Validity and acceptability of a laser fluorescence device compared to conventional methods for detection of proximal caries in primary teeth

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ABSTRACT

Aim: Accurate detection and diagnosis of caries in primary molars is challenging, especially for proximal lesions where direct visual examination is difficult. Therefore, the aim of this *in-vivo* and *in-vitro* study was to assess the validity, reproducibility and acceptability of a laser fluorescence pen and compare these outcomes with those of conventional methods of proximal caries detection in primary molar teeth.

Methods: Eighty-two children (aged 5-10 yrs) were recruited. Initially 1030 proximal surfaces were clinically examined using meticulous visual examination (ICDAS), bitewing radiographs, and a laser fluorescence device (LF pen). Temporary tooth separation (TTS) was achieved for 447 surfaces and these surfaces were re-examined visually (ICDAS) and by the LF pen. The teeth were subsequently extracted and serially sectioned for histological validation. Proximal surfaces were further assessed *in-vitro* using direct visual examination and the LF pen. The validity of all diagnostic methods was assessed. Results of both *in-vivo* and *in-vitro* assessments were compared. Intra- and inter-examiner reproducibility were assessed, the second examiner re-examined 10% of surfaces. Patient acceptability of the different diagnostic methods was measured using self-completed questionnaires.

Results: At D_1 (enamel and dentine caries) diagnostic threshold, the sensitivity of ICDAS visual examination, TTS, radiographic examination and LF pen examination was 0.52, 0.75, 0.14, 0.58 and the specificity at this threshold was 0.89, 0.88, 0.97, 0.85 respectively. At D_3 (dentine caries) diagnostic threshold, the sensitivity of the ICDAS examination, TTS, radiographic examination, and LF pen examination was 0.42, 0.49, 0.71, 0.63 respectively, while the specificity was 0.93 for both ICDAS examination and TTS, and 0.98 and 0.87 for radiographic and LF pen examinations respectively. ROC comparison of the different methods showed the radiographic examination to be superior at D_3 level. Intra-examiner reproducibility was 'substantial' to 'almost perfect' for all examinations, with the Kappa coefficient varying from K=0.75 at D_1 to K=0.95 at D_3. Inter-examiner reproducibility for ICDAS and radiographic examinations also demonstrated 'substantial' to 'almost perfect' agreement which varied from K=0.73 at D_1 to K=0.0.85 at D_3. The LF pen had significantly higher validity *in-vitro* than *in-vivo*. However, *in-vitro* LF pen readings were significantly different from the *in-vivo* readings ($P<0.05$). Regarding acceptability of these different approaches, children found TTS to be significantly less acceptable than the other methods.

Conclusions: Meticulous visual examination should be supported by radiographs. The LF pen did provide additional diagnostic information particularly at the D_1 threshold but not as much as

radiographs at the D_3 threshold. *In-vivo* LF pen readings do not relate to *in-vitro* readings. Children were least accepting of TTS, which would prove a barrier to routine clinical use.

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AAPD	American Academy of Paediatric Dentistry
BASCD	British Association for the Study of Community Dentistry
CCDH	Charles Cliffored Dental Hospital
CPITN	The community periodontal index of treatment needs
DD	DIAGNOdent
DIFOTI	Digital imaging fibre optic trans-illumination
dmft	Decayed/missing/filled primary teeth
DSTM	Dundee Selectable Threshold Method
DT	David Thompson
DV	Diagnostic value
EAPD	European Academy of Paediatric Dentistry
ECM	Electronic caries monitor
EDJ	Enamel dentine junction
ERK	Ekstrand/Ricket/Kidd
FGDP	Faculty of general dental practitioners
FOTI	Fibre optic trans-illumination
GA	General anaesthesia
GDP	General dental practitioners
ICC	Inter class correlation
ICCMS	International Caries Classification and Management System
ICDAS	International Caries Detection and Assessment System
IMD	Index of multiple deprivation
LF	Laser fluorescence
LLD	Lower left first primary molar
LLE	Lower left second primary molar
LRD	Lower right first primary molar
LRE	Lower right second primary molar
LSOA	Lower Super Output Area

Micro-CT	Micro-computed tomography
NCA	No care is advised
NHS	National Health Service
NHS REC	National Health Services Research Ethics Committee
OCA	Operative care is advised
PCA	Preventive care is advised
PMCs	Preformed metal crowns
PVN	Predictive value negative
PVP	Predictive value positive
QLF	Quantitative light-induced fluorescence
RE	Radiographic examination
RM	Robert Moorehead
ROC	Receiver Operating Characteristic
SCH	Sheffield Children's Hospital
SPSS	statistical package for social science
TTS	Temporary Tooth Separation
UK	United Kingdom
ULD	Upper left first primary molar
ULE	Upper left second primary molar
URD	Upper right first primary molar
URE	Upper right second primary molar
VAS	Visual Analogue Scale
VE	Visual examination
VM	Vanda Murray
WHO	World Health Organization

1 INTRODUCTION

Dental caries is the most common chronic disease in childhood. In the US, dental caries is five times more common than asthma and seven times more common than hay fever (U.S. Department of Health and Human Services, 2000). It has also been reported that 41% of American children aged 2-11 years had experience of dental caries (Beltran-Aguilar *et al.*, 2005).

Dental caries is a highly prevalent chronic disease amongst children and may cause considerable pain and suffering. Caries-related impacts may lead children to miss school days (Gift *et al.*, 1992) and may even adversely affect their body weight, growth and wellbeing (Sheiham, 2006). In addition, dental caries may result in acute infection which may require hospitalisation (Moles and Ashley, 2009). Severe untreated caries may even, rarely, cause the death of a child (Casamassimo *et al.*, 2009). Therefore, early diagnosis of the disease is important to prevent children from having to suffer from its consequences.

Diagnosis of dental caries has always been problematic, especially the diagnosis of proximal caries where the lack of accessibility and visibility makes it more difficult to detect caries at its early stages. Early diagnosis is paramount to allow evidence-based prevention of disease progression or early interventions (Deery, 2013).

Many methods have been used for the detection of dental caries. A systematic review of diagnostic methods has shown that visual examinations tend to have low sensitivity (Bader *et al.*, 2002). A standardised system, the International Caries Detection and Assessment System (ICDAS), has been developed. This system has been demonstrated to have high validity and reproducibility and therefore, appears better than other systems

(Ismail *et al.*, 2007; Jablonski-Momeni *et al.*, 2008; Shoaib *et al.*, 2009). Temporary tooth separation is used by some clinicians for the detection of cavitation and this adjunct to visual examination has been shown to be useful in identifying dental caries in the proximal surfaces of permanent premolars and molars (Deery *et al.*, 2000).

Bitewing radiographs provide an additional diagnostic yield especially for proximal caries where direct visual examination is not possible (Hopcraft and Morgan, 2005). However, in recent years, more concern about the effect of ionising radiation has increased awareness of the need to protect patients and avoid radiographs where other techniques exist. Guidelines have been set to minimise and avoid the unnecessary exposure of children to radiation (Espelid *et al.*, 2003).

A pen laser fluorescence device (LF pen, DIAGNOdent pen, Kavo Biberarch, Germany) was introduced specifically for the diagnosis of proximal caries. However, there is a paucity of research on the performance of the LF pen device in clinical settings. To date, no histological validation data have been published to compare the new device with more conventional methods (visual examination and bitewing radiographs) in the diagnosis of proximal caries in primary teeth clinically (*in-vivo*).

In addition, it is recognised that the fluorescence readings alter from *in-vivo* to *in-vitro* settings. Lussi et al (2001) have highlighted this phenomenon for permanent teeth, but there are currently no data on pre- and post-extraction fluorescence readings as measured by the pen laser fluorescence device in primary teeth.

There is increasing recognition that patients, including children, should be actively engaged in all areas of health research and service evaluation. The perspectives and experiences of children are important in gaining more meaningful insights into their acceptance of different treatments and their role in decision making. A literature review has revealed that dental research often fails to fully engage children, with the majority of studies being conducted *on* children (as objects of research) rather than *with* children (as active participants) (Marshman *et al.*, 2007).

Therefore, the aim of this study was to assess the validity, reproducibility and acceptability of a pen laser fluorescence device (DIAGNOdent-pen Kavo Germany) and compare these outcomes with those of conventional methods (visual examination, temporary tooth separation and bitewing radiographs) for proximal caries detection in primary molar teeth.

This thesis is structured as follows:

Chapter two commences with a narrative review of dental caries, its epidemiology, disease progression and histopathology. A detailed description of the diagnostic methods assessed in this study (visual examination, temporary tooth separation, radiographic examination and LF pen device) is provided. The acceptability of these diagnostic tests is also presented from the existing literature.

Chapter three presents the aim and objectives of the study.

Chapter four describes recruitment of participants and collection of experimental material for this clinical and laboratory study, together with a detailed description of the methodological approaches employed. The statistical methods and the data analysis strategy are also outlined.

Chapter five presents the research results including descriptive, validity and acceptability findings.

Chapter six reflects on the research findings including strengths and limitations of the study design. Recommendations are made for future research.

Chapter seven describes the overall conclusions reached from the study, with an emphasis on the clinical relevance and applications of this work.

2 LITERATURE REVIEW

2.1 Dental Caries

Dental caries is defined as the result of localised destruction of a susceptible tooth surface by products from microbial metabolism in the dental plaque covering the affected area (Fejerskov and Kidd, 2008). It is a very common preventable disease that can affect people at any time throughout their life (Pitts, 2004).

2.1.1 Prevalence of dental caries

Dental caries is a common chronic disease seen in childhood throughout the world. In the US, dental caries is five times more common than asthma and seven times more common than hay fever (U.S. Department of Health and Human Services, 2000). It has also been reported that 41% of American children aged 2-11 years have experience of dental caries (Beltran-Aguilar *et al.*, 2005).

Coordinated national surveys of child dental health have been taking place in the UK for the past 40 years. These surveys have been conducted by the British Association for the Study of Community Dentistry (BASCD) and the National Health Service (NHS). These surveys examine 5-year-old children in the UK and report their oral health status. The results are expressed using a standardised worldwide system which describes the prevalence of caries in the form of Decayed, Missing, and Teeth (abbreviated DMFT for the permanent dentition and dmft for the primary dentition) for the population (Knutson *et al.*, 1938).

The most recent report showed that 31% of 5-year-old children in England had evidence of dental caries in dentine ($d_3mft>0$) (BASCD, 2009). The average d_3mft of dental

caries in England was 1.11 in 5-year-old children in 2007-2008. The average varied from 0.9 in South East Coast with 24% of the population affected to 1.52 in North West with almost 40% of the population affected.

The average prevalence of caries in Yorkshire and Humber was close to the highest in England with an average d3mft of 1.51. The proportion of the population affected was also higher than average with 38.7% of children having at least one primary tooth which was decayed, missing due to caries or filled. The area with the lowest caries experience was York with an average d3mft of 0.74 while Bradford had the highest level of caries with an average d3mft of 2.42. Locally, Sheffield was found to have an average d3mft of 1.66 with 40.7% of the population affected.

2.1.2 Pathogenesis

Dental caries is a term that reflects signs and symptoms of an ongoing and past process. The carious process involves a dynamic de- and re-mineralisation process resulting from acidic by products of microbial metabolism on the tooth surface. This process may result in loss of minerals, and over time, may or may not lead to cavitation (Manji *et al.*, 1989). The caries lesion observed clinically is the accumulation of numerous episodes of pH fluctuations (de- and remineralisation) and at any stage of lesion development the physiologic balance may be restored and the lesion may be arrested (Manji *et al.*, 1991).

Dental caries develops in areas protected from mechanical wear of mastication, attrition and abrasion, where the biofilm does not get disturbed and is allowed to mature over time (Fejerskov, 2004). Formation of a cavity will further promote the dental caries process, and unless the patient cleans this area, the biofilm will remain undisturbed and the caries process will continue (Kidd and Fejerskov, 2004). Certain bacteria in the biofilm (*Streptococcus mutans* and *Lactobacilli*) produce acid when they metabolize fermentable carbohydrates (Loesche, 1986). This acid causes a decrease in the pH value causing demineralisation (loss of calcium, phosphate and carbonate). If this process is not stopped or reversed, cavitation will eventually take place (Featherstone, 2004).

Demineralisation can be reversed by calcium and phosphate in the presence of fluoride which acts as a catalyst for the diffusion of both minerals forming a new crystalline structure (fluoridated hydroxyapatite and fluorapatite) which is much more resistant to acid attacks than the original structure (Selwitz *et al.*, 2007).

Whether the lesion progresses to cavitation, remains the same or arrests is determined by the balance between pathological factors and protective factors (Featherstone, 1999).

Dental caries is a reflection of disturbance in a normal physiological balance between many factors which determine the plaque composition on the tooth surface. Therefore, the caries process cannot be prevented but the disease can be controlled and it's progression to cavitation can be prevented (Fejerskov, 1995).

2.1.3 Factors involved in caries development

Dental caries is a multifactorial disease which is caused by numerous biological factors that influence the outcome (net mineral loss) which ultimately result in cavity formation. The complex interaction between salivary composition and secretion, diet, pH fluctuations at different sites of the tooth and local immune responses in the oral cavity influence the composition of plaque and, in conjunction with other factors such as fluoride exposure, will consequently determine the net loss or gain of minerals at any surface covered by plaque (Fejerskov, 1997). The main determinants of caries activity will now be described below.

2.1.3.1 The host

Susceptible tooth

Factors affecting the caries process on tooth surface are; location, morphology, structure and composition, and posteruptive age (Zero, 1999). Fejerskov argues that relative caries resistance of teeth does not exist (Fejerskov, 1997), although a number of factors affect the acid solubility and thus increase the caries resistance of teeth. These include inorganic factors that determine enamel solubility, crystal shape and size, and proximity

of crystals. Fluoride when present as fluorapatite produces a more stable crystalline structure than hydroxyapatite and less soluble in acid. Therefore, it is more resistant to dental caries (Zero, 1999).

Another modifying factor of caries susceptibility is that of tooth maturity. Caries susceptibility is greatest immediately after eruption and it decreases with age (Kotsanos and Darling, 1991). Teeth undergo a post eruptive maturation process which involves re-precipitation of mineral that is less soluble than the original mineral it replaced. The re-precipitated crystals are rich with the less soluble fluorapatite crystals. These crystals may grow larger creating hypermineralised areas of enamel. These features explain the increased resistance of teeth to caries with age (Zero, 1999).

Alternatively, it has also been suggested that it is plaque stagnation on the partially erupted teeth, due to favourable conditions of plaque accumulation such as being out of the functional plane and the difficulty of brushing of these surfaces, rather than the stage of maturation of the tooth, that predisposes to caries (Carvalho *et al.*, 1989).

Saliva

Saliva flow rate and composition are important factors in reducing dental caries. Salivary protective mechanisms include flushing carbohydrate from the teeth, buffering and dilution of plaque derived acids, antimicrobial properties and providing organic and inorganic components that enhance remineralisation and inhibit demineralisation such as fluoride, calcium and phosphate (Featherstone, 2004).

Fluoride

Fluoride from extrinsic factors prevents caries in three ways:

- Fluoride inhibits demineralisation if present at the crystal surface at the time of acid attack (Tencate and Featherstone, 1991). So, if fluoride is present in the plaque fluid at the time that the bacteria produces acid, the fluoride will travel

with acid into the subsurface of the tooth, bind to the crystal surface and protect it against being dissolved (Featherstone, 1999).

- Fluoride enhances remineralisation by speeding up the growth of new crystals on the partially demineralised subsurface crystals in the caries lesion. The new crystals are fluorapatite with much lower solubility and higher resistance to caries (Featherstone, 1999).
- Fluoride inhibits plaque bacteria by inhibiting essential enzyme activity in the cariogenic bacteria. Fluoride is taken up by bacteria in the acidic media (Van Loveren, 1990).

2.1.3.2 The agent (the cariogenic microorganism)

The composition of the micro-flora is very diverse. *Streptococcus mutans* and *Lactobacilli* species have been found at higher concentrations in plaque covering early enamel caries and deep caries (Duchin and Vanhoute, 1978). No one single micro-organism can cause either enamel or root caries (Nyvad, 1993). A systematic review conducted to assess the microbiological involvement in dental caries (Tanzer *et al.*, 2001) supported the view that *Streptococcus mutans* and *Lactobacilli* species

play a major role in the initiation and progression of coronal and root caries in both children and adults. A more recent study (Corby *et al.,* 2005) found that in children with active caries, in addition to the above species, there was an abundance of other bacteria. These include; *Cardiobacterium, Fusobacterium, Actinomyces, and Haemophilus Parainfluenza*. However, their role in the initiation of dental caries is unknown. The composition of plaque is different according to its site in the mouth (Aas *et al.*, 2005) and depth of carious lesion (Munson *et al.*, 2004).

2.1.3.3 Diet (the environment)

The presence of fermentable carbohydrates and plaque on the tooth surface for a minimum amount of time is necessary to cause acid production and consequently demineralisation of enamel (Fejerskov, 1995).

Not all types of carbohydrates are equally cariogenic. Complex carbohydrates such as starch are less cariogenic while low molecular weight sugars such as sucrose, fructose and glucose are more cariogenic as they can be easily metabolised by bacteria forming acid (Barker *et al.*, 1981).

Sucrose is argued to be the most cariogenic sugar because of the ability of *S. mutans* to produce intracellular and intercellular storage polysaccharides from it. In addition, it is the source of energy for the most cariogenic bacteria (Jensen, 1999).

The frequency of carbohydrate ingested has been strongly associated with dental caries. The Vipeholm study showed clearly that increasing the frequency of eating sugary food increases the caries experience in human subjects (Gustafsson *et al.*, 1954).

Mechanical properties of food such as adhesiveness, hardness, cohesiveness and viscosity have also been suggested to have a role in the cariogenicity of food (Jensen, 1999).

Patients who use non-fermentable sweeteners, such as xylitol show marked reduction in caries incidence (Jensen, 1999). The use of other polyols as sweeteners, such as sorbitol, manitol and maltitol, in beverages and food to prevent caries is widespread. Nevertheless, the evidence supporting the role of xylitol in reducing the number of S. *mutans* in plaque and saliva and in reducing caries has influenced its use (Ly *et al.*, 2006).

Artificial sweeteners such as saccharine and aspartame are also considered non cariogenic and are used widely where a sweet taste is necessary (Rugg-Gunn, 1990).

Starch is the major source of carbohydrate in diet. Raw starch granules are slowly fermented in the oral cavity by salivary amylase because of their insoluble form. Cooked starch is degradated and more retentive which allows the cariogenic bacteria to use as a substrate (Jensen, 1999). Cooked starch when combined with sugar has been shown to be more cariogenic than sugar alone (Rugg-Gunn, 1990).

2.1.4 Caries pathology and histology

2.1.4.1 Enamel caries

Enamel is a fully mineralised structure. 95% of enamel is mineral and only 5% is water and organic matrix. Normal sound enamel consists of hydroxylapatite crystals tightly packed and arranged in rod and inter-rod enamel. Crystals are separated from each other by tiny inter-crystalline spaces which together form a fine network of diffusion pathways called micropores in the enamel. The outermost layer is porous due to the opening of the Striae of Retzius at the surface. Larger diffusion pathways are in the form of perikymata grooves (Fejerskov and Kidd, 2008).

Surfaces, such as proximal surfaces, are not more susceptible to caries because of their composition; they are more susceptible because they are out of the effect of mechanical wear of mastication and therefore present a plaque stagnation area (Weatherell *et al.*, 1984). A study conducted (Black, 1932), to look at the effect of undisturbed plaque on enamel surface for days and weeks showed that after one week of undisturbed plaque accumulation on enamel, there was no change in the enamel surface macroscopically. Microscopic examination showed a slight increase in the microporosity. The increase in microporosity leads to a change in the refractive index of enamel. After 14 days, the enamel changes can be seen as whitish opaque changes after air drying. After 3-4 weeks, complete dissolution of thin perikymata occurs and the intercrystalline spaces of involved enamel surface are enlarged and hence microporosity of enamel increases and further reduction in the refractive index of enamel occurs. At this stage, the clinical changes can be seen without air-drying and this is known as the white spot lesion.

Histological zones of enamel caries

A white spot lesion is the first enamel carious lesion to be detectable clinically. It may be seen clinically with or without air drying. On the proximal and buccal surfaces, histologically, the lesion appears as wedge shaped defect with the base at the enamel surface and the apex at the enamel-dentine junction following the direction of the

enamel prisms. The opposite is seen in the occlusal caries, where the caries lesion becomes wider as it approaches the underlying dentine following the prisms direction (Kidd and Joyston-Bechal, 1997).

The white spot lesion can be divided to four distinct histological zones as described below in Figure 2.1.

1. The intact surface zone: the outer most layer of the lesion and varies in depth from 20-50 µm.
2. The body of the lesion: observed beneath the enamel surface and extending in a triangular shape in to the tissue.
3. The dark zone: 90-95% of carious lesions have this zone. It is usually very wide in slowly progressive caries.
4. The translucent zone: this happens in the advancing front of the lesion. It varies in depth from 5-100 µm. There is slight loss of minerals in this zone.

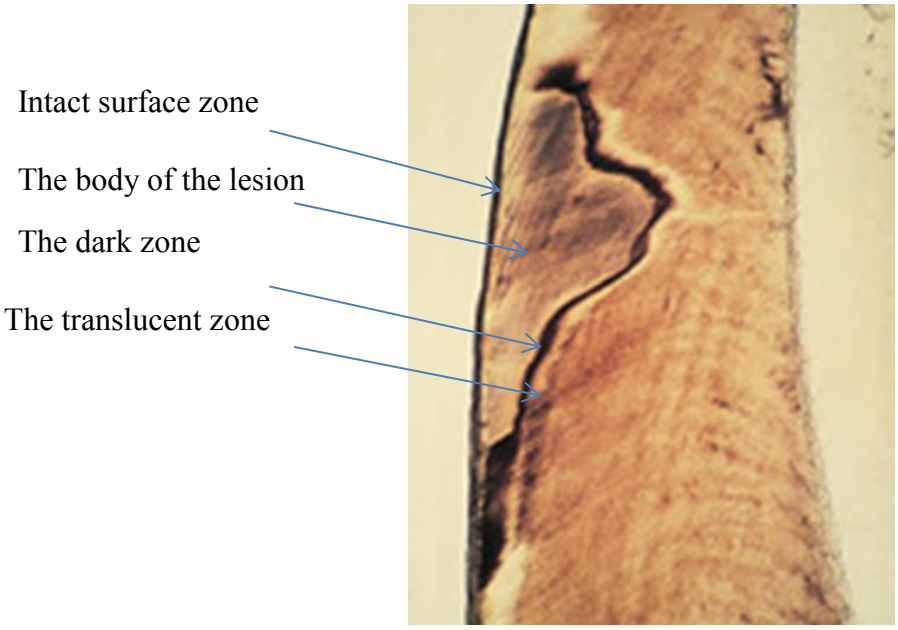

Figure 2.1 Histological zones of enamel caries reproduced from Soames and Southam, Oral Pathology, (2005)

2.1.4.2 Dentine caries

Although enamel is avascular and acellular and cannot actively respond to injuries, the dentine and the dentinal cells are vital tissues and possess specific reactions to external stimuli. The most common defence reaction by the pulpo-dentinal complex is the formation of sclerotic dentin along the dentinal tubules causing their gradual occlusion.

Histological zones of dentin caries

Dentin caries has been described to have four zones (Figure 2.2).

Zone of sclerosis

The sclerotic or translucent zone is located beneath and at the sides of the caries lesion. The dentinal tubules are obliterated by calcification of the odontoblast process itself. Therefore, sclerosed dentine has a higher mineral content. Dead tracts may be seen running through the zone of sclerosis. These are the result of death of odontoblasts at an early stage of the caries process. The empty dentinal tubules provide access of bacteria to the pulp. To prevent this, the pulpal end of the dead tract is occluded by a layer of hyaline calcified material derived from pulpal cells. Beyond this, often very irregular reactionary dentine forms following differentiation of odontoblasts from the pulp (Soames and Southam, 2005).

Zone of demineralisation

In this zone, the inter-tubular matrix is affected by the acid produced by bacteria in the zone of bacterial invasion. The dentine in this zone therefore is sterile. It is difficult to differentiate between the zone of demineralisation and the zone of bacterial invasion clinically (Soames and Southam, 2005).

Zone of bacterial invasion

In this zone, the bacteria penetrates and multiplies within the dentinal tubules. The bacterial invasion occurs in two stages. In the first stage, acidogenic bacteria, mainly

lactobacilli, produce acid which diffuses to the demineralised zone. In the second stage, mixed acidogenic and proteolytic microorganisms attack the demineralised matrix. The walls of the tubules are softened by the action of proteolytic bacteria. The process results in liquefaction foci which run parallel to the direction of the tubules (Soames and Southam, 2005).

Zone of destruction

In this zone, the liquefaction foci become larger and increase in number. Cracks containing bacteria and necrotic tissue appear at right angles to the dentinal tubules forming transverse clefts. Little of the normal dentine structure remains and cavitation occurs from the amelodentinal junction (Soames and Southam, 2005).

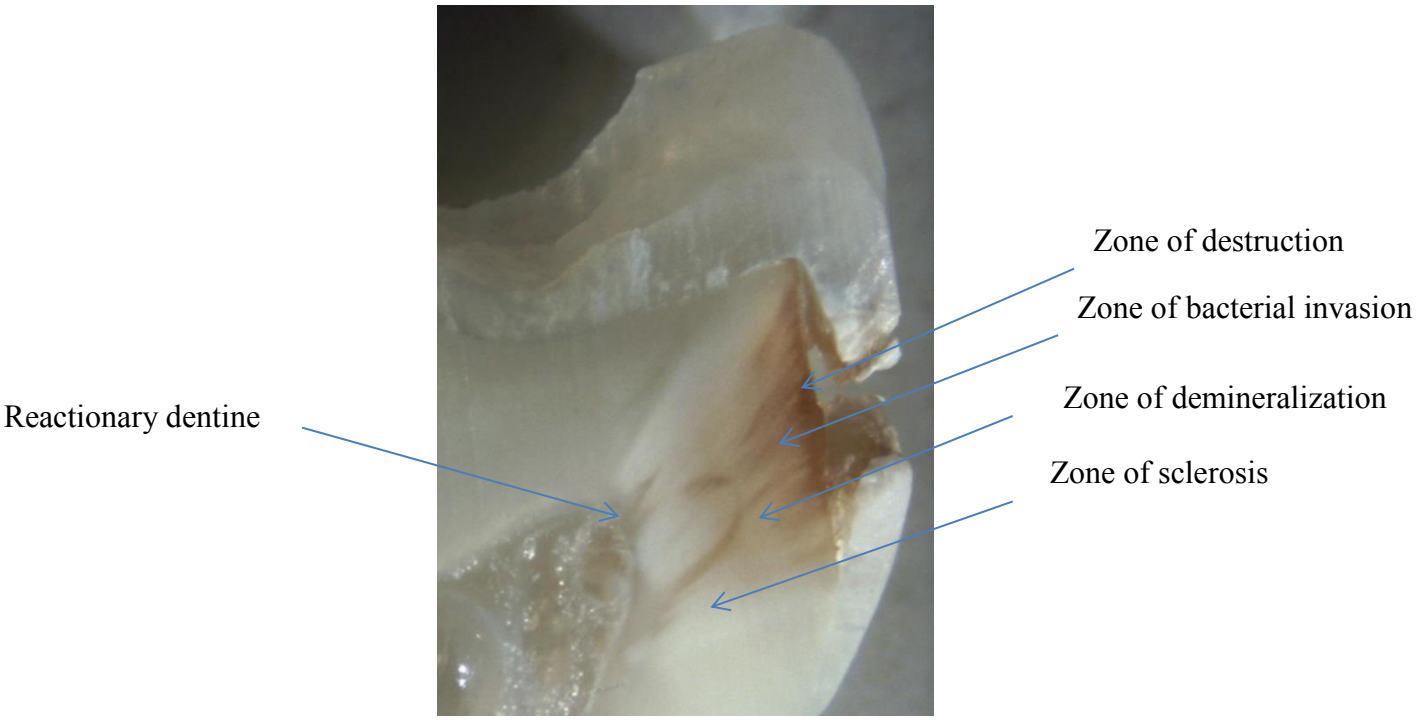

Figure 2.2 Histological zones of dentine caries

2.1.5 Dental caries in the primary dentition

There is no difference in the histological characteristics of caries affecting the primary or permanent dentition. However, anatomical variation between primary and permanent teeth results in a difference in early detection and diagnosis of dental caries.

Crown morphology

The crowns of primary teeth are smaller in general and more bulbous than their permanent successors. The primary crowns are wider mesiodistally than they are occlusogingivally (van Beek, 1983). The occlusal table of primary teeth is narrow in a buccolingual plane due to the convergence of labial and lingual walls (Curzon *et al.*, 1996).

The contact area

The contact area between primary molars is wide and gingivally located (Berkovitz *et al.*, 1992) which means that the diagnosis of interproximal caries is difficult before the lesion becomes extensive and a gray shadow appears beneath the marginal ridge (Curzon *et al.*, 1996).

Tooth Structure

The enamel of primary teeth is thinner than that of the permanent teeth. The pulp chambers of primary teeth are larger and the pulp horns are more prominent than those of permanent teeth. These together mean that there is very small distance between the outer surface of enamel and the pulp, which means that failure of early detection of lesions leads to penetration of caries to pulp especially in proximal lesions, where the distance between the mesial surface of the first mandibular primary molar and the pulp may be as little as 1.6mm (Wheeler, 1965).

2.2 Caries diagnosis

The term caries diagnosis has been used interchangeably with the term caries detection in the literature. However, it has been proposed that the two are distinct and should not be used interchangeably. Caries diagnosis is defined as "the art or act of identifying a disease from its signs and symptoms" while caries detection relates to identification of signs and symptoms (Nyvad, 2004). The process of caries diagnosis or detection requires a means of measuring the extent of the disease.

Caries is not a single state of disease but a continuous process of demineralisation starting from a microscopic mineral loss from the hard tissue to a total destruction of both hard tissue and the pulp. This process has been presented as the "iceberg of dental caries" shown in Figure 2.3 (Pitts, 2004). The base of the iceberg presents the initial lesions that can only be detected by more sensitive techniques followed by enamel lesions which can be seen as white lesions (D_1) or small cavitations in enamel (D_2). The top of the iceberg shows cavitated dentine lesions (D_3) and large lesions in to the pulp (D_4).

The prevention and management of dental caries relies on determining the presence of disease and identifying its stage (Pitts, 2004). Since prevention is the corner stone of any health programme, early detection of lesions is important for their reversal. Caries diagnosis is also important for risk assessment and treatment planning for individual patients (Kagihara *et al.*, 2009). The diagnosis of caries is also an integral part of the epidemiologist's role when conducting cross sectional caries prevalence surveys for planning and evaluation of services provided. Accurate caries diagnosis is also fundamental in clinical trials testing caries preventive agents (Kidd *et al.*, 1993). Furthermore, diagnosis of caries is important for research in terms of outcomes. Diagnostic criteria should be standardised in order for results to be compared (Jackson, 1950; Ismail, 2004)

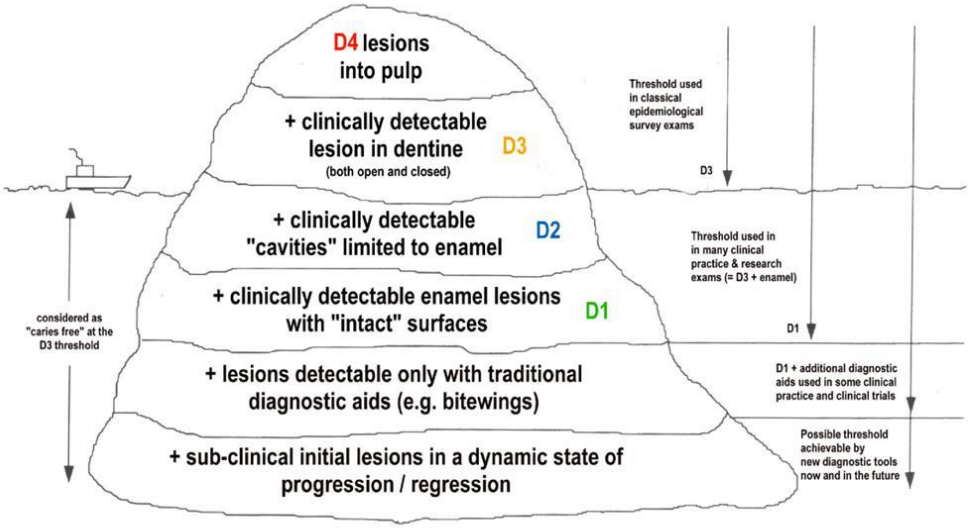

Figure 2.3 Pitts "Iceberg of Dental Caries": diagnostic thresholds in clinical trials and practice adapted from Pitts (2004).

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2.3 Methods of diagnosis of dental caries

The main methods of caries diagnosis that will be discussed in more details in this literature review are:

- Visual examination
- Visual examination after temporary tooth separation
- Radiographic examination
- Laser fluorescence pen (LF pen, DIAGNOdent pen)

2.4 Visual and visuotactile examination

2.4.1 History of caries diagnosis

It has been recognised for more than half a century that clinical detection of early dental caries is problematic. Deatherage and colleagues stated in 1939 that "*Dentists would probably disagree for about one third of the time in diagnosing the condition of the same child, unless improvements were made in the technique of diagnosis".*

Scientists have been trying to develop a reliable and reproducible diagnostic system for the detection and diagnosis of dental caries by clinical visual examination. Since at least 1954, there have been systems which included codes for the diagnosis of non cavitated caries as well as the cavitated (Backer Dirks *et al.*, 1961).

Other systems have applied cavitation diagnostic criteria for epidemiological studies such as studying disease prevalence (Jackson, 1950; Radike, 1968; WHO, 1997). The existence of a large number of different systems, using different thresholds for caries diagnosis, has led to problems in comparing results between studies.

Jackson (1950) stated that "*It will at once be realised that in order to compare the results of one worker with those of another, there must be a sufficient degree of*

homogenicity, otherwise comparisons are not possible". Therefore, he proposed criteria for the standard clinical procedure as follows:

- Teeth must be clean and any extrinsic stain or food debris must be removed before dental examination.
- Examinations must be carried out under good illumination.
- A clear mirror and a sharp probe should be used.
- Each tooth must be dried thoroughly and every surface examined.
- A pit or fissure is considered carious if the tip of the probe sticks without doubt and requires a definite pull for its removal. Anything which is doubtful is not included.
- Stained pits and fissures are not considered as carious unless they satisfy the previous test.
- The probe must be used in all pits and fissures and in different angles.
- Approximal caries are considered carious only if Ash's No. 12 probe catches a definite cavity or a roughened surface.
- Stained or opaque areas on smooth surfaces are only considered carious if the enamel shows clear evidence of dissolution.
- Arrested caries and exposed dentin in hypoplastic teeth are only considered carious if they show evidence of softening.

Although Jackson only considered the cavitated lesions as carious, he did mention later that the white opaque spots and lines are areas of hypocalcification that may easily become carious. He argued that these manifestations offer possibilities of preventive treatment and because these lesions can remineralise, they should not be considered as carious but still should be recorded for standardisation of procedure.

To determine whether the proposed standard procedure improved the consensus of diagnosis between examiners or not, three examinations were made (Jackson, 1950). Three examiners of the same competence were asked to examine a group of children. In the first two examinations, only one examiner followed the standard procedure while the other two examiners used whatever procedure they normally adopted. In the third

examination, the three examiners followed the same standard procedure. The analysis of the results showed that the variances between examiners for investigations 1 and 2 were significantly high, while in investigation 3, these variances were small and not of significance.

The investigator concluded that a well-defined protocol for caries diagnosis could improve inter-examiner reproducibility and suggested that the procedure is applicable for field surveys.

In 1954, Parfitt proposed another standard examination for caries, which included the precavitation stage of caries. Parfitt argued that, although counting gross lesions only as carious reduces errors, investigations such as preventive trials depend on the appearance of new carious lesions, thus these lesions must be counted.

In his standard examination he divided the progress of dental caries into four grades:

- Grade 1= slight discoloration of enamel surface with loss of lustre.
- Grade 2= roughness and pitting of surfaces, a condition which can be explored by explorer point.
- Grade 3= further loss of tissue and penetration causing pitting to reach dentine.
- Grade 4= further extension involves loss of dentine with cavitation.

In 1966, Marthaler introduced a standardised system for recording dental conditions. In his system, probes were only used when in doubt. He also divided his system to grades.

- Grade 1= slightly brown narrow line or (on smooth surface) white spot with hard surface, smaller than 2 mm.
- Grade 2= clearly brown or black line or (on smooth surface) white spot extends more than 2 mm.
- Grade 3= cavity, discontinuity of the enamel surface.
- Grade 4= cavity with the narrowest extent of the entrance broader than 2mm.

In 1973, Moller and Poulsen developed a more comprehensive system which could be used in different situations such as epidemiological studies and clinical trials as shown below.

The criteria of diagnosis for pits and fissures

- 0= sound
- 1= Discoloration, no definite sticking
- 2= Sticking with or without discoloration, no dentin involvement
- 3= Definite cavity with dentine involvement
- 4= Probable pulp complication

The criteria for vestibular and lingual smooth surfaces

- 0= Sound
- 1= White opaque area with loss of lustre, no loss of substance
- 2= Discontinuity in the enamel, loss of lustre, no loss of substance
- 3= Dentin involvement
- 4= Probable pulp complication

From their study they concluded that the classification system could be used without major changes in almost any study, although the authors stated that more studies should be conducted to define the diagnostic criteria in order to reduce the inter-examiner and the intra-examiner variability.

In the early 1970s, the World Health Organisation (WHO) started publishing its reports about basic methods for oral health surveys (WHO, 1977, 1997). The WHO system is one of the most widely used systems. Their diagnostic threshold for caries diagnosis is the cavitation level.

The current WHO (1997) diagnostic criteria are:

- Caries is recorded as present when a lesion in a pit or fissure, or on a smooth tooth surface, has a detectably softened floor, undermined enamel, or softened wall.
- A tooth with a temporary filling should also be included in this category.
- On approximal surfaces, the examiner must be certain that the explorer has entered a lesion.
- When any doubt exists, caries should not be recorded as present.

Over the last three to four decades, it has been well documented that caries prevalence has been reduced in industrialised Western countries because of the increasing availability of fluoride supplements (Marthaler, 1990). This decline in caries experience in children and adolescents has meant that more sensitive diagnostic criteria are required for recording caries (Marthaler, 1990, 1996) . Recording caries at its early stages allows the prevention of its progression (Deery, 2013).

Furthermore, it has been shown that the diagnosis of caries at the cavitated level results in an underestimation of caries levels in the population studied (Groeneveld, 1985; Pitts and Fyffe, 1988; Manji *et al.*, 1989). It has been documented in a study of prevalence of enamel lesions in a fluoridated and non-fluoridated area that there was a large difference in the numbers of lesions recorded in the test group and control group when caries was recorded at the dentinal levels. However, when all lesions (including caries at the enamel levels) were included, the total numbers of caries lesions were almost similar in both groups (Groeneveld, 1985).

Following on from this, Pitts and Fyffe (1988) conducted a study to test the effect of inclusion (or exclusion) of initial and enamel lesions on the results of a clinical examination in a low caries prevalence group. The criteria used for clinical examination was based on guidelines proposed by the WHO (WHO, 1977, 1997). The examinations were made on 287 dental undergraduate students between 1981 and 1984.

The examination conditions in this study were as follows:

- Teeth cleaned (by brushing).

- Compressed air was used to dry teeth.
- Adequate illumination by the use of a dental operating light.

The examination took place while the student was in a supine position and the examiner seated. The diagnostic criteria used for the visual examination were:

- Sound surface: no evidence of treated or untreated clinical caries (slight staining allowed in an otherwise sound fissure).
- Initial caries: no clinically detectable loss of substance. For pits and fissures, there may be significant staining, discoloration, or rough spots in the enamel that do not catch the explorer, but where loss of substance cannot be positively diagnosed. For smooth surfaces, these may be white, opaque areas with loss of lustre.
- Enamel caries: demonstrable loss of tooth substance in pits, fissures, or on smooth surfaces, but no softened floor or wall or undermined enamel. The texture of the material within the cavity may be chalky or crumbly, but there is no evidence that cavitation has penetrated the dentine.
- Caries of dentine: detectably softened floor, undermined enamel, or a softened wall, or the tooth has a temporary filling. On proximal surfaces, the explorer point must enter a lesion with certainty.
- Pulp involvement: Deep cavity with probable pulpal involvement. Pulp should not be probed.

Examinations were conducted by three examiners. Two examiners were trained and calibrated by the third examiner. The data were analysed by the use of a program called CARIES software package. This software can recalculate DMF and indices and their components according to three different diagnostic thresholds: D_1, D_2, D_3 (D_1 includes all clinically detected lesions, D_2 excludes initial caries, D_3 excludes initial and enamel caries) thus allowing for the exclusion or inclusion of different diagnostic levels.

The results showed that the use of different diagnostic thresholds can dramatically change the level of the reported dental caries. The level of decay reported was almost

doubled when the initial caries and enamel caries were included and the number of caries free subjects was reduced to approximately one quarter.

The investigators concluded that it is necessary in the future to re-examine the diagnostic thresholds used for surveys to choose the appropriate threshold for the survey's objective. They also concluded that by the use of less sensitive diagnostic thresholds, the disease may be underestimated and the results may be misinterpreted by the health workers and health planers.

Efforts of scientists thus continued in order to provide a more reliable system for caries diagnosis (Neilson and Pitts, 1991; Ekstrand *et al.*, 1998; Nyvad *et al.*, 1999; Fyffe *et al.*, 2000a).

In 1998, Ekstrand and colleague investigated the validity of a visual scoring system to detect occlusal caries against a histological 'gold standard'. They conducted an *in-vivo* study in Copenhagen, Denmark. Thirty five teeth from thirty five patients were included in this study. Teeth were cleaned using a rotating bristle brush and copious water. Visual examinations were conducted by two examiners, using a slightly modified version of a system described by Ekstrand et al (1997). Table 2.1 shows the criteria used for the visual examination.

Teeth then were extracted and examined histologically according to the criteria described by Eksrand et al (1997), shown in Table 2.2.

The examiners found that visual examination had a strong relationship with lesion depth and concluded that these criteria were able to detect occlusal caries, assess depth and diagnose activity.

Table 2.1 Criteria used in the visual examination by Ekstrand et al (1997).

Code	Criteria
0	No or slight change in enamel translucency after prolonged air drying
1	Opacity (white) hardly visible on the wet surface, but distinctly visible after air drying
1a	Opacity (brown) hardly visible on the wet surface, but distinctly visible after air drying
2	Opacity (white) distinctly visible without air drying
2a	Opacity (brown) distinctly visible without air drying
3	Localised enamel breakdown in opaque or discoloured enamel and/or greyish discoloration from the underlying dentin
4	Cavitation in opaque or discoloured enamel, exposing the dentin beneath

Table 2.2 Criteria used in the histological examination by Ekstrand et al (1997).

Code	Criteria
0	No enamel demineralisation or a narrow surface of opacity
1	Enamel demineralisation limited to the outer 50% of the enamel layer
2	Demineralisation involving between 50% of the enamel and one third of the dentine
3	Demineralisation involving the middle third of dentine
4	Demineralisation involving the inner third of dentine

Due to the increased interest in studying the effect of various caries preventive interventions, the recording of the effect of such interventions on teeth require a diagnostic system which is sensitive and able to record lesion progression. This requirement continued to drive investigators to develop more sensitive caries diagnostic criteria and protocols.

Therefore, Nyvad et al (1999) developed clinical diagnostic criteria which attempted to differentiate between active and inactive caries. The distinction between active and inactive caries was based on both visual and tactile criteria. Explorers were used to remove plaque from examined tooth surfaces, check for discontinuity of surfaces and check texture of surfaces (soft, rough, and leathery). The study included a group of 889 children between the ages of 9 to 14 years with high caries prevalence living in Lithuania. Examinations were conducted by two examiners who were extensively calibrated through clinical training. Children were examined for three consecutive years. Each year, 50 children were selected for assessment of inter- and intra-examiner reproducibility.

Before examinations, teeth were cleaned by brushing. Examinations were carried out under standardised conditions using the dental chair's operating light, compressed air and suction device. The criteria used for caries diagnosis are described in Table 2.3.

Nyvad and colleages found that the probability of confirming the diagnosis of sound, non-cavitated active and non-cavitated inactive was 98, 69 and 73% respectively. The inter- and intra-examiner reproducibility showed perfect agreement for diagnosis, which always exceeded 94%, and the Kappa values always exceeded 0.7.

