% and 76-100% for ethyl chloride and Endo ice, respectively.[23]

The correct use of cold tests is important in improving accuracy, reliability and reproducibility of these tests. Patients need to fully understand the feeling of cold tests as well as when and how to respond to the stimulus. Applying the cold stimulus to unaffected teeth before using the tests on affected teeth (with questionable pulp status) so that patients are aware of the cold stimulus sensation is important in reducing false results. The use of dry cotton pellets to test patient compliance and understanding of the test is also recommended. [10]

The application of cold tests requires a carrier such as a cotton pellet saturated with the sprayed agent applied with direct contact to the tooth tested.[22] Larger pellets have larger surface areas than smaller cotton pellets, thus allowing better thermal conduction. Cotton buds with wooden handles and small cotton pellets have smaller surface areas are therefore less efficacious in thermal conduction.[23] The application of the cotton pellet to the middle third of the labial/buccal surface of the crown for 5-8 seconds is recommended.[24,25] Avoiding contact with the gingival tissues is also important to reduce false positive results.

When using EPT, a positive response is the result of an ionic shift in the dentinal fluid within the tubules causing local depolarisation and thus the generation of action potential from intact nerves.[26] A positive response simply indicates that there are sensory fibres present within the pulp that can respond to the electrical stimulus. However, necrotic pulp tissue can leave electrolytes in the pulp space, which are able to conduct the electricity to the nerves further down the pulp space, simulating a normal pulp response.[27] In general, EPT is more reliable in detecting vital teeth rather than non-vital teeth. The sensitivity of EPT ranges between 67 and 100% while the specificity ranges between 88 and 100%.[21]

Applying the EPT on unaffected teeth prior to use to enhance patient understanding is also needed. Drying the tooth is essential in preventing false positive results due to electrical conduction to the adjacent teeth, or periodontium.[28] If possible, the contralateral tooth should be tested in order to establish a baseline response. Teeth should be tested at least twice to confirm the results and ensure consistency.[29] Changing the sequence of the teeth being tested has been reported to increase the reliability of EPT.[11] Another method is to change the speed of the current applied so that a faster current is applied. However, the numerical values of EPT have significance only if there is a high difference between the traumatised tooth and the vital

control teeth. The numerical value of the responses should be recorded for each tooth. The electrode should be applied to the middle third of the facial/ labial surface of the tooth with direct contact with the tooth structure. [30]

The value of sensibility tests is highly dependent on a number of factors including patient's understanding, compliance and cooperation and the degree of root development. Therefore, limiting their use in children, patients with learning disabilities and patients with limited communication. Such limitations were reported by respondents showing good understanding and appreciation of these limitations. Therefore, recording the results of such techniques with a comment on the reliability of the results and/or any limiting factors should be encouraged.

The ability of LDF in measuring the tooth's pulp blood flow rather than innervation lead to its use as a pulp vitality tester. The objectivity of this test (lack of dependence on patient's response) further supported its use.[31] The laser light reaches the pulp through the dentinal tubules acting as a guide. When light enters the tissue, it gets absorbed and scattered by the moving and circulating red blood cells. Laser photons are then shifted against moving red blood cells and backscattered/reflected back into a photodetector leading to a signal production.[32]

It has been reported that LDF is able to determine pulp vitality and offers a better chance of evaluating traumatised teeth than other pulp tests. Clinical studies have shown that LDF has higher sensitivity and specificity when compared to other pulp tests.[33,34] However, the cost of the equipment is considered to be high when compared to other pulp tests. Moreover, it is technique sensitive. Thus, careful interpretation of the results should also be considered.[9]

Recommendations

Although the use of pulp sensibility tests was relatively high within the cohort selected for this study when assessing traumatised teeth in children, GDPs and specialists should:

1) Routinely use sensibility tests with all traumatised teeth mainly at baseline and key review appointments as per IADT guidelines. [12,13]
2) Use a standardised technique able to reduce false results as described above and in order to be accurately compared with future pulp test results.

