The association between clinical and radiographic findings in carious primary molar teeth

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“Having a dream is what keeps you alive. Overcoming the challenges makes life worth living.”

Mary Tyler Moore

For my beloved husband, Omda, and my little heroes, Darin & Saif:

Your faith in me was infinite, your support was eternal and your sacrifices were countless.

Having you in my life is the greatest blessing from God.

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ABSTRACT

AIM

This project undertook two cross-sectional studies involving the primary dentition. The aim was:

Study A: to characterise the relationship between the radiographic appearance of early approximal carious lesions and cavitation threshold in primary molar teeth.

Study B: to correlate the radiographic and histological measurements of the Remaining Dentine Thickness (RDT) beneath deep caries lesions in primary molar teeth.

MATERIALS AND METHODS

Patients requiring extraction under general anaesthesia at the Leeds Dental Institute were asked to participate in the study. For Study A, teeth were examined visually (both in-vivo and in-vitro) for presence/absence of cavitation and radiographically according to two radiographic criteria (i) ICDAS radiographic scoring system and (ii) the extent of the lesion as < or > 0.5mm from the enamel-dentine junction (EDJ) into dentine. For Study B, RDT was measured radiographically and histologically (in mm).

RESULTS

For Study A, 72 primary molars with approximal carious lesions extending radiographically into enamel, outer and inner dentine were included. Teeth showed a mixture of first and second primary molars (30 and 42 respectively), maxillary and mandibular teeth (36 teeth each), and mesial and distal lesions (34 and 38 respectively). Regarding radiographic ICDAS, no cavitation was associated with score 0 and 1. For score 2, 3 and 4, cavitation was reported in 11%, 45% and 86% of the cases respectively. According to the radiographic extension from EDJ, there was a statistically significant increase ($p<0.05$, chi-

square) in the probability of cavitation (92%) with the radiographic lesions extended >0.5mm beyond the EDJ compared to the lesions extended < 0.5mm (29%).

For study B, 50 primary molars were collected. Teeth showed a mixture of 21 first and 29 second primary molars of which 23 were maxillary and 27 were mandibular teeth with approximal and occlusal lesions (28 and 22 respectively). Radiographic RDT overestimated the histologic RDT by approximately 0.4 (±0.2) mm. The overestimation was consistent across all primary molars and both proximal and occlusal lesions.

CONCLUSION

This study has given an additional insight into the radiographic interpretation in the primary dentition. It identified noticeable increase in the probability of cavitation when carious lesions extend >0.5mm beyond EDJ. In addition, it showed that digital bitewing radiographs overestimate the remaining dentine thickness in carious primary molars. These are significant findings when considering different treatment options for both early and deep carious lesions in primary molars.

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ABBREVIATION	DESCRIPTION
CPP-ACP	Casein Phosphopeptide- Amorphous Calcium Phosphate
EDJ	Enamel-Dentine Junction
GA	General Anaesthesia
ICDAS	International Caries Detection and Assessment System
LDI	Leeds Dental Institute
NHS	National Health Service
PIS(s)	Patient Information Sheet(s)
R&D	Research and Development
RDT	Remaining Dentine Thickness
REC	Research Ethics Committee
WTBB	Welcome Trust Brenner Building

Chapter 1 Introduction and Literature Review

1.1 Anatomical and physiological characteristics of dental tissue

Prior to investigating any disease process, a thorough understanding of the characteristics of the tissues is required.

1.1.1 General characteristics

Human teeth consist of four major components each with individual properties; enamel, cementum, dentine and pulp (Figure 1.1). Enamel forms the outermost hard tissue of the coronal aspect of the tooth and it is a highly mineralised acellular tissue (Nanci, 2007). Cementum, a mineralised and avascular connective tissue, forms the outermost layer of the apical portion of the root of the tooth and, conjointly with periodontal ligament and alveolar bone, provides tooth-supporting connective tissue which attaches teeth to the bones of the jaw (Nanci, 2007). A less mineralised, avascular, more elastic vital connective tissue, dentine, forms the second layer of both coronal and apical portions of the tooth and is covered by enamel, in the coronal portion, and by cementum in the radicular part of the tooth. Dentine encloses the central part of the tooth, the pulp, which is formed of vital soft connective tissue (Nanci, 2007). The dentine is formed and supported by the dental pulp; therefore, the two tissues are often known as the dentine-pulp complex due to their common origin (Nanci, 2007).