These results are similar to those published by other researchers who have reported on non-cavitated caries diagnosis (Pitts and Fyffe, 1988). However, these findings contradict the assumption made by some authorities (WHO, 1997) that the inclusion of non-cavitated lesions makes the reproducibility of the results poor.

Table 2.3 Description of the diagnostic criteria used by Nyvad et al (1999).

Score	Category	Criteria
0	Sound	Normal enamel translucency and texture
1	Active caries (intact surface)	Surface of enamel is whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved gently across the surface; generally covered with plaque. No clinically delectable loss of substance. Intact fissure, lesion extending along the walls of the fissure.
2	Active caries (surface discontinuity)	Same criteria as score 1. Localised surface defect (microcavity) in enamel only. No undermined enamel or softened floor detectable with the explorer.
3	Active caries (cavity)	Enamel/dentin cavity easily visible with the naked eye; surface of cavity feels soft or leathery on gentle probing. There may or may not be pulpal involvement.
4	Inactive caries (intact surface)	Surface of enamel is whitish, brownish, or black. Enamel may be shinny and feels hard and smooth when the tip of the probe is moved gently across the surface. No clinically detectable loss of substance.
5	Inactive caries (surface discontinuity)	Same criteria as score 4. Localised surface defect (microcavity) in enamel only. No undermined enamel or softened floor detectable with explorer.
6	Inactive caries (cavity)	Enamel/dentin cavity easily visible with the naked eye; surface of cavity may be shiny and feels hard when probed with gentle pressure. No pulpal involvement.
7	Filling (sound surface)	
8	Filling+active caries	Caries lesion may be cavitated or non cavitated.
9	Filling+inactive caries	Caries lesion may be cavitated or non cavitated

Thus, Nyvad and colleages (1999) concluded that these diagnostic criteria allow the diagnosis and assessment of caries activity reliably, although the non-cavitated caries lesions were included in the scoring system.

Fyffe and colleagues (2000a) conducted an *in-vivo* study to develop a method for recording dental caries at the D_1 diagnostic threshold (without loss of D_3 information), which was referred to as the Dundee Selectable Threshold Method for caries diagnosis (DSTM). The investigators then assessed its reliability and validity against a benchmark examiner. Twenty examiners participated in the study, ten of whom, although experienced clinicians, had never participated in caries prevalence surveys, therefore, were referred to as the 'novice examiners'. The remaining ten examiners had previously been trained for participation in prevalence surveys, therefore, were referred to as 'experienced examiners'. Both groups were trained and calibrated to use the diagnostic criteria shown in Table 2.4.

Examinations took place under standardised conditions. Teeth were cleaned by brushing and children were examined supine on school tables using portable dental lights, mirrors, ball ended CPITN probes and portable compressors with 3-in-1 syringes for drying teeth. Examiners recorded caries at the D_1 and D_3 diagnostic thresholds to investigate inter-examiner agreement at each threshold. The results showed that, for the experienced examiners, there was no significant difference in the inter-examiner agreement between the D_1 and D_3 levels except for one assessment when the inter-examiner agreement was higher at the D_3 diagnostic threshold than the D_1.

Assessed against a benchmark examiner, there was no significant loss of sensitivity in D_1 diagnostic threshold when compared to D_3. Although there was significant loss of specificity at D_1 threshold, the specificity was considered to be high.

Therefore, the authors concluded that the modification of the diagnostic criteria commonly used for surveys to include enamel caries, which could benefit from prevention and early intervention, did not affect the reliability or the benchmark validity of experienced examiners to a significant degree.

Table 2.4 Summary of the Dundee Selectable Threshold Method for caries diagnosis (Fyffe *et al.*, 2000a).

Permanent surface code	Criteria
G	Good, sound surface - a surface is recorded as sound if, in the opinion of a trained examiner, it shows no signs of treated or untreated dental caries.
W	White-spot lesion - visual assessment of dried tooth indicates intact surface, no clinically detectable loss of substance, with a white or cream- coloured area of increased opacity presumed carious by the trained examiner.
B	Brown-spot lesion - visual assessment of dried tooth indicates intact surface, no clinically detectable loss of substance, with a brown/black discoloration, presumed carious by the trained examiner.
E	Enamel cavity - in the opinion of the trained examiner, there is a lesion with demonstrable loss of surface but no visual, clinical evidence of the lesion penetrating dentin.
D	Dentin lesion (uncavitated) - Surfaces are regarded as falling into this category if, in the opinion of the trained examiner, there is a caries lesion into dentin but no visible evidence of cavitation.
C	Dentin cavity - surfaces are regarded as falling into this category if, in the opinion of the trained examiner, there is a carious cavity into dentin.
P	Pulp involved - surfaces are regarding as falling into this category if, in the opinion of the trained examiner, there is a carious cavity that involves the pulp, necessitating an extraction or pulp treatment.
A	Arrested dentinal decay - surfaces are regarding as falling into this category if, in the opinion of the trained examiner, there is arrested caries in dentin.

In 2002, Bader conducted a systematic review of the performance of different methods for identifying carious lesions, one of which was the visual examination. His review revealed that the visual and visuotactile methods for caries detection have low sensitivity and relatively high specificity. He also found that the strength of evidence available for estimation of the validity of the visual examination for caries diagnosis is poor.

Ismail (2004) concurred with this view and stated "*The dilemma is that while several solutions have been proposed, we still do not have consistent and valid systems for clinical caries detection"* in his paper which evaluated the validity of published visual and visuotactile caries detection system. His review revealed considerable variation between the systems used for diagnosis. Analysis of these data emphasised the need for one diagnostic criteria for visual detection of dental caries which should be based on the present scientific evidence and consensus of experts in this field.

2.4.2 The International Caries Detection and Assessment System (ICDAS)

In order to develop an integrated clinical detection and assessment system of dental caries, a group of caries researchers, restorative dentists, paediatric dentists and epidemiologists assembled to update the caries detection and assessment criteria and to put together all different definitions. A new system thus was developed in 2002, which was named the International Caries Detection and assessment System (ICDAS), following two development meetings in Dundee, Scotland (April, 2002) and Ann Arbor, Michigan (August, 2002) (Larato).

The development of ICDAS I and ICDAS II criteria was based on the research conducted by Ekstrand et al (1995; 1997), combined with work by Nyvad and colleagues (1999) and concepts from the Dundee Selectable Threshold Method (DSTM) for caries diagnosis (Fyffe *et al.*, 2000a), in addition to other caries detection systems which were described by Ismail (2004) in his systematic review.

ICDAS is a clinical visual scoring system which was developed for use in clinical practice, research, epidemiology and dental education. The main aim for the development of the ICDAS was to provide an international system which allows standardisation of data collection and enables comparability between studies (Topping and Pitts, 2009). It also provides clinicians and researchers with diagnostic criteria that show clear stages of caries process to enable them to decide at which stage of disease (cavitated or non cavitated) and severity they want to measure dental caries (Ismail *et al.*, 2007).

The examination must be carried out in the presence of compressed air to detect the earliest signs of caries. Teeth should first be cleaned with a tooth brush or a prophylaxis cup prior to examination, and the proximal surfaces should be flossed to remove dental plaque. A ball ended explorer can be used as an aid to remove any remaining plaque, and the examiner should lightly check for surface discontinuity and the presence of any tooth coloured restorations (Ismail *et al.,* 2007).

The use of sharp explorers for caries diagnosis has been discontinued as its effect can be harmful and damaging to teeth (Ekstrand *et al.*, 1987). Furthermore, it fails to add any information for diagnostic benefit (Lussi, 1991). In addition, it can act as a vehicle for transmission of infection from one fissure system to another (Loesche *et al.*, 1979).

2.4.2.1 Development of ICDAS criteria

During the first workshop for the development of ICDAS criteria in 2002, all participants examined 57 occlsal surfaces of extracted teeth. The clinical status of these surfaces was defined from a consensus of all participants. Then, teeth were sectioned and examined under 10x magnification using Ricketts et al (2002) histological scoring. Histological examination was carried out by two examiners. The histological validation showed that the percentage of tooth surfaces scored with ICDAS code 3 which had caries extending in to dentine (88%) was higher than that for tooth surfaces with score 4 (77%) as shown in Table 2.5.

Therefore, the decision of the ICDAS II workshop to switch the original code 3 and 4 of ICDAS I was taken in Baltimore, USA in 2005. The change represents a more accurate sequence of caries progression (Topping and Pitts, 2009).

Table 2.5 Percentage of tooth surfaces with caries extending in to dentine in all codes of ICDAS criteria adapted from ICDAS II criteria manual (2005)

Code	Number of teeth	Percentage into dentine (%)
0	2	0
1	11	9
2	18	50
3	8	88
4	13	77
5+6	5	100
Total	57	

Since that time, there has been no further change in codes of ICDAS, therefore, the suffix II has been dropped from the name (Pitts *et al.*, 2013).

Recently, the International Caries Classification and Management System (ICCMS) was developed and used in conjunction with ICDAS. The ICCMS provides dentists with information which enables them to stage the caries process and manage it appropriately. It also enables dentists to assess caries risk status and review caries in clinical and public health practice (Pitts *et al.*, 2013).

ICDAS criteria

The recording of dental caries using the ICDAS system is a two stage process. The code consists of two digits, the first digit is the restorative status of the tooth and the second digit is for the caries severity. ICDAS codes for restoration and caries severity are shown in Tables 2.6 and 2.7 (Ismail *et al.*, 2007).

Table 2.6 ICDAS codes for restoration status (Ismail *et al.*, 2007).

Code	Description
0	Un-restored and unsealed
1	Partial sealant (a sealant which does not cover all pits and fissures of the tooth surface
2	Full sealant
3	Tooth coloured restoration
4	Amalgam restoration
5	Stainless steel crown
6	Porcelain, gold or preformed meta crown or veneer
7	Lost or broken restoration
8	Temporary restoration

Table 2.7 ICDAS codes for caries severity (Ismail *et al.*, 2007).

0	**Sound tooth surface**
1	First visual change in enamel after air drying
2	Distinct visual change in enamel without air drying
3	Localised enamel breakdown with no visible dentine
4	Underlying dark shadow from dentin
5	Cavity with visible dentine
6	Extensive cavity with visible dentine

2.4.2.2 The validity of ICDAS visual examination

Many studies have been conducted to validate the ICDAS visual examination. Shoaib et al (2009) conducted an *in-vitro* study to assess the validity and reproducibility of the ICDAS II in the detection and assessment of the proximal and occlusal caries in primary teeth. Three trained examiners independently examined the proximal and occlusal surfaces of 121 extracted primary molars. The conditions of teeth varied from clinically sound to cavitated dentine, but extensively broken down teeth were excluded from the study. Teeth were cleaned then mounted in groups of four in pink impression putty to mimic their intra-oral anatomical position.

Examinations were carried out under standard conditions in a dental surgery, using the ICDAS II criteria (Table 6), with a dental light, blunt probe (CPITN) and 3:1 syringe to dry and wet teeth as required by the criteria. All examinations were conducted blind to previous examination scores with a gap of at least one week, to assess intra-examination reproducibility. The intra-examiner reproducibility ranged from 0.74 (ICDAS code ≥ 3) to 0.84 (ICDAS code ≥ 1) for both occlusal and proximal surfaces. The inter-examiner reproducibility ranged from 0.66 for approximal surfaces at ICDAS ≥ 3cut off to 0.73 for occlusal surfaces at ICDAS ≥3 cut off.

The investigators also found that, throughout the whole study, the specificity levels were higher than those for sensitivity. Using the ERK criteria for validation (Ekstrand *et al.*, 1997) (Table 2.2), the mean specificity level ranged from 85.5% for approximal surfaces (D_1 ERK_1 threshold) to 90.0% for occlusal surfaces (D_1 ERK_1 threshold). While the mean sensitivity ranged from 61.4% for proximal surfaces (D_1 ERK_1 threshold) to 77.9% for occlusal surfaces (ERK_3 threshold).

Although the ideal diagnostic method should provide high sensitivity as well as high specificity, it has been accepted that where the caries prevalence is low and the progression of caries is slow, high specificity is required at the expense of sensitivity (Downer, 1989). Therefore, the researchers concluded that the ICDAS II criteria for

diagnosis are appropriate when applied to primary teeth for the diagnosis of both proximal and occlusal caries.

Martignon et al (2007) later confirmed these results when assessing proximal surfaces of both primary and permanent teeth. They used the ICDAS criteria to determine lesion severity in relation to histological depth of sound and carious proximal surfaces of 140 permanent teeth and 108 primary teeth. Teeth were cleaned then examined under clinical conditions using a head light, air drying and the use of a WHO probe. A second examination was conducted eight days later to determine intra-examiner reproducibility, which was found to be 0.86 and 0.92 for the permanent and primary teeth respectively. Afterwards, teeth were examined histologically for demineralisation according to the EKR criteria (Ekstrand *et al.*, 1997) (Table 2.2). The Spearman correlation coefficient was 0.87 and 0.92 for the permanent and primary teeth respectively. The examiners concluded from their study that both the correlation between ICDAS scores and histological changes and the intra-examiner reproducibility for both primary and permanent proximal lesions was excellent.

Scientists have continued to assess the ICADS in clinical and experimental settings. Jablonski et al (2008) conducted an *in-vitro* study to assess the reproducibility and accuracy of the ICADS II criteria for the detection of occlusal caries. Four examiners examined the occlusal surfaces of 100 permanent teeth. The examination was repeated three weeks later for intra-examiner reproducibility. Then teeth were examined histologically using two different histological systems (Downer, 1975; Ekstrand *et al.*, 1997) (Table 2.8, Table 2.2).

The weighted Kappa values for inter- and intra-examiner reproducibility were 0.62 and 0.83 respectively. The relationship between the visual and both histological examinations was moderate to strong (r_s=0.43-0.72). The specificity at the D_1 diagnostic threshold (enamel and dentine) was 0.74-0.91 and the sensitivity was 0.59-0.73. At the D_3 diagnostic threshold, the specificity was 0.82-0.94 and the sensitivity was 0.48-0.83 for the four examiners. The results of this study are comparable to previously reported data which confirm the reproducibility and accuracy of the ICDAS II system for

diagnosis of dental caries at different stages. It can therefore be concluded that the ICDAS II system provides the current optimum methodology for visual caries diagnosis in both the primary and permanent dentitions.

Table 2.8 Criteria used in the Downer histological examination (Downer, 1975).

code	Criteria
0	No enamel demineralisation or a narrow surface of opacity
1	Enamel demineralisation limited to the outer 50% of the enamel layer
2	Demineralisation involving the inner 50% of the enamel
3	Demineralisation involving the outer 50% of dentine
4	Demineralisation involving the inner 50% of dentine

2.5 Visual examination after temporary tooth separation

The use of temporary tooth separation (TTS) has a long history. Since 1870, McQuillen recognised that the detection of proximal caries requires a thorough and careful examination. He claimed that "*even the most careful and experienced practitioners are sometimes deceived in their opinions of the teeth, and are led by the general appearance of integrity to pronounce organs sound which are very far from being so*" (McQuillen, 1870). Therefore, McQuillen suggested that in case of doubt, tooth separation should be undertaken to confirm the diagnosis. The use of TTS for caries diagnosis was supported by others but became increasingly less popular in clinical practice to the point of disappearance. Pitts and Longbottom (1987) strongly recommended in their review of the history of use of TTS, that this method should be utilised for caries diagnosis because the method is inexpensive, non-destructive and reversible.

In 1990, Rimmer and Pitts conducted a study to assess the diagnostic value of temporary tooth separation compared to visual examination alone and radiographic examination in a general practice situation. In this study, 211 children, aged 5-15 years were recruited.

Only 146 children had separators. The investigators found that TTS revealed additional proximal lesions (703 lesions compared to 479 lesions detected by visual examination alone). However, most of these lesions were in the pre-cavitation stage. The large dentinal lesions that were detected by TTS were also confirmed by radiographs while many of the initial lesions were not. Therefore, they concluded that TTS can be used as a diagnostic aid together with radiographic examination but should not replace it (Rimmer and Pitts, 1990).

In 2000, Deery and colleagues also conducted a study to assess the value of TTS compared to visual examination and other diagnostic aids. In this study, 182 Latvian children aged 11-15 years were examined twice, one week apart, before and after TTS. The key finding was that TTS detected 170 additional carious lesions at D_1 level (56.1% of all D_1 lesions detected), of which, 159 lesions were enamel lesions. They also found that TTS detected 20 lesions more at the D_3 level (36.3% of all D_3 lesions detected). These findings agree with the findings of Rimmer and Pitts (1990).

However, Novaes and colleagues (2012a) found that temporary tooth separation did not add to the diagnostic performance of methods used for detection of caries lesions. In addition, the maximum space achieved by TTS was less than 1mm.

2.6 Radiographic examination

2.6.1 Historical background

Dental radiographs are the most commonly used diagnostic aid for caries diagnosis. The development of dental radiographs began with the discovery of x-rays back in 1895 by a Bavarian physicist, Wilhem Conard Roentgen. The first X-ray tube was developed in 1913. In the same year, the first pre-wrapped intraoral films were manufactured by the Eastman Kodak Company. Films produced these days require less than 2% of the initial exposure required in 1920. Intraoral techniques used in dentistry include the paralleling technique, the bisecting technique and the bitewing technique. The paralleling technique

was first introduced in 1896 by C Edmond Kells. A few years later Weston Price, a Cleveland dentist, introduced the bisecting technique in 1904. The bitewing technique was introduced by Howard Raley Raper as a modified form of the original bisecting technique in 1925. The extra-oral technique used most frequently in dentistry is panoramic radiography, which was first developed in Japan in 1933 (Iannucci and Howerton, 2012).

2.6.2 Risk of ionising radiation

Dental radiography is frequently used as an adjunct to caries diagnosis, particularly for surfaces where visibility is poor or impossible. Its importance is reflected by the high number of dental radiographs taken annually in general dental practice in the UK, which was found to be at least 18 million every year (Hirschmann, 1995). However, this investigation involves risks associated with ionising radiation. This risk is highest for the young, especially for children under the age of ten where the risk is three times more than the risk for those at the age of 30 years, and least for the elderly (ICRP, 1991). Therefore every effort should be made to avoid unnecessary exposure to ionising radiation, especially for children. Dental radiography is acknowledged to incur small doses and risks as shown by smith (1992) who calculated a risk estimate and found that five-year-old children may be expected to have one induction of malignant disease following exposure to one million dental exposures. However, two studies in the United States have shown a possible association between dental radiography and brain and parotid tumours (Prestonmartin and White, 1990; Neuberger *et al.*, 1991).

To minimise these risks, numerous guidelines have been put in place to regulate the amount and frequency of exposure to ionising radiation (Espelid *et al.*, 2003; ADA, 2012). It is the responsibility of every dentist to make every effort to minimise this risk, by use of the correct technique and the right clinical judgment. Therefore, every patient should be examined carefully before the prescription of any radiographic examination, to ensure that radiographs are essential to aid diagnosis.

2.6.3 Radiographic caries diagnosis

For the diagnosis of caries in children, it has been shown that bitewing radiographs are an important supplement to clinical examination (Kidd and Pitts, 1990) not only for proximal caries but also for occlusal caries (Pitts, 1996; Weerheijm, 1997). Bitewing radiographs have many benefits for the diagnosis of dental caries; they can detect carious lesions which cannot otherwise be detected, monitor lesion progression and estimate extension of caries. Nevertheless, there is no justification for taking radiographs for routine screening for low risk populations (Hintze *et al.*, 1994; Pitts, 1996). During the last few decades, some changes related to the prescription of bitewing radiographs have occurred; these are due to a decrease in the prevalence of caries in industrialised countries, slower rates of caries progression due to exposure to fluoride (Mejare *et al.*, 1999) and increased concerns regarding the risks associated with ionising radiation (Valachovic and Lurie, 1982). These factors should, however, be balanced carefully against the consequences of failing to achieve an accurate caries diagnosis because of a reluctance to use a special investigation which has been shown to have an additional diagnostic yield (Pitts, 1996).

Dentists should be aware of those risks and patients should only be exposed to ionising radiation after careful examination and assessment of caries risk.

2.6.4 Caries risk assessment

A number of factors may be taken into consideration when making a decision about the need for radiographic assessment of caries. Caries risk assessment of each individual patient is important before taking such decision. A number of systems have been suggested to assist with this process. Two well recognised systems have been described, these are; a computer-based risk assessment model for caries (Cariogram) (Petersson, 2003) and caries management by risk assessment (CAMBRA) (Featherstone *et al.*, 2012).

2.6.5 The frequency of radiographic examination according to caries risk

The frequency of taking bitewing radiographs is largely dictated by an individual's caries risk status (Pitts and Kidd, 1992). Individuals with low caries risk require lower frequency of dental radiographic examination. Indeed, careful visual examination combined with other non-ionising caries diagnostic devices may be sufficient (Neuhaus *et al.*, 2009). In higher risk patients, the available evidence for the balance between the risk of ionising radiation and the additional diagnostic yield of radiographs is strong enough to justify individualised radiographic examination (Pitts, 1996), particularly for areas where direct visual examination is difficult or in some instances impossible such as proximal surfaces (Kidd and Pitts, 1990). There is no evidence that a 'blanket' regimen of automatic radiographic screening will benefit populations (Pitts, 1996) especially those with low caries experience (Hintze and Wenzel, 1994).

Hintze and Wenzel (1994) conducted a study to compare the value of a clinical examination compared to radiographic screening in a group of Danish children with a mean age of 14 years and mean caries experience of DMFT=1.2. Children were examined by three examiners under standard clinical conditions, visually and radiographically. They found that radiographs detected more than 94% of all lesions detected irrespective of lesion size. Of the occlusal surfaces diagnosed as sound, only 2.1% were subsequently found to have dentine caries radiographically, two of which were found to involve the inner half of dentine. These lesions would have been missed if the radiographic examination had not been conducted.

Of the proximal surfaces assessed as sound, 1.1% had dentine caries radiographically, one of which was in the inner half of dentine. These lesions also would have been missed if bitewing radiographs had not been taken. The small number of undetected dental caries requiring restorative treatment and the change in the behaviour of dental caries resulting in a slow progression meant that there was no convincing evidence that screening would be of additional clinical benefit for children with low caries experience (Hintze and Wenzel, 1994).

There is good evidence that an initial posterior bitewing radiographic examination is clinically justified in all new patients over the age of five whose posterior teeth have closed contact (Espelid *et al.*, 2003). This, however, should be conducted in conjunction with careful clinical examination to detect proximal and occlusal caries.

Subsequent radiographic examinations are prescribed in accordance to individual caries risk. The Faculty of General Dental Practitioners (1998) recommends six month intervals between radiographic examinations for high caries risk individuals until the caries risk status changes. This period is extended to one year for moderate caries risk and to 12-18 months for low caries risk in primary teeth. For patients with permanent teeth, a longer period of two years between radiographic examination is recommended for low risk individuals. Longer interval periods for subsequent radiographs have been recommended by the European Academy of Paediatric Dentistry (EAPD) (Espelid *et al.*, 2003), who recommend an interval of one year for high risk individuals and a period of 2-4 years for individuals with low caries risk according to their age. For initial bitewing examination, the Academy recommends that all 5-year-old children, even with no evidence of caries, should be considered for a baseline bitewing examination.

Another recommendation took into account the age of individuals in relation to caries risk as well as lesion progresion rate of children and adolescents in areas with low caries prevaence (Mejare, 2005). These recommendations identified key ages for taking bitewing radiographs, which were 5, 8-9, 12-13, and 15-16 years. These recommendations agree with the European Academy of Paediatric Dentistry guidelines regarding the periods of subsequent radiographic examination. They also recommended that 5-year-olds have an initial radiographic examination even those with no evidence of caries. This was justified by two studies of 5-year-old children which compared the diagnostic yield of radiographic examination compared to clinical examination. The authors found that, on average, radiographic examination revealed 1.2-1.8 more lesions than visual examination alone (Skold *et al.*, 1997; Anderson *et al.*, 2005). The identification of caries free 5-year-olds was also an indication that those children have a very small risk of developing a new carious lesion within the next 3-4 years (Mejare,

2005). A more recent guideline by the American Academy of Paediatric Dentistry recommended radiographic examination for all new patients with first permanent molars. Children with a primary dentition (before the eruption of first permanent molars) who had no clinical evidence of dental caries may not require radiographic examination (AAPD, 2009) which differs to the EAPD guideline (Espelid *et al.*, 2003). For high caries risk patients, subsequent radiographic examination periods of 6-12 months are recommended if proximal caries can not be examined visually or by probe. For low caries risk individuals, recall periods of 12-24 months are recommended for children in the mixed dentition. An extended period of 18-36 months is recommended for recall examination of patients in the permanent dentition (AAPD, 2009).

Despite the presence of guidelines, an important clinical consideration is the identification of lesions which are prone to more rapid caries progression in order to apply the most appropriate timing for radiographic examination (Mejare, 2005). It remains the dentist's resposibility to consider the benefits of radiographic examination against its risks. Furthermore, an individual caries risk assessment together with an evaluation of the rate of lesion progression should always preceed any bitewing radiographic examination (Mejare, 2005).

2.6.6 Progression of caries

The risk of overlooking a carious lesion at initial examination may be less critical in some patients than others. For example, Mejare et al (1999) described the outcomes of a non-operative treatment strategy in Swedish teenagers where the threshold for restoration of a lesion was clinical cavitation or evidence of a radiographic radiolucency extending through the outer half of dentine. The authors noticed that over 11 years, proximal enamel caries showed slow progression: 50% of lesions did not reach the inner half of enamel by the end of the study and 75% of these lesions took more than six years before they progressed to the inner half of enamel. Caries progression in proximal dentine, when the lesion had radiographically reached the enamel-dentine junction was found to be four times faster than the progression of lesions which had radiolucency

evident in the inner half of enamel. However, 50% of lesions at the enamel dentine junction did not show any progression after 3.1 years (Mejare *et al.*, 1999).

This agrees with the findings of another longitudinal study (Lith *et al.*, 1995) where the investigators followed children between the age of 8-18 years and found that only 2% of proximal lesions progressed to the inner half of dentine over 20 months, indicating that proximal caries progression can be very slow. However, it is acknowledged that some individuals show faster lesion progression. It has been shown that 20% of lesions in the inner half of enamel on the mesial surface of the first permanent molars in children aged 6-12 years progressed to dentine within a year (Mejare and Stenlund, 2000). Further, the rate of progression of enamel caries for proximal carious lesions in primary molars, which takes 2-2.5 years to progress into dentine, is twice as fast as in permanent molars (Shwartz *et al.*, 1984).

Moreover, the higher the number of lesions present, the greater risk that one of these lesions will progress to dentine caries (Mejare *et al.*, 1999). Proximal surfaces which are adjacent to recently restored surfaces have a four time higher risk of developing caries compared to contralateral teeth which did not have a restoration in adjacent surfaces (Qvist *et al.*, 1992). It has also been shown that enamel or dentine caries in the distal surface of second primary molars increases the risk of caries progression in the mesial surface of first permanent molars by about 15 times (Mejare and Stenlund, 2000).

2.6.7 The relationship between radiographic appearance and cavitation

It is very important to understand the relationship between radiolucency depth of carious lesions and clinical cavitation, as this is the threshold which dentists use when deciding whether or not to restore a tooth. Restoring a proximal lesion, which is subsequently found not to be cavitated, may be deemed unnecessary destruction of tooth tissue (Anusavice, 1992). Previous research has failed to identify a clear relationship between radiolucency depth on radiographs and a clinical cavitation of tooth surface. However, it has been shown that an increase of depth of radiolucency increases the probability of a lesion being cavitated (Ratledge *et al.*, 2001; Mariath *et al.*, 2007).

Most investigators would concur that there is a very small probability of cavitation where the radiolucent lesion is within enamel (Pitts and Rimmer, 1992; Akpata *et al.*, 1996; Hintze *et al.*, 1998). Hintze and co-workers (1998) examined 53 dental and dental hygiene students, with a mean age of 24 years, and found that 8% of lesions with a radiolucency within the inner half of enamel were cavitated.

This agrees with the findings of Pitts and Rimmer (1992) who found that 10% of permanent tooth surfaces and only 3% of primary tooth surfaces were cavitated when lesion radiolucency was confined to enamel.

The likelihood of cavitation, when the radiolucency extends to the outer half of dentine, appears to vary considerably between studies. Early studies suggested that radiolucencies in outer half of dentine were highly likely to be cavitated. Rugg-Gunn (1972) reported that there was a 100% possibility of cavitation when the radiolucency reached the outer half of dentine. Cavitation was assessed by direct visual examination of surfaces where pre-existing spacing was present. The same figure (100%) was reported by Mejare et al (1986) who assessed cavitation in premolars and adjacent teeth by direct visual examination following orthodontic extractions of premolars in teenage patients. However, it should be recognised that both these studies involved small numbers of carious surfaces and employed different methods for assessing cavitation. More recent studies have reported cavitation in around 80-90% of permanent proximal surfaces with radiolucencies extending to the outer half of dentine (Mejare and Malmgren, 1986; De Araujo *et al.*, 1992; Akpata *et al.*, 1996; Ratledge *et al.*, 2001).

A similar trend has also been shown for primary tooth surfaces. Nielsen et al, (1996) conducted an *in-vitro* study involving 72 proximal surfaces from 46 primary molars. Teeth were examined radiographically using two methods: a storage phosphor system (Digora) and Ekta speed plus film. The investigators found that the majority of lesions with a radiolucency in dentine were actually cavitated. This agrees with the findings of Mariath and colleagues (2007) who examined 51 children, aged 4-10 years, who had primary molars with radiolucencies in the outer half of dentine of proximal surfaces and found a high likelihood of associated cavitation.

However, Pitts and Rimmer (1992) conducted a large study in Scotland involving 211 children between the age of 5 and 15 years. In this study 1,468 permanent and 756 primary posterior proximal surfaces were assessed radiographically for radiolucency depth and clinically for the presence of cavitation following temporary tooth separation. The investigators found that only 40% of permanent and 28% of primary proximal surfaces with radiolucency in the outer half of dentine were clinically cavitated. These findings were comparable with these of previous studies where cavitation was seen in 52% of surfaces with a radiolucency in the outer half of dentine (Bille and Thylstrup, 1982; Thylstrup *et al.*, 1986). It could be concluded from these investigations that cavitation occurred at a later stage than previously shown and, interestingly, primary teeth underwent cavitation at a later stage than permanent teeth. The difference in cavitation reported from different studies may be due to differences in techniques used for taking radiographs, study design or water fluoridation of areas studied.

In summary, radiolucency in the outer half of dentine is highly indicative of associated tooth surface cavitation. However, it is not an absolute predictor of cavitation and, radiographs should, therefore be used in conjunction with other diagnostic methods in decision-making about the need for restorative intervention versus preventive only measures.With respect to a radiolucency in the inner half of dentine, studies have almost always, shown this to indicate cavitation (Pitts and Rimmer, 1992; Akpata *et al.*, 1996; Nielsen *et al.*, 1996; Hintze *et al.*, 1998). A summary of the main studies which looked at the relationship between radiographic depth and cavitation is described in Table 2.9.

Table 2.9 A summary of studies looking at the relationship between radiographic depth and cavitation

Study	Type of study	Type of teeth	Radiographic criteria	Validation method	Results
Mariath etal, 2007	*In-vivo* 51 children 4-10 years	Primary molars	Radiolucency into outer ½ of dentine	Elastomeric impressions after TTS	65% of lesions with radiolucency in outer ½ of dentine were cavitated.
Ratledge etal, 2001	*In-vivo*	Permanent molars 54 surfaces	Radiolucency into outer ½ of dentine	Elastomeric impressions after TTS	Cavitation present in 85% of surfaces with radiolucency into outer 1/2 of dentine.
Hintze etal, 1998	*In-vivo* 53 patients	Permanent molars 338	0= Sound 1= Radiolucency in outer ½ of enamel 2= Radiolucency in inner ½ of enamel 3= Radiolucency in outer ⅓ of dentine 4= Radiolucency in inner ⅔ of dentine	Visual examination after TTS	R0= 2.6% cavitated R1= 0% cavitated R2= 2% cavitated R3= 37% cavitated R4= 80% cavitated
Akpata etal, 1996	*In-vivo*	Permanent 108 molars and premolars	0= Sound 1= Radiolucency in outer ½ of enamel 2= Radiolucency in	Cavity preparation of carious tooth surfaces	R0= 0% cavitated R1= 0% cavitated R2= 19.3% cavitated

			inner ½ of enamel and EDJ 3= Radiolucency in outer half of dentine 4= Radiolucency in inner half of dentine		R3= 79.1% cavitated R4= 100% cavitated
Nielsen etal, 1996	*In-vitro*	Primary molars 46 teeth, 72 surfaces	0= No radiolucency 1= Radiolucency in outer ½ of enamel 2= Radiolucency in inner ½ of enamel 3= Radiolucency in dentine	Direct visual examination *in-vitro*	Code 0= 0%cavitated Code 1= 10.7% Code 2= 14.2% Code 3= 83%
Pitts and Rimmer, 1992	*In-vivo* 211 children 5-15 years	Primary molars 756 surfaces Permanent teeth 1468 surfaces74	R0= No radiolucency R1= Radiolucency in outer ½ of E R2= Radiolucency in inner ½ of dentine R3= Radiolucency in outer ½ of dentine R4= Radiolucency in inner ½ of dentine	Direct visual examination after TTS	Permanent teeth R1= 0% cavitated R2= 10.5% cavitated R3= 40.9% cavitated R4= 100% cavitated

As can be seen from the above studies (Table 2.9), the percentage of cavitated surfaces in relation to the depth of radiolucency varies between studies. This can be due to the difference in the nature of studies for example, *in-vitro* studies allow for the detection of cavitation in all surfaces while *in-vivo* studies may not be able to detect cavitation in some surfaces where direct visibility is not possible. In addition, the criteria used for radiographic depth of radiolucency is a very important factor, as most of studies divided the dentine radiolucency into two halves while some studies divided dentine radiolucency into three thirds. This certainly will affect the percentage of cavitation reported (outer third compared to outer half of dentine). Studies should be consistent in the criteria they use in order to be able to compare results of different studies.

2.6.8 The value of radiographic examination in the detection of caries

It is beyond doubt that intra-oral radiographs are valuable as a diagnostic aid in the detection of dental caries, especially in areas where direct clinical inspection is difficult or impossible. The literature has a wealth of studies showing the additional diagnostic yield for radiographic examination above that of clinical examination. However, this value differs between different studies because of the variability of many factors including: the population studied; caries prevalence; the method used for clinical examination; the threshold used for detection of caries; type of teeth examined and surfaces examined. Therefore, direct inter-study comparisons are complicated. The following section will attempt to explain the value of radiographs, taking into account these different factors.

A comprehensive review of the literature published between 1933 and 1987, which examined the value of radiographs in the diagnosis of proximal caries, included 29 relevant studies (Pitts, 1996). Most of these studies related to children and the clinical diagnostic threshold was invariably cavitation. The review found that in primary teeth, radiographs consistently revealed an additional diagnostic yield of 40-469% above that revealed by clinical examination alone, regardless of the diagnostic threshold and patient's age. In permanent teeth, radiographs also showed a high additional yield of 50-

250% more lesions than were detected by clinical examination only. It was concluded that clinical examination alone detected less than 50% of lesions while bitewing radiographs alone detected more than 90% of the total number of lesions. The accuracy or rigour of the clinical examination was one of the factors found to affect the additional value of radiographs; not surprisingly, the more meticulous the clinical examination, the less the additional value offered by radiographs.

In 1993, Ketley and Holt conducted an *in-vitro* study to compare the validity of visual examination in relation to radiographic examination for the diagnosis of occlusal caries in 100 second primary molars and 100 first permanent molars extracted from children under general anaesthesia in areas with suboptimal water fluoridation. Teeth were extracted because of caries or for orthodontic reasons. Teeth included were those with no obvious caries or restoration in occlusal surfaces.

For the visual examination, teeth were examined under a standard operating light using compressed air. Standardised bitewing radiographs were taken and examined using a light box without magnification. Teeth were then sectioned using a diamond saw. Sections were dried and examined visually for the presence and extent of carious lesions.

Inter- and Intra-examiner reproducibility was good ranging from a Cohen's Kappa statistic of 0.68-0.88 and 0.77- 0.92 respectively. The sensitivity and specificity of each method and both combined in primary and permanent teeth is shown in Table 2.10.

The results of the study showed that a combination of clinical and radiographic examination increased the sensitivity and detected the majority of lesions. However, their visual examination showed a lower sensitivity than reported previously in the literature. The authors explained this by the fact that the included teeth in the study were all seemingly free of caries and therefore more difficult to diagnose. The sensitivity of both methods was higher for primary teeth than permanent teeth.

Although this study was conducted almost three decades ago, it is comparable to more recent studies (Neuhaus *et al.*, 2011). The histological validation of the caries diagnosis makes the findings more valid than those of previous studies where radiographs were used as a validation method for the clinical diagnosis.

Table 2.10 The sensitivity and specificity of caries diagnosis methods in both primary and permanent teeth (Ketley and Holt, 1993).

Method	Primary teeth		Permanent teeth	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
Visual examination	0.45	1	0.31	0.98
Radiographic examination	0.93	0.89	0.67	0.92
Combination	0.93	0.89	0.75	0.9

The more recent studies have assessed the validity of radiographic examination in the diagnosis of occlusal caries in primary teeth at both the enamel and dentine levels of caries (Attrill and Ashley, 2001; Lussi and Francescut, 2003; Rocha *et al.*, 2003; Neuhaus *et al.*, 2010). The sensitivity of radiographs for diagnosis of enamel and dentine caries was found to range from 0.29 to 0.62 and from 0.54 to 0.96 respectively. The specificity of radiographs for caries diagnosis was found to range from 0.72 to 1. Taken together, these studies showed radiographs are more reliable at diagnosing sound occlusal surfaces than the detection of carious occlusal lesions.

Other studies have assessed the validity of radiographic examination for the diagnosis of proximal caries in primary teeth, which is the interest focus of this research. It would appear that this topic has received increasing attention over the past decade, as evidenced by the number of published studies. Some of these key papers will now be described.

Newman and colleagues (2009) looked at 611 school children, aged 6-13 years, from a non-fluoridated area with low socioeconomic status in Australia. Proximal surfaces of primary molars were assessed clinically, by four examiners who were calibrated by examining six children on two different occasions, one week apart. The intra- and inter-examiner reproducibility for visou-tactile examination and radiographic examination was 0.76. Bitewing radiographs were exposed using standard techniques. These radiographs were interpreted on radiographic viewers without magnification. The criteria used for both examinations are shown in Table 2.11.

The specificity of radiographs was more than 0.9, irrespective of the diagnostic threshold. The reference method used in this study was the total number of lesions detected by both methods, thus the results cannot be verified in the absence of histological validation.

Interestingly, although this study was conducted recently, the investigators used visual/tactile criteria for their clinical examination of the surfaces despite current evidence that suggests probing may damage early carious lesions, which could otherwise remineralise (Ekstrand *et al.*, 1987; van Dorp *et al.*, 1988; Yassin, 1995).

In addition the diagnostic threshold they used (Newman *et al.*, 2009) as a restorative threshold (C_3/R_3) is not actually the threshold that clinicians use for restoration. Although, C_3/R_3 normally relates to this threshold, the criteria employed in this study started from 1 as 'sound' instead of 0 as 'sound'. Thus the notation of 3 was given at an earlier stage (in enamel) instead of dentine, and, the threshold could not be considered a restorative threshold.

Table 2.11 Clinical and radiographic criteria for caries diagnosis (Newman et al., 2009).

Clinical criteria	Radiographic criteria
C1 – Sound surface	R1 – Sound
C2 – Discoloured surface which the sickle explorer could not enter	R2 – Radiolucency in outer half of enamel
C3 – Decayed surface which the sickle explorer withdrew with some resistance	R3 – Radiolucency in inner half of enamel
C4 – Decayed lesion, not involving pulp, in which the explorer moved freely	R4 – Radiolucency in the dentine
C5 – A lesion involving pulp	R5 – Radiolucency with obvious spread in the outer half of the
C6 – Restoration present-amalgam	R6 – Radiolucency with obvious spread in the inner half of the dentine
C7 – Restoration present-plastic	R7 – Filled surface and sound
C8 – Restored with recurrent caries-amalgam	R8 – Filled, with secondary caries (radiolucency and filling on the same surface)
C9 – Restored with recurrent caries-plastic	R9 – Extracted due to caries
C10 – Fractured amalgam restoration no caries-needs redoing	
C11 – Fractured plastic restoration no caries-needs redoing	
C12 – Extracted due to caries	
C13 – Fractured teeth-trauma	

Although of greatest diagnostic value in high-caries children, bitewing radiographs have also been shown to have additional diagnostic value in low risk populations. Poorterman et al (2010) conducted a study in the Netherlands to assess the value of bitewing radiographs for the detection of proximal caries in 6-year-old children with low caries

experience. Fifty children were examined by two calibrated dentists who had previous experience of comparable projects.

The clinical examination was conducted according to WHO criteria under standard clinical conditions. The diagnostic threshold was the presence of discoloration into dentine or enamel discontinuity (D_3). Bitewing radiographs were taken on the same day of the survey by one of the examiners using film holders. Radiographs were examined on an x ray desk viewer without magnification. The criteria adopted for both clinical and radiographic examination are shown in Table 2.12.

The inter-examiner reproducibility for clinical examination and bitewing radiographs was calculated using Cohens Kappa and was found to be good for both methods (0.94 and 0.87 respectively). The investigators found that, in this group of children, clinical examination alone significantly underestimated the amount of dental caries present as it only detected 44.8% of lesions. Radiographs detected about 50% of lesions which were not clinically identified. Bitewing radiographs had a 97% additional diagnostic yield at the D_3 level of diagnosis. Furthermore, 38% of children who were clinically diagnosed as caries-free had one or more dentine lesions requiring restoration. This finding obviously has important clinical relevance.

The results of this recent study support those of a previous survey in a low risk population undertaken by Anderson et al (2005). The investigators reported that bitewing radiographs detected a mean of 1.2 proximal lesions above those detected by clinical examination alone in 5-year old children.

It is important to note that none of the above studies used histological examination to validate their results. The number of lesions detected by both clinical and radiographic examinations is usually combined to provide the total number of lesions, as the reference point. Hence, results would tend to show a higher sensitivity for radiographic examination than it actually is as there is no way of identifying false positive and negative findings.

Table 2.12 Clinical and radiographic criteria for caries diagnosis (Poorterman *et al.*, 2010).

Clinical criteria	Radiographic criteria
0 = sound tooth (no evidence of treated or untreated clinical dentine caries)	0 = no radiolucency visible in enamel and/or dentine
3 = dentine caries	1 = radiolucency confined to the enamel
6 = filled surface without decay	2 = a circumscribed radiolucency visible in the dentine (D_3-level)
7 = filled surface with decay not connected to the restoration	3 = an adequate restoration
8 = filled surface with decay connected to the restoration	4 = an inadequate restoration (a missing, partly missing or fractured restoration, marginal over- or under extension, open proximal contact with chance of food impaction)
9 = filled surface with decay into the pulp	5 = a restoration in combination with a circumscribed radiolucency visible in the dentine
I = inadequate restoration (a missing, partly missing or fractured restoration, marginal over- or under extension, open proximal contact with chance of food impaction)	6 = a missing tooth surface

This hypothesis has been supported by the results of studies comparing the value of radiographs with other diagnostic methods which validated their results histologically (Virajsilp *et al.*, 2005; Braga *et al.*, 2009; Novaes *et al.*, 2009). In these studies, bitewing radiographs had lower sensitivity in the detection of proximal caries in primary teeth. The sensitivity of bitewing examination in these studies was found to be 0.16-0.5 at the enamel level of diagnosis and 0.4-0.7 at the dentine level of diagnosis. However, the specificity of bitewing radiographs was still found to be as high as previous studies with no histological validation (0.9-1). These studies actually compared bitewing radiographs

with a laser fluorescence device for the diagnosis of dental caries. The specific details will be explained further in a later section on the value of a laser fluorescence device.

2.6.8 Radiographic yield for the permanent dentition

The literature on the value of bitewing radiographs for the detection of proximal caries in permanent teeth concurs with that for primary teeth. Hopcraft and Morgan (2005) examined 879 adults from a low caries risk population to assess the value of radiographs compared to clinical examination for the diagnosis of proximal caries in permanent teeth. They found that bitewing radiographs provided a significant additional diagnostic yield of 204-336% with two thirds of lesions being detected by radiographs only.

These results are in line with the findings of Civera et al, (2007) who examined 30 adult patients from a low caries risk population clinically and radiographically with both digital and conventional radiography. They found that both radiographic techniques showed similar results, with three times higher caries detection than achieved from clinical examination alone. The lack of histological validation of both these studies may, however, have overestimated the value of radiographic examination.