3) Record the reliability of the results depending on their assessment of patient understanding, cooperation and response to contralateral healthy teeth and repeated measurements.
4) Interpret the results of the sensibility tests within the overall clinical assessment due to the inherent limitations of these tests.

Conclusion

The use of pulp sensibility tests was relatively high, but inconsistency in technique and recording of results was evident within the cohort selected for this study. Several barriers usually associated with the child patient, including cooperation, understanding and age were identified. The use of vitality tests and especially LDF was extremely low. It appears that there is a need to encourage vitality testing, including possibly the use of LDF in clinical practice for a better evaluation of the dental pulp. The high cost, the difficulty of the technique and training as well as limited knowledge about LDF are certainly a limiting factor in its widespread use.

Declaration of interests:
The authors declare no conflicts of interest nor did any funding attain in the conduct of this questionnaire study.

Acknowledgements:
The authors would like to thank the BSPD for distribution of the questionnaire survey to their members and all BSPD members who took the time to complete the survey.

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Fig. 1 Bar chart showing the number of children with traumatised permanent teeth per group of ndents seen in a month.

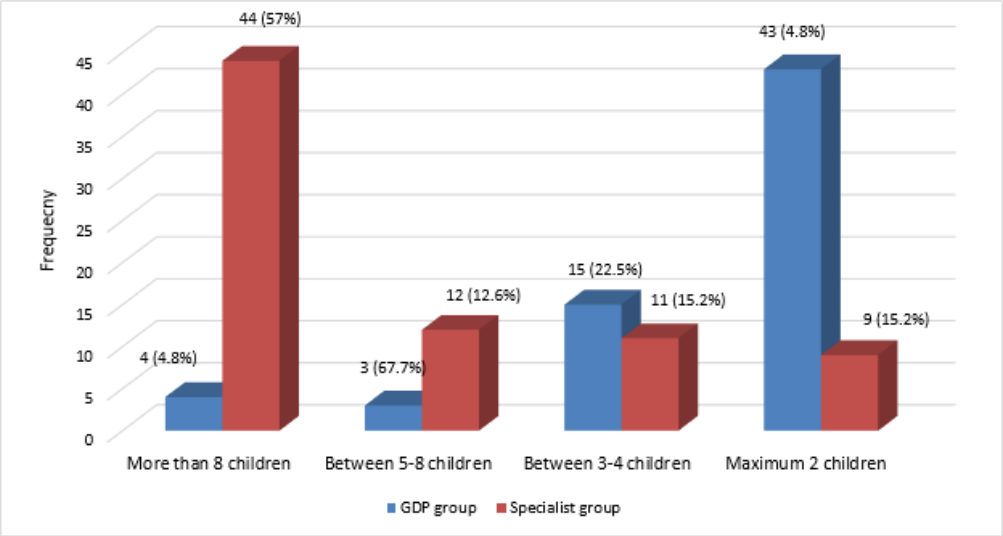


Table 1 General use of dental pulp tests

The overall frequency of using dental pulp tests			
		Frequency	Percentage %
Yes, routinely	GDPs	50/62	80.6
	Specialists	74/79	93.7
Sometimes	GDPs	12/62	19.4
	Specialists	5/79	6.3
No	GDPs	0	0
	Specialists	0	0
The timing of using dental pulp tests following traumatic dental injuries			
On initial presentation and at specific intervals	GDPs	52/62	83.9
	Specialists	78/79	98.7
At review appointments	GDPs	5/62	8.1
	Specialists	0	0
Only initially at the time of trauma	GDPs	3/62	4.8
	Specialists	0	0
Only when new symptoms arise	GDPs	2/62	3.2
	Specialists	1/79	1.3

Fig. 2 Bar chart showing types of sensibility/vitality tests used by respondents per group