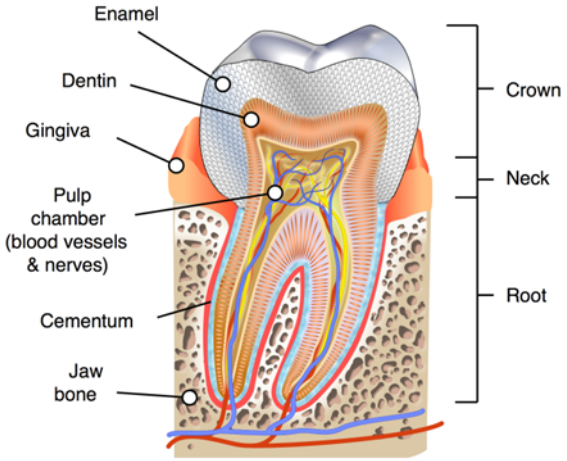


Figure 1-1 Diagram of a healthy human molar showing the enamel, dentine, cementum and pulp
(By KDS4444, Own work [CC BY-SA 4.0 (http://creativecommons.org/licenses/by-sa/4.0)], via Wikimedia Common)

1.1.2 Differences between primary and permanent teeth

Primary teeth demonstrate several chemical, morphological, and physiological differences from permanent teeth (Mortimer, 1970; Sønju Clasen and Ruyter, 1997). These differences may affect the clinical presentation, radiographic appearance and treatment decision of carious primary teeth. Table 1.1 summarises the main differences between primary and permanent teeth.

Table 1-1 Summary of differences between primary and permanent teeth

Characteristics	Difference in primary teeth compared to permanent teeth (Arnim, 1959; Koutsi et al., 1994; Nanci, 2007)
Size	• Smaller buccolingual and mesiodistal dimension compared to permanent dentition.
Colour	• More opaque due to a reduced dentine thickness.
Shape of crown	• Mesiodistal dimension greater than cervicoocclusal dimension. • Buccal and lingual surfaces converge occlusally. • Prominent cervical constriction. • More bulbous crown. • Contact areas are broad and flattened.
Enamel	• Reduced thickness. • Less mineralised. • Greater porosity and diffusion coefficient. • Higher numerical density of enamel rods. • Wider aprismatic zone. • Cervical enamel rods slope occlusally, while in permanent teeth these rods run perpendicular to the long axis of the tooth.
Dentine	• Reduced thickness. • Less mineralised. • Reduced dentinal permeability caused by reduced concentration and diameter of dentinal tubules.
Pulp	• More prominent pulp horns. • Larger pulp: tooth-area ratio.
Root	• Divergent. • Thin and tapered.

Anatomical and physiological characteristics of the coronal hard dental tissue in primary dentition are of particular relevance to this research project, therefore, will be discussed in more detail.

1.1.3 Enamel characteristics and implication in primary dentition

When it is compared to other tissue in the human body, enamel is the most mineralised tissue consisting of approximately 96% inorganic components, in the form of hydroxyapatite crystals (Nanci, 2007). At a macroscopic level, these crystals are packed tightly and extend from the enamel-dentine junction (EDJ) to the enamel surface. The remaining enamel composition consists of water (3.5%) and organic components (0.6%) (Ehrlich et al., 2009) which fill the inter-crystalline spaces (Garnett and Dieppe, 1990).

During enamel development, the ameloblasts which are the cells responsible for enamel formation, move from the EDJ towards the outer surface of the enamel. With the completion of enamel formation, the outer surface of the enamel is covered by ameloblasts which are destroyed during tooth eruption. This process renders enamel an acellular, insensitive tissue and consequently unable to regenerate when destroyed (Nanci, 2007).