Summary

All studies cited so far, irrespective of the type of teeth examined (primary or permanent) or type of surfaces (proximal or occlusal) agree that clinical examination alone is unable to detect all carious lesions. An additional diagnostic tool is required if all carious lesions are to be reliably identified. There is a compelling body of evidence to date to suggest that bitewing radiographs offer this additional diagnostic benefit, in both high and low caries risk populations.

2.6.9 Prescription of radiographs for children by general dental practitioners

Despite indisputable evidence for the value of radiographs in the diagnosis of clinically undetectable lesions (Kidd and Pitts, 1990; Pitts and Rimmer, 1992), and the development of clinical guidelines which recommend the taking of radiographs for early detection of carious lesions (Espelid *et al.*, 2003), these guidelines do not seem to be followed in some dental settings.

A survey of general dental practitioners (GDPs) in Scotland found that only 72% of dentists would routinely use radiographs as a diagnostic tool for children. Moreover, only 17% considered taking radiographs for children under the age of six years (Taylor and Macpherson, 2004). The results of this study were echoed by a survey conducted in Sheffield to determine the use of radiographs by GDPs before referral of children for carious extractions under general anaesthesia (Young *et al.*, 2009a). The authors found that only 12.4% of children referred had previous radiographic examination prior to hospital referral. They concluded that radiographs are not taken routinely for caries diagnosis by GDPs in the UK. A more recent study (Mauthe and Eaton, 2011), investigating the use of bitewing radiographs and adherence to guidelines by GDPs, found that NHS GDPs were significantly less likely to prescribe bitewing radiographs to children and adults as advised by the Faculty of General Dental Practitioners (UK) (Horner *et al.*, 2013) than were private GDPs.

However, this trend is not only seen in the UK. A survey in the Netherlands compared the taking of radiographs by GDPs and paediatric dentists, for a cohort of children who were under the same Dutch insurance company. The investigators found that radiographs were rarely used by GDPs for children under the age of six years, slightly more for children between the ages of six and eight, and significantly less frequently compared to paediatric dentists (Schorer-Jensma and Veerkamp, 2010).

The question that thus arises, is why do some general dental practitioners fail to take radiographs for caries diagnosis in their young patients? What is the reason for the

apparent reluctance of GDPs to take radiographs, despite the strong evidence-base for their value in caries diagnosis?

There are a number of possible barriers to undertaking a dental radiographic examination of children, as itemised below:

- Lack of knowledge of the usefulness of radiographs for young patients: only 15% of GDPs in Taylor and Macpherson's study (2004) considered bitewing radiographs very important for the diagnosis of caries in children.
- Lack of knowledge of existing clinical guidelines: a survey of the prescription of radiographs for children by GDPs in the South West of England found that more than half of GDPs did not have access to guidelines (Patel *et al.*, 2006).
- Lack of compliance by children themselves: a study assessed the acceptance of conventional type of bitewing radiography in 211 children between the ages of 3-15 years found that all children under the age of five years refused radiographs. Furthermore, 31% of children aged 5-9 years were unable to accept a radiographic examination (Pitts *et al.*, 1991). This lack of compliance may be attributed to many reasons including: anxiety, difficulties during placement of the film holder or a severe gag reflex (Poorterman *et al.*, 2010).
- Inadequate remuneration: a previous survey reported that 92% of GDPs thought that there should be a specific element of payment for taking radiographs for children. Half of the respondents stated that appropriate remuneration would increase the frequency of taking radiographs (Taylor and Macpherson, 2004).
- Risk of radiation: it has been reported that more than one third of GDPs believed the risk of radiation was an important or very important factor in the decision whether or not to take radiographs (Taylor and Macpherson, 2004).
- Time available: GDPs have acknowledged that treating children under the age of 6 years is stressful and time consuming (Van Dam *et al.*, 2003) and taking radiographs would increase both time and stress. Almost half of GDPs in Taylor and Macpherson's study (2004) thought that time was a key determinant factor in the taking of radiographs.

Clearly there are important clinical implications from inadequate caries diagnosis. The failure of GDPs to use radiographs may lead to a delay in the detection of dental caries and failure to provide appropriate treatment planning for prevention of early lesions and restoration of carious lesions before pulp involvement (Rodd *et al.*, 2006). Undoubtedly, efforts must be made to find a caries diagnostic aid that can overcome these barriers and gain a greater acceptance by both children and clinicians.

2.7 Laser fluorescence

2.7.1 Laser Fluorescence Device – DIAGNOdent 2095

Laser fluorescence (LF) has largely been used as a diagnostic aid for caries detection over the last decade. The first available laser fluorescence device was the Kavo DIAGNOdent 2095 device (DD) (Kavo, Biberarch, Germany). It contains a laser diode which uses a 655nm monochromatic light as the excitation light source, and a photo diode combined with a filter, which transmits light with a wave length longer than 680nm.

The device works on the basis that caries induced changes in the tooth structure lead to increased fluorescence at certain excitation wave lengths. The intensity of the fluorescence depends on the tooth structure and the wave length of the light hitting the tooth surface. The laser light (red) is transmitted to the tooth surface through an optical fibre. Around this fibre, a bundle of nine fibres is concentrically arranged to collect the fluorescence from the tooth surface. The back scattered light and ambient light is absorbed by the filter. The photo diode measures the amount of fluorescence passing through the filter. A digital display on the machine shows both a real time value and a maximum value.

The DIAGNOdent 2095 comes with two types of fibre optic tips, A and B. Tip A is a tapered tip, specifically designed for fissure caries diagnosis and tip B is a flat tip for smooth surface caries diagnosis (Lussi *et al.*, 1999). During the process of caries

detection, the red light beam enters the tooth surface and either passes unhindered into dentine or is partially scattered depending on the enamel structure. Regular crystalline enamel is more transparent, thus, transmits light with minimum deflection. A less homogenous enamel layer will lead to more diffracted and scattered light which then excites either the dental hard substance itself or excites so called fluorophores. The fluorophores are particles with the ability to fluoresce when excited by light at a specific wave length. The fluorophores in this case were identified as bacterial protoporphyrins excited by 655nm laser light. Thus the amount of back scattered fluorescence is proportional to the pore volume, the amount of bacteria in the lesion and the lesion depth (Neuhaus *et al.*, 2009).

The measurement on the digital display can vary from 0 (minimum fluorescence) to 99 (maximum fluorescence), thus making caries detection objective rather than subjective and making quantitative caries monitoring possible (Lussi *et al.*, 2001). The cut off-values for caries diagnosis for each depth of caries provided by the manufacturer is shown in Table 2.13 (Kavo, 2000).

Table 2.13 Interpretation of LF readings according to the manufacturer (Kavo, 2000).

	Manufacturer cut-off values	**Caries status**
Sound	0-4.9	No caries
Enamel caries	5-25	Caries in enamel
Dentine caries	25.01-35	Caries through enamel and into the outer half of dentine
Deep dentine caries	>35	Caries through enamel and into the inner half of dentine

2.7.1.1 Validity of DIAGNOdent (2095) for the detection of occlusal caries in permanent teeth

The LF device was first validated by Lussi and colleagues (1999). They conducted an *in-vitro* study to assess the LF performance and reproducibility for the detection of occlusal caries. They examined 105 teeth extracted by dental practitioners in Bern, Switzerland (a region with no water fluoridation). Teeth were first stored in 5% neutral buffered formalin, then were cleaned with a toothbrush and fluoride free pumice before examination.

All teeth had no signs of clinical occlusal caries. The assessment with the laser fluorescence system was as follows: first, the device was calibrated using the ceramic standard provided by the manufacturer, then, a baseline measurement for the tooth was taken by measuring the fluorescence of a sound spot on a smooth surface of the tooth. This value was then subtracted from the fluorescence value of the site tested. The selection of the site to be tested on the occlusal surface was based on it being either suspected to have caries or, in case of the absence of any suspicions, being the most susceptible point for caries.

A hand drawing of the occlusal site was produced indicating where the test site was done. The tip of the laser device was placed on the tested site and rotated around a vertical axis until the highest reading achieved. Teeth were measured after moistening with a drop of artificial saliva and after brief drying with a 3-in-1 syringe. After the teeth had been assessed with the laser device, they were prepared histologically. Ground sections were photographed at a magnification of x3.2. When the test area was reached, teeth were embedded in methylmethaacrylate and sectioned perpendicular to the occlusal surface producing slices with the test sites. Slices then were stained with acetic light green for 2-3 minutes then photographed. The cut-off limits were determined using histological and statistical methods where the highest likelihood ratios were found. The optimal cut-off limits for the laser device were found to be as follows:

- 0-4: no caries or histological enamel caries limited to the outer half of enamel thickness (D_1)
- 4.01-10: histological caries extending beyond the outer half, but confined to the enamel (D_2)
- 10.01-18: histological dentinal caries limited to the outer half of the dentine thickness (D_3)
- ≥ 18.01: histological dentinal caries extending into the inner half of dentine thickness (D_4)

According to these cut-offs, the sensitivity was found to range from 0.76 (moist teeth, D_3) to 0.87 (moist teeth, D_2). The sensitivity of the device for dry teeth was not significantly different from that of moist teeth (0.83). Furthermore, there was no difference in the specificity of the machine for wet and dry surfaces. The specificity values ranged from 72% (dried teeth, D_3) to 87% (moist teeth, D_3).

For measuring reproducibility, 83 molars (not the same ones used in the original study) were used (Lussi *et al.*, 1999). Photographs of the occlusal surfaces were taken and the test site was marked by a black dot. Eleven dentists assessed the teeth twice using the laser device. Dentists were asked to assess the fluorescence of the test site. The same procedure was repeated later in the same session. The intra- and inter-examiner reproducibility were calculated using Cohen's Kappa test. The average intra-examiner reproducibility was 0.88 (D_2 level) and 0.90 (D_3 level).

The reproducibility of the device was excellent despite the very short training period that the dentists had undergone. The inter-examiner reproducibility values were good with an average of 0.65 at the D_2 level and average of 0.73 at the D_3 level. These values may have been improved if the dentists had received longer training. Nevertheless, the high performance of the device shown in this study suggested that the device could be used as a diagnostic adjunct or as second opinion to test sites of clinical uncertainty. In addition, its high reproducibility suggested that it may be a valuable device for

longitudinal monitoring of caries and for assessing the outcome of preventive trials (Lussi *et al.*, 1999).

Further studies

The results of the previous study were supported by Shi et al (2000b), where they conducted another *in-vitro* study to assess the validity and reproducibility of the DIAGNOdent device compared to radiography. The occlusal surfaces of 76 premolars and molars with no macroscopic caries were assessed with the laser device (wet and dry), radiographically and histologically. The investigators found that the diagnostic accuracy of the DIAGNOdent device was significantly higher than that of bitewing radiographs. It was also reported that the LF device was better in detecting dentine caries (sensitivity of 0.82) than enamel caries (sensitivity of 0.46). Interestingly, the specificity was high for both enamel and dentine levels of diagnosis, being 0.95 and 1 respectively. The method had excellent reproducibility with a correlation coefficient of 0.97 and 0.96 under wet and dry conditions. However, as these results related to *in-vitro* studies, further testing was necessary before recommendations could be made for clinical use.

Clinical findings

Therefore, Lussi and colleagues (2001) conducted an *in-vivo* study to assess the clinical performance of the device. Seven dentists in Switzerland and Germany examined 332 occlusal surfaces in 240 patients. All dentists had participated in similar studies previously; therefore, they were familiar with the diagnosis of occlusal caries. Nevertheless, they had training sessions regarding the techniques and problems of fissure caries diagnosis, as well as, the principles of the use of the DIAGNOdent (2095) (Kavo, Biberach, Germany). In addition, the dentists used this device regularly for two months before this investigation to familiarise themselves with its use.

Teeth were not professionally cleaned before the study, and plaque, if present, was removed with an explorer without apical force. Only teeth with no visual signs of caries

were included. Where available, radiographs were assessed with a magnifying aid on a back-lit screen to determine the presence of dentine caries. Then teeth were assessed with the DIAGNOdent in the same way as explained in a previous study (Lussi *et al.*, 1999).

As the cut-off values for use *in-vivo* were not known, the decision to operatively treat the teeth was based on clinical and radiographic examination. The validation of results and extent of caries were determined after clinical intervention. The presence of caries was classified as follows: enamel caries (D_1, D_2), superficial dentinal caries (D_3), or deep caries (D_4). To assess reproducibility of the DIAGNOdent, the examination with the device was repeated at the same site in the same session by the same examiner. The cut-off points for different levels of diagnosis were determined in points where the optimal performance of the device, compared to the caries depth assessed by clinical intervention, was achieved. Accordingly, the cut-offs were found to be as follows: 0-13 (no caries); 14-20 (enamel caries); values >20 (dentinal caries).

At the dentine level, visual examination detected only 31% of dentine carious lesions compared to 63% and 92% of dentine caries for radiography and the laser device respectively. DIAGNOdent showed a better performance than both visual and radiographic examination ($P<0.001$). In addition, it showed an additional diagnostic yield of 117% compared to 45% from bitewing radiography. However, more meticulous visual inspection using ICDAS II has shown to perform better than a laser fluorescence device for the detection of occlusal caries (Braga *et al.*, 2009; Jablonski-Momeni *et al.*, 2011).

The specificity of DIAGNOdent (0.86) was found to be lower than that for bitewing radiographs (0.99). The reproducibility of the DIAGNOdent was excellent, supporting previous *in-vitro* studies (Lussi *et al.*, 1999; Shi *et al.*, 2000a). Thus, for clinical applications, the values suggested were as follows: 0-13 no active care is advised (NCA); for values 14-20, preventive care is advised (PCA); for values 21-29, preventive or operative care is advised depending on the patient's caries risk and clinical

presentation (PCA or OCA); values higher than 30 suggest that operative care is advised (OCA).

A higher cut-off value for intervention reduces the sensitivity but increases the specificity of the device in order to act as a safety net for teeth with calculus, stains or plaque where it has been shown that they increase the fluorescence and give rise to false positive results (Lussi *et al.*, 1999). It was concluded that a meticulous visual examination should be conducted first, followed by the laser device as an adjunct when uncertainty exists, as the combined advantage of higher sensitivity of the laser device with the higher specificity of visual examination may assist accurate diagnosis (Lussi *et al.*, 2001).

A systematic review of the use of laser fluorescence for *in-vivo* diagnosis of occlusal caries found that the device was accurate for the clinical diagnosis of occlusal caries, especially if used simultaneously with meticulous visual examination (Pinheiro *et al.*, 2004). A more general systematic review (Bader and Shugars, 2004) of the performance of a laser fluorescence device for the diagnosis of occlusal caries, included 25 studies sixteen of which were *in-vitro* studies for the assessment of the laser fluorescence device for the detection of occlusal caries in permanent teeth (12 studies) and primary teeth (four studies), and four were *in-vivo* studies for similar assessment in primary teeth (two studies) and permanent teeth (two studies). The other five studies assessed the performance of the DIAGNOdent for the detection of smooth surface caries, secondary caries and residual dentinal caries. The review concluded that the DIAGNOdent is more sensitive than traditional diagnostic methods. However, the lower specificity compared with visual methods limits its validity as a principle diagnostic method.

2.7.1.2 Validity of DIAGNOdent (2095) for detection of occlusal caries in primary teeth

The first study to determine the validity of the device for the detection of occlusal caries in primary teeth was by Lussi and Francescut (2003). They conducted an *in-vitro* study to compare the performance of DIAGNOdent with different conventional diagnostic techniques. Ninety-five primary teeth were examined visually, radiographically, with DIAGNOdent and histologically. Since there was no existing interpretation of the scale of DIAGNOdent for primary teeth, cut off values were determined where optimal performance of DIAGNOdent was achieved.

The histological assessment showed the optimal cut-off limits to be as follows: 0-4 no caries or caries in the outer half of enamel (D_0, D_1); 5-12 caries in the inner half of enamel (D_2); >12 dentinal caries (D_3, D_4). The study showed that DIAGNOdent had higher sensitivity (0.82) than visual (0.35) and radiograph (0.7) examination in both levels of diagnosis (D_2, D_3). However, the specificity of DIAGNOdent (0.85) was lower than that of bitewing radiographs (0.88) and visual examination (0.98). The overall performance of DIAGNOdent in primary teeth was similar to that found for *in-vitro* and *in-vivo* studies in permanent teeth (Lussi *et al.*, 1999; Shi *et al.*, 2000a; Lussi *et al.*, 2001).

Primary teeth have different macro and micro morphological characteristics to permanent teeth (Wilson and Beynon, 1989), thus these differences could affect their physical properties and hence the performance of the laser device. In addition, enamel of primary teeth is thinner than that of permanent teeth (Avery, 2002), thus showing more fluorescence of the underlying dentine. Interestingly, cut-off levels for the use of DIAGNOdent for the detection of occlusal caries in primary teeth were similar to those found previously in permanent teeth (Lussi *et al.*, 1999). The reproducibility was also very good suggesting that the device can be used for longitudinal monitoring of caries in primary teeth and for assessing the outcome of preventive interventions. Although this was an *in-vitro*study, the authors suggested a cut-off value for clinical intervention similar to that found in the *in-vivo* study in permanent teeth (>30). These values were

later proven to be accurate by Anttonen et al (2003) who validated the DIAGNOdent device *in-vivo* in primary teeth.

Most studies, conducted after Bader and Shugars' systematic review (2004), which have assessed the validity of the DIAGNOdent for the diagnosis of occlusal caries in primary teeth have shown the device to have higher specificity than sensitivity especially at the dentine level of diagnosis, as shown in Table 2.14 (Braga *et al.*, 2008; Kavvadia and Lagouvardos, 2008; Apostolopoulou *et al.*, 2009; Rodrigues *et al.*, 2009; Neuhaus *et al.*, 2010).

Interestingly, the majority of these studies have also shown the device to be more sensitive in the detection of enamel caries than dentine caries, which contradicts previous studies which found the converse to be true (Cortes *et al.*, 2003a; Lussi and Francescut, 2003; Rocha *et al.*, 2003).

As also can be seen in Table 2.14, cut-off values for caries diagnosis varied between studies which further indicates the overlap between different bands of diagnostic levels for the machine, and reinforces the limitations of DIAGNOdent as a principle method of caries diagnosis as there are no clear demarcation lines between each level of caries diagnosis. In summary, it has been shown that the combination of DIAGNOdent with visual examination gives the highest validity for the diagnosis of occlusal caries in primary teeth (Neuhaus *et al.*, 2010; Kavvadia *et al.*, 2011; Souza *et al.*, 2013).

Table 2.14 Studies conducted after 2004 examining the validity of laser fluorescence device for the detection of occlusal caries in primary teeth.

Study (year)	Method/ Validation	Sensitivity	Specificity	Cut-off values
Souza et al (2013)	*In-vitro*/ Histology	D_1:0.63 D_3:0.73	D_1:0.84 D_3:0.92	Sound: 0-15 Enamel: 16-30 Dentine: ≥ 31
Kavvadia et al (2011)	*In-vitro*/ Histology	D_1: 0.87 D_3: 0.39	D_1: 0.38 D_3: 0.87	Sound: 0-9 Enamel: 10-29 Dentine: ≥30
Neuhaus et al (2011)	*In-vitro*/ Histology	D_1: 0.74 D_3: 0.68	D_1: 0.81 D_3: 0.84	Sound: 0-9 Enamel: 10-16 Dentine: ≥17
Apostolopoulou et al (2009)	*In-vitro*/ Histology	D_1: 0.90 D_3: 0.36	D_1: 0.36 D_3: 0.91	Sound:0-15 Enamel: 16-58 Dentine: ≥59
Rodrigues et al (2009)	*In-vitro*/ Histology	D_1: 0.24 D_3: 0.20	D_1: 0.92 D_3: 0.93	Sound: 0-7 Enamel: 7.1-24 Dentine: ≥25
Kavvadia & Lagouvardos (2008)	*In-vivo*/ Biopsy	D_1: 0.43 D_3: 0.78	D_1: 0.88 D_3: 0.63	Sound: 0-9 Enamel: 10-29 Dentine: ≥30
Barbria (2008)	*In-vivo*/ Visual	0.89	0.89	Sound: 0-4 Enamel: 5-25 Dentine: ≥26

2.7.1.3 Validity of DIAGNOdent (2095) for the detection of proximal caries

The detection of proximal caries with DIAGNOdent 2095 was not possible *in-vivo* as there was no tip available that was able to penetrate proximal spaces (Lussi *et al.*, 2006). However, Virajsilp et al (2005) conducted an *in-vitro* study to assess the validity of the original laser fluorescence device (DIAGNOdent 2095) for the detection of proximal caries in primary teeth and compared its validity with bitewing radiography.

Two examiners, who had been previously trained on 10 primary teeth to standardise proximal caries diagnosis by bitewing radiography, DIAGNOdent and histological examination, assessed 107 proximal surfaces from 107 primary molars, which were stored in 0.9% normal saline solution. Bitewing radiographs were taken under standardised conditions using film holders and interpreted using a standard view box at ×2 magnification using Pitts' criteria (Pitts, 1984).

DIAGNOdent examination was conducted on teeth with and without direct contact. For the direct examination of non-contacting teeth, probe A was used according to the manufacturer's instructions. The spot on the surface with the highest reading was marked on a photograph of the tooth. For the other examination of contacting teeth, two teeth were mounted, with proximal surfaces contacting each other, in a wax base. The DIAGNOdent with probe A was placed on the buccal and lingual embrasures of the proximal surfaces of teeth and on the marginal ridge of the occlusal surface. The highest value from each of these readings was recorded.

Both DIAGNOdent examinations were conducted by two examiners to assess intra- and inter-examiner reproducibility. For the subsequent histological examination, a line was drawn on the occlusal surface in a mesiodistal direction perpendicular to the marginal ridge, through the point on the proximal surface with the highest DIAGNOdent reading. The tooth was hemi-sectioned immediately adjacent to the line, with an Exakt cutting machine (EXAKT Apparatebau, Germany) then the extension of caries was determined under a stereomicroscope at ×25 magnification using criteria suggested by Russel and Pitts (1993).

The examiners found that the intra-examiner and inter-examiner reproducibility for DIAGNOdent with and without contacting teeth was excellent (0.97-0.99). The caries diagnostic sensitivity of DIAGNOdent without contacting teeth (0.93) was higher than the sensitivity with contacting teeth (0.85) and both were more sensitive than radiography (0.41). However, the specificity of both DIAGNOdent measurements were almost the same (0.78 and 0.89 respectively) and were lower than the specificity of bitewing radiographs (1.0).

The examiners concluded from their study that DIAGNOdent has higher validity for the detection of proximal caries in primary teeth than radiography and they recommended further *in-vivo*studies to confirm the validity of the method clinically (Virajsilp *et al.*, 2005). However, in the absence of a special tip that can penetrate the inter-proximal space, it is difficult to confirm that the tip A of DIAGNOdent is actually measuring the fluorescence of the intended surface or fluorescence from an adjacent surface.

2.7.2 Laser fluorescence pen (DIAGNOdent pen 2190)

In 2006, a new tip was developed for the detection of proximal caries (Lussi *et al.*, 2006). The new device, known as the DIAGNOdent pen 2190 (LF pen, Kavo, Biberach, Germany), as with the first LF system, is based on the fact that carious teeth lead to increased fluorescence at specific excitation wave lengths (Hibst *et al.*, 2001). The intensity of fluorescence depends on the tooth structure and the wave length of the light. The new device, as with the previous one, emits light of a wave length of 655nm.

The device has two different fibre tips, a conical tip, with a diameter of 0.7 mm at the measurement site for use on approximal surfaces and a cylindrical tip, with a diameter of 1.1 mm for use on occlusal and smooth surfaces. Each tip can rotate around its long axis to allow placement of the probe on the mesial and distal surfaces at the oral and facial sides in anterior and posterior teeth. A red point on it indicates the light direction.

The propagation of both the excitation light and fluorescence light occurs in the same single solid fibre tip in opposite directions. This is in contrast to the first LF system

where excitation light is transported through a central fibre while fluorescence light is collected from hard tissue through additional fibres which are concentrically arranged around the central fibre.

2.7.2.1 Validity of DIAGNOdent pen (2190) for detection of occlusal caries

The new device DIAGNOdent pen has been shown to be valid *in-vitro* for detection of occlusal caries in permanent teeth (Lussi and Hellwig, 2006). The cut-off values of the new device were found to be slightly different to those of the original device (Table 2.15). However, compared to the old device, it has been shown to have the same validity and reproducibility for detection of occlusal caries (Kuhnisch *et al.*, 2007).

Table 2.15 Optimal Cut-off values of the old and new DIAGNOdent devices for the detection of occlusal caries in permanent teeth (Lussi and Hellwig, 2006).

Histological assessment	**Old DD tip A**	**New DD cylindrical tip**	**New DD conical tip**
D_0: no caries	0-7	0- 6	0-7
D_1: caries in the outer half of enamel	7.1-14	6.1-13	7.1-12
D_2: caries in the inner half of enamel	14.1-24	13.1-17	12.1-19
D_3-D_4: in dentine	>24	>17	>19

A summary of studies which have assessed the validity of the DIAGNOdent pen for the detection of occlusal caries in primary molars with the reported optimal cut-off values is provided in Table 2.16.

It can be seen from this table that, to date, there has been only one study conducted *in-vivo* with histological validation of results (Teo *et al.*, 2014). This study clearly shows higher cut-off values than the previous *in-vitro*studies and also the *in-vivo* study. It also showed lower specificity than the other studies and it was the only study which reported

lower specificity than sensitivity. This may be due to the presence of plaque on the examined occlusal surface which gives false positive results as the examiners did not clean the teeth before assessment.

Table 2.16 Studies conducted for the validation of DIAGNOdent pen for the detection of occlusal caries in primary teeth.

Study (year)	Method/ Validation	Sensitivity	Specificity	Cut-off values
Teo et al (2014)	*In-vivo*/ Histology	D_1:0.87 D_3:0.95	D_1:0.44 D_3:0.64	Sound: 0-14 Enamel: 15-42 Dentine: >42
Matos et al (2012)	*In-vitro*/ Histology	D_1: 0.69 D_3: 0.78	D_1: 0.67 D_3: 0.90	Sound: 0-8 Enamel: 9-30 Dentine: >30
Novaes et al (2012)	*In-vitro*/ Histology	D_1: 0.78 D_3: 0.68	D_1: 0.71 D_3: 0.84	Sound: 0-9 Enamel: 10-29 Dentine: ≥30
Bittar et al (2011)	*In-vitro*/ Histology	D_1: 0.94 D_3:0.91	D_1: 0.55 D_3: 0.74	Sound: 0-9 Enamel: 10-30 Dentine: ≥31
Matos et al (2011)	*In-vivo*/ Histology	D_1: 0.68 D_3: 0.95	D_1: 0.81 D_3: 0.88	Sound: 0-4 Enamel: 5-34 Dentine: ≥35
Neuhaus et al (2011)	*In-vitro*/ Histology	D_1: 0.7 D_3: 0.76	D_1: 0.9 D_3: 0.8	Sound: 0-14 Enamel: 15-31 Dentine: >30

Due to the absence of histological validation of the results of the *in-vivo* study conducted by Matos and colleagues (2011), direct comparison between the *in-vivo* studies with and without histological validation is not possible due to the higher sensitivity and specificity reported for *in-vivo* studies which lack histological validation (Novaes *et al.*, 2009; Chu *et al.*, 2010).

2.7.2.2 Validity of DIAGNOdent pen for detection of proximal caries in permanent teeth

The new LF device (DIAGNOdent pen, Kavo, Biberach) was first validated for the detection of proximal caries by Lussi and colleagues (2006). They conducted an *in-vitro*study in which they used 75 permanent molars, frozen at -20°C until use, with a total of 150 proximal surfaces without macroscopic cavitation.

Teeth were cleaned with water for 15 seconds then with a prophyflex and sodium bicarbonate for 10 seconds. After that, teeth were scaled for removal of calculus on proximal surfaces. Photographs of occlusal and proximal surfaces were taken with a magnification of ×2.8 to identify the test areas. Teeth were embedded in composite in between two other teeth in a mannequin to simulate contact points. The whole block was stored at 100% humidity before measurements were undertaken. Bitewing radiographs were examined by five experienced dentists on a backlit screen to determine whether the surfaces examined showed: no radiolucency (D_0), radiolucency in the outer half of enamel (D_1), radiolucency in the inner half of enamel (D_2), radiolucency in dentine (D_3, D_4).

Teeth were then measured using the LF pen as follows: first, the device was calibrated for every tooth using a ceramic reference. The fluorescence of a sound spot on the buccal surface of the tooth (zero value) was recorded. For measuring the proximal surface, the tip of the LF pen was moved from the buccal side to the lingual or palatal side below the contact area. The highest value was registered. The same procedure was repeated from the lingual and palatal side. The point with the highest value was marked on the photograph as a reference point for later histological examination.

The same procedure was repeated using two sapphire fibre tips: i) a cylindrical tip with a thickness of 0.4mm and a width of 1.1mm; ii) a conical tip with a width of 0.7mm. To assess reproducibility, the measurements were repeated by the same person on the same day. After the assessment of teeth, they were histologically examined. Teeth were ground mesio- distally on a Knuth-Rotor polishing machine using silicon carbide papers

of grain size 60 µm under tap water for cooling. Grinding was continued until the periphery of the test site was seen under the microscope (magnification ×3.2). After that, papers with smaller grain sizes of 30, 18 and 10 µm were used. The surface was coloured with saturated rhodamine B and sections were photographed (magnification ×3.2, ×6.4).

As there was no interpretation for the scale of the DIAGNOdent pen available, the optimum cut-off limits were determined using histological and statistical methods where the highest sum of sensitivity and specificity was achieved. The optimum cut-off limits for both tips of DIAGNOdent pen were found to be as shown in Table 2.17.

The sensitivity values ranged between 0.84 and 0.92 and the specificity ranged between 0.81 and 0.93. Both tips showed a similar performance which was significantly higher than bitewing radiography ($P< 0.05$). The high sensitivity at all levels of caries diagnosis (D_1, D_2, D_3) indicated that the DIAGNOdent pen can differentiate between sound lesions and lesions in the outer half of enamel, in contrast to the original DIAGNOdent which did not have this capability (Mendes *et al.*, 2005; Braga *et al.*, 2008).

Table 2.17 Optimal cut-off values of two different tips of DIAGNOdent pen for caries measurement (Lussi *et al.*, 2006).

Histological assessment	**Cylindrical tip cut-off values**	**Conical tip cut-off values**
D_0: no caries	0-6	0-9
D_1: caries in the outer half of enamel	6.1-9	9.1-13
D_2: caries into the inner half of enamel	9.1-15	13.1-19
D_3-D_4: caries into dentine	>15	>19

Both tips of the DIAGNOdent pen showed high reproducibility (Kappa values of 0.74 for WDG, 0.85 for TWDG) suggesting that the new device could be used for longitudinal monitoring of caries and for assessing the outcomes of preventive interventions. The authors suggested that the device's main potential use is to facilitate preventive management of dental caries, rather than merely for the detection of dentinal lesions requiring restorations. Furthermore, they concluded on the basis of their study's findings that the new DIAGNOdent system could be a useful adjunct to proximal caries detection in the clinical setting (Lussi *et al.*, 2006).

Although, the investigators used frozen teeth in their study, which have been shown to have stable fluorescence values and hence stable cut-off values, the cut-off values from this study cannot be applied clinically as it was not an *in-vivo* study. In addition, the cut-off values found from this study, and previous studies, are not a precise value but rather a range of values. Furthermore, the histological involvement of dentine does not necessarily indicate the need for restorative intervention. As for all DIAGNOdent assessments, the decision for subsequent restorative intervention should be based on a range of variables, such as the patient's caries risk, fluoride status and caries activity.

A randomised clinical trial (Huth *et al.*, 2010) was conducted to clinically validate the previous results of the Lussi and colleagues study (Lussi *et al.*, 2006). This study included 117 patients, each with one proximal surface which was either sound or had a non-cavitated carious lesion. Teeth were cleaned, rinsed and air dried then visually examined with a dental light and mirror.

A radiographic examination of all sites was performed, then based on the radiographic scores, the decision was made to either treat the tooth operatively (opening the lesion) or preventively (topical fluoride application). The DIAGNOdent measurements were then undertaken by four experienced and calibrated examiners according to the manufacturer's instructions. Then teeth that radiographically showed dentine caries were opened and lesion depths were determined by inspection. Radiographic validation of the DIAGNOdent pen readings of all sites was conducted in addition to the clinical validation of the opened lesions.

According to the radiographic lesion depth assessment, DIAGNOdent pen cut-off values were suggested to be as follows: sound=0-7; enamel caries=7.1-16; dentine caries>16. To distinguish between radiographically sound and carious lesions, a cut-off of 7 was suggested, which revealed a sensitivity of 0.68 and a specificity of 0.70. For the differentiation between enamel and dentine caries, a cut-off of 16 was suggested which showed a sensitivity of 0.60 and a specificity of 0.84. The suggested cut-off values were almost the same as those found in an *in-vitro* model shown in Table 2.17 (Lussi *et al.*, 2006). However, the sensitivity in this study (Huth *et al.,* 2010) was much lower at these cut-off values.

Considering the clinically prepared teeth, there was a fair positive correlation between the actual clinical lesion depths and DIAGNOdent pen measurements. Therefore, the investigators concluded that the new device may be used as an additional diagnostic tool for detection of proximal caries in permanent teeth (Huth *et al.*, 2010). The study intended to validate the results of Lussi et al's *in-vitro* study (2006). Although the same cut-off values as the *in-vitro* study were proposed, the limitations of the *in-vivo* study make it difficult to confirm the clinical application of these values for many reasons. Firstly, there was no true histological examination, which is the gold standard for the validation of results. The method used for validation in this study was radiography which has been shown to be useful for the detection of proximal caries (Wenzel, 2004) but not perfect. Secondly, the clinical validation was only able to test the false positives but was not able to test for false negatives. Thus further *in-vivo* studies with histological validation are warranted to confirm the cut-off values suggested by previous studies.

2.7.2.3 Validity of DIAGNOdent pen for detection of proximal caries in primary teeth

The validity of DIAGNOdent pen for detection of proximal caries in primary teeth was first assessed clinically in 2009 (Novaes *et al.*, 2009). Novaes and colleagues (2009) conducted an *in-vivo* study to assess the validity of the new device for the detection of proximal caries in primary teeth and compare its performance with that of visual

examination and bitewing examination. They included 50 children, with 621 surfaces, aged 5-12 years living in Brazil (in an area with water fluoridation of 0.7 ppm).

They excluded surfaces which had approximal restorations, hypoplasia, or large carious lesions on proximal surfaces or any other surfaces on the same tooth. In addition, surfaces were excluded in the absence of the adjacent tooth. Two examiners were involved, who had been previously trained by a bench mark examiner using three patients for each method. No calibration was performed. All proximal surfaces were cleaned with a rotating brush with pumice and with dental floss. Visual examination was conducted under standard clinical conditions using a mirror and WHO periodontal probe. Teeth were visually examined using ICDAS II criteria (Ismail *et al.*, 2007).

For radiographic examination, bitewing radiographs were taken using film holders and interpreted on a backlit screen at x2 magnification using Ekstrand's criteria (Ekstrand *et al.*, 1997). For the DIAGNOdent pen examination, probe tip 1 (for proximal surfaces) was used. Calibration against a ceramic surface was conducted first. Then, on every tooth, the device was calibrated on a sound surface of the same tooth to record the zero value, which was later subtracted electronically from the readings of approximal surfaces under assessment. After drying the proximal surface for 5 seconds, the tip was introduced into the interproximal space beneath the contact area, from the buccal side then from the lingual/palatal side. The highest reading from the two measurements of each surface was recorded. Then, teeth were separated using orthodontic elastic modules for 7 days to achieve a space of 0.5-1mm. If adequate space was not achieved, the procedure was repeated.

The surface was classified, after direct clinical examination with a mirror and a WHO probe, as:

- sound (score 0) = no change in enamel translucency after air drying
- white spot lesion (score 1): white or brown discolouration in wet and dry surfaces
- cavitation (score 2): loss of surface integrity visually detected or by the WHO probe.

This examination was also conducted by two examiners and it was used as the standard reference point for previous examinations. The inter-examiner reproducibility for the reference standard examination was high (k = 0.93). The cut-off values were determined using the reference standard method where the highest accuracy was achieved.

The optimum cut-off values were found to be: sound= 0-5; white spot caries= 5.1-16; cavitation >16. The sensitivity of all methods was low for detecting white spot lesions ranging from 0.16 to 0.23. However, the specificities were high ranging from 0.94 to 1. Concerning the detection of proximal caries at cavitation level, both the DIAGNOdent pen and bitewing radiography had significantly higher sensitivity than visual examination. However, specificities at the cavitation level were again high for all methods (0.99-1). All three methods showed similar reproducibility values ranging from 0.72 to 0.77. The examiners concluded from the study that both the DIAGNOdent pen and radiography performed better than visual examination for the detection of proximal caries in primary teeth. However, treatment decisions should not be based simply on the results of either examination.

Although the study was *in-vivo* and conducted under standard clinical conditions, the results of the study cannot be confirmed because of the absence of histological validation. The method used for validation (temporary tooth separation) has been shown not to be valid as a standard reference method due to its low reproducibility but it can be used as a supplementary method for diagnosis (Hintze *et al.*, 1998; Deery *et al.*, 2000). Although the reproducibility of the reference method was high in this study, the results need to be confirmed histologically.

Following on from this, Braga and colleagues (2009) conducted an *in-vitro* study to assess the validity of the DIAGNOdent pen for the detection of proximal caries in primary teeth compared to visual examination and bitewing radiography with the use of histological validation in addition to direct visual examination as a reference method.

In this study, 131 proximal surfaces were examined by two examiners visually, radiographically and with the DIAGNOdent pen. One bench mark examiner trained

them using 15 surfaces. No calibration was performed. Teeth were then frozen at -20 ℃ until use. Before conducting the examination, teeth were defrosted for four hours at room temperature. Teeth were then placed in an arch model in such a way to simulate the contact points as best as possible. Surfaces with proximal restorations, hypoplasia, proximal cavitations or large cavitations on smooth or occlusal surfaces were excluded. Proximal surfaces were assessed visually, with the aid of light and with no magnification at a distance of 30 cm from the examiner's eye, using the ICDAS criteria (Ismail *et al.*, 2007). For radiographic examination, bitewing radiographs were taken using film holders and interpreted on a backlit screen at x2 magnification using Ekstrand's criteria (Ekstrand *et al.*, 1997).

After all examinations, teeth were removed from the arch model and examined directly as a first reference standard method. Surfaces were classified as sound, white spot lesions or with cavitation (Novaes *et al.*, 2009). Subsequently, teeth were serially sectioned in 250 µm thick slices using a 0.3-mm-thick diamond saw mounted in a microtome. Then, sections were examined by both examiners under a stereomicroscope at x16- 40 magnification. Lesions were classified according to lesion depth (D_0-D_4).

The DIAGNOdent results were compared using two different validations. First, using direct visual examination as the reference method and second, using the lesion depth as a reference standard method. The cut-off values obtained using direct visual examination where the highest accuracy was achieved were: 0-4= sound; 4.1-38= white spot caries; and >38= cavitation. The cut-off values obtained using histological depth were: 0-8- sound; 8.1-30= enamel caries; >30= dentine caries. There was a significant correlation between lesion depth and direct visual examination ($p<0.0001$). Considering both reference methods, the DIAGNOdent pen showed higher sensitivity (0.82-0.87) and significantly lower specificity (0.25-0.47) than visual examination (sensitivity: 0.72, specificity: 0.80) and radiography (sensitivity: 0.55, specificity: 0.80) in detecting initial caries. However, at the cavitation threshold, similar sensitivities (0.47-0.59) were obtained by all methods.

Visual examination showed higher specificity (0.98), at cavitation threshold, than other methods (0.71-0.87). Considering the area under the receiver operating characteristic (ROC) curves, visual examination showed the highest values at both levels of caries diagnosis. Visual examination also showed higher accuracy than the DIAGNOdent pen and radiography at both levels of diagnosis. However, the DIAGNOdent pen and bitewing radiography showed similar accuracy and area under the ROC curve values, which agrees with the findings of the *in-vivo* study (Novaes *et al.*, 2009) but differs to the findings of another *in-vitro* study in permanent teeth (Lussi *et al.*, 2006) which found the DIAGNOdent pen to perform better than bitewing radiography for the detection of proximal caries.

The conflicting results of these studies may be due to differences in the structure of primary and permanent teeth. Braga and colleagues concluded from their study that both DIAGNOdent pen and bitewing radiography showed good performance, especially in detecting deeper lesions. However, visual examination performed better for the detection of proximal caries *in-vitro* in primary teeth than the DIAGNOdent pen and bitewing radiography (Braga *et al.*, 2009).

Again, the limitations of this study (Braga *et al.*, 2009) make it difficult to confirm its results. Although the investigators tried to simulate proximal contacts, it is not possible to simulate the soft tissues around teeth and the oral cavity conditions. Furthermore, access and visibility are facilitated in an *in-vitro* model. Therefore, it is difficult to directly compare the results of a visual examination from *in-vitro* and *in-vivo* approaches, especially in children, where poor cooperation may be modifying factor (Novaes *et al.*, 2010). Interestingly, Novaes and colleagues (2010) found that the number of false positive results was higher with visual examination in children who reported higher discomfort.

The same study (Novaes *et al.*, 2010) reported results similar to the previous *in-vivo* study (Novaes *et al.*, 2009) where the DIAGNOdent pen and radiography had a similar performance (sensitivity of 0.52 for both, specificity of 0.95-0.97) for detection of proximal caries in primary teeth, which was better than ICDAS II (sensitivity of 0.23,

specificity of 0.99). The standard reference in this *in-vivo* study was also temporary tooth separation.

In 2012, Chen and colleagues conducted a clinical investigation to assess the validity of the LF pen for the detection of proximal caries in primary molars. Two hundred and fifty six surfaces from 216 primary molars of 96 children were examined visually, radiographically and with the LF pen. The results of the study were validated clinically by opening the cavity if radiographs showed an indication for operative treatment. If not, surfaces were evaluated visually and re-examined three months later. The investigators found that visual examination had very low sensitivity of 2.5% at the white spot level of diagnosis and a sensitivity of 70.7% at cavity level. Radiographic examination had good sensitivity of 64.1% at the white spot level and very high sensitivity of 97.7% at cavity level. For the LF pen, the sensitivity was 56.4% and 92.1% at white spot and cavity levels respectively. The specificity of all examinations was high ranging from 93% to 97%. The examiners concluded from their study that LF pen examination and bitewing radiographs can detect cavitation on proximal surfaces of primary molars. Therefore, the LF pen could be used as an alternative to radiographic examination. However, with the absence of histological validation it is difficult to confirm the reliability of these results.

To date, none of the *in-vivo* studies have used histological validation. The results of the histologically validated *in-vitro* studies should be applied with caution in the clinical setting. Therefore, further *in-vivo* studies with histological validation would seem to be warranted to assess the validity of the DIAGNOdent pen for the detection of proximal caries in primary teeth and compare its performance to visual examination and bitewing radiographs. A summary of studies conducted to validate diagnostic methods of proximal caries in primary teeth is described in Table 2.18.