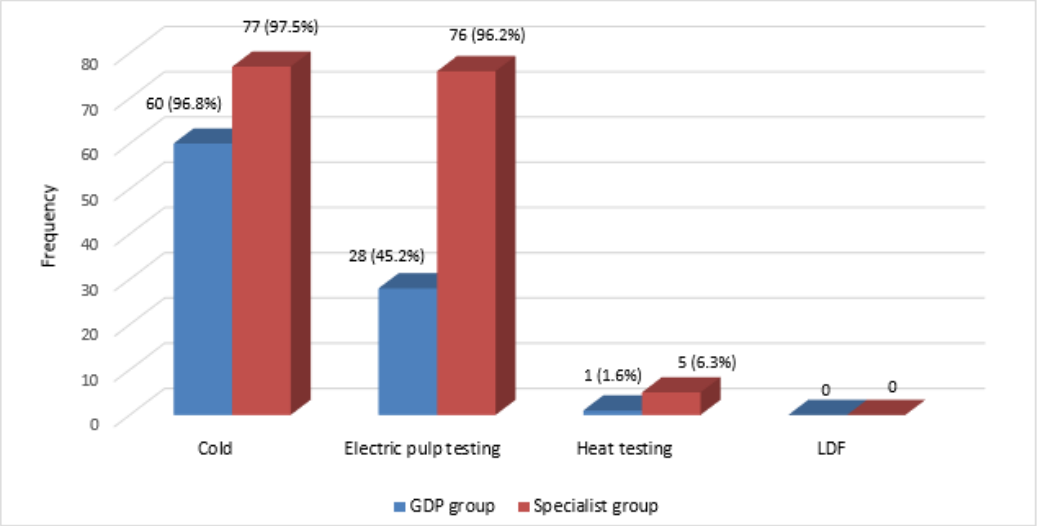


Table 2 Reliability of sensibility tests

Perception of the reliability of sensibility tests by respondents			
Chart Area		Frequency	Percentage %
Yes,	GDPs	16/62	25.8
	Specialists	18/79	22.8
Sometimes	GDPs	32/62	51.6
	Specialists	50/79	63.3
No	GDPs	14/62	22.6
	Specialists	11/79	13.9
Practical techniques performed by respondents in improving the reliability of sensibility tests			
I use a control tooth for the child to experience the desired sensation	GDPs	56/62	90.3
	Specialists	69/79	87.3
I repeat the test on each tooth	GDPs	40/62	64.5
	Specialists	57/79	72
I apply a false positive reading such as applying a dry cotton pledget.	GDPs	17/62	27.4
	Specialists	37/79	46.8
I do not do anything in specific	GDPs	1/62	1.6
	Specialists	2/79	2.5