Table 2.18 A summary of studies conducted to validate diagnostic methods of proximal caries in primary teeth

Study	Type of study	Type of teeth	Visual examination	Radiographic examination	LF pen examination	Level of diagnosis	Validation method
(Chen et al, 2012)	*In-vivo* / 96 children	Primary teeth 216 teeth 256 surfaces	Ekstrand's criteria 1,2= Enamel caries 3,4= Dentine caries Cut-offs for white spot lesions and cavitations were not specified.	Wenzel's Criteria 1,2= Enamel caries 3,4= Dentine caries Cut-offs for white spot lesions and cavitations were not specified.	0-7= Sound 8-16= Enamel ≥ 17 Dentine	White spot/ Cavitation	Invasive treatment
(Novaes et al, 2010)	*In-vivo* / 76 children	Primary teeth 592 surfaces	ICDAS criteria 1,2= Enamel caries 3,4,5,6= Dentine caries Cut-off thresholds for non-cavitated lesions 0 v 1,2,3,4,5,6 and for cavitated lesions 0,1,2 & 3,4,5,6.	Ekstrand's criteria 1,2= Enamel caries 2,3,4= Dentine caries Cut-off thresholds for non-cavitated lesions 0 v 1,2,3,4, and for the cavitated lesions 0,1 v 2,3,4	0-5=Sound 5-15=Enamel ≥16=Dentine	Non-cavitated/ cavitated	Temporary Tooth Separation

(Huth et al, 2010)	*In-vivo* / 117 patients	Permanent teeth/117 surfaces	Criteria for visual scoring V1=white spot V2=brown spot V3=greyesh discoloration from underneath. n.a for sites not assessable by visual examination. Cut-offs determined according to clinical caries depth D0 v D1-4 D1,2 v D3,4	Pitts criteria (1988) R1,R2= Enamel caries R3,R4= Dentine caries Cut-off thresholds R0 V R1-4 R1,2 v R3,4	0-7=sound 8-16=enamel ≥ 17= Dentine	Sound versus carious/ enamel versus dentine	Radiographic + clinical intervention
(Novaes et al, 2009)	*In-vivo* / 50 children	Primary teeth/621 surfaces	ICDAS criteria 1,2= initial caries 3,4= enamel discontinuity +/- underlying shadow 5,6= caviation Cut-off for white spot lesions is 0 v 1,2,3,4,5,6 and for cavitations is 0,1,2 v 3,4,5,6	Modified Ekstrand's criteria R1=Enamel R2= outer 1/3 of Dentine R3= middle 1/3 of dentine R4= inner 1/3 of dentine Cut-off for white spot lesions is	0-5=sound 6-16=enamel ≥ 17= Dentine	White spot/ cavitation	Temporary Tooth Separation

				0 v 1,2,3,4 and for cavitations is 0,1, v 2,3,4			
(Braga et al, 2009)	*In-vitro*	Primary teeth / 85 teeth 131 surfaces	ICDAS criteria 1,2= initial caries 3,4= enamel discontinuity +/- underlying shadow 5,6= caviation The cut-off for white spot & D1 is 0 v 1,2,3,4,5,6 The cut-off for cavitation & D3 is 0,1,2 v 3,4,5,6	Modified Ekstrand's criteria R1=Enamel R2= outer 1/3 of Dentine R3= middle 1/3 of dentine R4= inner 1/3 of dentine	Cut-off obtained by DVE was 0-4=sound 5-38=white spot ≥ 39= cavitation Cut-off by histology 0-8=sound 9-30=enamel ≥ 31= Dentine	White spot/ cavitation	Direct visual examination criteria is white spot lesions/ cavitation Histological validation Criteria is Downer D0=sound D1,2=E D3,4=D
Lussi et al, 2006)	*In-vitro*	Permanent teeth 75 teeth 150 surfaces	Not examined	D0= no radiolucency D1= outer half of enamel D2= inner half of enamel D3= outer half of dentine D4= inner half of dentine	0-9= sound 9.1-19=enamel ≥20= dentine	D1 D2 D3	Histological validation Downer criteria

The table above (Table 2.18) summarises the main studies looking at the validity of the LF pen compared to visual examination and radiographic examination for the detection of proximal caries in primary and permanent teeth. The table shows clearly the inconsistency of the use of the diagnostic criteria for the different examinations tested, which makes it difficult to compare the results of these different studies due to the use of different diagnostic criteria.

In-addition, some studies use the wrong terminology for their levels of diagnosis such as (Novaes et al, 2010), (Novaes et al, 2009) and (Braga et al, 2009) where the levels of diagnosis were at cavitation while the data analysis was conducted at initial dentine caries. Other studies such as (Chen et al, 2012) did not clarify at what level the data analysis was conducted which makes it difficult to interpret the results. These variations in the terminology makes the interpretation of the results to the readers who are not fully aware of this dilemma confusing may lead to a misunderstanding of the results.

2.7.3 Factors affecting the DIAGNOdent fluorescence values

A number of factors have been shown to influence DIAGNOdent measurements as listed below.

- The presence of deposits such as stain, plaque, calculus, toothpaste or prophylactic paste may give rise to false positive results. Thus, some investigators suggested that thorough cleaning is an essential prerequisite to accurately diagnose caries using the DIAGNOdent device (Lussi *et al.*, 1999; Shi *et al.*, 2000a; Cortes *et al.*, 2003a; Bamzahim *et al.*, 2004; Bamzahim *et al.*, 2005; Lussi *et al.*, 2005; Lussi and Reich, 2005). However, this effect seem to be limited to occlusal surfaces as it has been found that there was no significant difference between the performance of the LF pen on the cleaned and non-cleaned proximal (Bittar *et al.*, 2012).
- Drying time is one of the factors that has been found to have an effect on the LF device's readings. Lussi and colleagues (2005) found that occlusal surfaces should be dry in order to achieve optimal results with the LF device. However, Bittar and colleagues (2012) in their *in-vitro* study of the drying effect on both proximal and occlusal surfaces found that drying had no significant effect on the performance of the LF pen except in case of dehydration where the readings were higher.
- Disturbed tooth development or mineralisation may lead to increased fluorescence in the absence of caries (Lussi *et al.*, 1999; Shi *et al.*, 2000a; Farah *et al.*, 2008).
- The presence of fissure sealants affects the diagnosis of dental caries by a laser fluorescence device, and modified cut-off diagnostic values should be used to identify carious lesions (Deery *et al.*, 2006).
- In the case of *in-vitro* studies, the storage solution, such as chloramines, formalin or thymol have been found to cause significant reduction in fluorescence of extracted teeth stored in these solutions. In contrast, teeth which have been frozen at -20 ℃ without a storage solution showed no significant change in fluorescence over 2 years (Francescut *et al.*, 2006).

2.8 Other methods for caries detection and diagnosis

2.8.1 Fibre optic transillumination

Fibre optic transillumination (FOTI) has been used as an alternative method for the detection of proximal caries (Friedman and Marcus, 1970). In this method, a very high intensity white light is shone from an intra-oral fibre optic light probe on the lingual or buccal surfaces of the examined teeth to enhance visual detection of caries. Sound enamel is comprised of densely packed hydroxyapatite crystals producing an almost transparent structure. A sound tooth absorbs a minimal amount of light whilst a carious lesion absorbs and scatters the light due to its lower mineral content. Therefore, a sound tooth appears translucent while a carious lesion appears darker with transillumination (Cortes *et al.*, 2003b). FOTI has been compared to other diagnostic methods in many studies (Deery *et al.*, 2000; Mialhe *et al.*, 2009). A recent systematic review (Gomez *et al.*, 2013) showed the sensitivity of FOTI in detecting non-cavitated carious lesions ranged from 0.21 to 0.96, and the specificity ranged 0.74 to 0.88. FOTI can be used for the detection of caries in all surfaces (Cortes and Ellwood, 2000). However, it is particularly useful for proximal lesions. A limitation of FOTI is the lack of the ability to record images. In order to overcome this limitation, digital imaging fibre optic transillumination (DIFOTI) has been developed. This system comprises of a camera which can be fitted with one of two heads; designed for either smooth surfaces or occlusal surfaces. Images are displayed on a computer screen where they can be saved for future reference (Pretty, 2006). Both methods are subjective rather than objective. Therefore, the interpretation relies largely on the examiner's training.

2.8.2 Electronic caries monitor

Sound tooth structure has very high electrical resistance. When demineralisation occurs, the surface becomes porous. The pores become filled with fluid and ions from saliva. These act as conductors to electric current, hence, reducing the electrical resistance of the tooth structure. The difference in electrical conductance can be measured by the

electronic caries monitor (ECM) on a surface level or at a specific site (Longbottom and Huysmans, 2004). The reliability of the ECM has been shown to be variable in the literature. A review (Huysmans *et al.*, 2005) of the performance of the ECM for caries detection showed the sensitivity and specificity of the ECM for site specific measurements to be 74% (± 11.9) and 87.6% (± 10) respectively. While for surface measurements, the sensitivity was 63% (± 2.8) and the specificity was 79.5% (± 9.2). However, other studies have shown the ECM to be less reliable than other methods for detection of dental caries (Wicht *et al.*, 2002; Huysmans *et al.*, 2005) due to the tendency to produce false positive values.

2.8.3 Quantitative light-induced fluorescence

Quantitative light-induced fluorescence (QLF) is a method of detection, quantification and monitoring of early enamel caries. Demineralisation of enamel decreases its auto fluorescence. QLF operates on the principle of quantifying the loss of fluorescence of demineralised enamel (Pretty, 2006). This technique uses a blue light (370nμ), which when applied to enamel, creates auto fluorescence detectable by a small intra-oral camera. QLF light passes through enamel into the EDJ where the fluorescence eminates. When a lesion exists, it appears as a dark spot on a bright green background. The fluorescent image is recorded and the fluorescence is analysed quantitatively (van der Veen and de Jong, 2000). The fluorescence of healthy enamel is assumed to be 100%. Any area with a loss of fluorescence of more than 5% is considered carious (Pretty, 2006). Studies comparing the validity of QLF to other caries detection methods showed QLF to be sensitive in detecting carious lesions especially early caries (Meller *et al.*, 2006; Kühnisch *et al.*, 2007). QLF has also shown high sensitivity *in-vivo*(Ferreira Zandoná *et al.*, 2010). This supports the *in-vitro* results of Tencate & colleagues (2000) who assessed the sensitivity and specificity of QLF with histological validation and found it to be 79% and 75% respectively.

2.9 Histological validation

Any new diagnostic test should be validated against a true diagnosis often called the ‘gold standard’. For the validation method to be of ‘gold standard' it should fulfil three criteria: i) it should be reproducible; ii) it should reflect the pathoanatomical appearance of the disease it is intended to measure, and iii) it should be independent from the diagnostic method to be validated (Wenzel and Hintze, 1999).

For the validation of caries diagnostic methods, different approaches have been used. Direct visual examination after tooth separation has been used for the validation of radiographic examination (Ekstrand *et al.*, 1997; Deery *et al.*, 2000). However, using visual examination as a ‘gold standard’ may be subject to criticism as it does not fulfil one of the criteria, since it has been shown that visual examination is not reproducible (Ekstrand *et al.*, 1997; Hintze *et al.*, 1998; Eggertsson *et al.*, 1999). Moreover, visual inspection may not be able to determine how deep a lesion is in case of non-cavitated carious lesions (Hintze and Wenzel, 2003).

Another method for validation of caries diagnosis has been radiography (Wenzel *et al.*, 1991). Radiography has also fallen short of a ‘gold standard’ since it is also poorly reproducible (Hintze and Wenzel, 2003).

Alternatively, micro-computed tomography can be used for the validation of caries diagnostic methods. Micro-computed tomography (micro-CT) is a laboratory method for examining the morphology and dimensions of dental hard tissue (Dowker *et al.*, 1997). In addition, micro-CT has been shown to successfully monitor the degree of mineralisation of dental tissue and assess the amount of mineral content in carious tissues (Huang *et al.*, 2007).

This method has been used to validate the diagnostic performance of radiography (Young *et al.*, 2009b). Micro-CT is able to examine multiple sections, by producing serial digital images. The duration of the scan time ranges from 1.5-2 hours depending on the size of the section.

Mitropoulous and colleagues (2010) conducted an *in-vitro* study to investigate the potential of micro-CT as an alternative to histological examination as a 'gold standard'. Forty proximal surfaces of 20 teeth (12 premolars and 8 molars) were examined with micro-CT and histologically by bisecting them with a microtome disk in the centre of the suspected carious lesions. The investigators found that micro-CT was not able to detect early demineralisation of teeth. Thus, despite the high diagnostic accuracy achieved (0.85), micro-CT did not fulfil the criteria required of a 'gold standard'.

So far, histological validation remains the only 'gold standard' for validation of caries diagnosis. Deery and colleagues (2006) set out to determine the reproducibility of histological validation. They examined the occlusal surfaces of 37 teeth (25 molars, 12 premolars) with differing caries status varying from apparently sound to cavitation. Each tooth was mounted in a block of polymethyl metha acrylate and was serially sectioned longitudinally in a mesiodistal direction using a water-cooled diamond disc on a microtome to achieve three cuts. This resulted in four sections with a thickness of approximately 1.5-2 mm and six surfaces to examine per tooth.

Each surface was photographed, under magnification of x15 using a light microscope, with a digital camera. Images of sections were viewed on a computer screen by all three examiners independently. Examiners were trained and calibrated to use the criteria established for histological examination (Downer, 1975). Each examiner assessed the sections twice and they were found to have almost perfect intra-examiner reproducibility, ranging from 0.82-0.92 and 0.96-1.0 at the D_1 and D_3 levels respectively. Inter-examiner reproducibility varied from substantial at D_1 to almost perfect at D_3. Therefore, the examiners concluded that the method is highly reproducible and reliable.

Serial sectioning of teeth to slices of a thickness of 250-300μm followed by microscopic examination at a magnification of x16-40, has been used to validate the diagnostic performance of ICADS II for the detection of occlusal caries and proximal caries in primary teeth (Braga *et al.*, 2009). Serial sectioning has also been used to validate the diagnostic performance of a laser fluorescence device for the detection of caries in both

primary and permanent teeth (Rocha *et al.*, 2003; Deery *et al.*, 2006; Aljehani *et al.*, 2007; Jablonski-Momeni *et al.*, 2011).

Another approach to histological validation involves cutting a single section through the centre of the carious lesion (hemi-sectioning). However, this method has been shown to be less accurate than serial sectioning. Deery and colleagues (1995) conducted a study to compare the outcomes of histological examination following serial sectioning and hemi-sectioning. One hundred and twelve permanent molars were first cut through the centre of the occlusal lesion (if visible). After hemi-sectioning, both halves were examined under a binocular microscope using a magnification of x2.5 by two examiners, according to the criteria listed in Table 2.19. Subsequently, teeth were serially sectioned and re-examined by both examiners as before.

The investigators (Deery *et al.*, 1995) found the results of both hemi-sectioning and serial sectioning to be different on 12 occasions (10.7%). Two teeth which were originally judged to be sound by hemi-sectioning were found to have enamel caries and ten teeth which were thought to have enamel caries, were found to have dentine caries.

Table 2.19 Diagnostic criteria used for serial sectioning and hemisectioning (Deery *et al.*, 1995).

Code	Category	Diagnosis
0	Sound	No caries
1	Enamel caries	Carious lesion in outer half of enamel only
2	Enamel caries	Carious lesion in inner half of enamel only
3	Dentine caries	Carious lesion into outer half of dentine
4	Dentine caries	Carious lesion into inner half of dentine

These results were confirmed by Hintze and Wenzel (2003) who found that serial sectioning identified significantly more carious lesions than hemi-sectioning irrespective of lesion depth. Interestingly, 35% of lesions recorded as sound by hemi-sectioning were found to be carious after serial sectioning, with most of these lesions being proximal. The intra-examiner reproducibility for histological examination was almost perfect (serial sectioning=0.98; hemi-sectioning=1). The investigators concluded that generally, serial sectioning detected a significantly higher number of carious lesions than hemi-sectioning. More importantly, histological examination was highly reproducible, thus, fulfilling the universal criterion that any 'gold standard' assessment should be highly reproducible.

Hemi-sectioned tooth samples have been used to validate proximal caries detection by a laser fluorescence device and bitewing radiography in primary teeth (Mendes *et al.*, 2005; Virajsilp *et al.*, 2005). This approach has also been used to validate the performance of a laser fluorescence device and visual examination for the detection of occlusal caries in permanent teeth (Rodrigues *et al.*, 2009).

Tooth grinding has also been employed to carry out histological examination of caries lesions (Lussi and Francescut, 2003; Lussi *et al.*, 2006; Neuhaus *et al.*, 2010). In these studies, teeth were ground longitudinally, under constant water, on a polishing machine with silicone carbide paper of grain size 60µm. Teeth were monitored microscopically (magnification of x3.2) to check the progression of grinding. Once the periphery of the lesion was reached, polishing papers of smaller grain size were used (30, 18, 10µm), and the surface was coloured with rhodamine B and photographed at a magnification of x3.2 and x6.4.

A number of protocols have been used for histological scoring of the presence and extension of caries. The most commonly employed criteria is that proposed by Downer (Downer, 1975). This criteria uses the enamel-dentine junction as an important land mark between enamel and dentine caries in the assumption that once caries reaches the EDJ it spreads laterally and undermines the enamel surface (Table 2.8).

In 1997, Ekstrand and colleages developed a more detailed histological scoring system (Table 2.2). This system combined deep enamel caries with initial dentine demineralisation in score 2 based on the close relationship that was found between enamel caries and reactions in underlying dentine on proximal lesions (Bjorndal and Thylstrup, 1995) and on occlusal lesions (Ekstrand *et al.*, 1995). Ekstrand et al (1997) found that soft dentine corresponded to demineralisation in the middle third of dentine or more.

Although histological validation has shown to be the 'gold standard', it is not without limitations. It requires considerable time and effort, and can only be conducted on extracted teeth (Hintze and Wenzel, 2003).

To conclude, histological validation of caries diagnosis has been shown to fulfil the optimum criteria for a 'gold standard' assessment and is the most frequently used method for validation.

2.10 Children's acceptability of caries diagnostic procedures

2.10.1 Children's involvement as service users

In the United Kingdom, there are more than 13 million children and young people under the age of 18-years, and they represent almost a quarter of the population (Woodfield, 2001). Children are very important users of health services, accounting for almost a quarter of general practitioner consultations and up to 30% of accident and emergency patients (Hart and Chesson, 1998). Within dentistry, children accounted for 26% of the courses of treatments provided by GDPs in England in 2011/12 (Health and Social Care Information Centre, 2012).

2.10.1.1 Importance of involving children

It is thus imperative that children are actively involved in service evaluation, audit and medical research to address their needs, opinions, expectations and experiences as

service users (Woodfield, 2001). Although parents and carers have been used as proxies, their opinions may not always reflect children's perceptions (Osman and Silverman, 1996). It has been shown that children are perfectly able to express their opinions, if these are sought using appropriate methods. Furthermore, children's views can be actively used in decision-making and the process of change (Woodfield, 2001).

A systematic review of the child dental literature from 2000-2005 assessed the extent to which contemporary oral health research involved children and found that 87% of the research used children as objects of research, 5.7% involved parents as a proxy, 7% involved children in some way and 0.3% of research actively involved children (Marshman *et al.*, 2007). Another systematic review of child dental case reports published between 2000 and 2005 has also shown that only few reports involved children directly (10%) or through a proxy (2%) (Morgan *et al.*, 2008). Both reviews concluded that, traditionally, dental professionals have not fully engaged children in oral health research and most research was "on" children rather than "with" children (Marshman *et al.*, 2007; Morgan *et al.*, 2008). There is, therefore, great scope to involve children and young people more fully in dental research and service evaluation.

There is now a growing expectation that health care services should actively involve children, listen to their opinions, and value their views, especially when related to decisions about their treatment planning, outcomes and service evaluation (Department of Health, 2004). More recent policy from the UK Children's National Service Frame work (Department of Health, 2010) stated that '*Children and young people must also be offered opportunities to speak of their experiences and to say what in their views has and has not made a difference to their lives*'. Moreover, there is an expectation from any funding body that the 'user' has been actively involved in the study right from design to dissemination. In view of these recommendations, this research has sought to involve children. Although the focus of the research is on clinical outcome measures, the perspectives of children will also be sought as these may have important relevance to the study's recommendation.

2.10.2 Measuring acceptability

Children are able to express insightful views and opinions if appropriate methods are adopted (Woodfield, 2001). Research approaches for children within the context of oral health have been well described by Marshman and Hall (2008). These include both quantitative and qualitative methods. Quantitative techniques include questionnaires and scales. For questionnaires to be truely child-centred, they should be developed and validated with children. Response format in questionnaires tend to involve 3- or 5-point likert tick box style. Scales offer an alternative method to responding in a questionnaire manner and may include:

i) Visual Analogue Scale (VAS), where children mark their response on a 10cm line with 0cm indicating for example, the most positive result and 10cm indicating the most negative result.

ii) Faces (Pictorial) scale, where children are shown faces with different expressions varying from negative, neutral to positive.

iii) Coloured Analogue Scale, which varies in colour from pink to red.

To date, no validated measure of acceptability for diagnostic procedures has been developed for use with children in dentistry. Indeed, a review of the literature suggests that the acceptability of dental investigations has generally received little attention within paediatric dentistry-related studies. Although the laser fluorescence device has been well described as a diagnostic method, its acceptability has not been widely investigated. Furthermore, the acceptability of radiographic examination has received surprisingly scant attention. Pierro et al (2008) conducted a study to evaluate a modified bitewing film holder in preschool children and considered the acceptability of the method and the quality of the radiographic images. The investigators radiographed 66 children, aged 3-5 years, with the modified film holder. 73% of all participating children accepted the radiographic examination with the modified film holder. The 4- and 5-year-old children's perceptions were assessed using a visual face scale to determine: A) satisfaction; B) indifference; and C) dissatisfaction. The authors found that 74% of children involved in this evaluation (N=43) were satisfied after the examination (Pierro

et al., 2008). Although the investigators assessed acceptability in terms of managing to undertake the radiographic examination, in addition to assessing satisfaction after the procedure, they did not assess the procedure's acceptability in terms of how easy it was or how pleasant the experience was for these children.

Another aspect to consider in terms of the acceptability of caries diagnostic procedures is the use of temporary tooth separation to facilitate visual examination of proximal lesions. The acceptability of TTS has previously been assessed in terms of managing to undertake the examination (Rimmer and Pitts, 1990). The investigators found that, out of 146 children who required separators, 12 children refused or removed them. Most of studies in the literature that have involved children have simply assessed pain intensity after the placement of orthodontic separators rather than assessing the acceptability of the procedure itself (Bergius *et al.*, 2002; Giannopoulou *et al.*, 2006).

Some studies have considered acceptability as part of a wider investigation. Bell and colleagues (2010) assessed the acceptability of preformed metal crowns (PMCs), in conjunction with TTS, in young children. Questionnaires were developed with children. Ninety eight children with mean age of 6.6 years, who had PMCs on primary molars within the paediatric clinic at Sheffield Dental Hospital, were invited to rate their views and experience of having PMCs, using a 3-point pictorial Likert scale (positive, neutral, or negative). Sixty two questionnaires were returned (63% response rate). Most of children reported that the clinical procedure was really easy indicating a high acceptance of the procedure (Bell *et al.*, 2010).

To date, only one study has sought to compare children's acceptability (by virtue of reported levels of discomfort experienced) of different methods for caries detection (Novaes *et al.*, 2012b). In this Brazilian study, 76 children, aged 4-12 years were subject to a visual examination, bitewing radiographs and LE pen (DIAGNOdent). Immediately after these diagnostic procedures, children were asked to indicate their level of discomfort using the 6- points Wong-Baker FACES pan rating scale (Wong and Baker, 1988). The key finding was that radiographs, the LF pen and temporary tooth separation

caused more discomfort than visual examination, but overall discomfort was low. The child's age was found to be an important variable in the outcome.

Clearly, if children experience an unacceptable level of discomfort during a procedure, they may be unwilling to have this procedure again. Furthermore, movement or poor compliance during the procedure may negatively impact on the quality of the diagnostic outcome. It is proposed, therefore, that the present study, should build on these preliminary findings by Novaes and colleagues, in order to gain a greater insight into children willingness to accept a variety of caries diagnostic procedures. Findings relating to young patients' experiences have to be taken into consideration when making recommendations for future clinical practice.

In summary, an extensive review of the literature has not shown clear evidence for the superiority of one method of caries detection over another. Therefore, there is a need for a study to examine the validity and acceptability of the most commonly used caries detection methods and compare them to the new laser fluorescence pen.

3 AIMS AND OBJECTIVES

3.1 Aim

The overall aim of this study is to assess the usefulness of a pen laser fluorescence device for the detection of proximal caries in children's primary teeth.

3.2 Objectives

The specific objectives of this study are as follows:

- to assess validity and reproducibility of a pen laser fluorescence device
- to compare outcomes for a pen laser fluorescence device with these from conventional methods: visual examination with and without tooth separation and bitewing radiographs
- to assess children's acceptability of a pen laser fluorescence device in comparison to visual examination with and without tooth separation and bitewing radiographs

3.3 Null hypothesis

- The null hypothesis for this experimental study is that there is no difference in the validity, reproducibility and acceptability of a pen laser fluorescence device compared to conventional methods (visual examination with and without tooth separation and bitewing radiographs) for the detection of proximal caries in children's primary teeth.

4 METHOD AND MATERIAL

4.1 Study registration and ethical approval

The study was conducted within the School of Clinical Dentistry and the Charles Clifford Dental Hospital, Sheffield. The study obtained ethical approval from the National Health Services Research Ethics Committee (NHS REC) on 13th August, 2012 (Reference 12/YH/0214). The study also received approval from Sheffield Teaching Hospitals Research Governance Department (protocol number STH16301) (Appendix 1). Written consent was obtained from all parents or guardians of young participants to participate in the study. Specific consent was also given to collect children's teeth after extraction for use in the histological part of the study. Clinical training in the clinical aspects of the study and calibration started in September, 2012. Recruitment of participants and clinical data collection commenced in December, 2012 and was completed in September, 2013. Laboratory studies were completed by March 2014. All tooth samples were obtained and stored in accordance with the Human Tissues Act (Human Tissue Act, 2004).

4.2 Examiners involved in the study

Two researchers were involved in conducting the study. Their results were assessed for inter-examiner reproducibility. The reference examiner (CD), a Professor of Paediatric Dentistry, has been involved in numerous published studies in caries diagnosis of children, has helped develop the ICDAS criteria, and has many years of experience using histological scoring of teeth sections, radiographic scoring and other diagnostic systems (Fyffe *et al.*, 2000a, 2000b). The chief investigator (SS), a PhD student, although not familiar with dental examinations under epidemiological or trial conditions, had previous clinical experience in the examination of paediatric patients.

4.3 Study design

The study involved a mixed method approach, incorporating both clinical and laboratory components. The project was primarily a prospective *in-vivo* study for the validation of a laser fluorescence pen (LF pen) method of proximal caries diagnosis in primary teeth compared to visual examination with and without temporary tooth separation (TTS) and radiographic examination.

An *in-vitro* study using the LF pen was also conducted after collection of extracted primary molars, to gain an insight into the potential extrapolation of *in-vitro* measurements to those using an *in-vivo* approach. Results of both *in-vivo* and *in-vitro* studies were validated using a histological 'gold standard' following serial sectioning of teeth to examine caries status. The investigator sought to incorporate the views of children where possible in the study design. Thus young service users were consulted to determine the acceptability of the different caries diagnostic methods.

4.3.1 Overview

The Department of Paediatric Dentistry at the Charles Clifford Dental Hospital runs daily new patient assessment clinics where about 2550 patients are seen annually, of which around 1500 are aged between 5 and 11 years. About 25% of these patients are referred for extractions under general anaesthesia (GA). Therefore, the study sought to recruit patients from these clinics for participation in the *in-vivo* study. There was then the opportunity to collect those teeth which subsequently were extracted under GA in order to conduct the *in-vitro* study and histological validation. A flow chart summarising the main stages of the study design is illustrated in Figure 4.1.

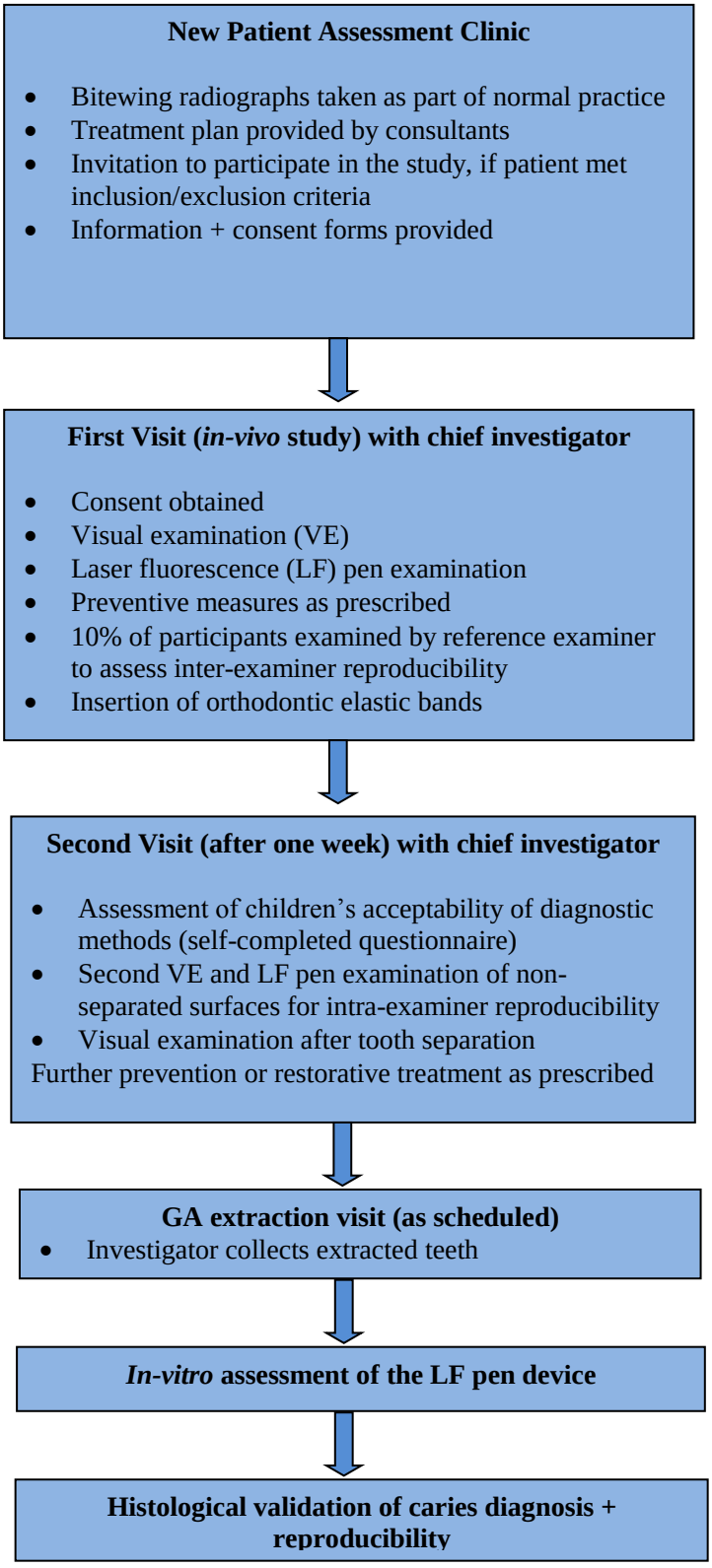

Figure 4.1 A flowchart showing the general outline of the research study

Outcome measures

The two main outcome measures for the study are as follows:

- The validity and reproducibility (including an *in-vitro* versus an *in-vivo* comparison) of a laser fluorescence pen for detection of proximal caries in primary molars compared with finding from bitewing radiographs and visual examination, including the use of temporary tooth separation.
- Patient acceptability of a laser fluorescence pen for detection of proximal caries in primary molars compared with results for bitewing radiographs and visual examination, including the use of temporary tooth separation.

4.3.2 Recruitment of patients

Ninety children, aged 5-11 years, who attended the paediatric dentistry clinic of the Charles Clifford Dental Hospital, Sheffield, and who were assessed by a consultant and found to meet the inclusion criteria and need extractions under GA, were invited to participate in the study. These potential participants were introduced to the chief investigator, after their assessment had been completed, and the project was explained. Parent and child information sheets, together with consent/assent forms (Appendices 2-5) were provided. Children were then booked for the necessary pre-GA prevention or restorative treatments with the chief investigator. At this subsequent visit, written consent for study participation was obtained, thereby allowing participants a period of one to two weeks reflection as to whether or not they wanted to participate in the study. Their necessary prevention/restorative treatment was to be provided by the chief investigator, irrespective of whether they chose to participate in the study or not.

The following inclusion and exclusion criteria were applied by the consultant who assessed the patient, prior to introducing the patient to the chief investigator.

Inclusion criteria

- Children aged 5-11 years of age at recruitment
- Children with caries in one or more primary molars

- Children having extraction of one or more teeth
- Teeth with closed contacts between primary molars
- Children who required intra-oral bitewing radiographs as part of their normal clinical assessment

Exclusion criteria

- Children with severe learning disabilities who were unable to participate even with additional support from the research group
- Children or parents who did not wish to participate in the study
- Patients who were experiencing symptoms and required urgent extractions
- Children with medical conditions which put them at risk when having a dental procedure, such as immunocompromised patients

4.3.3 Tooth sample selection

A power calculation was conducted using nomograms for calculation of sample size in diagnostic studies as shown in Figure 4.2 (Jones *et al.*, 2003). The power calculation was based on existing studies of the validity of laser fluorescence pen device where the sensitivity was found to vary from 0.65 in an *in-vivo* study (Novaes *et al.*, 2009) to 0.95 in an *in-vitro* study (Braga *et al.*, 2009). Therefore, the sensitivity used for the power calculation was 0.80 (an average of both values). Because data were collected from the clinic, the disease prevalence used for the calculation was 0.7. The significance level used was p=0.05. The number of surfaces required to answer the research question was found to be 262 surfaces. It was estimated that 80 patients needed to be recruited, which made this study potentially larger than any previously conducted study.

In addition to the patient exclusion criteria, the following exclusion criteria were applied to tooth samples:

Exclusion criteria for individual surfaces

- The presence of frank cavitation interproximally (absence of a marginal ridge)

- The presence of a large carious cavity on occlusal or smooth surfaces
- The presence of a large occlusal or proximal restoration
- The absence of the adjacent tooth
- The presence of enamel or dentine defects
- The presence of extensive non carious tooth surface loss

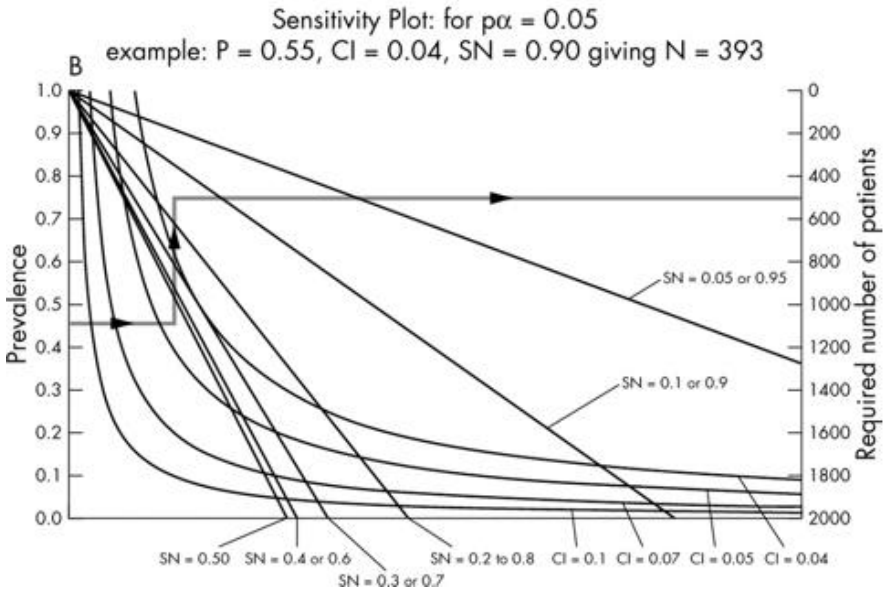

Figure 4.2 Sample size calculation nomogram adapted from Carson et al., 2005

4.4 Examination methods

Caries diagnosis was undertaken for participants by the chief investigator using the four approaches listed below:

- Radiographic examination
- Meticulous visual examination
- Meticulous visual examination after temporary tooth separation (TTS)
- Laser fluorescence pen (LF pen) examination

The chief investigator was trained in the use of ICDAS codes and criteria and radiographic examination system by the reference examiner (CD) who had previous

experience in the use of these systems (Deery *et al.*, 2000; Shoaib *et al.*, 2009) For the laser fluorescence pen, an expert from the Kavo Company visited the Dental Hospital in order to train the chief investigator. One hundred percent of radiographs and approximately 10% of participants were re-examined by the reference examiner using the ICDAS criteria to assess the inter-examiner reproducibility of the radiographic examination and the meticulous visual examination, which was assessed using the Kappa statistic (Landis and Koch, 1977). The level of agreement was interpreted as follows:

- K < 0, less than chance agreement
- K= 0.01–0.20, slight agreement
- K= 0.21– 0.40, fair agreement
- K= 0.41–0.60, moderate agreement
- K= 0.61–0.80, substantial agreement
- K= 0.81–0.99, almost perfect agreement

4.4.1 Radiographic examination

Left and right-sided digital bitewing radiographs were taken of children's upper and lower primary molars, as part of their routine new patient assessment when clinically indicated (Figure 4.3). These radiographs are taken for new patients for caries diagnosis according to established clinical guidelines and good practice (FGDP, 1998; Espelid *et al.*, 2003). They were not taken for the specific purpose of the study, thus children who did not require radiographs or who were unable to tolerate intra-oral films, were excluded from the study. All radiographs were taken by a qualified radiographer within the Hospital's radiography department. The digital x-ray machine (Sirona Heliodent DS intraoral X-ray generator, Bensheim, Germany) was set to 60KV, 7mA and the exposure time was 0.08s. Digital sensor holders (Rinn, XCP-DS) were used to take radiographs, and the focus to film distance was 40cm. Intra oral sensors (Durr VistaRay) were scanned and processed in a scanner (VistaScan). The hospital uses a software package known as IMPAX (AGFA Healthcare's IMPAX X-Ray Angio Analysis R1.0) for

viewing diagnostic images in the radiology domain. Images were examined on the clinic's computer screens at x5 magnification.

Figure 4.3 Radiographic examination was undertaken as part of normal practice at the new patient assessment clinic.

Radiographs were interpreted for caries existence using a modified Ekstrand System (Ekstrand *et al.*, 1997) (Table 4.1). This modified protocol involved the separation of code 2= caries involving inner 1/2 of enamel + outer 1/3 of dentine into two codes: code 2a= caries involving the inner 1/2 of enamel to the enamel dentine junction (EDJ) and code 2b= caries involving outer 1/3 of dentine. This approach permitted comparison with studies undertaken with the Ekstrand system and back comparison with studies using the EDJ as a significant landmark (Mendes *et al.*, 2004; Deery *et al.*, 2006; Lussi *et al.*, 2006; Rodrigues *et al.*, 2009). The radiographs of all patients were examined by both the chief investigator and the reference examiner. All teeth were scored and recorded in a data sheet starting from the upper right second primary molar and finishing at the lower right second primary molar (Appendix 6).

4.4.1.1 Training and calibration

Scores for radiographs (Ekstrand *et al.*, 1997) were reviewed ahead of the training (Table 4.1). The reference examiner (CD) reviewed the codes with the chief investigator and explained how to apply the scores on radiographs of 10 patients. Another set of radiographs of 10 patients were scored by both examiners independently, then scores were reviewed and any disagreement were discussed and resolved.

Table 4.1 A modified Ekstrand (1997) system showing the diagnostic criteria for radiographic examination of caries.

Score	Extension of caries in radiographs
0	Sound tooth surface
1	Radiolucency limited to the outer half of enamel
2a	Radiolucency involving inner half of enamel
2b	Radiolucency extending up to one third of dentine
3	Radiolucency extending to middle third of dentine
4	Radiolucency extending to inner third of dentine

4.4.1.2 Reproducibility assessment of training

For the purpose of training, intra-examiner reproducibility was assessed using bitewing radiographs of ten patients chosen randomly from the hospital computered system (IMPAX) which had been taken in the previous week excluding any radiographs used previously in the training sessions. Radiographs were examined and scored by the chief investigator on two occasions, one week apart. The intra-examiner reproducibility was calculated using the Kappa statistic and was found to be 0.69.

Although reproducibility was acceptable, a higher reproducibility was felt to be desirable. Therefore, a further set of radiographs of 10 different patients was chosen in

the same way and examined twice, one week apart, after reviewing the scoring criteria with the reference examiner. The intra-examiner reproducibility was calculated and was found to have improved to 0.82.

To assess inter-examiner reproducibility, the reference examiner scored the same set of radiographs as scored previously by the chief investigator. Inter-examiner reproducibility was calculated and the Kappa value was found to be 0.62. Further training in the form of scoring a new set of 10 pairs of radiographs was undertaken in order to achieve better agreement. Re-scoring the same set of radiographs as scored previously by the chief investigator was undertaken, which took the Kappa to 0.79.

To confirm this high inter-examiner reproducibility, another set of 10 pairs of radiographs was examined and scored by both examiners (CD & SS). The Kappa value was found to be 0.67. Taken overall, inter-examiner reproducibility for the 20 sets of radiographs was found to be K=0.71, which was considered acceptable to start the study.

Radiographic training and calibration was conducted over a period of 6 months whilst awaiting for ethical approval.

4.4.2 Meticulous visual examination

The meticulous visual examination was carried out in a dental unit with an operating light, a plane mirror, a 3-in-1 syringe, and access to a blunt probe (CPITN), as required by the criteria (Figure 4.4). Teeth were examined both wet and after drying with compressed air. Visual examination was carried out using the International Caries Detection and Assessment System (ICDAS) criteria (Ismail *et al.*, 2007). ICDAS codes and criteria are shown in Table 2.7. Examiners (SS & CD) separately recorded codes for each surface on a score sheet.

4.4.2.1 Training and calibration

First, the chief investigator reviewed information available on the ICDAS website (www.icdas.org). Then training was conducted in the form of a lecture given by the reference examiner (CD) who discussed and reviewed the codes and criteria. Initial scoring was undertaken using pictures of 60 teeth on PowerPoint slides. This was followed by a practise session in the paediatric clinic where caries status of all tooth surfaces of primary teeth of five patients was scored by both examiners. Scores were recorded on a scoring sheet. Any disagreement in the scoring was discussed and resolved.

Figure 4.4 Meticulous visual examination of primary molars was undertaken in a clinical environment.

4.4.2.2 Reproducibility assessment of training

For the purpose of training, reproducibility of application of the ICDAS codes was first assessed using digital photographic images of extracted primary molar teeth prepared by the reference examiner. A total of 30 photographs, presenting 72 surfaces were coded. The gold standard assessment was undertaken by the reference examiner ahead of the training. Intra- and inter-examiner reproducibility were calculated using Kappa statistic and were found to be 0.79 and 0.42 respectively. The intra-examiner reproducibility was substantial. However, the inter-examiner reproducibility was low. Scores were reviewed by both the chief investigator and the reference examiner. The disagreement was found to relate to a difficulty in distinguishing the different surfaces on the photographic images, rather than erroneous application of the ICDAS codes.

The chief investigator repeated the scoring after understanding the images representation of each surface, and reproducibility was recalculated. The Kappa statistic for inter-examiner reproducibility was repeated and was found to be slightly better at 0.58. However, it was still low.

Therefore, the reproducibility was reassessed by examining 10 patients following receipt of ethical approval. Each examiner was blind to the scores of the other examiner. Inter-examiner reproducibility was calculated and was found to be 0.79 which was acceptable to start the main study.

4.4.3 Meticulous visual examination after temporary tooth separation (TTS)

Orthodontic elastic separators (3M Unitek separator modules) were placed between primary molars which met the study inclusion criteria as shown in Figure 4.5. They were left in situ for 7 days to create temporary tooth separation (Figure 4.6) and thus allow subsequent direct visual examination of the inter-proximal surfaces. The same criteria (ICDAS) were used for visual examination of caries status of every test surface after TTS.

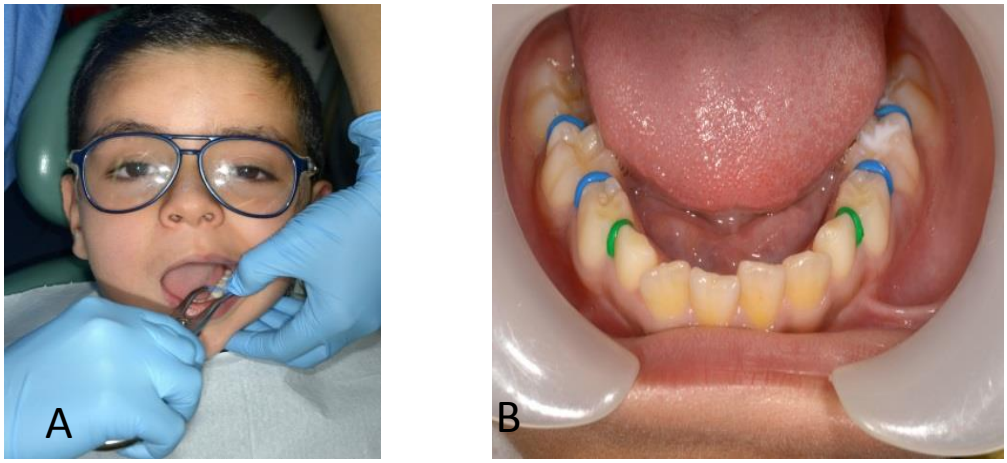

Figure 4.5 Placement of orthodontic separators between test teeth to achieve separation (A, B).

Figure 4.6 Space created by orthodontic separators to allow direct visualisation of proximal molar surfaces.

4.4.4 Laser fluorescence pen examination (LF pen, DIAGNOdent 2190)

The final method for caries assessment of the test teeth employed a DIAGNOdent pen (LF pen, Kavo, Biberach, Germany), which was developed in 2006 for the detection of proximal caries (Lussi *et al.*, 2006). The device is non-invasive and in current clinical use. It is commercially available with no reported risks (Figure 4.7).

The device operates on the basis that carious teeth produce increased fluorescence at specific excitation wave lengths (Hibst *et al.*, 2001). The intensity of fluorescence depends on the tooth structure and the wave length of the light.

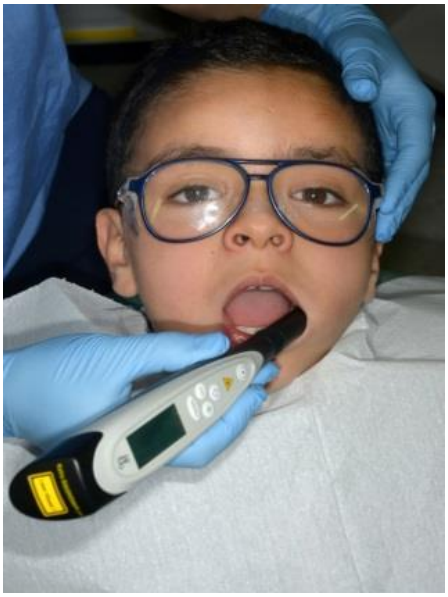

Figure 4.7 Caries examination using a pen laser fluorescence device.

The device can be fitted with two different fibre tips, a conical tip, with a diameter of 0.7mm with minimum thickness of 0.4mm at the measurement site (tapered wedge shaped tip TWDG) and a cylindrical tip, with a diameter of 1.1mm (wedge shaped tip WDG). Each tip can rotate around its long axis to facilitate placement of the probe on the mesial and distal tooth surfaces at the oral and facial sides in anterior and posterior teeth. A red point on the tip itself indicates the light direction. The propagation of both the excitation light and fluorescence light occurs in the same single solid fibre tip in opposite directions.

In this study, a tapered wedge shaped tip TWDG (probe tip 1 for proximal surfaces) was employed for all measurements. First, the machine was set to zero, then the tip was

calibrated against a porcelain reference before examination. On examination, the device was recalibrated on a sound tooth surface to represent the background fluorescence of teeth in that individual patient (zero value) according to the manufacturer's instructions. Proximal surfaces were then assessed by inserting the probe tip underneath the contact area from the buccal and the lingual/palatal sides with the red dot directed towards the surface to be recorded and moved around until the peak value was reached. The number was shown on the digital screen as well as on the pen's screen (Figure 4.8).

Figure 4.8 Digital screen displaying the reading on the LF pen.

The highest value of the two measurements (buccal and lingual) was recorded and the zero value was then subtracted from the highest measurement (Rodrigues *et al.*, 2009). LF pen values for each tooth surface were entered on a paper data recording sheet (Appendix 7) before they were entered on to the computer data base, statistical package for social science (IBM SPSS statistics 21) spread sheet.

The values were translated in analysis according to manufacturer's instructions as follows:

- 0-7= sound surface
- 8-15= enamel caries
- ≥16= dentine caries

4.4.4.1 Training and calibration

For the laser fluorescence pen, an expert from the Kavo Company (VM) trained the chief investigator on two occasions. The first session took place prior to obtaining ethical approval, thus a demonstration of how to use the machine was conducted on extracted teeth. However, following ethical approval, a second training session was provided by the same person (VM) and involved the use of the LF pen on patients after obtaining their consent.

4.5 Acceptability of different diagnostic methods

The views of children about different diagnostic methods were assessed by asking them to complete a pre-piloted self-complete questionnaire which asked them how easy or difficult they had found the test, and whether they would be happy to have it again. They completed a 5-point pictorial scale as shown in appendix 8.

The questions used in the questionnaire were chosen after conducting a small qualitative survey where 20 patients (9 girls, 11 boys) aged 5-11 years were asked their views about radiographs and separators. A number of open questions were asked, in different ways, and it was found that children understood and responded well to the following two key questions:

- What was it like to have the x –ray pictures (or the elastic bands between your teeth)?
- Would you be happy to have them again?

The pilot questionnaire was given to five children to check if they could understand the language and the response format. All managed to do so and no changes were thus needed for the final questionnaire.

4.6 Conduct of main study

4.6.1 *In-vivo* study

After obtaining ethical approval, the chief investigator provided all the clinicians who were working in the Paediatric Dentistry Department of the Charles Clifford Dental Hospital with a letter explaining the project and asking for their assistance in patient recruitment (Appendix 9). In addition, a colourful poster describing the project was placed in the waiting area with the aim of raising awareness of the study and increasing recruitment (Appendix 10).

The chief investigator was responsible for recruiting participants from the Paediatric Dentistry Department. All patients, who satisfied the inclusion criteria, were invited to participate in the study. The project was explained to participants and their parents/guardians and consent forms were provided along with adult and child information sheets. Patients were approached at their first attendance at the clinic, following their consultant-led new patient assessment. All children who were approached by the chief investigator were booked with her for a subsequent pre-GA preventive visits, which is the standard clinical protocol within the department. In some cases, children also required items of restorative treatment such as placement of a preformed metal crown, prior to their dental GA. These items of treatment were also provided by SS.

Those children who consented to participate in the study received the diagnostic caries tests during the same visit as having the prescribed preventive interventions (oral hygiene instruction, topical fluoride varnish application, diet analysis and/or fissure sealants) or restorative treatment. For children, whose parents did not wish them to

participate in the study, the preventive and/or restorative treatments were provided by SS as normal.

Details regarding the sequence of events during these visits are provided below.

First visit

Patients who agreed to participate in the study

Consent/assent forms were collected from parents and children respectively. Then, children were asked to choose number one or two in order for SS to know which examination method to start with, the visual examination or the LF pen. Each visit SS changes the meaning of those numbers. Visual examination was conducted using the ICDAS criteria for caries detection by SS. The examination was conducted in the dental clinic with normal operating light illumination, 3-in-1 syringe, a mirror and a WHO periodontal probe. Teeth were examined wet, then re-examined after drying for 5 seconds with compressed air. Scores were recorded by the dental nurse on a recording sheet. SS was blind of scores of other examinations.

Inter-proximal surfaces of primary molars were also assessed for the presence of dental caries using the pen laser fluorescence device (DIAGNOdent pen, Kavo Biberarch, Germany) according to the manufacturer's instructions. Scores were also recorded by the dental nurse and SS was blind of scores of other examinations.

Orthodontic elastic separators were then placed between the primary molars which met the study inclusion criteria. They were left in situ for 7 days (or a maximum of 14 days in some occasions where the patients failed to attend their appointment) to create temporary tooth separation to allow a subsequent direct visual examination of the inter-proximal surfaces. Caries preventive measures, including oral hygiene instruction and topical fluoride application, were provided at this first visit.

Second visit

A second visit was scheduled for one week later. At this visit, the acceptability of the different diagnostic methods (bitewing radiographs, temporary tooth separation, visual examination and LF pen device) was assessed by asking children to complete the short acceptability questionnaire. Questionnaires were given to patients when they first arrived to complete in the waiting room before receiving any interventions.

A repeat caries diagnosis was then undertaken on the clinic after removal of any orthodontic separators using the same method used in the first visit for selection of method to start with. All inter-proximal surfaces of primary molars (the separated and the non-separated surfaces) were re-examined using the ICDAS system (Ismail *et al.*, 2007) and the LF pen. The separated surfaces were examined to assess the effect of separation on caries diagnosis, and the non-separated surfaces were re-examined to assess the intra-examiner reproducibility.

Further preventive measures, such as fissure sealants or diet analysis, were then conducted according to the patient's treatment plan. Patients who required further treatment, such as fillings or crowns, were booked for extra sessions with SS. All participants were then scheduled for dental extractions under general anaesthetic at Sheffield Children's Hospital, in accordance with their initial treatment plan. GA appointments were booked directly by SS to make sure appointments are not booked later than 3 weeks after examinations.

In order to assess inter-examiner reproducibility of the visual examination, 10% of participants were examined by the bench mark examiner (CD) during their first or second visit.

4.6.2 Collection of teeth

Extracted teeth were transported from the Sheffield Children's Hospital to the Oral Pathology Department of the Charles Clifford Dental Hospital in labelled specimen pots.

There they were stored at -20℃ (Francescut *et al.*, 2006) in a locked laboratory until use.

4.6.3 *In-vitro* study

Frozen teeth were defrosted for 16 hrs. To ensure 100% humidity, a wet paper towel was placed at the bottom of each sample pot ensuring that there was no direct contact between the tooth and the paper towel. Then all teeth were cleaned with a tooth brush and running tap water for 15 seconds. Any remaining soft radicular tissue was removed using a surgical curette with care not to scratch the crown.

Inter-proximal surfaces of extracted teeth were reassessed (*in-vitro*) for the presence of dental caries using the LF pen to gain insight into the potential extrapolation of *in-vitro* findings to *in-vivo* ones. Teeth were also examined using the ICDAS criteria to assess the relationship between the radiographic depth and clinical cavitaion of proximal surfaces.

Each tooth was dried with a tissue and left exposed to air at room temperature for 5 minutes before taking the caries measurements. First, the ICDAS score of the surface was recorded, then the LF pen measurement was taken. Prior to each measurement, the LF pen was calibrated according to the manufacturer's instructions. After that, standardisation of the fluorescence (measuring the zero value) was carried out by registering the fluorescence value on a sound area of enamel. This value was then subtracted from the value of fluorescence of the site to be measured. Four consecutive measurements were taken from each site, the mean and the highest values were calculated and used later for analysis. Each tooth was then stored in formalin (10% concentration) in a separate labelled specimen pot prior to tooth sectioning.

4.7 Histological examination

In order to validate the results obtained from the previous caries diagnostic tests, histological assessment of caries status was undertaken as the gold standard.

4.7.1 Tooth preparation

Each tooth was dried in acetone for 2-3 minutes to allow the surface to bond properly to wax during sectioning (Figure 4.9). The dry mesial surface was then marked with a black permanent marker (STAEDTLER permanent Lumocolor) to aid identification of tooth surfaces after sectioning (Figure 4.10).

Figure 4.9 Acetone pot in which the tooth is dried before marking the mesial surface.

Figure 4.10 The mesial surface of a first primary molar marked with a permanent marker.

4.7.2 Tooth sectioning

Each tooth was mounted on wax then serially sectioned longitudinally in a mesio-distal direction using a water cooled band saw 0.2 mm thick (EXAKT-Apparatebau GmGH, Norderstedt, Germany) to achieve 5-8 cuts (Figure 4.11). Each section was approximately 500 microns (μm) thick. No teeth were lost during sectioning (Figure 4.12).

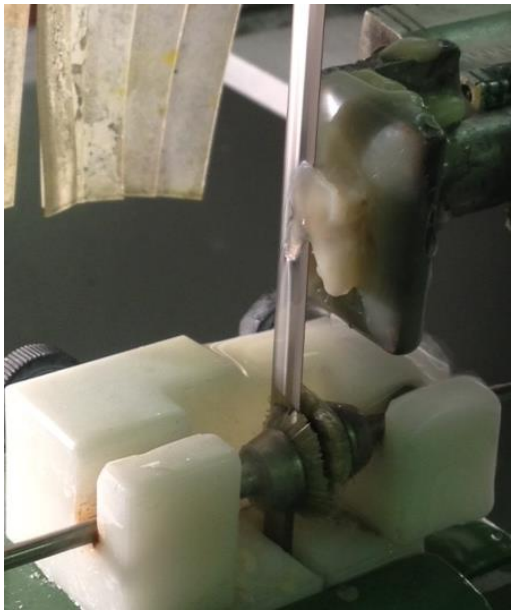

Figure 4.11 A mounted primary molar during sectioning

4.7.3 Examination of tooth sections

After sectioning the tooth, each section was examined from both sides, by SS, under a magnification of x15 using a stereo-microscope (Figure 4.13) to confirm the presence and depth of any carious lesion. Digital images of histological sections were taken, and scoring was done on a computer screen by both examiners (SS and CD). The criteria proposed by Ekstrand (1997) were used as these are the basis for the development of the ICDAS criteria and corresponded to the visual ranking system. However, they do not provide a specific cut-off between enamel and dentine like most other studies do. Therefore, the criteria were modified. The alteration made being the separation of code

2= caries involving inner 1/2 of enamel + outer 1/3 of dentine into two codes: code 2a= caries involving inner 1/2 of enamel to the enamel dentine junction (EDJ) and code 2b= caries involving the outer 1/3 of dentine (Table 4.2) (Figure 4.14). This permitted comparison with studies undertaken using the Ekstrand system (Rocha *et al.*, 2003) and back comparison with studies using the EDJ as a significant landmark (Mendes *et al.*, 2004; Deery *et al.*, 2006; Lussi *et al.*, 2006; Rodrigues *et al.*, 2009).

Table 4.2 Codes and criteria used for histological examination adapted from Ekstrand et al (1997).

Score	Histological extension of caries
0	No enamel demineralisation or a narrow surface zone of opacity (edge phenomenon)
1	Enamel demineralisation limited to the outer half of the enamel layer
2a	Enamel demineralisation involving inner half of enamel
2b	Demineralisation involving outer third of the dentine
3	Demineralisation involving the middle third of the dentine
4	Demineralisation involving the inner third of the dentine

After scoring all sections of each tooth, the highest score was given to the tooth. Scores were first recorded on a scoring paper sheet then they were transferred to an SPSS spread sheet. For inter-examiner reproducibility, an on-line random sequence generator was used, http://stattrek.com/statistics/random-number-generator.aspx, to randomly select 68 surfaces from the 680 to be examined by both the reference examiner and SS to assess the reproducibility of the histological examination.

Figure 4.12 A histological section of a primary molar as examined under the stereomicroscope (x15)

Figure 4.13 Stereomicroscope used for the histological examination of sections

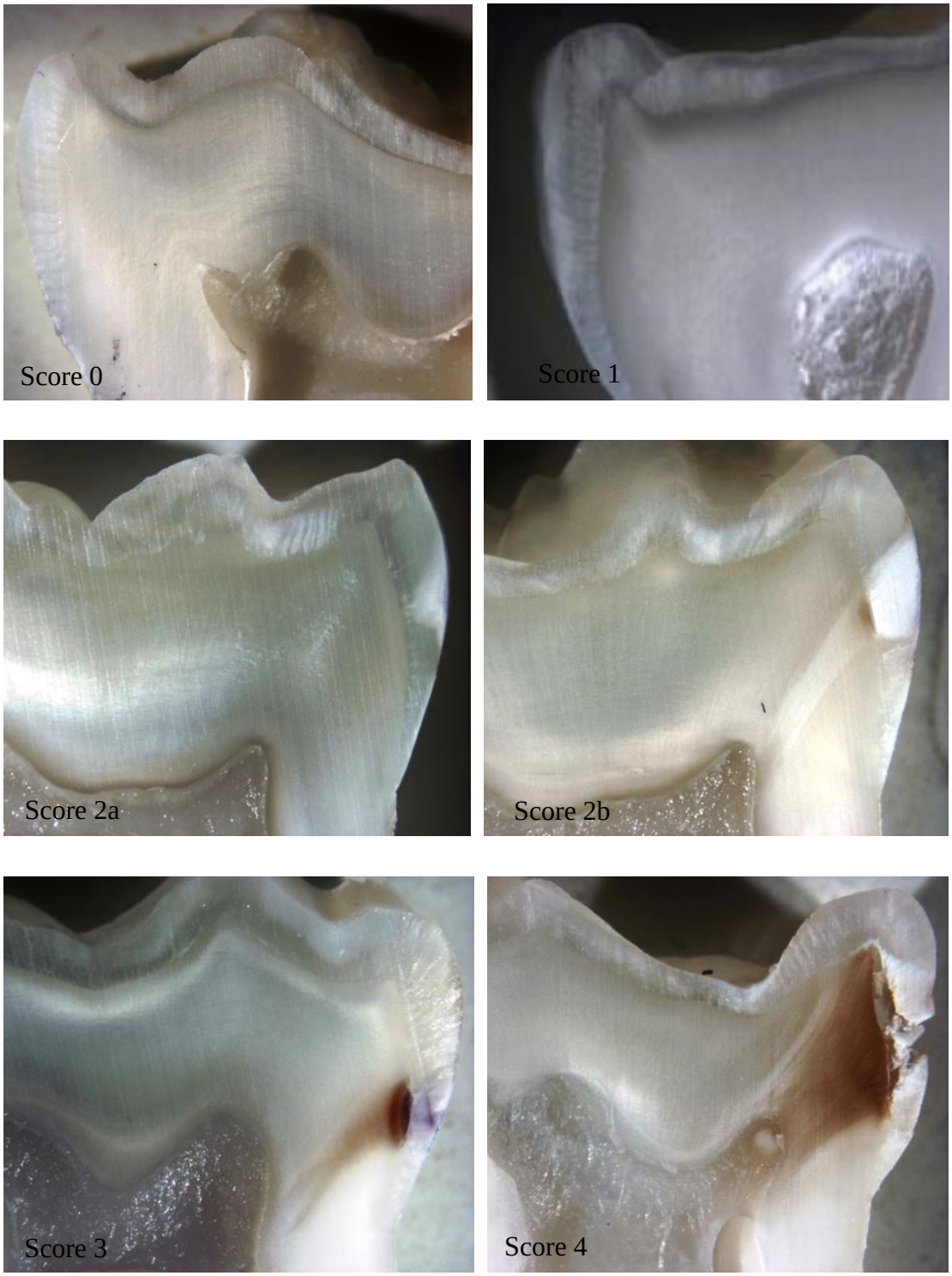

Figure 4.14 Digital images of histological sections with different histological extension of caries adapted from Ekstrand et al (1997)

4.7.4 Training and calibration

4.7.4.1 Tooth sectioning training

The SS was trained by an expert technician (DT) in the oral pathology laboratory. The training was done over three sessions. Each session was at least two hours to ensure correct use of the machine and to achieve high quality tooth sections.

4.7.4.2 Training and calibration for histological scoring

SS was first trained in the use of the stereo-microscope and taking quality digital images by an expert (RW). Training for scoring the histological sections was done by the reference examiner. Training was conducted over two sessions. In the first session, ten teeth (5-8 sections each) were examined under the stereo-microscope. Scores were discussed and agreed by both examiners. In the second session, another set of ten teeth (5-8 sections each) were examined both under the stereo-microscope and on digital photographs on a computer screen. Inter-examiner reproducibility was assessed using the Kappa statistic and was found to be 0.79 which was acceptable to start the study.

4.8 Statistical analysis

The aim of the study was to assess the validity and acceptability of the laser fluorescence device, and to compare the results with those of bitewing radiographs and meticulous visual examination, with and without temporary tooth separation.

IBM SPSS statistics 21 and SAS 9.2 programmes were used to analyse data. Simple descriptive analysis of all the variables was first conducted using SPSS statistics 21.

Validity of the pen both *in-vivo* and *in-vitro* was assessed by calculating the sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratios positive and negative, and the area under the receiver-operating characteristics curves for each diagnostic method using the SAS 9.2 programme. Data was analysed at three diagnostic levels (D_1, D_3, ERK_3). The ROC comparisons were performed by using a contrast

matrix to take differences of the areas under the empirical ROC curves (DeLong *et al.*, 1988).

The correlation between the LF pen scores *in-vitro* (highest and average), and between the *in-vitro* and *in-vivo* scores is measured using Spearman's correlation. The difference between the LF pen scores *in-vivo* and *in-vitro* was assessed using one sample T-Test.

Inter- and intra-examiner reproducibility was assessed using the Kappa statistic (at D_1 and D_3 thresholds).

Acceptability of the methods was analysed using simple descriptive statistics. Repeated measure analysis of variance test was used to determine any differences in patient acceptability between the three diagnostic approaches where the significance level is set at $p<0.05$. P-Values used the Sidak Correction for multiple comparisons. A statistics clinic, through the Clinical Research Office, confirmed the appropriateness of the study design and proposed statistical tests.

5 RESULTS

5.1 Introduction

A total of 82 children were recruited to the study over 9 months (December 2012-September 2013). All children attended on two occasions during which they had their teeth examined using four different caries detection methods. Then their views about these different detection methods were assessed using a pre-piloted self-complete questionnaire. Teeth were reassessed *in-vitro* by the LF pen and by direct visual examination of the proximal surfaces prior to histological validation where possible.

The results in this chapter are presented in four sections:

Section 5.2 Provides details for the participants with regards to gender, age, and socioeconomic status. In addition, clinical data are provided for the teeth/surfaces which were subject to the caries diagnostic methods including histology. Simple descriptive analysis is presented for all variables.

Section 5.3 reports the validity of the different diagnostic methods including sensitivity, specificity, positive and negative predictive values and area under the ROC. Intra- and inter-examiner reproducibility will also be reported in this section.

Section 5.4 presents the results of the *in-vitro* assessment of the extracted teeth.

Section 5.5 presents the results for patient acceptability of the diagnostic methods used in this study.

5.2 Participants and study material

5.2.1 Response rate

Ninety patients met the inclusion criteria and were invited to participate in the research project. However, six of these patients subsequently failed to attend their appointments with the investigator and they were referred to other staff members to follow up on their attendance. One patient had an emergency GA extraction before his second examination, therefore was excluded from the study. One child, although agreed to participate in the study, was too anxious to cope with an examination, giving an overall response rate of 91% (82/90).

5.2.2 Participants

A total of 82 children aged 5-10 years (mean age=6.4; SD=1.3 years) participated in the study. As can be seen from Figure 5.1, there was greatest representation from younger children, with almost 80% aged between 5 and 7 years. The proportion of boys (54%, N=44) was slightly higher than that for girls (46%, N=38). All the children who attended their appointments agreed to participate in the study. Almost 50% of the participants were from an ethnic minority group.

The socio-economic status of the participants was examined using GeoConvert software, which was used to convert postcodes to an Index of Multiple Deprivation (IMD) rank which is used to measure deprivation in England (http://geoconvert.mimas.ac.uk). The IMD is based on the Lower Super Output Area (LSOA). There are 32,482 LSOAs in England. The most deprived LSOA for each Index is given a rank of 1 and the least deprived LSOA is given a rank of 32,482. These ranks fit in to five categories of deprivation with 1 being the most affluent and 5 being the most deprived. The study population showed a range of IMD rank of 81 to 28,779 (mean=8,925; SD=8,660). This showed that there was a high representation (71%) from socially disadvantaged families (Figure 5.2).

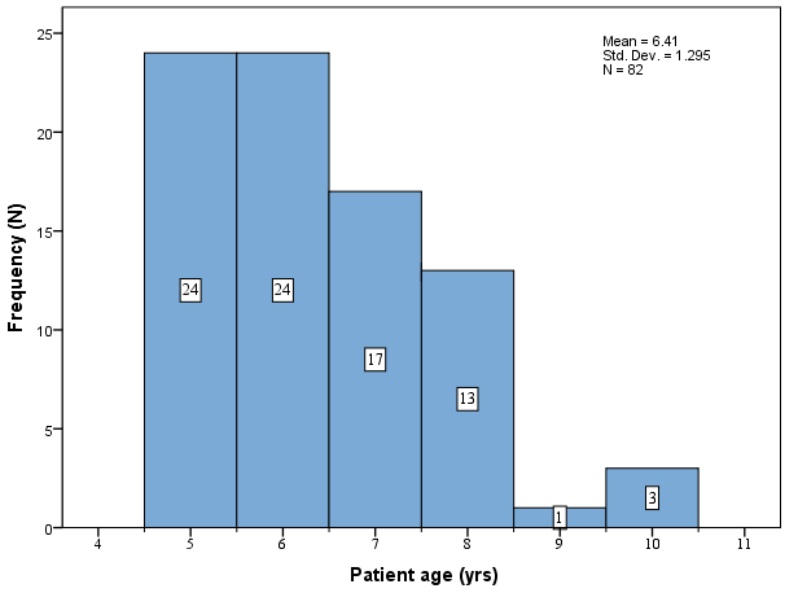

Figure 5.1 The frequency (N) of children who participated in the study according to their age (n=82).

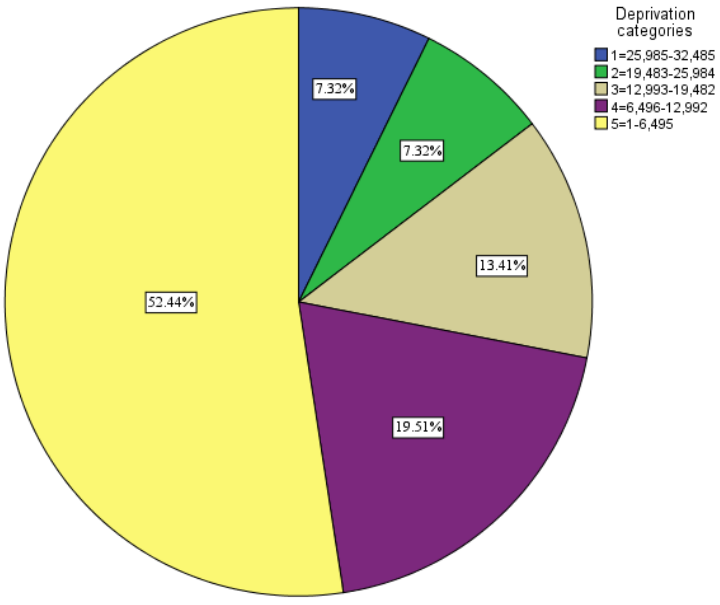

Figure 5.2 The distribution of the study population (N=82) according to their IMD rank (LSOA), with 1 being the most affluent and 5 being the most deprived.

5.2.3 Study material

Each child had a mean of 15.7 surfaces subject to examination (SD=1.1; range=10-16) using the different diagnostic methods tested in this study. A total number of 1225 surfaces were examined, of which 195 surfaces (16%) had frank cavitation, and therefore were excluded from subsequent analysis. Of those included in the final analysis, 447 surfaces were temporarily separated and 542 were histologically validated.

5.2.3.1 Clinical data before temporary tooth separation (first visit)

Meticulous visual examination (ICDAS)

Meticulous visual examination before temporary tooth separation (TTS) showed 63% of the examined surfaces to have no visible sign of caries. Dentine caries was found in 17.9% of the surfaces (ICDAS code 3–5). Surfaces with ICDAS code 6 (N=195, 16% of total surfaces examined) were excluded from subsequent analysis (Table 5.1).

Table 5.1 ICDAS scores for proximal surfaces examined visually in the first visit (caries prevalence of the participants).

ICDAS code	Number of surfaces	Percentage (%)
Code 0 = Sound	649	63
Code 1 = opacity shows after drying	22	2.1
Code 2 = opacity shows without drying	175	17
Code 3 = enamel cavitation	11	1.1
Code 4 = underlying darkness	63	6.1
Code 5 = small dentine cavity	110	10.7
Total	1030	100
Code 6 = large dentine cavity	195	Excluded

Radiographic scoring analysis

All radiographs of 82 patients were scored by both the investigator and the reference examiner. The inter-examiner reproducibility was found to be substantial (Kappa=0.64). agreed. The results of this examination were used for the validity analysis. A descriptive analysis of all radiographic examinations is provided below.

Radiographic examination conducted by the investigator found 56% of the surfaces to be sound. Enamel caries was found in 13.7% of the surfaces examined while dentine caries was found in 28.4% of the surfaces examined (Table 5.2).

Table 5.2 Radiographic scores of surfaces determined by the investigator.

Radiographic score	Number of surfaces	Percentage (%)
Score 0 = sound	577	56
Score 1 = caries in outer enamel	63	6.1
Score 2 = caries in inner enamel	78	7.6
Score 3 = caries in outer dentine	149	14.5
Score 4 = caries in middle dentine	81	7.9
Score 5 = caries in inner dentine	62	6.0
Can’t be seen	20	1.9
Total	1030	100
Cavitated surfaces	195	Excluded

Radiographic examination conducted by the reference examiner found 61.7% of surfaces to be sound. Enamel caries was found in 13.5 of surfaces while dentine caries was found in 23.3% of surfaces (Table 5.3).

The third examination found 58.3% of surfaces to be sound. Both examiners agreed that 14% of surfaces had enamel caries and 25.1% of the surfaces had caries into dentine (Table 5.4).

Table 5.3 Radiographic scores of surfaces determined by the reference examiner.

Radiographic score	Number of surfaces	Percentage (%)
Score 0 = sound	636	61.7
Score 1 = caries in outer enamel	93	9.0
Score 2 = caries in inner enamel	46	4.5
Score 3 = caries in outer dentine	136	13.2
Score 4 = caries in middle dentine	53	5.1
Score 5 = caries in inner dentine	52	5.0
Can't be seen	14	1.4
Total	1030	100
Cavitated surfaces	195	Excluded

Table 5.4 Radiographic scores of surfaces agreed by both examiners.

Radiographic score	Number of surfaces	Percentage (%)
Score 0 = sound	600	58.3
Score 1= caries in outer enamel	79	7.7
Score 2 = caries in inner enamel	65	6.3
Score 3 = caries in outer dentine	155	15
Score 4 = caries in middle dentine	62	6.0
Score 5 = caries in inner dentine	42	4.1
Can't be seen	27	2.6
Total	1030	100
Cavitated surfaces	195	Excluded

LF pen examination

Examination with the LF pen before TTS found 58.5% of examined surfaces to be sound and 28.2% to have caries extending into dentine interpreting the cut-off values as recommended by manufacturer (Table 5.5).

Table 5.5 LF pen scores for surfaces examined at the first visit.

Pen code	Pen readings	Number of surfaces	Percentage (%)
Code 0	0-7 = sound	603	58.5
Code 1	8-15 = enamel caries	137	13.3
Code 2	16-99 = dentine caries	290	28.2
Total		1030	100
	Cavitated surfaces	195	Excluded

5.2.3.2 Clinical data for surfaces with temporary tooth separation

A total number of 447 surfaces were temporarily separated. A descriptive analysis of a meticulous visual examination and LF pen examination of the separated surfaces before and after temporary tooth separation is described below.

Meticulous visual examination

A total number of 447 surfaces were temporarily separated. A descriptive analysis of a meticulous visual examination and LF pen examination of the separated surfaces before and after temporary tooth separation is described below.

Meticulous visual examination of the separated surfaces before TTS found 65.1% of surfaces to be sound. Only 20.5% of surfaces had enamel caries and 14.3% of surfaces had dentine caries (Table 5.6).

Meticulous visual examination after temporary tooth separation showed only 37.1% of surfaces to have no visual signs of caries. However, enamel caries was found in almost 40% of surfaces and dentine caries was found in 23.5% of surfaces (Table 5.7).

Table 5.6 ICDAS scores for proximal surfaces examined visually in the first visit prior to temporary tooth separation.

ICDAS code	Number of surfaces	Percentage (%)
Code 0	291	65.1
Code 1	6	1.3
Code 2	86	19.2
Code 3	4	0.9
Code 4	29	6.5
Code 5	31	6.9
Total	447	100

Table 5.7 ICDAS scores for proximal surfaces examined visually in the second visit after temporary tooth separation.

ICDAS code	Number of surfaces	Percentage (%)
Code 0	166	37.1
Code 1	17	3.8
Code 2	161	36
Code 3	10	2.2
Code 4	20	4.5
Code 5	73	16.3
Total	447	100

LF pen examination

LF pen examination before temporary tooth separation showed 51.2% of the proximal surfaces examined to be sound. Dentine caries was found in 30.4% of the surfaces (Table 5.8).

Table 5.8 LF pen scores of the separated surfaces examined before TTS.

Pen code	Pen scores	Number of surfaces	Percentage (%)
Code 0	0-7 =sound	229	51.2
Code 1	8-15=enamel caries	82	18.3
Code 2	16-99=dentine caries	136	30.4
Total		447	100

Tooth surfaces were examined with LF pen after TTS. The LF pen failed to give appropriate results due to technical problems at the second visit for six participants. Therefore, pen readings of 30 surfaces are missing. The pen examination after TTS found 49.6% of surfaces to be sound. Dentine caries was found in 34.1% of surfaces (Table 5.9).

Table 5.9 LF pen scores of separated surfaces examined after TTS.

Pen code	Pen scores	Number of surfaces	Percentage (%)
Code 0	0-7=sound	207	49.6
Code 1	8-15=enamel caries	68	16.3
Code 2	16-99=dentine caries	142	34.1
Total		417	100

5.2.3.3 Clinical data for surfaces with histological validation

For the histological validation, a total of 356 primary molars were available (mean=4.34 per child, SD=2.09), of these 213 (60%) were first primary molars (mean=2.6 per child; SD=1.3) and 143 (40%) were second primary molars (mean=1.7 per child; SD=1.4). In addition, 167 (47%) of these teeth were from the upper jaw and 189 (53%) were from the lower jaw. These teeth were collected from 78 patients. The number of teeth extracted from each quadrant is shown in Table 5.10. Extracted teeth of four patients were lost for the following reasons; accidental disposal of teeth (N=2), bringing the GA appointment forward due to an emergency (N=1) and missing the collection date due to multiple cancellations of the GA appointment (N=1). Teeth were sectioned so that each tooth provided 5-8 sections. Each section was approximately 500 microns thick. No teeth were lost during sectioning.

Table 5.10 Description of the extracted primary molars collected.

Teeth		**Right**	**Left**	**Total**
First primary molars (N=213)	**Upper**	47	49	96
	Lower	60	57	117
Second primary molars (N=143)	**Upper**	35	36	71
	Lower	38	34	72
Total		180	176	**356**

A total of 684 proximal surfaces were histologically examined, of which 142 surfaces were score 6 (ICDAS), therefore these were excluded from the subsequent analysis, leaving 542 proximal surfaces. Histological examination found 21.7% of surfaces (N=118) to be sound. Caries into enamel was found in 35.5% of surfaces while dentine caries was found in 42.5% of surfaces (N=231) (Table 5.11).

Table 5.11 The number of surfaces according to histological extension of caries.

Histological score	Number of surfaces	Percentage (%)
Score 0	118	21.7
Score 1	60	11
Score 2	133	24.5
Score 3	61	11.2
Score 4	36	6.6
Score 5	134	24.7
Total	542	100

The following tables present the descriptive data for each caries detection method which were subsequently validated using histological assessment.

Meticulous visual examination before TTS of surfaces with histological validation

Meticulous visual examination before TTS of the histologically validated surfaces showed 56.8% of surfaces to be sound and only 20.7% and 22.5% of the proximal surfaces to have enamel and dentine caries respectively (Table 5.12).

Meticulous visual examination after TTS of surfaces with histological validation

Meticulous visual examination after TTS of the histologically validated surfaces showed 33.3% of surfaces to be sound and 27.8% of surfaces to have dentine caries (Table 5.13).

Table 5.12 The number of surfaces which were visually examined before TTS and validated histologically.

ICDAS code	Number of surfaces	Percentage (%)
Code 0	308	56.8
Code 1	13	2.4
Code 2	99	18.3
Code 3	4	.7
Code 4	39	7.2
Code 5	79	14.6
Total	542	100
Code 6	142	Excluded

Table 5.13 The number of surfaces visually examined after TTS and histologically validated.

ICDAS code	Number of surfaces	Percentage (%)
Code 0	79	33.3
Code 1	5	2.1
Code 2	87	36.7
Code 3	2	0.8
Code 4	13	5.5
Code 5	51	21.5
Total	237	100

Radiographic examination of surfaces which were histologically validated

Radiographic examination showed 52% of surfaces to be sound, only 11.3% with enamel caries and a further 34.2% with dentine caries (Table 5.14).

Table 5.14 The number of surfaces which were examined radiographically and validated histologically.

Radiographic score	Number of surfaces	Percentage (%)
Score 0 = sound	282	52
Score 1 = caries in outer enamel	33	6.1
Score 2 = caries in inner enamel	28	5.2
Score 3 = caries in outer dentine	73	13.5
Score 4 = caries in middle dentine	64	11.8
Score 5 = caries in inner dentine	48	8.9
Not scorable	14	2.6
Total	542	100

LF pen examination before TTS of surfaces with histological validation

Examination of surfaces, which were histologically validated, with the LF pen before TTS showed 50.7% of surfaces to be sound and 34.1% of surfaces to have caries extending it to dentine (Table 5.15).

Table 5.15 The number of surfaces which were examined with the LF pen before TTS and validated histologically.

Pen code	Pen scores	Number of surfaces	Percentage (%)
Code 0	0-7=sound	275	50.7
Code 1	8-15=enamel caries	82	15.1
Code 2	16-99=dentine caries	185	34.1
Total		542	100

LF pen examination after TTS of surfaces with histological validation

Examination of proximal surfaces, which were histologically validated, with the LF pen after TTS showed 44.7% of surfaces to be sound and 38.1% of surfaces to have caries into dentine (Table 5.16).

Table 5.16 The number of surfaces which were examined with the LF pen after TTS and validated histologically.

Pen code	Pen scores	Number of surfaces	Percentage (%)
Code 0	0-7=sound	101	44.7
Code 1	8-15=enamel caries	39	17.3
Code 2	16-99=dentine caries	86	38.1
Total		226	100

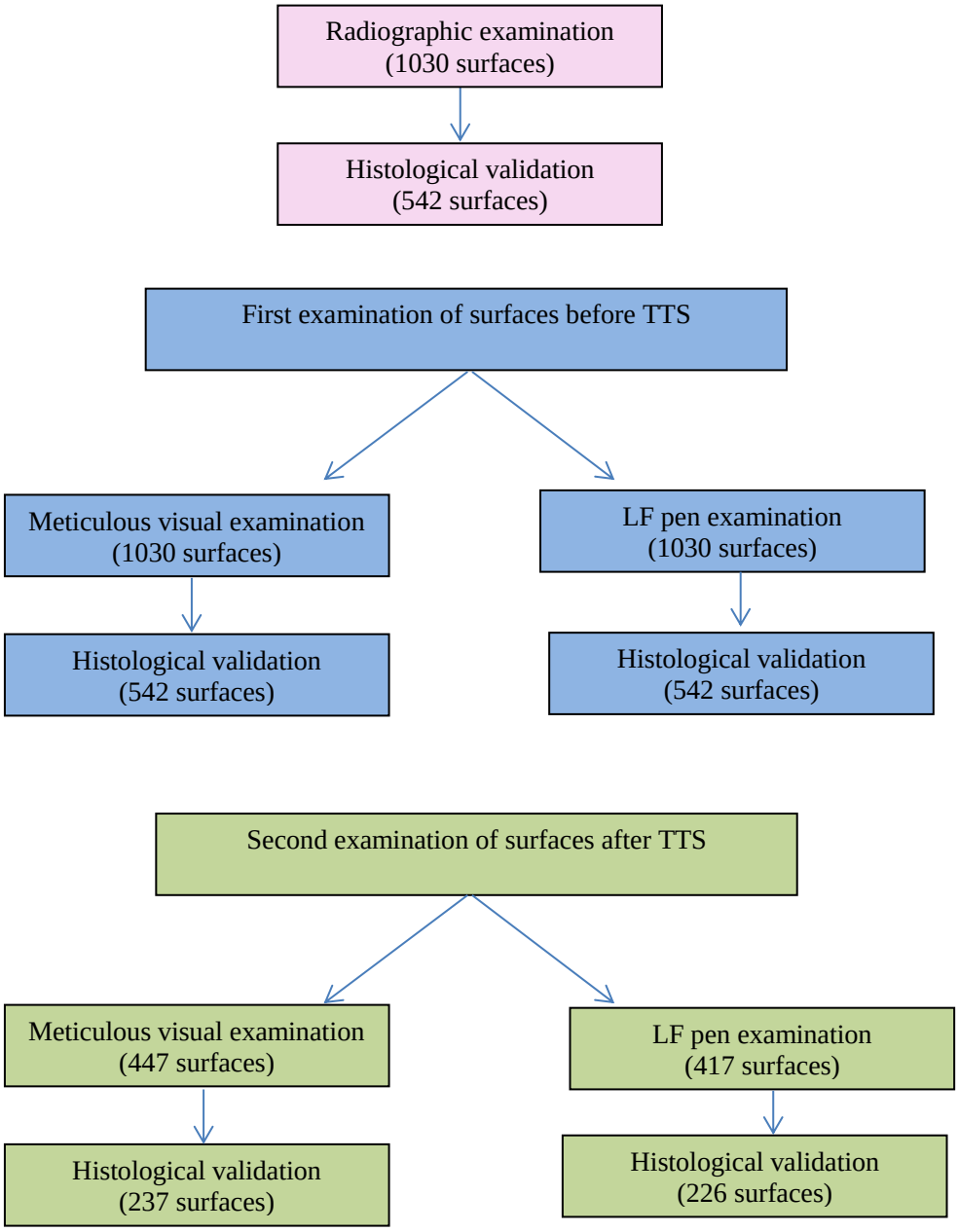

Figure 5.3 A schematic diagram to demonstrate the number of tooth surfaces analysed at each stage of the study.

5.3 Validity of diagnostic methods

The validity of different diagnostic methods is presented at three diagnostic thresholds.

- D_1 threshold includes all lesions (enamel and dentine)
- D_3 diagnostic threshold is the threshold which uses the EDJ as a land mark to be able to compare our results with studies that used this threshold. In this threshold all dentine lesions are included
- ERK_3 diagnostic threshold is the threshold that corresponds with the ICDAS criteria. At this threshold, lesions in the outer third of dentine are added to the enamel lesions. Therefore, only deep dentine lesions are included

5.3.1 Validity of detection methods *in-vivo*

5.3.1.1 Validity of meticulous visual examination without temporary tooth separation

At D_1 diagnostic threshold, the sensitivity of visual examination was 0.52. At D_3 and ERK_3 levels the sensitivity was 0.43 and 0.55 respectively.

The specificity of visual examination was higher at all diagnostic thresholds and varied from 0.89% at D_1 level to 0.93at both D_3 and ERK_3 levels (Table 5.17).

Receiver operating characteristic curve and area under the curve

The area under the ROC curve for visual examination at D_1 level is 0.709 compared to 0.761 and 0.795 at D_3 and ERK_3 respectively (Table 5.20) (Figures 5.4, 5.5, 5.6).

5.3.1.2 Validity of meticulous visual examination after TTS

Table 5.17 presents the validity of the visual examination after TTS. The sensitivity of the visual examination after TTS was found to be 0.75 at D_1 level, which is noticeably higher than that for visual examination without TTS. However, at D_3 and ERK_3 diagnostic thresholds the sensitivity did not increase much and the diagnostic value of visual examination after TTS was identical to that before temporary tooth separation at

72% and 81% respectively. The specificity of visual examination after TTS was as high as that achieved before TTS and varied from 0.88 at D_1 level to 0.93 at D_3 level.

The positive likelihood ratio for visual examination with TTS was higher than that for visual examination without TTS at both D_1 and D_3 levels of diagnosis. However, at D_1 level, the visual examination showed high predictive value positive and low predictive value negative with and without TTS (Table 5.17)

Receiver operating characteristic curve and area under the curve

Visual examination with TTS at D_1 diagnostic threshold has an area under the ROC of 0.831 which is the highest of all diagnostic methods at the same diagnostic threshold. This implies that visual examination with TTS is the best method for the detection of enamel caries in proximal surfaces of primary molars (Table 5.20) (Figures 5.7, 5.8, 5.9).

Table 5.17 The diagnostic parameters of the visual examination (VE) before (N=542) and after (N=237) temporary tooth separation (TTS) for proximal surfaces of primary molars.

Method	Diagnostic threshold	Sn	Sp	PVP (%)	PVN (%)	DV (%)	Likelihood ratio	
							+ve	-ve
VE	D_1	0.52	0.89	94	34	60	4.69	0.54
	D_3	0.43	0.93	82	69	72	6.39	0.61
	ERK_3	0.55	0.93	77	82	81	7.64	0.48
VE+ TTS	D_1	0.75	0.88	97	35	77	6	0.29
	D_3	0.49	0.93	88	65	72	7.37	0.54
	ERK_3	0.63	0.90	77	83	81	6.6	0.41

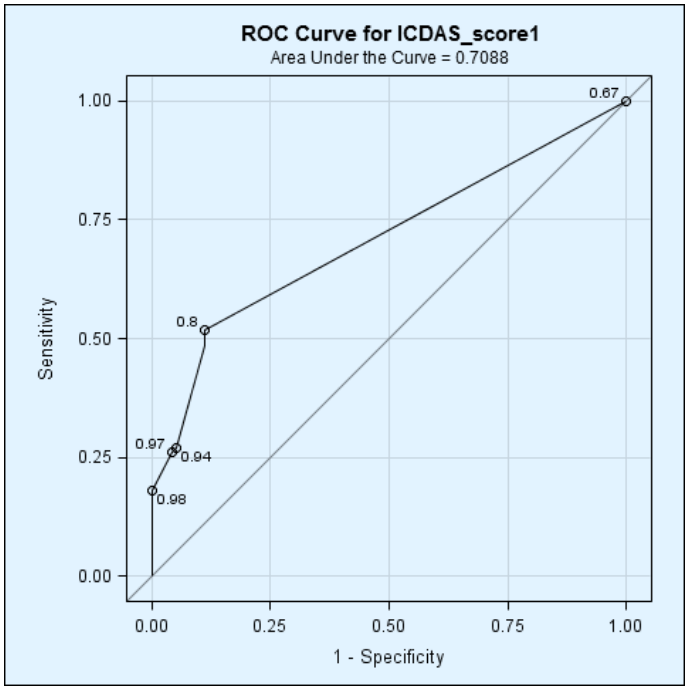

Figure 5.4 curve for visual examination at D_1 diagnostic threshold.