Fig. 3 Bar chart showing types of cold tests used

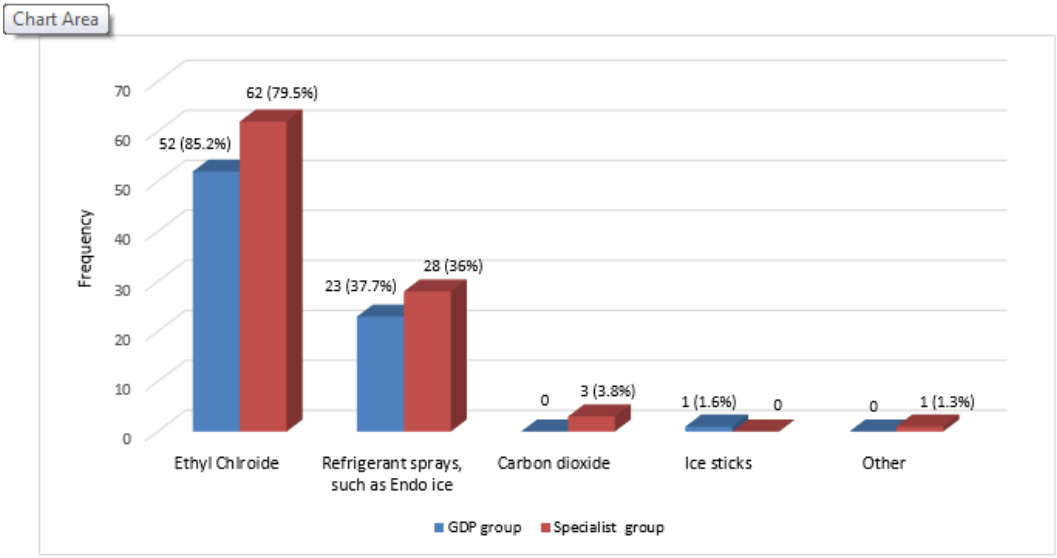
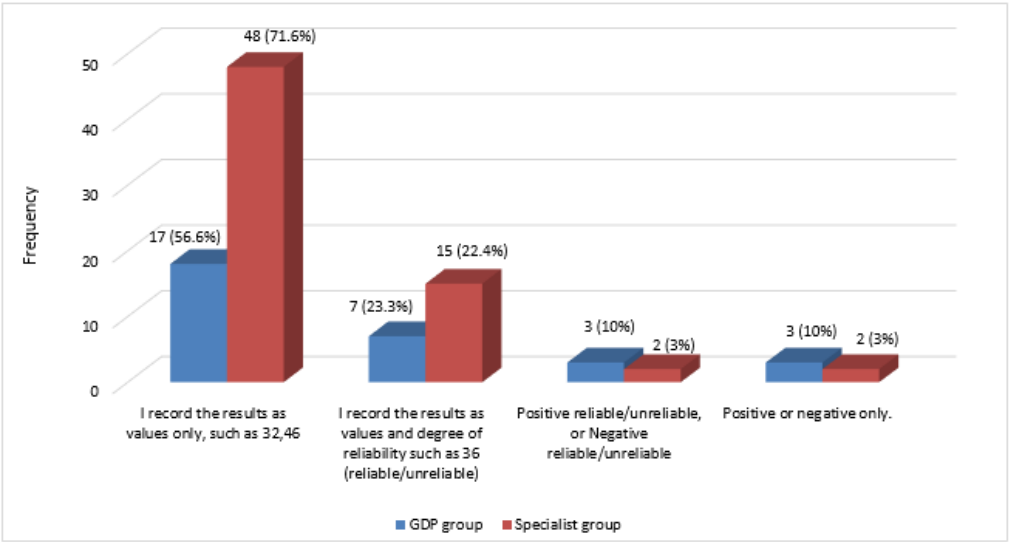


Fig. 4 Bar chart showing a) different methods used in documenting the results of the EPT per group, and b) different techniques in choosing the EPT value reading recorded

a)



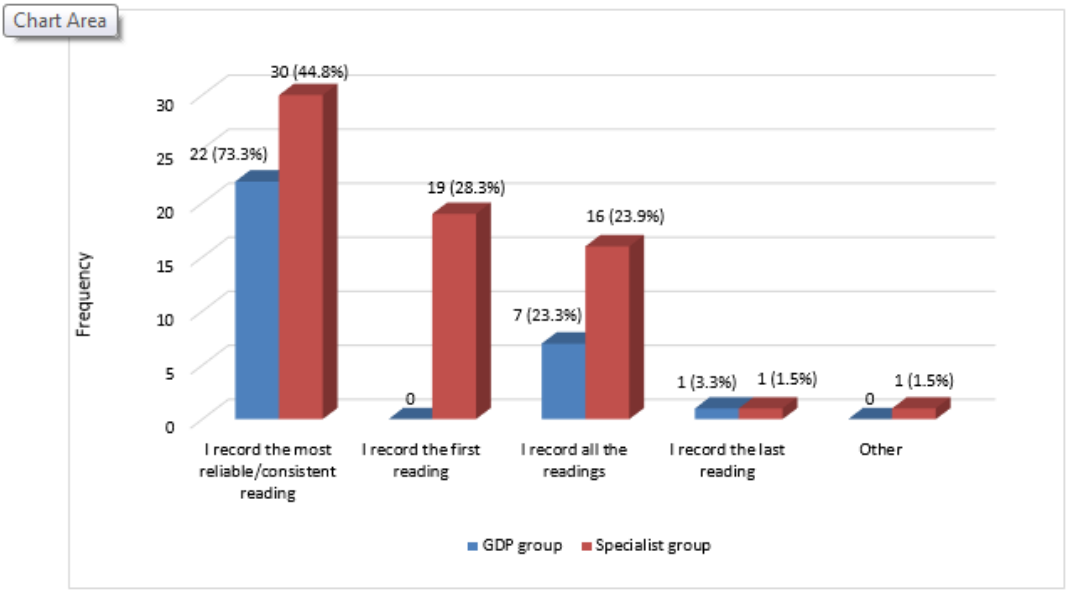
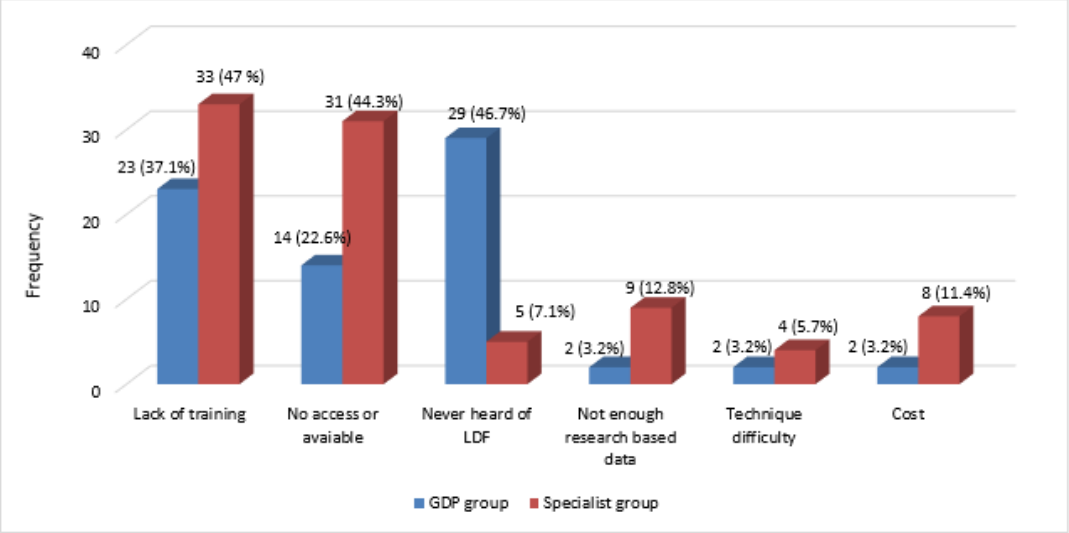


Fig. 5 The reasons/barriers in using LDF in dental trauma



Appendix 15: Manuscript submitted for publication

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).

Test group (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. A 2x2 contingency table for LDF based on a cut-off ratio of 0.6 is presented in Table 4. 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)

The outcomes of EPT and ethyl chloride are presented in Table 3 and a 2x2 contingency table are presented in Table 5.

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 ≥ 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.

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Figure 1 Photograph showing the LDF's splint, made using Vinyl Polysiloxane impression material, with two holes drilled in order to guide the LDF probes.

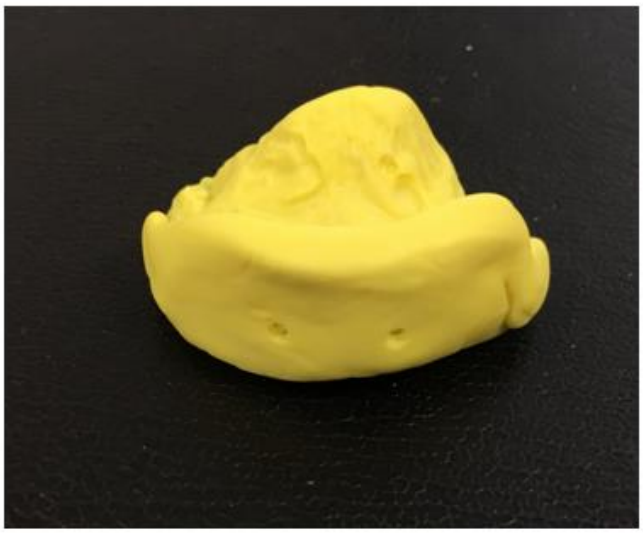

Table 1 Baseline demographics and clinical characteristics of the subjects included in the study showing no difference between the groups