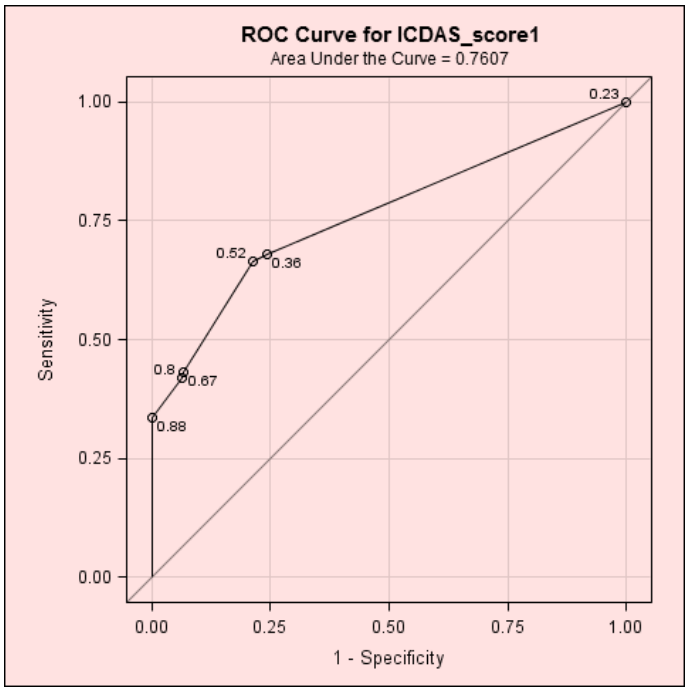

Figure 5.5 ROC curve for visual examination at D_3 diagnostic threshold.

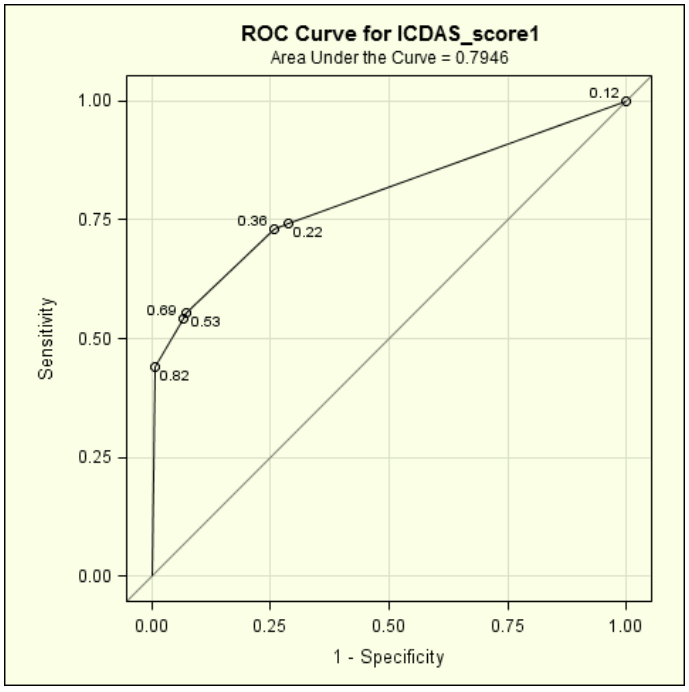

Figure 5.6 ROC curve for visual examination at ERK_3 diagnostic threshold.

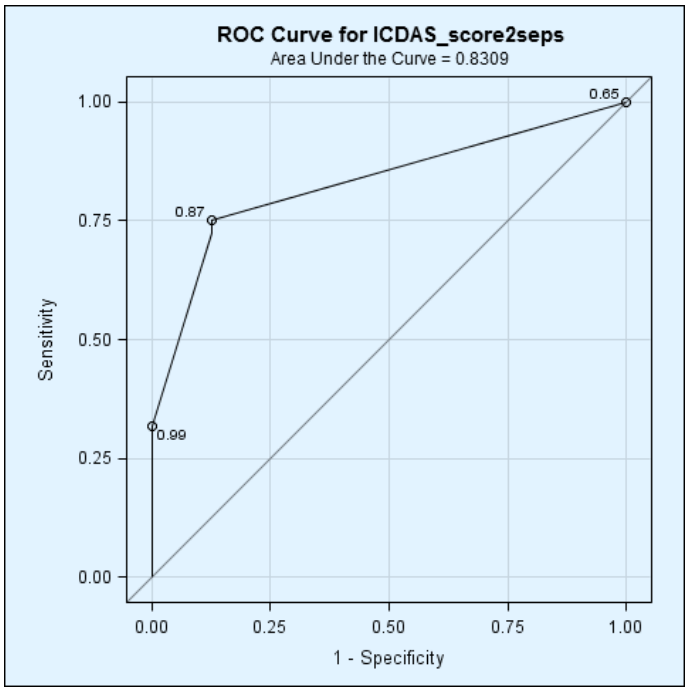

Figure 5.7 ROC curve for visual examination after TTS at D_1 threshold.

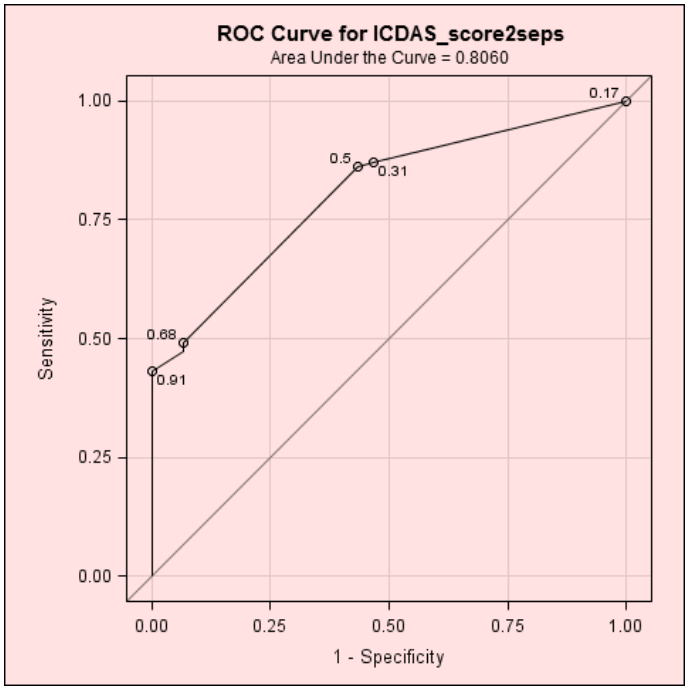

Figure 5.8 curve for visual examination after TTS at D_3 threshold.

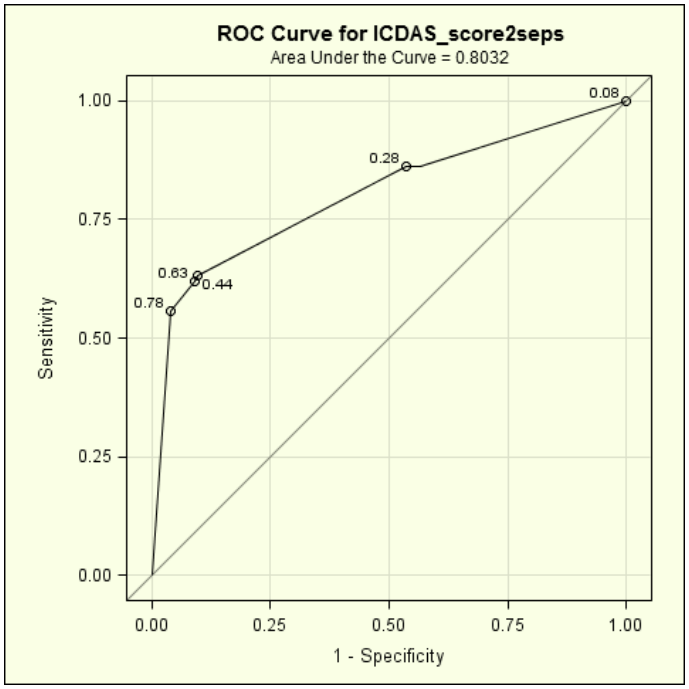

Figure 5.9 ROC curve for visual examination after TTS at ERK_3 threshold.

5.3.1.3 The validity of radiographic examination

Table 5.18 presents the validity of radiographic examination. At D_1 level the specificity of radiographic examination was high at 0.97 with a low sensitivity of only 0.14.

At the EDJ level (D_3) the sensitivity was higher at 0.71 with a constant high specificity of 0.98.

At the ERK_3 threshold, radiographic examination had the highest sensitivity and specificity of all methods at 0.86 and 0.94 respectively. It also had the highest positive likelihood ratio of all the diagnostic methods assessed (35.67) at D_3 level of diagnosis.

Receiver operating characteristic curve and area under the curve

The area under the curve for radiographic examination at D_1 level was 0.754. Radiographic examination had the highest area under the ROC curve at D_3 and ERK_3 diagnostic thresholds of 0.898 and 0.923 respectively (Table 5.20) (Figures 5.10, 5.11, 5.12).

Table 5.18 The diagnostic parameters of radiographic examination (RE) (N=542) for proximal surfaces of primary molars.

Method	Diagnostic threshold	Sn	Sp	PVP (%)	PVN (%)	DV (%)	Likelihood ratio	
							+ve	-ve
RE	D_1	0.14	0.97	95	25	33	5.41	0.88
	D_3	0.71	0.98	96	82	87	35.67	0.3
	ERK_3	0.86	0.94	88	94	92	15.37	0.15

Sn=sensitivity, Sp=specificity, PVP=predictive value positive, PVN=predictive value negative

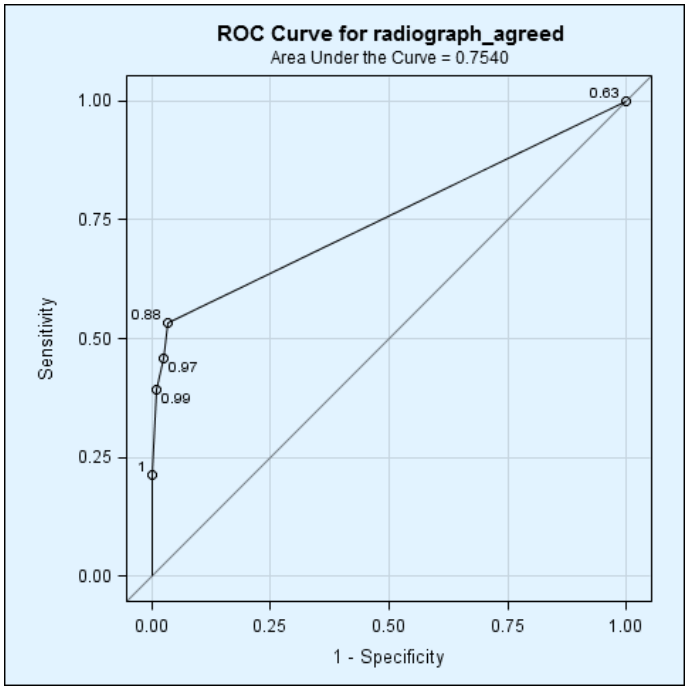

Figure 5.10 ROC curve for radiographic examination at D_1 threshold.

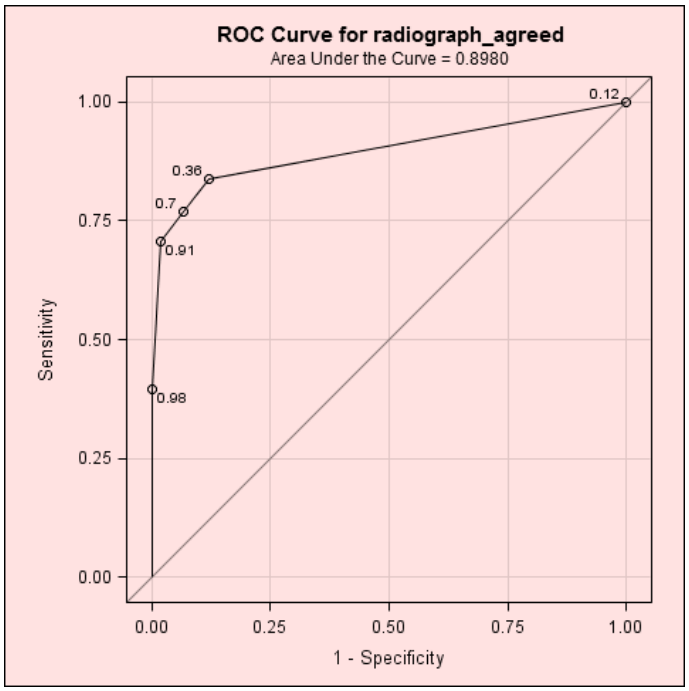

Figure 5.11 ROC curve for radiographic examination at D_3 threshold.

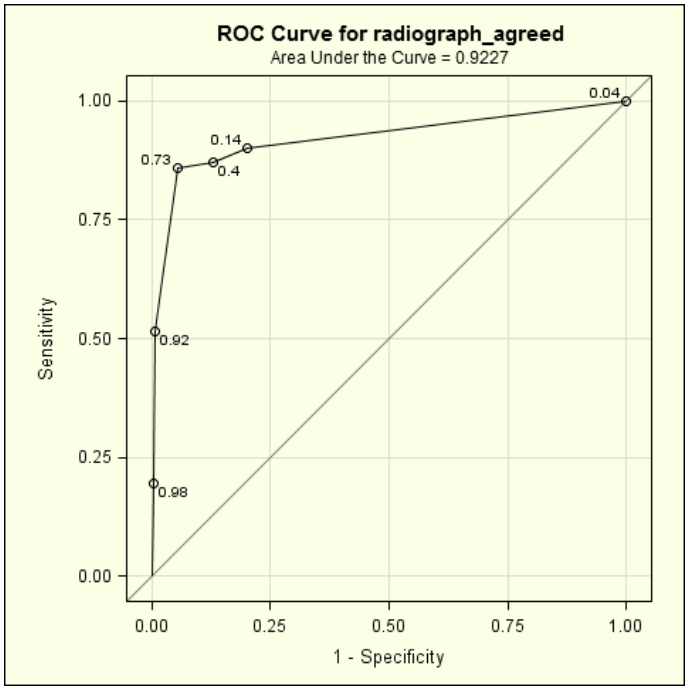

Figure 5.12 ROC curve for radiographic examination at ERK_3 threshold.

5.3.1.4 The validity of LF pen examination without TTS

Table 30 provides data for the validity of LF pen examination. The sensitivity of the LF pen examination at D_1 level was 0.58 with a specificity of 0.85.

At D_3 and ERK_3 the sensitivity was slightly better at 0.63 and 0.71 respectively, while the specificity was as high as for D_1 at 0.87 and 0.83 at D_3 and ERK_3 thresholds (Table 5.19).

Receiver operating characteristic curve and area under the curve

The area under the ROC for the LF pen examination at D_1 level (0.788) was slightly higher than that for the radiographic examination at the same diagnostic threshold. However, this area is smaller than that for radiographic examination at D_3 and ERK_3 diagnostic levels at 0.835 and 0.861 (Table 5.20) (Figures 5.13, 5.14, 5.15).

5.3.1.5 The validity of LF pen examination with temporary tooth separation

The sensitivity of LF pen examination with TTS at D_1 level was 0.60 while the specificity was 0.85. At D_3 and ERK_3 the sensitivity was slightly higher at 0.65 and 0.77 respectively. The diagnostic value of the LF pen examination after TTS was very similar to that without TTS at 63%, 76%, and 80% at D_1, D_3 and ERK_3 thresholds respectively.

The positive likelihood ratio of the LF pen with TTS was higher than that for the LF pen examination without TTS at both D_3 and ERK_3. At D_1 level of diagnosis, the LF pen had high predictive value positive and low predictive value negative with and without TTS (Table 5.19).

Receiver operating characteristic curve and area under the curve

The area under the ROC curve for LF pen examination after TTS is also similar to that for the examination without TTS at all diagnostic thresholds (Table 5.20) (Figures 5.16, 5.17, 5.18).

Table 5.19 The diagnostic parameters of LF pen examination before (N=542) and after (N=226) temporary tooth separation.

Method	Diagnostic threshold	Sn	Sp	PVP (%)	PVN (%)	DV (%)	Likelihood ratio	
							+ve	-ve
LF pen	D_1	0.58	0.85	93	36	64	3.82	0.49
	D_3	0.63	0.87	78	76	77	4.86	0.43
	ERK_3	0.71	0.83	65	87	79	4.13	0.35
LF pen + TTS	D_1	0.60	0.77	94	24	63	2.67	0.51
	D_3	0.65	0.88	84	72	76	5.30	0.40
	ERK_3	0.77	0.81	66	88	80	4.2	0.29

Sn=sensitivity, Sp=specificity, PVP=predictive value positive, PVN=predictive value negative

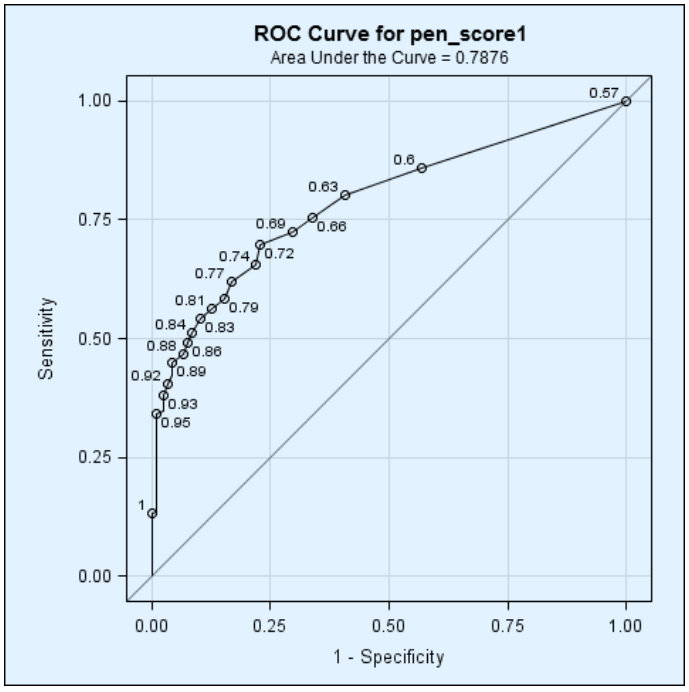

Figure 5.13 ROC curve for LF pen examination at D_1 threshold.

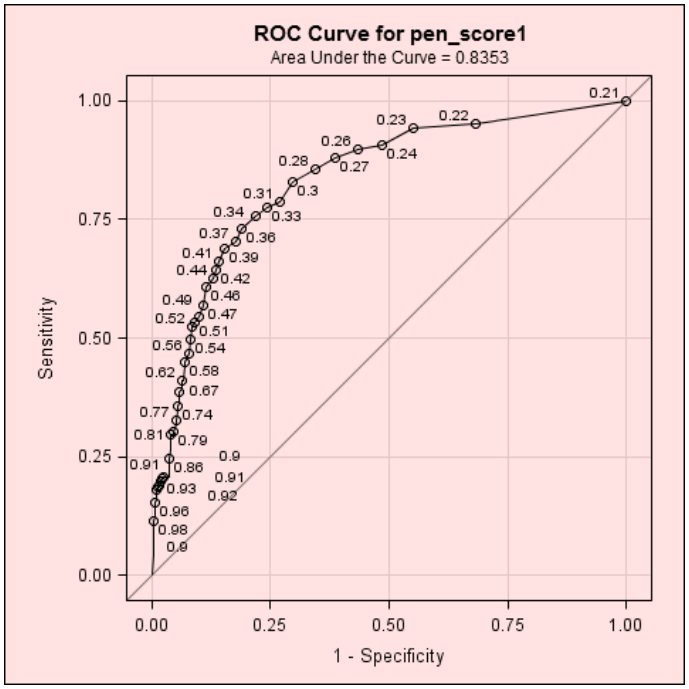

Figure 5.14 ROC curve for LF pen examination at D_3 threshold.

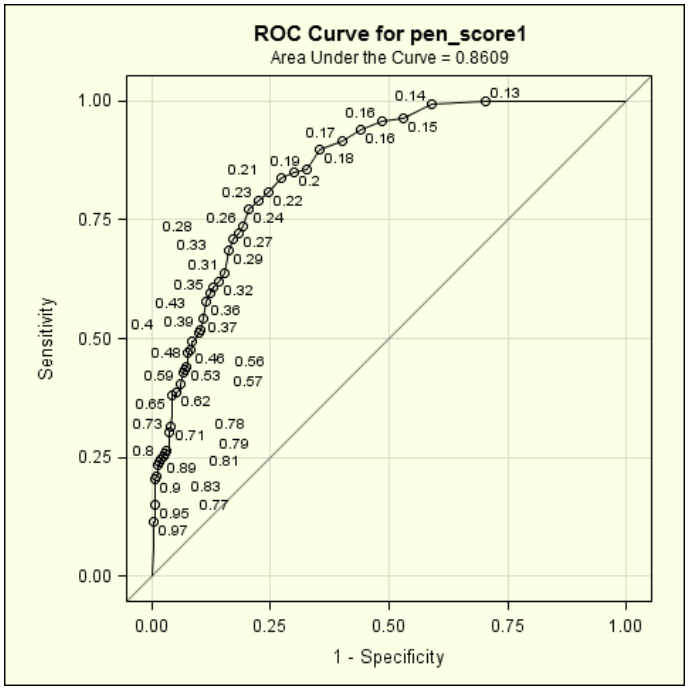

Figure 5.15 ROC curve for LF pen examination at ERK_3 threshold.

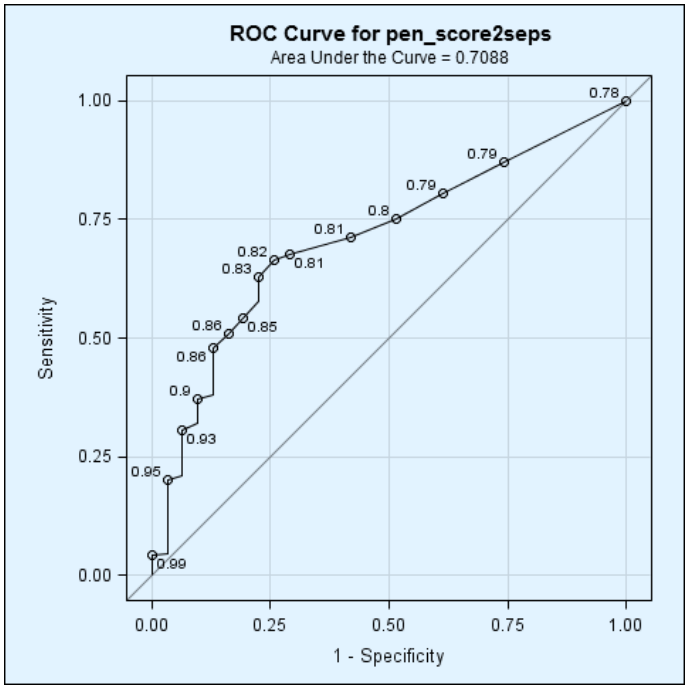

Figure 5.16 ROC curve for LF pen examination after TTS at D_1 threshold.

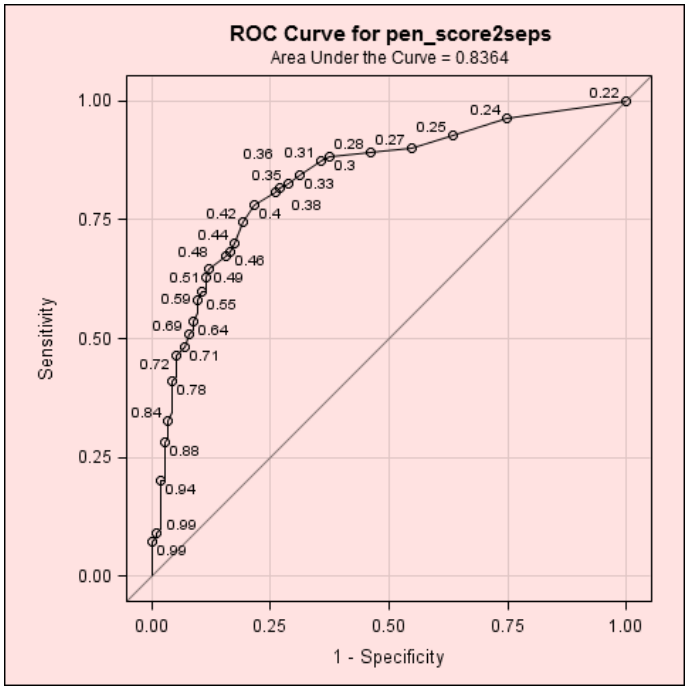

Figure 5.17 ROC curve for LF pen examination after TTS at D_3 threshold.

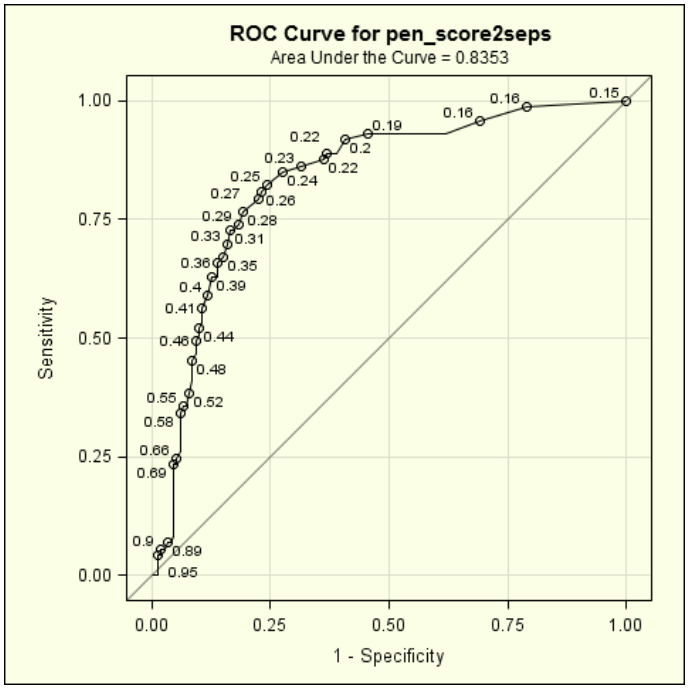

Figure 5.18 ROC curve for LF pen examination after TTS at ERK_3 threshold.

Table 5.20 Receiver operating characteristic statistics for the different diagnostic methods at three diagnostic thresholds.

Examination method	Diagnostic threshold	ROC Model			
		Area	Standard Error	95% Wald Confidence Limits	
VE	D_1	0.709	0.018	0.673	0.744
	D_3	0.761	0.019	0.722	0.799
	ERK_3	0.795	0.022	0.752	0.837
VE+ TTS	D_1	0.831	0.026	0.779	0.883
	D_3	0.806	0.026	0.754	0.858
	ERK_3	0.803	0.032	0.740	0.866
Radiographic	D_1	0.754	0.014	0.726	0.781
	D_3	0.898	0.014	0.870	0.926
	ERK_3	0.923	0.014	0.894	0.951
LF pen	D_1	0.788	0.020	0.747	0.828
	D_3	0.835	0.018	0.800	0.87
	ERK_3	0.861	0.016	0.830	0.892
LF pen + TTS	D_1	0.709	0.046	0.618	0.799
	D_3	0.836	0.027	0.783	0.889
	ERK_3	0.835	0.029	0.779	0.892

VE=visual examination, RE=radiographic examination, TTS=temporary tooth separation

For information, details regarding the number of surfaces, sound and affected, detected by each diagnostic approach for three diagnostic thresholds are provided in Table 5.21.

Table 5.21 The number of surfaces, sound and affected, detected by each diagnostic approach for three diagnostic thresholds.

Measure	Level	TN	FP	FN	TP	Diseased	Sound
visual	**D_1**	105	13	203	217	420	118
	D_3	290	21	129	98	227	311
	ERK_3	345	27	74	92	166	372
Visual + TTS	**D_1**	28	4	51	153	204	32
	D_3	112	8	59	57	116	120
	ERK_3	142	15	29	50	79	157
LF pen	**D_1**	100	18	175	245	420	118
	D_3	271	40	85	142	227	311
	ERK_3	308	64	48	118	166	372
LF pen + TTS	**D_1**	24	7	77	117	194	31
	D_3	101	14	39	71	110	115
	ERK_3	123	29	17	56	73	152
Radiographic	**D_1**	114	3	348	56	404	117
	D_3	297	6	64	154	218	303
	ERK_3	338	20	23	140	163	358

TN= true negative, FP= false positive, FN= false negative, TP= true positive

5.3.1.6 Optimum cut-off values for the LF pen *in-vivo*

Optimum cut-off values for detection of proximal caries in primary teeth by the LF pen were identified using Youden's J statistic (Youden's index). The optimum cut-off value at D_1 was found to be 10. Optimum cut-off value at D_3 was found to be 14. The optimum cut-off value at ERK_3 was found to be 15. Therefore the suggested cut-off values for use for proximal caries detection in primary teeth are as follows:

- Sound= 0-10
- Enamel caries= 10.1-14
- Outer dentine caries= 14.1-15
- Inner dentine caries >15

5.3.1.7 ROC comparison between different diagnostic methods *in-vivo*

ROC comparison of the validity of different diagnostic methods assessed in this study is presented in Table 5.22 with the radiographic examination having the best performance of all the methods at D_3.

Table 5.22 A ROC comparison of the validity of the different detection methods for proximal caries in primary teeth.

Detection methods compared	At D_1 diagnostic level		At D_3 diagnostic level	
	Difference	**P value**	**Difference**	**P value**
VE x VE+TTS	VE+TTT is better	0.00	VE+TTT is better	0.00
VE x LF pen	LF pen is better	0.00	No difference	0.09
VE x RE	RE is better	0.04	RE is better	0.00
LF pen x RE	No difference	0.60	RE is better	0.00
LF pen x LF pen + TTS	No difference	0.36	LF pen + TTS is better	0.02
VE+TTS x LF pen	No difference	0.72	No difference	0.25
VE+TTS x RE	No difference	0.60	RE is better	0.00
VE+TTS x LF pen+ TTS	VE+TTS is better	0.01	No difference	0.432

VE=visual examination, RE=radiographic examination, TTS=temporary tooth separation

5.3.2 Reproducibility of detection methods *in-vivo*

Inter- and intra-examiner reproducibility were calculated using the Cohen's Kappa statistics at D_1 and D_3 thresholds. This measures the level of agreement above chance.

5.3.2.1 Intra- examiner reproducibility

Visual examination showed substantial intra-examiner agreement at D_1 with a Kappa statistic of 0.76 and almost perfect agreement at D_3 with a Kappa statistic of 0.83. Intra-examiner reproducibility for radiographic examination was almost perfect at both levels of diagnosis ranging from 0.91 at D_1 to 0.95 at D_3 (Table 5.23).

The intra- examiner reproducibility for the LF pen examination was substantial at both levels of diagnosis with a Kappa statistic ranging from 0.75 at D_1 to 0.77 at D_3. Histological examination showed perfect agreement at D_1 with a Kappa statistic of 1 and almost perfect agreement at D_3 with Kappa statistic of 0.88.

Table 5.23 Intra-examiner reproducibility of caries detection for visual, radiographic, LF pen, and histological examination at D_1 and D_3 diagnostic thresholds.

Examination method	Diagnostic threshold	Kappa value (95% confidence interval)
Visual examination	D_1	0.76 (0.70-0.81)
	D_3	0.83 (0.77-0.89)
Radiographic examination	D_1	0.91 (0.84-0.98)
	D_3	0.95(0.89-1)
LF pen examination	D_1	0.75(0.70-0.79)
	D_3	0.77(0.72-0.82)
Histological exam	D_1	1(1-1)
	D_3	0.88(0.77-0.99)

5.3.2.2 Inter-examiner reproducibility

Inter-examiner reproducibility for visual examination showed substantial to almost perfect agreement with Kappa values ranging from 0.76 at D_1 to 0.85 at D_3 (Table 5.24).

For radiographic examination, inter examiner reproducibility was substantial at both diagnostic thresholds with Kappa values ranging from 0.73 at D_1 level to 0.79 at D_3 level.

Histological examination showed perfect agreement between examiners at D_1 level and almost perfect agreement at D_3 level with a Kappa value of 0.87.

Table 5.24 Inter-examiner reproducibility of caries detection for visual, radiographic and histological examination at D_1 and D_3 diagnostic thresholds.

Examination method	Diagnostic threshold	Kappa value (95% confidence interval)
Visual examination	D_1	0.76 (0.60-0.91)
	D_3	0.85 (0.71-0.99)
Radiographic examination	D_1	0.73 (0.69-0.77)
	D_3	0.79(0.76-0.83)
Histological exam	D_1	1(1-1)
	D_3	0.87(0.76-0.99)

5.3.3 Validity of detection methods *in-vitro*

5.3.3.1 Validity of direct visual examination of proximal surfaces *in-vitro*

Direct visual examination of proximal surfaces *in-vitro* has a high sensitivity and specificity for the detection of enamel caries and was found to be 0.90 and 0.97 respectively. The likelihood ratio positive of direct visual examination of proximal

caries *in-vitro* is 27.2. The optimum cut-off value for the detection of enamel proximal caries in ICDAS was found to be code 1.

At D_3 diagnostic threshold, the sensitivity of visual examination was 0.85 and the specificity was 0.97. The likelihood ratio positive was also high at 29.5. The optimum cut-off value for the detection of dentine caries using ICDAS criteria is code 3 (Table 5.25).

Table 5.25 *In-vitro* diagnostic parameters of direct visual examination of proximal caries at D_1 and D_3 diagnostic thresholds.

Examination method	Histological threshold	Optimum cut-off	Sn	Sp	PVP (%)	PVN (%)	Likelihood ratio	
							+V	-V
Direct VE	D_1	Code 1	0.90	0.97	99	69	27.2	0.09
	D_3	Code 3	0.85	0.97	97	85	29.5	0.15

Receiver operating characteristic curve and area under the curve

The area under the ROC curve for direct visual examination *in-vitro* at D_1 level is 0.94 compared to 0.96 at D_3 (Table 5.26).

Table 5.26 Area under the curve for direct visual examination of proximal *in-vitro* at D_1 and D_3 diagnostic thresholds.

Examination method	Diagnostic threshold	ROC Model			
		Area	Standard Error	95% Wald Confidence Limits	
Direct VE	D_1	0.94	0.00	0.93	0.96
	D_3	0.96	0.00	0.95	0.97

5.3.3.2 Percentage of tooth surfaces classified using ICDAS by histological status

Histological examination of surfaces directly examined *in-vitro* using ICDAS criteria showed that all surfaces with ICDAS code 5 or 6 have caries extending to dentine. While 92% of surfaces with ICDAS code 3 had dentine caries, only 50% of the surfaces with code 4 had caries extending to dentine (Table 5.27).

Table 5.27 The percentage of surfaces with dentine caries in relation to their ICDAS code.

ICDAS code	No of surfaces with dentine caries/total No of surfaces	Percentage of surfaces with cavitation (%)
0	2/167	1
1	3/40	7.5
2	50/151	33
3	23/25	92
4	7/14	50
5	132/132	100
6	155/155	100
Total No of surfaces	684	

5.3.3.3 The relationship between radiographic depth and cavitation of proximal surfaces

Direct visual examination of proximal surfaces *in-vitro* showed that 55% of surfaces with a radiographic radiolucency reaching the outer third of dentine were cavitated, whilst almost 100% of surfaces showing a radiolucency extending into middle or inner third of dentine were cavitated. Only a small percentage of surfaces with a radiolucency in enamel showed cavitation (Table 5.28).

Table 5.28 The percentage of surfaces with cavitation in relation to their radiographic score.

Radiographic score	No of surfaces with cavitation/total No of surfaces	Percentage of surfaces with cavitation (%)
0	13/306	4.2
1	2/31	6.4
2	5/30	16.6
3	42/76	55.5
4	57/58	98
5	168/168	100
Total No of surfaces	669	

5.3.3.4 Validity of the direct LF pen examination of proximal caries *in-vitro*

The LF pen showed very high sensitivity at D_3 diagnostic threshold where the sensisitivity of the highest pen score was 0.97 and the sensitivity of the average pen score was 0.95. However, the pen showed lower specificity for the detection of dentine caries where the specificity of the highest score was 0.68 and the specificity of the average score was 0.74. In addition, the Likelihood ratio reduced noticeably at a higher level of diagnosis because of the increase in the number of the false positives detected by the pen (Table 5.29).

Table 5.29 The validity of the highest and average LF pen score for the direct *in-vitro* detection of proximal caries

Examination method	Histological threshold	Sn	Sp	PVP (%)	PVN (%)	Likelihood ratio	
						+V	-V
Highest LF-pen score	D_1	0.85	0.89	98	56	8.20	0.16
	D_3	0.97	0.68	79	95	3.08	0.04
	ERK_3	0.99	0.55	67	99	2.40	0.01
Average LF-pen score	D_1	0.84	0.90	98	55	8.81	0.17
	D_3	0.95	0.74	82	94	3.79	0.06
	ERK_3	0.99	0.65	70	99	2.9	0.01

5.3.3.5 Receiver operating characteristic curve and area under the curve

The area under the ROC curve for direct examination with the LF pen *in-vitro* using the highest score at D_1 level is 0.91 compared to 0.93 at D_3 (Table 5.30). The area under the ROC curve for the average pen score is identical to that of the highest score at both levels of diagnosis (Figure 5.19, 5.20, 5.21).

Table 5.30 The area under the ROC for the LF pen highest and average s *in-vitro*

Examination method	Diagnostic threshold	ROC Model			
		Area	Standard Error	95% Wald Confidence Limits	
Highest LF-pen score	D_1	0.91	0.01	0.88	0.93
	D_3	0.93	0.00	0.92	0.95
	ERK_3	0.90	0.01	0.87	0.92
Average LF-pen score	D_1	0.91	0.01	0.88	0.93
	D_3	0.93	0.00	0.91	0.95
	ERK_3	0.90	0.01	0.87	0.92

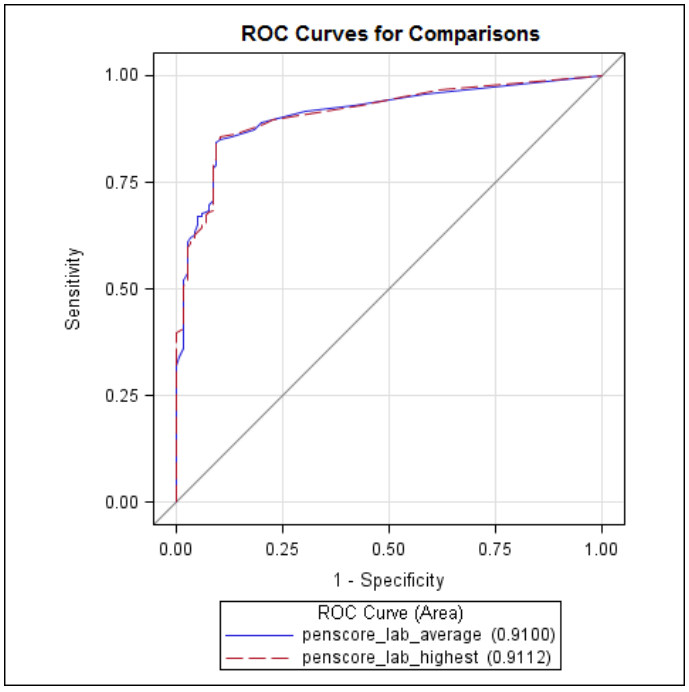

Figure 5.19 ROC curve for the LF pen highest and average scores *in-vitro* at D_1.

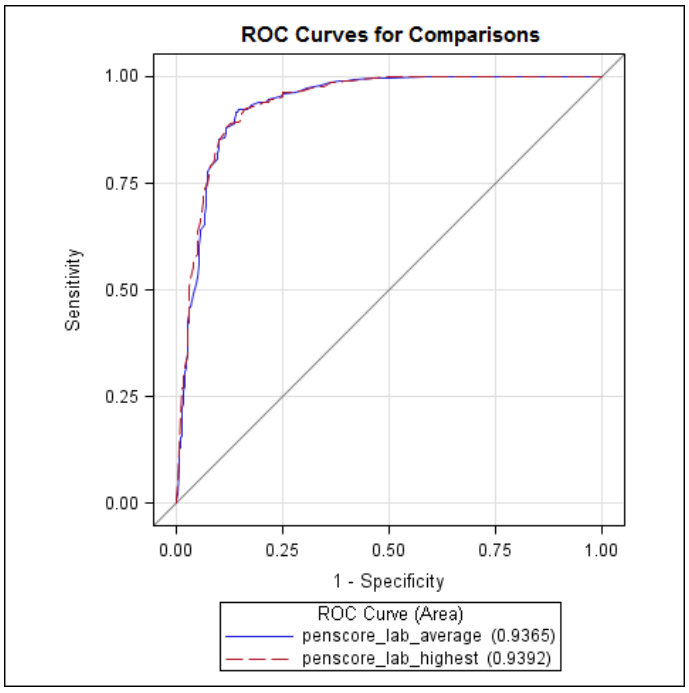

Figure 5.20 ROC curve for the LF pen highest and average scores *in-vitro* at D_3.

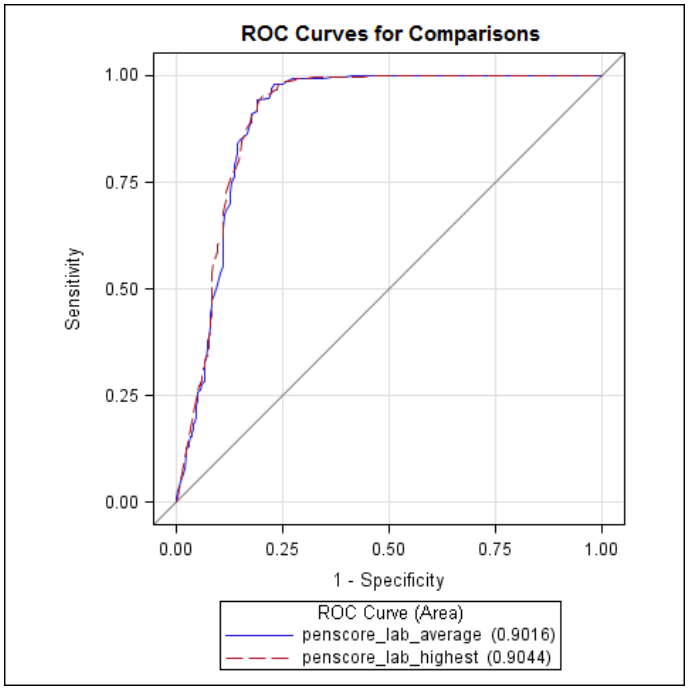

Figure 5.21 ROC curve for the LF pen highest and average scores *in-vitro* at ERK_3.

5.3.3.6 Correlation between *in-vitro* LF pen highest and average scores

High correlation of 0.98 (n=670, $p<0.05$) was shown between both the highest and the average LF pen scores for the direct detection of proximal caries *in-vitro* (Figure 5.22).

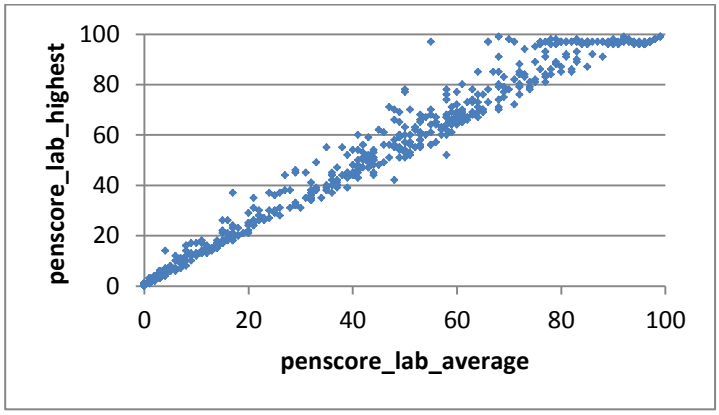

Figure 5.22 The correlation between the LF pen average and highest scores *in-vitro*.

5.3.3.7 The optimum cut-off values of the LF pen *in-vitro*

The optimum cut-off values for the LF pen highest and average scores are shown in Table 5.31.

Table 5.31 The optimum cut-off values for the LF pen highest and average scores.