Variable		Test group n (%)	Control group n (%)	Total	P-value
Age (years)					
	Mean (SD)	12.1 (2)	12.7 (2)	--	0.25
Gender					
	Male	21 (56.7)	25 (67.6)	46 (62.2)	
	Female	16 (43.3)	12 (32.4)	28 (37.8)	
	Total	37	37	74	0.47
Type of traumatic dental injury					
Enamel-dentine fracture		14 (38)	15 (40.5)	29 (39.2)	
Complicated crown fracture		--	6 (16.2)	6 (16.2)	
Concussion		1 (2.7)	--	1 (2.7)	
Subluxation		3 (8.1)	1 (2.7)	4 (10.8)	
Extrusive luxation		1 (2.7)	3 (8.1)	4 (10.8)	
Intrusive luxation		1 (2.7)	--	1 (2.7)	
Avulsion		13 (35)	7 (19)	20 (27)	
Lateral Luxation		3 (8.1)	3 (8.1)	6 (16.2)	
Enamel-dentine fracture with lateral luxation		1 (2.7)	--	1 (2.7)	
Mid root fracture		--	1 (2.7)	1 (2.7)	
Enamel fracture with subluxation		--	1 (2.7)	1 (2.7)	
	Total	37	37	74	0.18
Tooth type (Teeth with vital pulps)					
Central incisor		23 (62.2)	19 (100)	42 (56.7)	
Lateral incisor		14 (37.8)	18 (48.7)	32 (43.2)	
	Total	37	37	74	0.48
Stage of root development (Teeth with vital pulps)					
Full root length and wide open apical foramen (diameter >2mm).		0	1 (2.7)	1 (2.7)	
Full root length and half open apical foramen		5 (13.5)	1 (2.7)	6 (8.1)	
Full root length and closed apical foramen		32 (86.5)	35 (94.6)	67 (90.5)	
	Total	37	37	74	0.15

Table 2 Mean Flux values of LDF's first and second recordings showing Flux range, final mean Flux and the results of the paired sample t-test comparing the LDF's results of teeth with vital and non-vital pulps.

Status	Recording 1 (Flux)	Recording 2 (Flux)	Flux range	Mean Flux (SD)	*P* value
Non-vital pulp	6.36	7.40	1.7 - 27.8	6.88 (5.4)	0.00
Vital pulp	9.87	10.61	3.4 - 34.9	10.24 (5.6)	

Figure 2 ROC curve of the Flux values of LDF showing poor estimation of sensitivity and specificity with a small area under the blue curve. The green line represent a reference line indicating that a test is useless in differentiating between diseased and non-diseased. The red circle indicates the best cut-off value.