Diagnostic threshold	Optimum cut-off value LF pen highest score	Optimum cut-off value LF pen average score
D_1	10	8
D_3	31	26
ERK_3	38	33

5.3.3.8 ROC comparisons of *in-vitro* examinations

The ROC comparison of LF pen highest and average score showed no difference between the validity of the scores (p=0.253) (Figure 5.19, 5.20, 5.21). However, the ROC comparison of the validity of direct visual examination of the proximal caries *in-vitro* compared to the validity of LF pen examination showed that direct visual examination is significantly better for the detection of proximal caries at both levels of diagnosis; D_1 and D_3 (p=0.002) (Figure 5.23, 5.24).

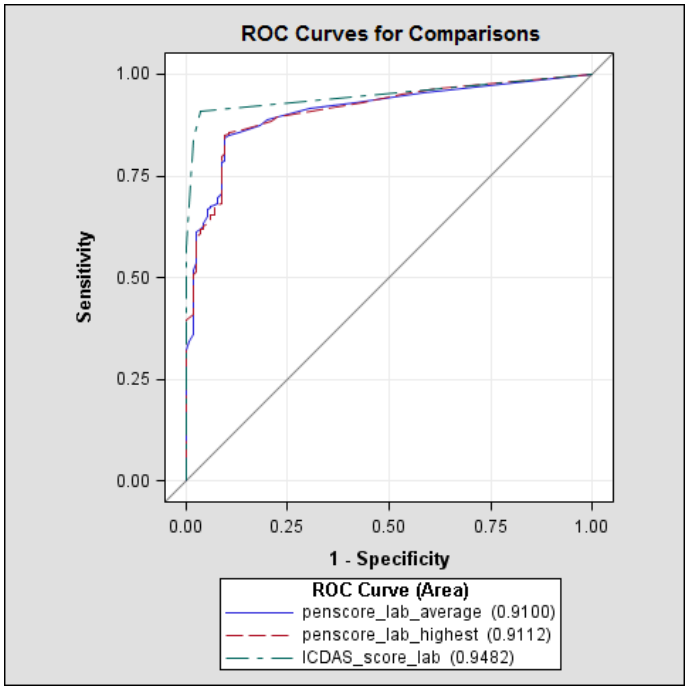

Figure 5.23 ROC comparison of direct visual examination and LF pen highest and average scores *in-vitro* at D_1.

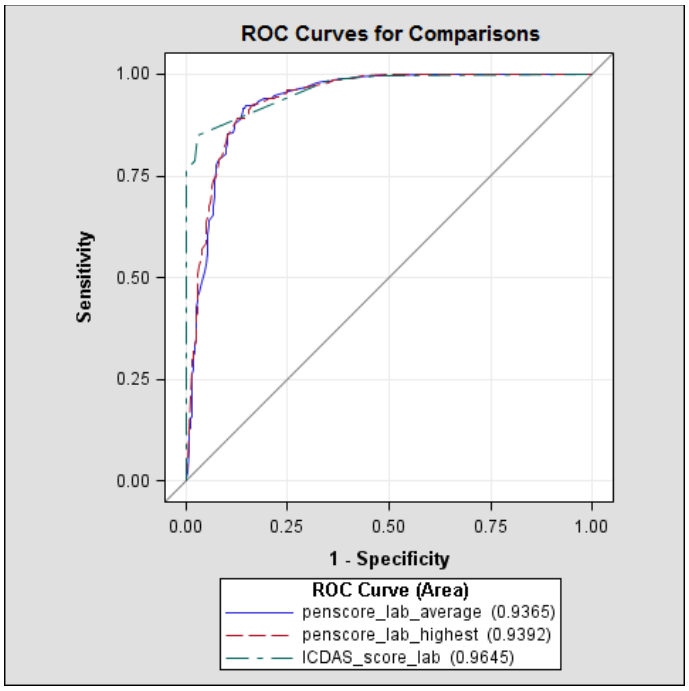

Figure 5.24 ROC comparison of direct visual examination and LF pen highest and average scores *in-vitro* D_3.

5.4 The validity of the LF pen *in-vivo* versus *in-vitro*

The ROC comparison of the validity of the LF pen *in-vivo* and *in-vitro* showed the LF pen to have significantly higher validity *in-vitro* than *in-vivo* at all diagnostic levels ($p<0.05$).

5.4.1 Correlation between *in-vivo* and *in-vitro* scores

Spearman's correlation between *in-vivo* and *in-vitro* highest and average LF pen scores was found to be 0.64 ($N=670$, $p<0.05$) (Figure 5.25).

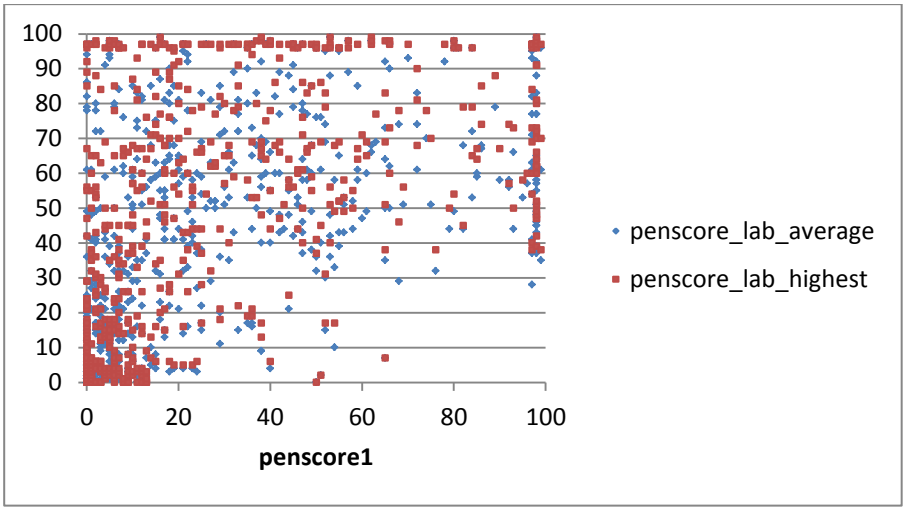

Figure 5.25 Correlation between LF pen scores *in-vivo* (pen score1) and *in-vitro* (pen score lab highest and average).

5.4.2 Difference between LF pen scores *in-vivo* and *in-vitro*

One sample T-Test was used to separate the random variation from the systematic component of the variation. The difference between the LF pen scores *in-vitro* (highest and average) and *in-vivo* follows the normal distribution (Figure 5.26, Figure 5.2.7). This test showed a significant difference between the mean value of the *in-vivo* scores and the mean value of the average LF pen score *in-vitro* ($p=0.00$). There is a systematic variation of 13.2. The average LF pen scores were 13.2 higher than the *in-vivo* score.

However, the random variation is demonstrated by the standard deviation (SD), this was +/-29.83 on a scale of 0 to 99. The difference between the LF pen scores *in-vivo* and the highest LF pen scores *in-vitro* showed the same trend seen for the difference between the LF pen scores *in-vivo* and the average LF pen scores (Table 5.32).

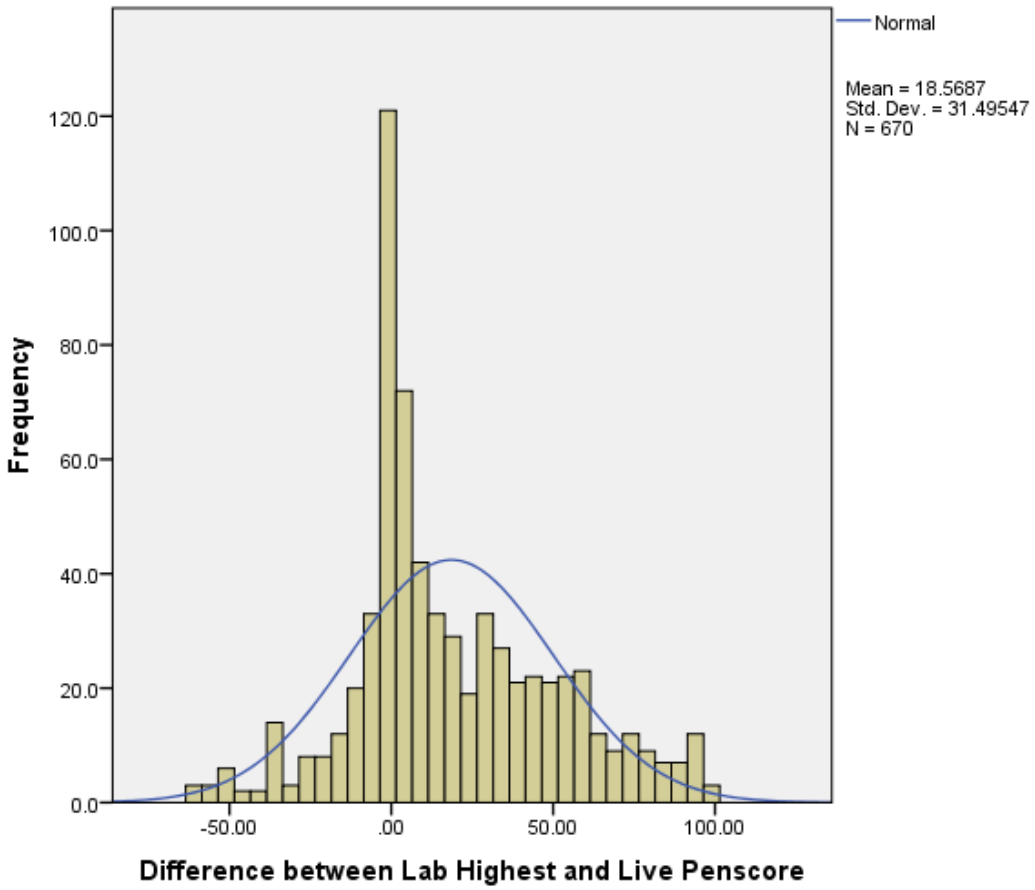

Figure 5.26 The distribution of the difference between *in-vitro* (lab) highest scores and *in-vivo* (live) pen scores

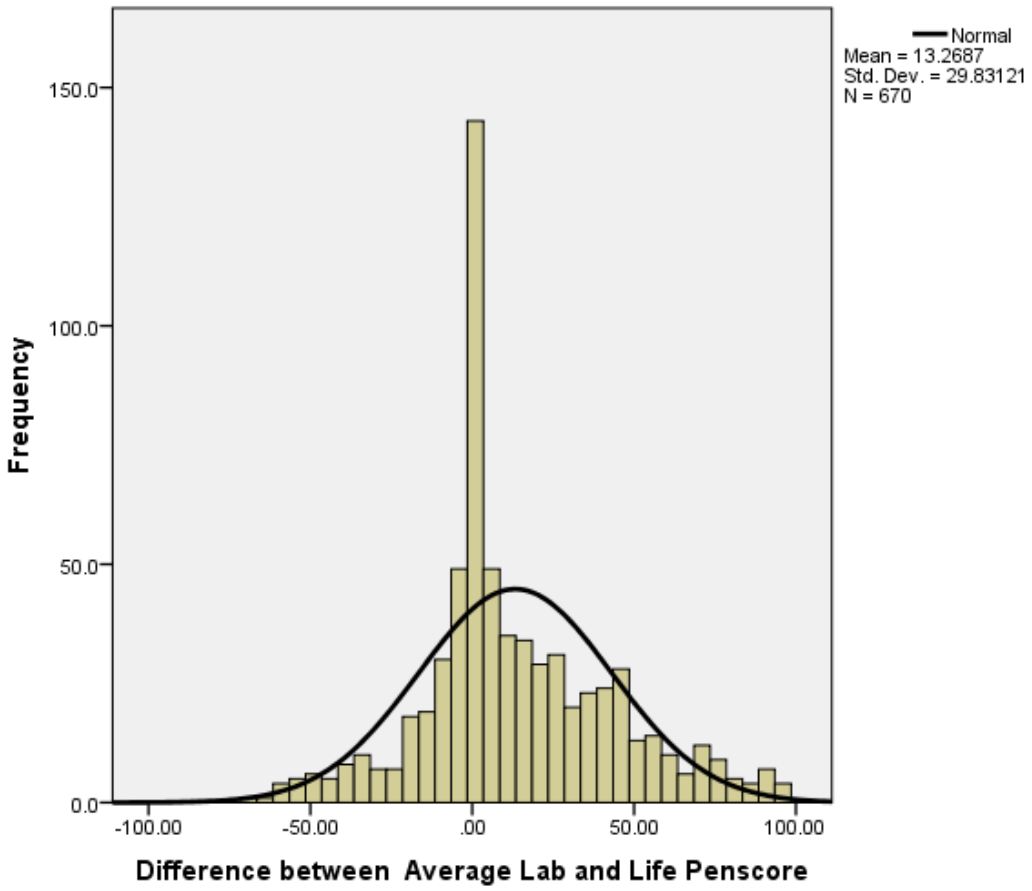

Figure 5.27 The distribution of the difference between *in-vitro* (lab) average scores and *in-vivo* (live) pen scores.

Table 5.32 The difference between the LF pen scores *in-vivo* and *in-vitro.*

Difference between LF pen scores *in-vivo* and the average LF pen score *in-vitro*				
Mean difference	**Std. Dev**	**Std. Error Mean**	**95% CI Std. Dev**	**sig**
13.26	29.83	1.15	28.31-31.52	0.000
Difference between LF pen scores *in-vivo* and the highest LF pen score *in-vitro*				
18.56	31.49	1.21	29.89-33.27	0.000

5.4.3 The optimum cut-off values for the LF pen *in-vivo* and *in-vitro*

The optimum cut-off values for the highest LF pen scores *in-vitro* are similar to the optimum cut-off value of the LF pen *in-vivo* at D_1 level. However, at D_3, the optimum cut-off values of the LF pen *in-vitro* are much higher than the optimum cut-off values for the LF pen *in-vivo* (Table 5.33).

Table 5.33 The optimum cut-off values for the LF pen *in-vivo* and *in-vitro.*

Diagnostic threshold	*In-vivo* cut-off values	*In-vitro* cut-off values	
		highest score	average score
D_1	10	10	8
D_3	14	31	26
ERK_3	15	38	33

5.5 Acceptability of different diagnostic methods

To assess the acceptability of the different diagnostic methods used in this study, all 82 participants were given a self-complete questionnaire. Children engaged well in this enquiry and there was a 100% response rate.

Table 5.34 provides details of the number (and percentage) of children who rated each method according to a hierarchical 5-point visual and verbal scale from 'very easy' to 'very hard'. The most acceptable approach was the use of the mirror with 90% of children reporting it was 'very easy' or 'easy'. The remaining 10% were ambivalent and, notably, no child found it 'hard'. The next most acceptable test was radiographic examination with 75% finding it 'very easy' or 'easy'. Although in the case of radiographs, a small percentage of children (10%) did find it to be 'hard/very hard'. The LF pen was again generally well received by the young participants; 71% perceiving it to be 'very easy' or 'easy', but the percentage finding it difficult increased to 16%. Finally, the least acceptable test was TTS, with the majority (43%) reporting it to be 'hard' or 'very hard'. Conversely, around a third (33%) actually said that TTS was 'very easy' or 'easy'.

Table 5.34 Children's acceptability of diagnostic methods.

	Acceptability category *Respondents N (%)*				
Examination method	Very easy 	Easy 	Didn't mind it 	Hard 	Very hard 
Mirror	43 (52)	31 (38)	8 (10)	0 (0)	0 (0)
X-ray	42 (51)	20 (24)	12 (15)	4 (5)	4(5)
LF pen	34 (42)	24 (29)	11 (13)	9 (11)	4 (5)
TTS	17 (21)	11 (13)	19 (23)	21 (26)	14 (17)

A second way of determining acceptability was to ask the children if they would be happy or not to have the tests done again (Table 5.35). All participants reported that they would be prepared to have an examination with a mirror again and 93% were amenable to the idea of radiographs again. Almost the same proportion of children who had found the LF pen 'hard/very hard' were not at all keen to have this investigation again (16%). A similar correlation was found for TTS, with 44% of children reporting that they would be 'very unhappy/unhappy' at having this procedure again.

Table 5.35 Children's preparedness to undergo a repeat diagnostic test.

	Acceptability category *Respondents N (%)*				
Examination method	Very happy 	happy 	Didn't mind it 	Unhappy 	Very unhappy 
Mirror	34 (42)	28 (34)	20 (24)	0 (0)	0 (0)
X-ray	19 (23)	35 (43)	22 (27)	5 (6)	1 (1)
LF pen	29 (35)	26 (32)	16 (20)	6 (7)	5 (6)
TTS	14 (17)	13 (16)	19 (23)	22 (27)	14 (17)

In addition to simply analysing the percentage of children who had rated each test according to its acceptability category, further statistical analysis was undertaken, using the mean score for each test. Scores could range from 1.0, which would represent the most positive score possible ('very happy') through to 5.0 which would represent the most negative score possible ('very unhappy'). As these data were found not to be normally distributed, non-parametric statistical tests were indicated. Furthermore, as multiple comparisons of means were required, repeated measure analysis of variance was undertaken with the level of statistical significance set at $p<0.05$. P-Values quoted here are using the Sidak Correction for multiple comparisons.

Table 5.36 provides the mean acceptability score for each test. The highest (most negative) score (mean=3.06, CI =2.69-3.44) was found for TTS. In keeping with the findings above, visual inspection was found to have the lowest (most positive) score, with a mean of 1.57 (CI=1.39-1.76). Statistical analysis confirmed that TTS was significantly less acceptable than the other three tests ($p<0.05$, repeated measures ANOVA).

Table 5.36 Mean scores for acceptability of diagnostic tests, where 1=most acceptable and 5=least acceptable (N=82).

Technique	Mean acceptability score	95% confidence Interval		Significance p
		Lower bound	Upper bound	
Radiographic	1.81	1.50	2.12	0.77
Visual	1.57	1.39	1.76	0.09
LF pen	2.03	1.71	2.36	0.88
TTS	3.06	2.69	3.44	0.00

Overall, there was no significant difference in the acceptability of the diagnostic tests according to gender ($p=0.73$). Neither was there any significant difference ($p=0.68$) in acceptability according to age, as mean acceptability scores were similar for the two age groups studied: 5 to 7-year olds and 8 to 11-year-olds.

In order to gain a more detailed insight into whether there were any intra-gender and intra-age group differences in the acceptability of the different tests, mean acceptability scores were analysed separately for boys and girls, and for the two age groups (Tables 5.37 to 5.40). Boys found TTS to be significantly more difficult than the other three tests (mean score=3.27, $p<0.05$). Girls also found that TTS was significantly more difficult than visual and radiographic examination ($p<0.05$), but there was no statistically significant difference between reported acceptability of TTS and the LF pen ($p=0.124$).

Analysis of mean acceptability scores for the younger age group of children revealed that they found TTS significantly more difficult that the other three examinations ($p<0.05$). In addition, they also found the LF pen to be significantly more difficult than a visual examination ($p<0.05$). This contrasted slightly with the older participants who did not find the LF pen to be more difficult than the visual examination ($p=0.89$), although they also found TTS to be significantly more difficult than the other three tests ($p<0.05$).

Table 5.37 Mean acceptability score of the different diagnostic tests according to gender.

Patient Gender	Technique	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Boy (N=44)	Radiograph	1.91	0.19	1.51	2.31
	Visual	1.55	0.11	1.31	1.78
	LF pen	1.87	0.20	1.45	2.28
	TTS	3.27	0.24	2.78	3.74
Girl (N=38)	Radiograph	1.71	0.20	1.30	2.11
	Visual	1.61	0.12	1.37	1.84
	LF pen	2.21	0.21	1.78	2.63
	TTS	2.87	0.24	2.38	3.36

Table 5.38 Mean acceptability score of the different diagnostic tests according to age group.

Age group	Technique	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
5-7 years (N=65)	Radiograph	1.91	0.14	1.62	2.19
	Visual	1.57	0.08	1.40	1.74
	LF pen	2.14	0.14	1.84	2.43
	TTS	3.01	0.17	2.66	3.35
8-11 years (N=17)	Radiograph	1.71	0.27	1.16	2.26
	Visual	1.59	0.16	1.26	1.91
	LF pen	1.93	0.29	1.35	2.51
	TTS	3.129	0.33	2.45	3.80

Table 5.39 Intra-gender differences in mean acceptability score for the diagnostic tests.

Patient gender	Technique (A)	Technique (B)	Mean difference (A-B)	Sig p	95% confidence interval for difference	
					Lower bound	Upper bound
Boys (N=44)	B/W	Visual	0.36	0.58	-0.28	1.01
		LF pen	0.04	1.00	-0.69	0.78
		TTS	-1.35	0.00	-2.12	-0.58
	Visual	B/W	-0.36	0.58	-1.01	0.28
		LF pen	-0.31	0.72	-0.96	0.33
		TTS	-1.71	0.00	-2.46	-0.96
	LF pen	B/W	-0.04	1.00	-0.78	0.69
		Visual	0.31	0.72	-0.33	0.96
		TTS	-1.40	0.00	-2.15	-0.64
	TTS	B/W	1.35	0.00	0.58	2.12
		Visual	1.71	0.00	0.96	2.46
		LF pen	1.40	0.00	0.64	2.15
Girls (N=38)	B/W	Visual	0.10	0.99	-0.56	0.76
		LF pen	-0.49	0.38	-1.25	0.25
		TTS	-1.16	0.00	-1.95	-0.37
	Visual	B/W	-0.10	0.99	-0.76	0.56
		LF pen	-0.59	0.09	-1.25	0.06
		TTS	-1.26	0.00	-2.03	0.49
	LF pen	B/W	0.49	0.38	-0.25	1.25
		Visual	0.59	0.09	-0.06	1.25
		TTS	-0.66	0.12	-1.43	0.10
	TTS	B/W	1.16	0.00	0.37	1.95
		Visual	1.265	0.000	0.498	2.031
		LF pen	0.666	0.124	-0.102	1.433

Table 5.40 Intra-age group differences in mean acceptability score for diagnostic tests.

Patient age group	Technique (I)	Technique (J)	Mean difference (I-J)	Sig	95% confidence interval for difference	
					Lower bound	Upper bound
5-7 years (N=65)	B/W	Visual	0.340	0.275	-0.126	0.805
		LF pen	-0.229	0.815	-0.758	0.299
		TTS	-1.098	0.000	-1.651	-0.545
	Visual	B/W	-0.340	0.275	-0.805	0.126
		LF pen	-0.569	0.008	-1.033	-0.105
		TTS	-1.437	0.000	-1.975	-0.899
	LF pen	B/W	0.229	0.815	-0.299	0.758
		Visual	0.569	0.008	0.105	1.033
		TTS	-0.868	0.000	-1.407	-0.329
	TTS	B/W	1.098	0.000	0.545	1.651
		Visual	1.437	0.000	0.899	1.975
		LF pen	0.868	0.000	0.329	1.407
8-11 years (N=17)	B/W	Visual	0. 125	0.999	-0.781	1.031
		LF pen	-0.219	0.993	-1.249	0.811
		TTS	-1.417	0. 004	-2.494	-0.341
	Visual	B/W	-.125	0.999	-1.031	0.781
		LF pen	-.345	0. 889	-1.248	0.559
		TTS	-1.543	0.001	-2.590	-0.495
	LF pen	B/W	.219	0. 993	-0.811	1.249
		Visual	.345	0. 889	-0.559	1.248
		TTS	-1.198	0. 017	-2.248	-0.149
	TTS	B/W	1.417	0.004	0.341	2.494
		Visual	1.417	0.001	0.495	2.590
		LF pen	1.198	0.017	0.149	2.248

Finally, in terms of willingness to have the test again, the same trends were seen. Children were reportedly significantly less happy to have TTS again than any of the other tests ($p<0.05$). Age and gender did not show any significant effect on the willingness to have a repeat test ($p>0.05$).

6 DISCUSSION

6.1 Overview

This was a complex multi-faceted study which sought an answer to one of the most common clinical challenges encountered by dentists: how best to detect and diagnose interproximal caries in young children, from both a scientific and patient-perspective? Overall, the study's aims and objectives were achieved and novel and clinically relevant data were produced. This discussion section will now consider the strengths, limitations, difficulties and rewards encountered during the study. The key findings will be appraised and compared with those of previous studies. Finally, the clinical relevance of the study will be presented and recommendations will be made for future related research.

6.2 Reflection on the study participants and design

Although comprehensive, this study was complex because it involved the planning of several different stages: recruitment of young patients to an *in-vivo* study; scheduling and collection of extracted teeth following a dental GA, and an *in-vitro* investigation of teeth following histological sectioning and microscopy. The study protocol also required the participation of two examiners at different stages of caries diagnosis which involved extensive testing of intra- and inter-examiner agreement. Although some difficulties were faced throughout the study, every effort was made to minimise bias and the effect of any confounding factors, such that the findings stand up to scientific scrutiny. More details relating to the study conduct and methods adopted are presented in the following sections.

6.2.1 Sample size calculation

Fundamental to the success of this study, in answering the research question, was the determination and achievement of an adequate sample size. The first step was to review the literature for any data provided by previous studies. Interestingly, this search revealed a deficiency in the rigour of many previous investigations as the majority of those seeking to validate proximal caries detection methods in primary and permanent teeth (*in-vivo* or *in-vitro*) failed to employ a sample size calculation (Lussi *et al.*, 2006; Braga *et al.*, 2009; Novaes *et al.*, 2009; Novaes *et al.*, 2010; Bittar *et al.*, 2012; Chen *et al.*, 2012).

The present study did undertake a sample size calculation, which showed the need for 262 surfaces, in order to answer the research question. Overall, the study sample provided 542 surfaces which were subject to histological validation. However, for those samples which were subject to visual examination and LF pen examination after temporary tooth separation, 237 and 226 surfaces respectively were validated histologically. These numbers were close to the number required to identify any statistically significant differences, if they existed. It should be remembered that the sample size calculations were based on a carious prevalence including code 6 lesions (n=142). However these surfaces were subsequently excluded because these easily seen large lesions inflate the sensitivity. If these surfaces had been included the sample size would have exceeded the initial sample size calculation. In addition, we used a sensitivity of 0.8 for the diagnostic method tested, which is in keeping with the sensitivity level reported by previously published studies. It should be noted, that the only other study to have used a power calculation for a sample size was one recently conducted by Teo et al (2014) which validated diagnostic methods for occlusal caries in primary teeth *in-vivo*. In this study, the authors used nomograms for their sample size calculation (Jones *et al.*, 2003), but adopted a high sensitivity of 0.95, which they assumed to be the required sensitivity of the diagnostic method. Assuming a high sensitivity for the diagnostic method leads to a corresponding decrease in the number of surfaces required to reach significance. Furthermore, assuming high sensitivity also

reduces the specificity of the diagnostic method, which has important clinical significance when assessing disease status, such as caries, as a false positive diagnosis leads to unnecessary treatment. Teo and colleagues' (2014) sample size calculation showed the need for only 100 surfaces which they subsequently failed to obtain for their *in-vivo* sample. It is therefore suggested that the number of surfaces validated in the present study was generally adequate to provide statistically meaningful results.

6.2.2 Recruitment of participants

Following on from the sample size calculation, the study then relied on the successful recruitment of participants to provide the required number of tooth samples. The difficulties of recruiting children to medical research are well recognised, particularly the recruitment of families from ethnic minority groups (Rice and Broome, 2004; Spears *et al.*, 2011). However, this proved to be one of most positive and rewarding aspects of the investigation as will be described.

Recruitment of the 82 participants to this clinical study went exceptionally well, with over a 90% response rate. Of the eight children who were invited but didn't participate, six of them were not approached by the investigator herself. The remaining two families initially agreed to participate but then were unable to subsequently attend and were therefore excluded. Furthermore, there was very high representation from Asian and other ethnic minority children, which ensured good generalisability of the findings. The majority (80%) of children in this study were from the younger age group, with ages ranging from 5 to 7 years. This agrees with findings of previous studies which have described the typical demographic profile of children referred to hospital settings for caries management (Young *et al.*, 2009a). Such active engagement of young children and their families to the present study was unexpected and therefore warrants explanation.

The first thing that may have encouraged participation was the fact that children and their parents/carers received visually attractive and simple study information leaflets, with time to reflect about whether they wished to join the study or not. They were also

given a personal and clear explanation about the study and why it was being undertaken by the investigator. This approach is in line with the recommendations of Marshman et al (2012) who undertook a qualitative study to explore recruitment of young children with caries to a randomised clinical trial. It was found that a clear explanation about the research from a dentist who was liked/trusted was a major factor in encouraging parents to consent to their child's participation.

A further factor prompting participation in this study was the fact that the investigator provided the necessary course of treatment for the child (prevention and restorations), whether they participated or not. Thus the families were not burdened with any extra visits, and in fact received the treatment more expediently by seeing the same clinician in designated clinic slots. Parents seemed genuinely motivated to consent to the study as they could see that their child was benefiting from a meticulous dental examination using extra methods to those normally used, which may have led to the detection of otherwise missed carious lesions. They were also provided with fast track GA appointments, which were booked at their convenience, which reduced the risk of them not attending.

The investigator also took every opportunity to praise children for their contribution to the research project and adopt child-friendly language. They were given the impression that they were actually 'heroes' by participating in this study because they were '*helping us to find the best way to find holes in children's teeth*'. Parents and children were also excited about finding out more about the '*clever power rangers' laser pen*' which gives numbers that correlate directly to the condition of the tooth examined. Having the digital screen in front of parents displaying the scores kept parents engaged in the detection of caries in their children's teeth. It was also more informative to parents about the condition of their child's teeth, without simply saying that their child had '*holes*' in their teeth, which some parents find insulting.

Children and their parents also enjoyed the oral hygiene session where young patients had disclosing solution brushed on their teeth showing different shades of purple. Children were curious to know whether their brushing was good or not and were more

motivated to learn the proper way of brushing after finding out they were not that good! Parents frequently expressed their appreciation for being in the study and felt that their child had learnt from it. An indirect benefit to service provision within the dental clinic was the high attendance rate by the study participants. Despite the fact that 71% of participants were from areas of high deprivation, the failure rate of attendance for treatment appointments with the investigator was only 10%. Failed or cancelled appointments are a major concern within the NHS, accounting for up to 25% of all appointments in the paediatric dentistry clinic in Sheffield. It is surmised, therefore, that participation in the study, and the rapport established with the investigator, encouraged better attendance than would otherwise have been achieved in this high caries experience patient group.

A final reflection on the high engagement of ethnic minority children (50% of the study group) within the study may, in part, relate to the ethnicity of the investigator herself, who is a fluent Arabic-speaking Libyan woman. It can only be surmised that some ethnic groups may have felt more comfortable in agreeing to participate in the study because they felt commonality with the investigator. A fascinating recent systematic review on the barriers and facilitators to minority clinical research participation reported that having research staff representative of the research participants' racial/ethnic group was key to successful recruitment (George *et al.*, 2014). It was found that patients from ethnic minority groups valued research staff that they could relate to culturally and communicate with in their first language. These issues should certainly be considered in future studies to ensure that ethnic minority groups are adequately represented in clinical research.

6.2.3 Tooth sample selection

In this study, participants underwent diagnostic caries testing of all primary molars, rather than only a pre-selected tooth/teeth being subject to the experimental testing. Furthermore, the investigator was blind to which teeth had been treatment planned for extraction, at the child's initial assessment with a consultant, thereby reducing any

potential bias. Surfaces which were then noted to not meet the inclusion criteria were subsequently excluded from the statistical analysis. It may be argued that testing all primary molars present in each participant's mouth may have presented an additional burden in terms of discomfort and time, but this was not the investigator's clinical impression. Furthermore, testing all teeth was more representative of a 'real life' clinical approach to comprehensive caries diagnosis.

Interestingly, this approach of full mouth testing has not been adopted by previous investigators who have tended to pre-select a single tooth/surface in their experimental design (Novaes *et al.*, 2010; Mendes *et al.*, 2012). For example, Novaes and colleagues (2010) examined only the distal surface of the first primary molar, excluding the mesial surface, but without giving any justification for this exclusion.

However, the present protocol did lead to considerable disparity between the number of surfaces examined in each stage and the number of surfaces actually validated. It was not possible to get equal numbers of surfaces for all stages, as may be the case in other studies where a single tooth/surface is pre-determined. For example, in the case of TTS, if the child lost or removed the separators, the data would be lost in the present study. In contrast, in other studies, the investigators would have reinserted the separators and brought the patient back for a third visit in order to obtain the necessary data (Novaes *et al.*, 2009; Novaes *et al.*, 2010; Mendes *et al.*, 2012).

Another reason for missing TTS-related data in the present study for some surfaces was that the interproximal contacts were already spaced so that even the largest separator would not remain *in-situ*. In addition, it was not always possible to place separators at the mesial surface of some first primary molars as the separator tended to 'protrude' above the contact point and be poorly tolerated by the child. The patient would either pull the separator out themselves or would request that the investigator removed them before they left the clinic. The difficulty encountered with separator placement between the canine and first primary molar may explain the reason why Novaes and colleagues (2010) did not include the mesial surfaces of the first primary molar in their study.

While the inclusion criteria of the present study were clearly defined to homogenise the samples (ie exclusion of teeth affected by fluorosis or enamel/dentine defects), patient factors may still have affected the performance of the diagnostic systems. This is because diagnostic systems may sometimes behave differently in different patients, due for example, to differing mineral densities of teeth from one patient to another. Thus, if too many teeth from too few patients were assessed, the external validity of the study would be affected. To minimise this possibility, the sample consisted of 542 surfaces collected from 82 patients. An average of six surfaces were collected from each patient to reduce bias. This differs to data collection in the study by Novaes et al (2009) where 621 surfaces were collected from 50 patients giving an average of 12 surfaces from each patient.

6.2.4 Caries prevalence of the sample

Initial clinical visual examination of all surfaces showed the caries prevalence at D_1 to be almost 50% and at D_3 to be 35% (including cavitated surfaces=16% and restored surfaces=6%).

However, following histological validation, the caries prevalence of the sample was found to be much greater, with 78% of surfaces carious at D_1 level and 43% of surfaces carious at D_3 level. This higher caries prevalence in the validated sample may have been expected as it reflects the poorer prognosis of those teeth which required extraction under GA. However, the experimental material did include teeth with a range of carious lesions, including some sound surfaces. Comprehensive treatment planning for a dental GA sometimes includes extraction of non-carious primary teeth for orthodontic reasons such as balancing extractions. In addition, teeth extracted due to caries affecting one proximal surface may have the other one as sound, or if the tooth had only a small occlusal lesion it may even have had two sound proximal surfaces. Therefore, the sample studied demonstrated a range of caries stages, representative of the general child population.

The caries prevalence of the validated sample did approximate the assumed prevalence employed in the initial sample size calculation, where the prevalence was estimated to be 80%.

Another point to consider, relating to the experimental sample, is the argument that excluding grossly carious teeth from the study affected internal validity, as teeth with extensive caries are still part of the diagnostic continuum. These teeth are represented by an ICDAS visual code of 6 (cavitation in dentine involving at least half the tooth surface). However, all score 6 surfaces were excluded from the present analysis which may potentially reduce the accuracy of representative caries prevalence within the sample. However, because the aim of the study was to assess the diagnostic methods for detection of proximal caries, the inclusion of surfaces with frank cavitation may have falsely increased the sensitivity of these methods. In addition, as discussed above, the sample demonstrated wide variation in the various caries stages, therefore exclusion of surfaces with frank cavitation was felt to be justified.

Clearly, the study group had a high caries experience, by virtue of the fact that they had been referred by their primary carers to a hospital setting for the management of their carious dentition, and/or behavioural/medical needs. The mean dmft of a British child who undergoes a DGA is reported to be around 7 giving a caries rate at least four times higher than the average British child (Hosey *et al.,* 2006). It is therefore acknowledged that the present study participants were not representative of the general population, but were representative of the thousands of children who are referred to secondary services for management of their carious primary dentition.

6.2.5 Methodological approach for the LF pen

The zero value of the LF pen was recorded according to the manufacturer's instructions. It was then subtracted from the pen scores before statistical analysis. Many previous investigators have assumed that the LF pen subtracts the zero value electronically and have not included this extra calculation (Braga *et al.*, 2009; Novaes *et al.*, 2009; Chen *et al.,* 2012). However, the manufacturers have stated that the machine does not subtract

the value automatically. It has also been shown that the sensitivity and specificity of the LF pen for the detection of occlusal caries (Rodrigues *et al.*, 2008) and for proximal caries (Rodrigues *et al.*, 2009) reduces significantly in the absence of the zero value subtraction. Hence it is recommended that clinicians should not eliminate this step from the procedure. Furthermore, findings from previous studies may not be directly comparable if they have not all adopted this same calculation.

6.2.6 Tooth storage

In keeping with the study's standard protocol, all tooth samples obtained from the operating theatres of Sheffield Children's Hospital, were subsequently stored frozen at -20℃ in the oral pathology laboratory at the Dental Hospital. Many methods for storage of extracted teeth have been used including immersion in: 1% chloramine; 10% formalin, and 0.02% thymol. However, it has been shown that these solutions cause a statistically significant decrease in the fluorescence of teeth (-72%, -60%, -54% respectively). In contrast, frozen teeth have been found to undergo a minimum increase in fluorescence of 5% (Francescut *et al.*, 2006). Therefore, this approach was adopted by the present study.

6.2.7 Histological scoring on digital images

Histological tooth sections were first examined under a stereomicroscope. Digital images of the histological sections were then taken and sections were subsequently scored by two examiners (SS and CD) on computer screens. It has been shown that this method produces comparable results to those obtained by scoring histological sections directly under a microscope (Jablonski-Momeni *et al.*, 2009). Furthermore, the use of digital images was much more convenient and time-efficient for the examiners, rather than scoring sections directly under the microscope. The excellent intra- and inter-examiner reproducibility (Kappa coefficient of 0.87-1) shown in the present study confirmed the suitability of this method.

6.2.8 The challenge of histological validation

This was an *in-vivo* study which compared the validity of a pen laser fluorescence device to three different diagnostic methods for proximal caries in primary molars including: radiographic examination and visual examination with and without temporary tooth separation. To date, this is the only *in-vivo* study which has attempted to validate findings from clinical caries diagnosis of proximal surfaces in primary molars with a subsequent histological examination (gold standard).

Three previous *in-vivo* studies have, however, been conducted to assess the same clinical diagnostic methods, two of these employed temporary tooth separation as the 'gold standard' against which to validate the findings (Novaes *et al.*, 2009; Novaes *et al.*, 2010). However, it has been shown that visual examination lacks one of the main criteria for any gold standard, that of reproducibility (Deery *et al.*, 2000). The third *in-vivo* study (Chen *et al.*, 2012) employed bitewing radiographs to validate the clinical diagnosis of caries; if lesions were shown to be cavitated they were subject to instrumentation using a handpiece and the penetration depth of the lesion was evaluated visually prior to restoration. However, radiographic examination has also shown poor reproducibility (Hala et al, 2006). Radiographic examination is also not independent of the methods to be validated, therefore lacking another of the cited criteria required of any gold standard assessment (Hintze and Wenzel, 2003).

The obvious limiting factor with histological validation is that the tooth must be sectioned after examination in order to validate the results of the clinical examination. Experimental teeth must therefore be collected following extraction or physiological exfoliation. Clearly unless clinically indicated, it would be unethical to extract teeth purely for research purposes, and the problem with awaiting natural exfoliation is that there is no control over the time period between the examination and exfoliation. The *in-vivo* study of Rocha and colleagues (2003) utilised teeth which had exfoliated up to 45 days after they had been tested with the DIAGNOdent device. It is argued that during this time period a sound surfaces could have progressed to a D_1 lesion, and a D_2 lesion

could have progressed to a D_3 lesion leading to an underestimation of lesion size by the method used for examination. While this may be unlikely in this short interval, investigators should bear in mind the rapid progression rate of some carious lesions in primary teeth which may reduce the sensitivity of the method assessed if there is an extended time lapse between examination and subsequent collection of the tooth sample.

To avoid this potential problem, all patients in the present study were booked for their dental GA by the investigator herself. This ensured that tooth extractions occurred expediently following caries diagnosis; the delay between initial examination and tooth collection was usually in the order of 2-3 weeks.

6.2.9 Statistical analysis

Most studies which have previously assessed the validity of caries diagnostic approaches have presented findings in terms of sensitivity and specificity. A recent systematic review of methods for caries detection found that only five studies reported predictive values (positive and negative), and only three studies reported likelihood ratios (Gomez *et al.*, 2013). Reference was therefore made to the Cochrane handbook for systematic reviews of Diagnostic Test Accuracy (DTA), which stipulates the standards which should be considered when analysing the quality of diagnostic studies (http://www.cochrane.org/handbook). Thus all appropriate tests recommended by the DTA, which included predictive positive and negative values and the likelihood ratios, were applied in the present study.

Statistical support for the present study was provided by Dr Zoann Nugent who is a senior health outcomes analyst at the Manitoba Institute of Cell Biology, Canada. She was previously employed as a statistician at the Dental Health Services Research Unit, University of Dundee, UK. As such, she has a deep understanding of epidemiological (Pitts *et al.*, 2004) and diagnostic dental caries research (Deery *et al.*, 1999; Deery *et al.*, 2006; Pitts *et al.*, 2007; Shoaib *et al.*, 2009). She is therefore one of the few people with the knowledge and understanding to handle this type of data. The large data set used in this research had to be manipulated to allow its analysis. All data were entered by the

investigator who also requested and interpreted the subsequent analysis. However, Dr Nugent performed the necessary statistical computations and advised on the most appropriate statistical methods.

6.2.10 Strengths of the study

The study had a number of strengths, both in terms of its design and conduct, which will be briefly appraised.

Study design

The present study is the only clinical study to date which has included a sample size calculation and histological validation for the detection of proximal caries in primary teeth. The large sample size makes the results of this study more reliable and generalisable than those in previous publications.

The study design also included some steps which ensured novel findings: it is the first study to report the optimum cut-off values for the detection of proximal caries in primary teeth by the LF pen, which has important clinical relevance. Furthermore, this is the first investigation of its kind to assess the validity of TTS histologically compared to the other diagnostic methods. TTS has been used as the validation method in many *in-vivo* studies (Novaes *et al.*, 2009; Novaes *et al.*, 2010; Mendes *et al.*, 2012) but these lacked a gold standard to show how robust the validation method was itself.

Training and calibration

The chief investigator had an extensive period of training in relation to caries diagnosis with her supervisor, CD, who has published widely in this field. A thorough assessment of intra- and inter-examiner reproducibility was undertaken before commencing the study. Although substantial agreement was achieved from the first time in most of the examinations, the reference examiner (CD) provided further training until the achievement of as close to perfect agreement as possible.

The application of scores following visual and radiographic examination is recognised to entail some subjectivity as they rely on the experience and judgement of the examiner and relate to descriptive criteria. Thus comprehensive training and calibration was warranted. The use of the LF pen, however, involves reading of a number which appears on the screen of the pen, thus data do not rely on the user's judgement. The readings, however, are technique-sensitive, and their accuracy rely on the competency of the investigator in using the LF pen. It was therefore imperative that the investigator received thorough training from the supplier and supervisor.

Kappa scores for intra- and inter-examiner reproducibility relating to all components of the study were high indicating substantial (K=0.73) to perfect (K=1) agreement in all examinations. These results demonstrate the value of thorough training in achieving good intra- and inter examiner reproducibility, as well as providing evidence for the excellent reliability of the methodological approaches.

Children's involvement

When treating children, behaviour management is key to successful outcomes. Dental health care professionals continually face the challenge of delivering the best evidence-based care whilst ensuring that any interventions are acceptable to the young patients themselves. In the context of the present study, if one caries diagnostic approach had proved to be vastly superior to another one, yet was more unacceptable to the patient, then its clinical application would be limited.

The present study therefore involved children and sought their views regarding different diagnostic methods. Although this was a very small part of the overall study, it was felt to be of importance. Previous investigations in this field have focused on the validity and reproducibility of the caries diagnostic methods (Bader *et al.,* 2002) but there has been a paucity of research from the patient perspective. If a diagnostic test provokes discomfort, this could be a major disadvantage, especially in children.

There is growing emphasis in paediatric healthcare to involve children and young people in both research and service evaluation and delivery. The deficiency of user involvement

in oral health research was first highlighted in a systematic review undertaken by Marshman and colleagues (2007). Since that time, there has been a steadily emerging literature which has involved children in dental research. Most recently, Santamaria et al (2015) reported on children's acceptability of different methods of caries management. In this study a more comprehensive assessment of acceptability was undertaken which involved an assessment of children's pain perceptions using a visual analogue scale as well as a behaviour rating using the Frankl scale. The intention of the present study, however, was to undertake a very simple and quick assessment of children's views using a Likert scale response and child-friendly language. The questionnaire was developed and pilot-tested with young patients and proved an effective way of seeking their feedback.