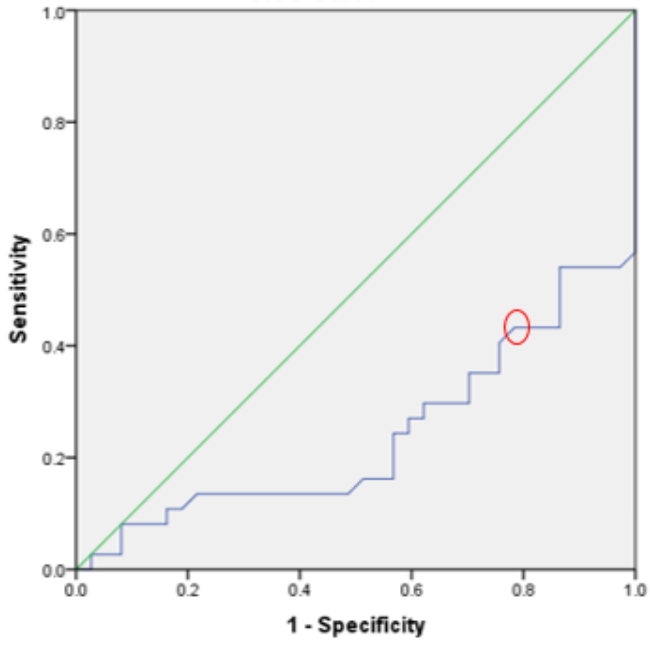

Figure 3 ROC curve for the Flux ratios of LDF showing poor estimation of sensitivity and specificity with a small area under the blue. The green line represents a reference line indicating that a test is useless in differentiating between diseased and non-diseased. The red circle indicates the best cut-off ratio.

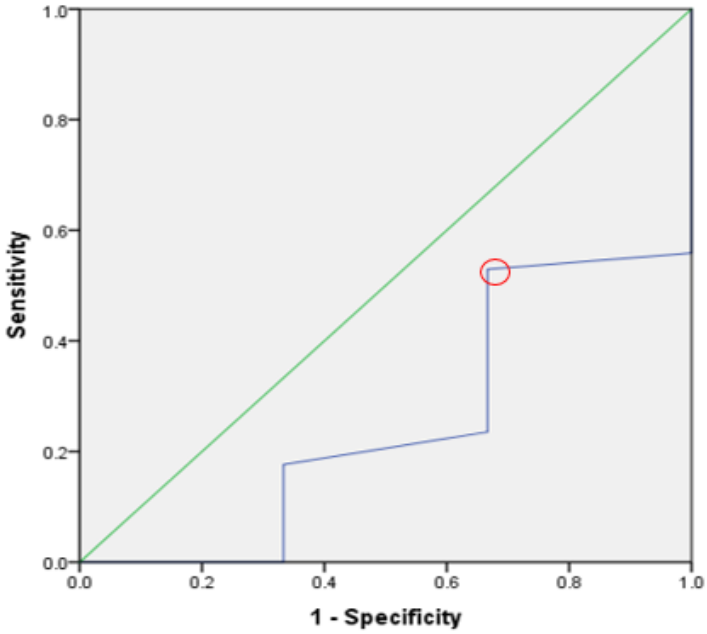

Table 3 A summary table showing the accuracy outcomes and repeatability of all three tests. The LDF's results shown under Flux value and Flux ration correspond to an LDF cut-off threshold of a value of 6.3 and a ratio of 0.6.

	LDF		EPT	Ethyl chloride
	Flux value	Flux ratio		
Sensitivity %	43.2	53	83.8 – 94.6	81.1 – 91.9
Specificity %	21	33%	89.2 – 97.6	73 – 81.1
Positive predictive value %	35.5	44.4	89.7 – 96.9	77.3 – 81.1
Negative predictive value%	16	41.2	85.7 - 94.3	81.1 – 90
Repeatability	0.85		0.86	0.81

Table 4 A 2x2 accuracy assessment table for LDF based on a cut-off ratio of 0.6.

	Pulp status		Total
	Non-vital	Vital	
Test non-vital	20[(a)]	25[(b)]	45
Test vital	17[(c)]	12[(d)]	29
Total	37	37	74

(a) True Positive (b) False positive (c) False negative (d) True negative

Table 5 A 2x2 accuracy assessment table for a) EPT and b)ethyl chloride

a) EPT	Pulp status		Total
	Non-vital	Vital	
Test non-vital	31[a]	1[b]	32
Test vital	2[c]	33[d]	35
Unreliable	4	3	7
Total	**37**	**37**	**74**
b) Ethyl chloride			
Test non-vital	30[a]	7[b]	37
Test vital	3[c]	27[d]	30
Unreliable	4	3	7
Total	**37**	**37**	**74**

(a)True Positive (b) False positive (c) False negative (d) True negative