It was interesting to note that the young children in the present study did not appear to find radiographic examination difficult. This contradicts the clinical impressions of many general dental practitioners who report poor patient compliance to be the main reason for not taking intra-oral radiographs for caries diagnosis in the primary dentition (Mauthe and Eaton, 2011) . Within the dental hospital setting, radiographs were taken by highly skilled radiographers which may account for the high acceptability found by this study. Furthermore, hospital staff may be prepared to spend more time in preparing children to have radiographs, which may not be the case in practice where there are greater time and financial pressures. Nonetheless, this study challenges the misconceptions commonly held by general practitioners that young children do not tolerate intra-oral radiographs, providing appropriate behaviour management and techniques are adopted.

It is also worth comment that younger children found the LF pen examination to be significantly less acceptable than visual examination ($p<0.05$), while older children did not. The investigator observed that it was more difficult to insert the LF pen tip between the contacts of younger children's teeth than the older children. This was attributed to the presence of tighter tooth contacts and more limited access in some young children, which led to insertion being more uncomfortable and causing more pressure on the

gingiva between the teeth. The development of a finer LF pen tip in the future would help in this respect.

Not surprisingly, TTS was found to provoke statistically higher discomfort than was the case for other examinations and fewer children were prepared to consider having TTS a second time. The use of orthodontic separators is commonplace for teenagers prior to the placement of fixed orthodontic treatment and is widely acknowledged to cause pain and discomfort at initial placement and peaks within the subsequent 24-48 hour period (Asiry *et al.*, 2014). More recently, the development of the non-invasive Hall Technique for placement of preformed metal crowns has also required the use of orthodontic separators in younger patients (Bell *et al.*, 2010). Clinical experience certainly supports the finding act TTS is an uncomfortable experience for many children, although thresholds to TTS vary widely. It may be concluded that poor acceptability of TTS may prove a barrier to its routine use for caries diagnosis in young children.

6.2.11 Limitations of the study

Although intra- and inter-examiner reproducibility have been described above as a strength of the study, there was one small area which could be considered an omission; inter-examiner reproducibility for use of the LF pen was not actually assessed. The reference examiner (CD), although had had previous experience in the use of the LF pen, felt that the investigator had received more recent training and practice. Thus he did not feel it appropriate for his results to serve as the gold standard for the study, as in the case of the other assessments. Furthermore, as the LF pen had been previously shown to have high inter-examiner reproducibility in the published literature, this was felt to be sufficient evidence.

In retrospect, an assessment of inter-examiner reproducibility for use of the LF pen would have been a useful exercise. This would have provided additional data to support or refute the reliability of the LF pen in detecting caries when used by different clinicians with differing levels of experience. The LF pen is not primarily a research tool but is marketed as a commercially available device for caries diagnosis. It would be

important to elucidate, therefore, how it performs by a range of clinicians, who have not necessarily had a prolonged training in its use.

Another suggested limitation was the *in-vitro* assessment of the LF pen where proximal surfaces were assessed directly by the LF pen without having a proximal contact with an adjacent surface as is the case for most of *in-vitro* studies. However, the aim of the study was to assess the ability of the pen to detect fluorescence from carious lesions outside the mouth and assess the optimum cut-off values of the pen *in-vitro* and compare them to the *in-vivo* cut-off values rather than try to mimic the clinical situation.

A further limitation, out with the investigator's control, was the fact that not all *in-vivo* data collected from patients could be validated histologically.

In addition, in hindsight it would have been preferable to have taken into account the exclusion of code 6 lesions from the sample size calculation, and analyse the data at the level of cavitation (ICDAS code 5), because this is the point where restoration is almost always needed.

Finally, the histological material produced from this study could have been more utilised and more analysis of the histological sections would provide further useful data.

6.3 Ethical considerations

Application for ethical approval through the National Research Ethics Service as well as obtaining local research governance proved a lengthy and complex process. Although ultimately successful, the process took almost 12 months and did delay the start of the project. However, following initial submission of the application for ethical approval, only minor amendments were required such as adding some further details to the parent's information sheet providing clarification for some of the steps of the study. No major amendment of the protocol was required. The lesson to be learnt from this experience is that ethical approval should be sought very early on during a finite period of research, such as PhD, so that progress is not compromised.

The study subsequently progressed extremely well and adhered to all good practices required for ethical research. Patients and parents had an adequate time to reflect whether or not to participate in the study. The high uptake of participation, may in part, relate to the user-friendly patient and parent information leaflets, as advocated by the Local Research Ethics Committee. No ethical concerns arose during the study and there were no patient complaints or untoward clinical incidents.

6.4 Key findings

6.4.1 Satisfaction of study aims and objectives

It is worth reviewing the study's original aims and objectives in order to consider whether they were fully met. The overall aim of this study was to assess the usefulness of a pen laser fluorescence device for the detection of proximal caries in children's primary teeth.

The specific objectives of this study were:

- to assess validity and reproducibility of a pen laser fluorescence device
- to compare outcomes for a pen laser fluorescence device with these from conventional methods: visual examination with and without tooth separation and bitewing radiographs
- to assess children's acceptability of a pen laser fluorescence device in comparison to usual examination with and without tooth separation and bitewing radiographs

It can be appreciated from the results provided, that both the aim and objectives were fully met by this study.

6.4.2 Validity of *in-vivo* examinations

Our results showed higher specificity than sensitivity for all diagnostic methods. The results had also a pattern of better performance at a more advanced level for all the diagnostic methods.

Visual examination

The findings from the present study showed the visual examination to have low sensitivity for the detection of early proximal caries (0.52). This agrees with the findings of previous systematic reviews of the performance of visual examination (Bader *et al.*, 2002; Ismail, 2004; Gomez *et al.*, 2013).

Visual examination without TTS had lower sensitivity for the detection of dentine caries than radiographic examination and the LF pen, which agrees with the findings of several previous clinical studies (Novaes *et al.*, 2009; Novaes *et al.*, 2010; Mendes *et al.*, 2012).

The specificity of visual examination was high at all diagnostic thresholds and was comparable with the specificity of the LF pen and radiographic examination which also agrees with the findings of previous studies (Novaes *et al.*, 2009; Novaes *et al.*, 2010; Mendes *et al.*, 2012).

However, the ROC comparison of the different diagnostic methods used in this study showed visual examination to be the least useful of all methods for the detection of proximal caries in primary molars at both levels of diagnosis.

Visual examination with temporary tooth separation

Visual examination with TTS had the highest sensitivity of all the diagnostic methods assessed in this study at the D_1 threshold. For the detection of enamel caries in proximal surfaces of primary molars, visual examination with TTS has been shown to be the best method with the highest area under the ROC curve. High sensitivity for detection of early caries lesions is more important when a preventive rather than a restorative intervention is the intention.

In the present study, ROC comparison of the different methods showed that temporary tooth separation added to the validity of dentine caries detection over visual examination without temporary tooth separation, and there was no difference in the validity of visual examination with TTS and the LF pen examination for the detection of dentine caries. However, the method was the least acceptable for children and radiographic examination was still significantly better that visual examination with TTS for the detection of dentine caries.

Radiographic examination

The sensitivity of radiographic examination for the detection of enamel caries was very low at 0.15 which agrees with the findings of Novaes and colleagues (2009) who found the sensitivity of radiographs at D_1 to be 0.16 and the findings of Novaes and colleagues (2010) who found the sensitivity to be 0.20.

Although histological examination showed 35% of surfaces to have enamel caries, radiographic examination showed only 11% of the surfaces to have enamel caries which shows that radiographic examination was only able to detect less than one third of the enamel lesions. A systematic review of the validity of methods for the detection of non cavitated carious lesion has also shown radiographic examination to have poor results for the detection of these lesions (Gomez *et al.*, 2014).

However, for the detection of dentine caries, radiographic examination has shown to have the highest sensitivity of all the diagnostic methods which agrees with the findings of (Novaes *et al.*, 2009), but disagrees with the findings of Shoaib et al (2009) and Braga et al (2009) who found the visual examination to be better than radiographic examination for the detection of proximal caries. Their findings (Braga *et al.*, 2009; Shoaib *et al.*, 2009) may be attributed to the fact that their study was *in-vitro* and it is impossible to simulate the *in-vivo* settings *in-vitro*. Visibility and mobility of the proximal surfaces is always higher *in-vitro* than *in-vivo*.

Radiographic examination also had the highest specificity of all the diagnostic method assessed at all levels of diagnosis.

ROC comparison of the different methods assessed in this study showed radiographic examination to be only better than visual examination without TTS for the detection of proximal caries at the D_1 level. Visual examination with TTS and the LF pen were both significantly better than radiographic examination at this level of diagnosis. However, radiographic examination was shown to be significantly better than the other methods for the detection of dentine caries in proximal surfaces of primary teeth.

LF pen examination

The results of the present study showed the specificity of the LF pen to be higher than the sensitivity for all thresholds which agrees with other studies conducted in primary teeth (Gimenez *et al.*, 2013).

These results also showed a trend of better performance at a more advanced threshold which agrees with previous studies on the LF pen in proximal surfaces (Lussi and Hellwig, 2006; Novaes *et al.*, 2009; Novaes *et al.*, 2010).

The LF pen showed a significantly better performance with TTS than without TTS. One may hypothesise that more space would give more accessibility for the pen to the lesion, and these results showed that this assumption is true.

In the present study, the LF pen showed a better performance for the detection of dentine caries than for the enamel caries. These findings agree with the findings of previous studies (Novaes *et al.*, 2009; Novaes *et al.*, 2010).

Since there is a poor correlation between the pen scores and the mineral content of the tooth, and a better correlation with the presence of infected dentine (Celiberti *et al.*, 2010) and the initial lesions are less infected than dentinal lesions (Kidd *et al.*, 2003), that may explain why the pen is better for the detection of dentine lesions since it detects bacterial metabolites (Lussi *et al.*, 2004).

Although the LF pen had higher sensitivity than visual examination at the D_3 threshold, its specificity was lower than that for visual examination at the same level of diagnosis.

Therefore, the ROC comparison of the two methods showed no difference in the performance of both methods at the D_3 threshold.

Finally, the optimum cut-off values *in-vivo* showed an increase in the sensitivity of the LF pen for the detection of proximal caries without compromising the specificity. Therefore, the new cut-off values should be recommended and the manufacturer's cut-off values should be modified for the better detection of proximal caries in primary teeth.

6.4.3 Validity of *in-vitro* examinations

6.4.3.1 Direct visual examination

Direct visual examination of proximal surfaces *in-vitro* showed the highest validity of all examinations implying that if proximal surfaces could be seen directly, the best method for proximal caries detection in primary molars would be the meticulous visual examination.

The relationship between direct visual examination and histology showed that 33% of surfaces with code 2 have caries extending into dentine, which confirms the suitability of Ekstrand's criteria for histological examination (Ekstrand *et al.,* 1997) where code 2 histology combines the inner surface of enamel with the outer one third of dentine. This is in contrast to the Downer's criteria where score 2 only includes the inner half of enamel and uses EDJ as a separating point between enamel and dentine caries. This project showed that clinically visible enamel caries does not always stop at the EDJ histologically. Therefore, the Ekstrand's criteria rather than Downer's criteria (Downer, 1975) should be adopted for the histological validation of studies validating meticulous visual examination. Most previous studies which compared the validity of different diagnostic methods including ICDAS visual examination used the Downer's criteria for their histological validation (Lussi *et al.,* 2006; Braga *et al.,* 2009; Novaes *et al.,* 2010).

However, there is some merit in including lesions up to the EDJ as a subgroup, as was done in this research, as it permits comparison with previous work. All the diagnostic methods assessed in this study showed better lesion detection at D_3 threshold of diagnosis. The optimum cut-off for the visual detection of enamel caries using ICDAS criteria is code 1, and the optimum cut-off value for the detection of dentine caries is code 2, which supports the argument above.

The original ICDAS I codes 3 and 4 were switched in ICDAS II based on the examination of 57 occlusal surfaces of permanent teeth during the development of ICDAS criteria, where they found that 88% of surfaces with the original code 3 ICDAS had caries into dentine while 77% of surfaces with code 4 ICDAS I had caries into dentine, therefore, the decision was made to switch the codes 3 and 4 in ICDAS II criteria (Topping and Pitts, 2009). The results of this study with 684 surfaces show that 92% of proximal surfaces with code 3 ICDAS have dentine caries while only 50% of surfaces with ICDAS code 4 have dentine caries. This suggests that the ICDAS I criteria is the most appropriate system for use in proximal surfaces in primary teeth, and therefore question this change.

Direct visual examination of proximal surfaces *in-vitro* showed that there is a slim chance (16% or less) that radiolucency in enamel would show cavitation, a finding which agrees with previous studies (Pitts and Rimmer, 1992; Akpata *et al.*, 1996; Hintze *et al.*, 1998). However, the possibility increases to 52% when the radiolucency extends to the outer third of dentine a finding which agrees with data reported by (Bille and Thylstrup, 1982; Thylstrup *et al.*, 1986). Cavitation was seen in 100% of surfaces with a radiolucency in the middle or inner third of dentine, which also agrees with previous studies which almost always, have shown this to indicate cavitation (Pitts and Rimmer, 1992; Akpata *et al.*, 1996; Nielsen *et al.*, 1996; Hintze *et al.*, 1998).

6.4.3.2 Direct examination with the LF pen

The LF pen showed higher validity *in-vitro* than *in-vivo*. A finding which is expected because of the design of the study, where the pen is applied directly to the carious lesion *in-vitro* while the accessibility was limited *in-vivo*. The *in-vivo* results of better validity for the pen with TTS than without also support this finding.

It has been noticed in this *in-vitro* evaluation of the LF pen that the tip of the device must be in direct contact with the carious lesion. The LF pen was not able to detect the fluorescence from a distance *in-vitro*. These observations trigger the question of whether the LF pen is actually detecting something different *in-vitro* than *in-vivo*.

Some studies recommended the use of the average pen score rather than the highest pen score (Francescut *et al.*, 2006) for the analysis without providing any evidence for this suggestion, although the manufacturer suggests the highest score to be taken for the detection of caries. The results of the present study showed no difference in the validity of the LF pen examination using the highest pen scores or the average pen scores for the analysis. Therefore, there is no need for this extra step which is a burden for the clinicians and difficult to adopt in routine practice.

6.4.3.3 *In-vitro* vs *in-vivo* findings of LF pen validity

The LF pen readings *in-vitro* were significantly different from those *in-vivo* and showed no linear relation, therefore, it is not possible to allow the application of a correction factor convert from *in-vitro* to *in-vivo* or vice versa.

The optimum cut-off values for the LF pen *in-vitro* were similar to the optimum cut-off values *in-vivo* at the enamel caries level. However, at outer dentine and inner dentine caries the optimum cut-off values for the LF pen *in-vitro* were much higher than the *in-vivo* ones.

The significant difference in the scores and cut-off values for both *in-vivo* and *in-vitro* settings suggest that the LF pen works differently in both settings, therefore, the results

of *in-vitro* studies are not representative of the clinical situation and cannot be applied clinically.

6.5 Clinical considerations

To date, much of the experimental work on the validity of different caries diagnostic approaches has been conducted *in-vitro,* thus the applications of the findings to real life practice remain open to question. The importance of the present study's *in-vivo* versus *in-vitro* design is therefore clear, as it has allowed the investigator to determine whether it is possible to extrapolate laboratory findings to the clinical setting. Furthermore, the inherent variances in children's compliance in accepting the different methods have been taken into consideration.

Accurate detection and diagnosis of dental caries is fundamental to evidence-based treatment planning for children. It is important to first fully determine each child's caries risk status in order to devise an appropriate prevention strategy, which may include the frequency of professionally applied topical fluoride applications. Early interventions are directed at arresting the caries process, and may obviate the need for restoration (Deery, 2013). The benefits of this approach are obvious in reducing the burden of disease for children and their families, reducing potential stress for the clinician, and saving costs for health services. Clinicians need to have the knowledge and skills to employ and interpret the most reliable caries diagnostic tests for their patients. In addition, the validity of any method also needs to be balanced against any potential risks to the patient as well as the acceptability of the test.

From the findings of this study, the ICDAS visual examination alone was suboptimal in the detection of dentine caries in proximal surfaces of primary molars. Furthermore, it was a time-consuming exercise to adopt this methodology for the whole dentition. Temporary tooth separation was the least acceptable method for children. Therefore, the use of ICDAS in conjunction with TTS is not recommended for routine clinical use in children's primary dentition.

The LF pen device, although not having as high validity as radiographic examination for the detection of dentine caries, did add to the validity of dentine caries detection. Therefore, it does have a place in clinical practice as an adjunct to visual examination. It also gives a degree of objectivity as its score is reproducible over time (Lussi and Francescut, 2003). The new cut-off values found in this study are recommended for use clinically for the detection of proximal caries in primary teeth. However, the main advantage of the LF pen, over radiographic examination, is the avoidance of ionising radiation. This allows a clinician to undertake frequent re-examinations of a particular site where caries progress requires close monitoring. There are occasions where parents refuse to consent to their child having dental radiographs because of heighted (unfounded) concerns about ionising radiation. There are also some rare medical conditions of DNA repair deficiencies where ionising radiation presents a real and increased risk of the patient developing cancer (Knoch *et al.*, 2012). The cost and training requirements of the LF pen would, however, need to be addressed as they may present a barrier to its uptake in general practice.

For the majority of patients, however, assuming that clinical guidelines and safe practice are adhered to, radiographic examination remains the optimum caries diagnostic aid. This study has confirmed that in order to detect the maximum number of carious proximal lesions, visual examination must be accompanied by radiographic examination. Furthermore, radiographs are helpful in decision-making as to which teeth require restorative intervention and which can be monitored, providing preventive strategies are put in place. Clinicians should bear in mind that more than 50% of lesions showing a radiolucency on bitewing radiographs extending into the outer third of dentine were actually found to be cavitated. All surfaces with a radiolucency extending into the middle or inner third of dentine were cavitated clinically and a restorative approach was indicated.

Most importantly, this study's recommendation that intra-oral radiographs are invaluable in caries diagnosis is supported by the fact that young children generally found them to be acceptable, which is positive finding. General dental practitioners need

to be supported and encouraged to be more proactive in taking radiographs for children in line with clinical guidelines (Horner *et al.*, 2013). In doing so they will identify caries at an early enough stage to instigate professional prevention and more importantly inform parents so they can improve tooth brushing and dietary habits. Early detection of cavitated lesions would allow less invasive restorative interventions, such as the use of preformed metal crowns using the Hall Technique, and thereby avoiding the need for pulp therapies, extractions and, potentially, dental general anaesthetics.

6.6 Further research

There is a need for more *in-vivo* studies to confirm the results of this study and further assess the validity of different detection methods for caries. Studies with both *in-vivo* and *in-vitro* elements, such as this study, are important to allow direct comparisons between the results achieved from clinical testing and experimental models.

Further research with the LF pen should focus on the development of clinically relevant cut-off values which will improve caries detection in both primary and permanent teeth. In addition, there is a need for the exploration of the mode the device uses to detect caries, as it can be seen that the LF pen works differently *in-vitro* than in clinical settings.

There is a need to examine the order of code 3 and 4 ICDAS for occlusal surfaces in primary teeth and proximal surfaces in permanent teeth.

It would also be interesting to develop the preliminary work conducted on patient acceptability of different caries diagnostic tests. More detailed qualitative enquires are indicated to seek the views and preferences of general dental practitioners, patients and parents in order to reach a fuller understanding of how caries diagnosis is valued and acted upon. The teaching of caries diagnosis should also be reviewed regularly to ensure that the future dental profession is equipped with the knowledge, skills, competencies and attitudes to diagnose caries accurately and safely. An understanding of all aspects of

caries is core to any undergraduate or postgraduate dental curriculum, but it should continually evolve in order to be more research-led (Schulte *et al.*, 2011).

Further research could be carried out using the histological material collected in this study provided ethical approval to retain and carryout further research is obtained. The histological sections could be further used to assess the mineral content across the carious lesions. It could also be used for the assessment of the dentine matrix for example the assessment of the extent of degradation across the lesion. In addition, mechanical tests could be carried out on these sections to assess the hardness of dentine across the lesion.

Finally, there is considerable scope to undertake a future economic analysis of the benefits of caries diagnosis for both children and adults. In an increasingly difficult financial climate, health care services must make efficiency savings whilst retaining high quality and evidence-based care. The time taken to undertake a meticulous caries diagnosis clearly has cost implications for both primary and secondary care practices and has to be balanced against the cost-benefits. A recent systematic review of economic evaluations of caries prevention programmes revealed a paucity of high quality studies (Marino *et al.*, 2013). But to date, there appear to have been no studies to determine the cost-benefits and economic implications relating to different modes of caries diagnosis. The cynic may argue that some clinicians would rather not know the extent of caries in their young patients, as this entails an ethical responsibility to provide more intensive courses of treatment. Nonetheless, there would be great value to those planning and commissioning future dental health services to have data to support the cost-benefit of early caries diagnosis in young children.

7 CONCLUSIONS

7.1 Conclusions

A number of conclusions can be made as a result of this comprehensive investigation as highlighted below:

- For the detection of proximal caries at D_1 and D_3, visual examination is the least useful of the diagnostic tests included in this investigation.
- Visual examination with temporary tooth separation does add to the value of the detection of caries at D_1 and D_3.
- The LF pen provided an added caries detection value over visual examination at D_1 but not at D_3.
- The use of the LF pen in conjunction with temporary tooth separation achieves better caries diagnosis that the LF pen without temporary tooth separation.
- Radiographic examination is the optimum test for dentine caries diagnosis.
- The validity of the LF pen *in-vitro* is higher than its validity *in-vivo*.
- There is a significant difference between the mean values of the *in-vivo* LF pen readings and the *in-vitro* readings and this difference is not linear. Thus the simple application of a correction factor is not supported.
- There is no difference between the validity of the LF pen using the average score or the highest score.
- More than 50% of surfaces with a radiolucency in the outer third of dentine are cavitated and 100% of surfaces with a radiolucency extending into the middle or inner third of dentine are cavitated.
- The vast majority of children found visual examination and radiographic examination acceptable.

- Small children (aged 5-7 years) found the LE pen to be significantly more difficult than a visual examination.
- Most of children found TTS to be significantly more difficult than all other methods.

7.2 Lay summary of the study

It is sometimes difficult for dentists to see tooth decay between children's first molars, as the teeth are close together. It is important that tooth decay is identified early so that dentists can give preventive advice to parents and children, and can treat any holes in teeth, before they cause a problem. So we wanted to carry out a study to find the best way of detecting tooth decay between first molar teeth in children aged 5- to 10-years.

Eighty-two children, who were patients at the Charles Clifford Dental Hospital, Sheffield took part in our study. On their first visit, children had a careful examination of all their teeth with a dental mirror. They also had some dental x-rays and a laser fluorescence device was shone between their teeth to see if there was any decay present. At the end of this visit, some small elastic bands were placed between their molar teeth which, over the next few days, pushed their teeth slightly apart (so that the dentist could see between the teeth more easily). At their second visit, the children had a check-up with the dental mirror again and the laser fluorescence device. We also asked the children what it felt like to have these different detection examinations.

Some of the children then needed to have some of their teeth removed as part of their treatment. After removal, theses teeth were cut and examined under a microscope to validate the clinical findings.

The results of our study found that just looking carefully with a dental mirror was not good enough to always find all the tooth decay present. The laser fluorescence device and the elastic bands did help to improve the accuracy of finding tooth decay, but the very best test for reliably finding tooth decay between first molar teeth was a dental x-ray.

When the children were asked their views about having these different examinations to their teeth, most of them said that the check up with the mirror and the x-rays were fine. The children reported finding the elastic bands not very nice and they would not like to have them again.

So we would recommend that, where necessary, dentists should use x-rays to be sure of finding tooth decay in children's teeth, so that they can provide the best advice and treatment for them.

7.3 Recommendation to clinicians

The following clinical recommendations are made in light of the key findings from this investigation:

- Meticulous visual examination is acceptable way of detecting interproximal caries in primary molars and should be adopted in routine clinical practice.
- Radiographs add significantly to meticulous visual examination and are well accepted by children. Therefore, they should be routinely used in line with existing good clinical practice guidelines.
- The LF pen does add some diagnostic value over meticulous visual examination alone but it is not as valuable as radiographs. However, as the LF pen does not produce the risks associated with ionizing radiation, it can be used more frequently than dental radiographs to monitor lesions. It may also aid clinicians in selecting the most appropriate time for taking radiographs.
- When using the LF pen, clinicians should use the highest score given for each surface rather than taking an average score of several different readings obtained for a particular tooth surface.
- TTS did provide additional diagnostic value over meticulous visual examination, but were poorly tolerated by children, thus have limited application in normal clinical practice.

8 References

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9 Appendices

Appendix 1	Ethical approval letter
Appendix 2	Parents information sheet
Appendix 3	Children information sheet
Appendix 4	Parents' consent form
Appendix 5	Children assent form
Appendix 6	Acceptability questionnaire
Appendix 7	Letter to clinicians
Appendix 8	Clinical poster for children and parents
Appendix 9	Scoring sheet for radiographs
Appendix 10	Scoring sheet for clinical examination (visit 1 & 2)
Appendix 11	Scoring sheet for *in-vitro* examination
Appendix 12	Scoring sheet for histological examination
Appendix 13	Conference paper: Children's acceptability of diagnostic methods for approximal caries detection in primary molars. (Winner of the poster prize, BSPD, 2013)
Appendix 14	Conference paper: *In-vivo* evaluation of methods of approximal caries detection in primary molars. (Winner of the Max Horsnell travel award, BSPD, 2014)

Appendix 1

NHS
Health Research Authority
NRES Committee Yorkshire & The Humber - Sheffield
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13 August 2012

Mrs Samiya Subka
Unit of Oral Health and Development
School of Clinical Dentistry
Claremont Crescent
SHEFFIELD
S10 2TA

Dear Mrs Subka

Study title:	**Validity and Acceptability of a Laser Fluorescent Device for Detection of Proximal Caries in Primary Teeth Compared with Conventional Methods**
REC reference:	**12/YH/0214**
Protocol number:	**STH16301**

Thank you for the letter of 31 July 2012 from Professor Helen Rodd, 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 Alternate Vice-Chair

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.

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).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of 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

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).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		03 April 2012
Investigator CV	S Subka	23 July 2012
Other: Upgrade report		12 August 2011
Other: Medical Physics Expert (MPE) assessment		08 February 2012
Other: Clinical Radiology Expert (CRE) assessment		13 March 2012
Other: Instructions for use of DIAGNOdent Pen		
Other: Academic supervisor CV: H Rodd		23 July 2012
Other: Academic supervisor CV: C Deerey		23 July 2012
Participant Consent Form: Parent/ guardian	2	23 July 2012
Participant Consent Form: Child: Assent	2	23 July 2012
Participant Information Sheet: Child	2	23 July 2012
Participant Information Sheet: Parent/Guardian	3	31 July 2012
Protocol	2	23 July 2012
Questionnaire	2	23 July 2012
REC application		03 April 2012
Referees or other scientific critique report		09 January 2012
Response to Request for Further Information		23 July 2012
Response to Request for Further Information		31 July 2012

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 NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

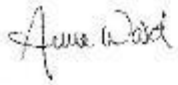
You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/YH/0214	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely



pp
Dr **Basil Sharrack**
Chair

Email: anne.ward7@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Professor Helen Rodd, University of Sheffield*
Dr Erica Wallis, Sheffield Teaching Hospitals NHS Foundation Trust

Appendix 2

Parent/ Guardian Information Sheet

What is the best way to find tooth decay?

We are inviting your child to take part in a research project. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you are happy for your child to take part. The information sheet will tell you the purpose of this study and what will happen if you take part.

What is the purpose of the study?

Early detection of tooth decay in children is important so the dentist can give advice on treatment to stop it getting worse. This helps to prevent children getting tooth ache or having more difficult treatment. A new way for detecting decay has been developed. This is a special laser pen which is simple to use. It involves the application of a red light to tooth surfaces, and then a beep sound gives a number on the screen of the pen indicating the level of decay in the tooth surface. The aim of our study is to investigate whether this device is useful for identifying decay between baby teeth in children and to see how acceptable it is for children compared to a normal examination and x-rays. The laser pen will be used during the first two visits of your child's course of treatment. We will not ask your child to come for any extra visits for the purpose of the study.

Why has my child been chosen?

Your child has been chosen because he/she attends the Charles Clifford Dental Hospital for treatment of tooth decay. A total of 80 children will be invited to participate in the study.

Does my child have to take part?

It is entirely up to you to decide whether or not you wish your child to join the study. Your decision will not affect your current or future care. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a

consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to my child if they take part?

If you decide to allow your child to take part then your child will receive two intra-oral x-rays as part of their normal clinical treatment. Your child will not receive any additional x-rays as a result of participating in this study. While all x-rays carry some risk, in this case it can be considered negligible. Then the researcher (Dr Samiya Subka) will examine your child first by mirror, then with the laser pen. Then your child will have one or more elastic bands fitted between his/her back baby teeth, which is not part of the routine examination and may cause some discomfort to some children during placement. These bands will be removed after seven days at your child's next visit to allow a direct examination of the surfaces between these teeth. Some children (your child may be one of these) will have a second examination in the same visit by the research supervisor (Prof Chris Deery) to see how repeatable the examination is. At the second visit, your child will be asked to complete a short questionnaire on his/her arrival which will ask them their opinion on having x-rays and the laser pen. Then your child will have a quick dental exam with a mirror after removal of the elastic bands, and another check with the pen laser. At these two visits, your child will also be given the preventive treatment that he/she requires.


Exam with the mirror


Elastic bands between baby teeth


Exam with the laser pen

What do I have to do?

If you would like your child to take part, simply sign one copy of the consent form and bring it with you to your child's next visit. You should keep the other signed copy of the consent form and this information sheet for your own records.

What are the possible risks of taking part?

There are no known risks for children participating in the study.

What are the possible benefits of taking part?

Your child will have a very thorough assessment of the possible decay in their back teeth. In addition, participants will be seen for sooner appointments and will be offered more flexibility in choosing appointments because they are being seen by the researcher herself. It is also hoped that the results of this study will improve our way of detecting tooth decay so that preventive treatment can be started sooner. The findings of the study may help us to reduce the need for dental x-rays in the future.

Will my child's taking part in this study be kept confidential?

All information that your child provides us for this study will be kept private.
To protect your child's privacy the following measures will be taken to ensure that no one apart from the main researchers will have access to your child's identity:

- Their name will not appear on any documents. They will be allocated a code number which will be used as identifier. Only the main researchers will know their name and code number.
- Your child's name will not be used in the analysis or writing up of the findings derived from the study. Their details will be kept in a locked cabinet and will only be reviewed by the researchers.

What will happen to the results of the research study?

Following completion of the study we will analyse the findings. The results will be included in the researcher's PhD thesis and will also be published in a scientific journal. We also plan to report our findings at national and international dental conferences so other dentists will benefit from
knowledge gained from this study.

Who is organising and funding the research?

The study has been organised by the unit of Oral Health and Development of the University of Sheffield, UK. Funding has been provided by the University of Sheffield and Dr Samiya Subka is a sponsored student by the Ministry of Education, Government of Libya for the duration of her PhD study.

Who has reviewed the study?

The study design and conduct has been reviewed and approved by the Research Ethics Committee in Sheffield.

What if I wish to complain about the way in which the study has been conducted?

If you have any cause to complain about any aspect of the way you have been approached or treated during the course of this study, the normal complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have any complaints or concerns please contact either the project coordinator: Name: Prof Chris Deery Tel: 0114 271 7885
Otherwise you can use the normal hospital complaints procedure and contact the following:
Name: Mrs Tracey Plant Tel: 0114 271 7832
OR
Otherwise you can use the normal university complaints procedure and contact the following: Name: Research Consultative Unit Tel: 0114 222 1469

What if I am harmed?

If you are harmed by your participation in this study, there are no special compensation arrangements. However, if you are harmed due to someone's negligence, then you may have grounds for legal action.

Who can I contact for further information?

Further information about the study is available from Dr Samiya Subka, unit of Oral Health and Development, School of Clinical Dentistry, Claremont Crescent, Sheffield S10 2TA. Telephone: 0114 271 7877, email: s.subka@sheffield.ac.uk.

Thank you for taking the time to read about the study

What if I don't want to join in anymore?

If you want to stop doing the research at any time, you can stop without giving a reason. Just tell me or your parent/guardian. No one will mind.

What do I do now?

There is a sheet to fill in. If you would like to join in, please tick the box on the sheet and bring it with you when you come to see me.

You might have some more questions to ask me about the research. You can call Samiya Subka on 0114 271 7877. You can call me if you would like to ask me anything. I can call you back if you like. You can e-mail me at: s.subka@sheffield.ac.uk

Thank you for reading this. I hope to meet you soon.

The University Of Sheffield.

What is the best way to find holes in teeth?

What do you think?

Who am I?

My name is Samiya Subka and I work in this hospital.
This is me.

What are we doing?

Research tries to find out the answer to an important question. We are doing some research about the best way to find holes in children's teeth. You have been asked to join in because you have some holes in your teeth and we want to find them all! We hope that it will be fun. We hope you will want to join in, but you do not have to.

What will happen?

If you would like to join in, this is what will happen.

1. You will receive two intra-oral x-rays as part of your normal clinical treatment

2. Then, I would like to look at your teeth with a mirror.

3. Then I will use a special pen that can look through teeth and tell me if they have any holes.

4. After that I will put an elastic band between your teeth.

5. When I see you again after seven days, I would like you to write on some paper to tell me what it was like to have the mirror, X-rays, special pen and elastic bands. It is fine for a grown up to help you with these questions.

6. Then, I will remove the elastic band and look at your teeth again quickly with the mirror and with the special pen.

What happens next?

I will look at all the results, and hopefully find out which is the best way for finding holes in children's teeth, then we can make them better and help children to look after their teeth.

Appendix 4

Participant Identification Number: _____

PARENT/GUARDIAN CONSENT FORM

Title of Project: What is the best way to find tooth decay?

Name of Researchers: Mrs Samiya subka, Professor Chris Deery, Professor Helen Rodd

Please tick box

1. I confirm that I have read and understand the information sheet dated for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected. ☐
3. I understand that all data will be treated confidentially. ☐
4. I agree for my child to take part in the study. ☐

_____	_____	_____
Name of Participant	Signature	Date
_____	_____	_____
Name of researcher	Signature	Date

(Please keep one copy and send one copy back)Fair Processing Notice

Your personal data will be used only in accordance with Sheffield Teaching Hospitals NHS Foundation Trust notification under the Data Protection Act 1998 and in compliance with the Freedom of Information Act 2000. The Trust will not disclose any personal information to any other third parties, except where required by law, without your express consent. Further details in relation to the use of personal data can be found on the Trust's web site http://www.sth.nhs.uk/info-gov/Data%20Protection.htm Any queries concerning Data Protection and Freedom of Information should be addressed to the Information Governance Manager, Sheffield Teaching Hospitals. Telephone 0114 2265153.

Appendix 5

Participant Identification Number: _____

The University Of Sheffield.

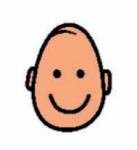
Participant assent form

Have you read the information sheet, or had it explained to you?

YES NO

Have you had time to ask questions and talk about the study?

YES NO



Are you happy with the answers you have been given?

YES NO

Do you understand that it is your choice to take part in the study?

YES NO

Do you understand that you can stop at any time? (You do not have to say why you want to stop.)

YES NO

Are you happy to take part in the study? **YES NO**

Your name……..Date……

Samiya Subka ……..Date……

Appendix 6

Scoring sheet for radiographs

Tooth	Surface	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9
URE	D									
URE	M									
URD	D									
URD	M									
ULD	M									
ULD	D									
ULE	M									
ULE	D									
LLE	D									
LLE	M									
LLD	D									
LLD	M									
LRD	M									
LRD	D									
LRE	M									
LRE	D									

Appendix 7

Scoring sheet for clinical examination (visit 1 & 2)

Clinical examination
Patient ID:
Date of examination:

Tooth	Surface	ICDAS score	LF Pen score
URE	D		
URE	M		
URD	D		
URD	M		
ULD	M		
ULD	D		
ULE	M		
ULE	D		
LLE	D		
LLE	M		
LLD	D		
LLD	M		
LRD	M		
LRD	D		
LRE	M		
LRE	D		

Appendix 8

What is the best way to find tooth decay?

Firstly, we want to learn a little bit about you.

1. I am a Boy Girl

2. I am ☐ **years old**

Next we want to know what you think about the different tests that you had
Please put a circle around your answer

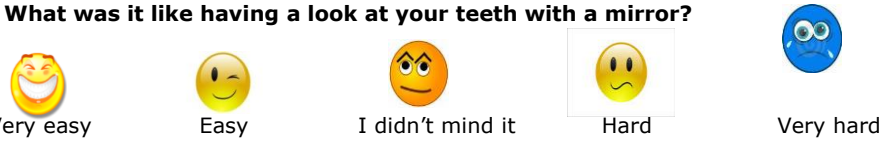
3. What was like having X-rays of your teeth?

Very easy Easy I didn't mind it Hard Very hard

4. How would you feel if you had to have X-rays again?

Very happy Happy I don't mind Unhappy Very unhappy

5. What was it like having a look at your teeth with a mirror?



Very easy Easy I didn't mind it Hard Very hard

6. How would you feel if you had to have the mirror again?

Very happy Happy I don't mind Unhappy Very unhappy

7. What was it like having your teeth tested with the special pen?

Very easy Easy I didn't mind it Hard Very hard

8. How would you feel if you had to have the special pen again?

Very happy Happy I don't mind Unhappy Very unhappy

9. What was it like having the elastic bands on your teeth?

Very easy Easy I didn't mind it Hard Very hard

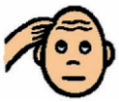
10. How would you feel if you had to have the elastic bands again?

Very happy Happy I don't mind Unhappy Very unhappy

Can you tell us what you think in this box, remember this is NOT a test!

Why is your dentist looking at your teeth?

Is there anything good or bad you want to tell us about your visit?



Thank you for your help! Please put your answers in the box in the waiting room

Appendix 9

Email: s.subka@sheffield.ac.uk

Date: 03/12/2012

Validity and acceptability of a laser fluorescence device for detection of proximal caries in primary teeth

Information for clinical staff in the paediatric dentistry clinic

Dear colleagues,

I am writing to let you know about my PhD research project which seeks to compare different caries diagnostic methods. The project is being supervised by Professor Deery and Professor Rodd. We are trying to find out more about a new diagnostic machine (pen laser fluorescence device, DIAGNOdent pen), used for the diagnosis of proximal caries by comparing the results of this machine to conventional methods of caries diagnosis such as oral examination and bitewing radiographs. The results of different diagnosis methods will be validated histologically.

I will need to recruit around 80 children to the study, aged 5-11 years, and who have caries in one or more primary molars and are going to have one or more primary molars extracted under general anaesthesia. Children with severe learning disabilities who are unable to participate even with additional support from the research group, children who are experiencing symptoms and require extractions as soon as possible, and children with medical conditions which put them at risk when having a dental procedure, such as immunocompromised children and children with heart disease will be excluded from the study. Ethical approval has been granted for this study.

With your permission and support, I would like to attend new patient clinics in order to identify and recruit appropriate study participants. If children meet the inclusion criteria I will book them with myself for any necessary preventive treatments, according to your treatment plan, so that I can conduct the caries diagnosis tests at the same time. The child will then have the extractions according to your treatment plan. Children participating in the study will have a sticker near the treatment plan to remind you to keep the teeth after extraction under GA. Labelled pots will be provided to keep the extracted teeth in.

A parent information sheet is attached to provide you with more information about the study. Please let me, or my supervisors, know if any concerns arise during this project.

Thank you for your time and cooperation

Samiya Subka
BDS, MClin Dent (Paed), PhD student
The Unit of Oral Health and Development.

Appendix 10

What is the best way to find holes in teeth?

Dr Samiya Subka, Professor Helen Rodd & Professor Chris Deery

School of Clinical Dentistry, University of Sheffield, UK

School of Clinical Dentistry, University of Sheffield, UK

Hello, my name is Samiya Subka, I am a dentist and work in this hospital.
This is me!

We are doing a research study to find out the best way of seeing if children have any decay (holes) between their back teeth. We may ask you to join in if you have some holes in your teeth and have had two special pictures of your teeth (called x-rays) taken.

What will happen in this project?

1. First, I would like to look at your teeth with a mirror.

2. Then I will use a special pen that can look through teeth and tell me if they have any holes.

3. After that I will put an elastic band between your teeth so I can see any holes.

I will look at all the results, and hopefully find out which is the best way for finding holes in children's teeth so that we can make them better and children wont get toothache.

We hope that it will be fun and you would like to join in, but you do not have to. Please ask at reception if you would like to know more.

Appendix 11

Scoring sheet for *in-vitro* examination

In-vitro examination
Case number:
Date:

Tooth	Surface	ICDAS score	Four LF pen scores				Highest LF score	Average LF score
URE	D							
URE	M							
URD	D							
URD	M							
ULD	M							
ULD	D							
ULE	M							
ULE	D							
LLE	D							
LLE	M							
LLD	D							
LLD	M							
LRD	M							
LRD	D							
LRE	M							
LRE	D							

Appendix 12

Scoring sheet for histological examination

Tooth	Surface	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9
URE	D									
URE	M									
URD	D									
URD	M									
ULD	M									
ULD	D									
ULE	M									
ULE	D									
LLE	D									
LLE	M									
LLD	D									
LLD	M									
LRD	M									
LRD	D									
LRE	M									
LRE	D									

Appendix 13

Children's acceptability of diagnostic methods for approximal caries detection in primary molars

Subka S, Rodd H.D., Deery C.

Unit of Oral Health & Development, School of Dentistry, University of Sheffield, UK.

Background: Accurate diagnosis of approximal caries in primary molars is difficult. Furthermore, children's acceptability of different diagnostic approaches may vary and negatively impact on the accuracy of the diagnostic test as well as clinician preferences.

Aim: This study aimed to assess the acceptability to children of four different methods of approximal caries diagnosis: visual inspection (ICDAS), intra-oral radiographs, laser fluorescence (DIAGNOdent pen) and temporary tooth separation.

Method: Thirty-five children (19 girls, 16 boys), aged 5-11 years (mean=6.4; SD= 1.39), were asked to complete a short questionnaire using a 5 face pictorial scale ranging from very happy to very unhappy to obtain children's views of the different diagnostic methods.

Results: All children agreed that visual examination was '*not hard'*. However, 12% (n=4) reported radiographs as being '*hard*' or '*very hard'*: 3 of them said they would not be happy to have them again. A quarter (26%; n=9) found the pen to be '*hard or very hard'* and indicated they would not be happy to have it again. With respect to elastic separators, 31% (n=11) stated that they found this '*hard'* or '*very hard'* and 12 children said they wouldn't be happy to have them again. There was no statistically significant difference in the level of discomfort reported according to gender or age ($p>0.05$; chi-squared test).

Conclusion: Poor acceptability of the laser fluorescence pen and temporary tooth separation, which were associated with most discomfort by these participants, may prove a barrier to their routine use for caries diagnosis in young patients.

Appendix 14

In-vivo evaluation of methods of approximal caries detection in primary molars.

Subka S[1], Rodd H.D$_1$., Nugent Z[2], Deery C[1].

[1]Unit of Oral Health & Development, School of Dentistry, University of Sheffield, UK.

[2] Cancercare Manitoba, Manitoba, Canada.

Background: Accurate diagnosis of caries in primary molars is challenging, especially for proximal lesions where direct visual examination is difficult.

Aim: This *in-vivo* study aimed to assess the validity and reproducibility of three methods of approximal caries detection in primary teeth: visual inspection (ICDAS), radiographs, and temporary tooth separation (TTS).

Method: Thirty children aged 5-11 years were recruited. Sixty-nine proximal surfaces were evaluated using: meticulous visual examination (ICDAS) before and after TTS, and radiographic examination. The teeth were subsequently extracted and serially sectioned for histological validation. Kappa was used to assess inter- and intra-examiner reproducibility (10%).

Results: At D_1 (enamel and dentine caries) diagnostic threshold, the sensitivity of radiographic examination, ICDAS visual examination and TTS was 69%, 51%, 78%, respectively. The specificity for all examinations at this threshold was 100%. At D_3 (dentine caries) diagnostic threshold, the sensitivity of the radiographic examination, ICDAS examination, TTS was 72%, 19%, 35% respectively, while the specificity was 100% for both ICDAS examination and TTS, and 92% for radiographic examination. Intra-examiner reproducibility was excellent for both ICDAS examination (K=0.79 at D_1, K=0.96 at D_3), and radiographic examination (K=1 at D_1, K=0.9 at D_3). Inter-examiner reproducibility for ICDAS and radiographic examinations demonstrated substantial agreement at K=0.79, K=0.75 respectively.

Conclusion: For the detection of approximal caries in primary teeth whether the lesion is in enamel or dentine meticulous visual examination should be supported by radiographs. TTS does assist the diagnosis of lesions in enamel but does not add to the diagnostic validity of dentine caries diagnosis.