Several studies have investigated the mineral content of enamel in primary and permanent teeth using different characterisation methods (He et al., 2011; Shellis, 1984; Targino et al., 2011). It has been reported that sound enamel of primary teeth is less mineralised and more porous compared to permanent successors (Hunter et al., 2000; Lippert et al., 2004; Nanci, 2007). Besides, investigations in healthy primary teeth showed that the mineral content differs between the inner and outer half of enamel within the same tooth (Wong et al., 2004). Further, studies have shown that mineral content of enamel can be affected by different systematic and environmental factors such as environmental trace elements, birth-term, pre- and postnatal complications and systemic diseases (Brown et al., 2004; Lakomaa and Rytömaa, 1977; Rythén et al., 2010). Studies in the primary dentition from different geographical locations showed variation in the environmental trace elements (such

as fluoride, calcium, phosphate, manganese and lead) content of enamel (Brown et al., 2004; Lakomaa and Rytömaa, 1977). Also, it was demonstrated that children born preterm have lower calcium and phosphate and higher carbon concentrations in the enamel compared to full-term children (Rythén et al., 2010). Moreover, systemic diseases such as diabetes, vitamin D deficiency and celiac disease were found to alter mineral content of enamel (Aine et al., 1990; Atar et al., 2007; Koehne et al., 2013). These factors may lead to profound and destructive impacts on developing enamel and alter its potential to de/remineralise.

The enamel of primary teeth was also found to be thinner than that of permanent teeth (Mortimer, 1970). An average thickness of enamel being approximately 0.5-1mm compared to a thickness of 0.5- 2mm in permanent teeth (Nanci, 2007).

From a clinical perspective, these physiological and anatomical features may increase enamel vulnerability to dental caries and wear. In the literature, it was found that caries progression rate from enamel into dentine is faster in primary teeth compared to permanent (Peyron et al., 1992; Vanderas et al., 2003), which could be explained by the above anatomical and physiological differences between both dentitions.

1.1.4 Dentine characteristics and implication in primary dentition

In contrast with enamel, dentine consists of approximately 69% hydroxyapatite and has a higher percentage of organic components occupying the dentinal tubules (Pizzi and Mittal, 2003). Its combined resilient and rigid nature supports the overlying brittle enamel and provides flexibility; thus, improves the tooth's ability to withstand masticatory forces (Nanci, 2007).

Three main types of dentine are identified; primary (pre-eruptive), secondary (post-eruptive) and tertiary (reactionary or reparative) dentine. Tertiary dentine is deposited from the odontoblasts in response to pulpal stimulation and, depending on the origin of the

odontoblasts it may be described as reactionary or reparative. Reactionary dentine is secreted by the original odontoblast cells following injury while reparative dentine is deposited by new odontoblast-like cells following the death of original odontoblast cells (Smith et al., 2003) .

The closely packed dentinal tubules, which contain odontoblastic processes and filled with fluid and organic materials, extend through the entire thickness of dentine. The tubule's pattern follows the course taken by odontoblasts during dentine formation (Chowdhary and Reddy, 2010) and their diameter in addition to their numerical density increases with distance from the EDJ (Sumikawa et al., 1999). The physiological and morphological features of these tubules enhance the hydrophilicity and permeability of the dentine surface (Nanci, 2007). The permeable surface of dentine facilitates diffusion of bacterial and chemical substances across the dentine to the pulp and peri-radicular tissues (Koutsi et al., 1994).

Several investigations reported definitive morphological differences between primary tooth dentine and permanent tooth dentine. The average thickness of dentine in primary teeth (2 mm) is lower than that in permanent teeth (4mm) (Stambaugh and Wittrock, 1977; Sweet, 1949). As a result of this reduced thickness, the pulp horns are more prominent in primary molars, and the pulp: tooth-area ratio is larger (Arnim, 1959).

Moreover, primary teeth present lower numerical tubule densities and wider diameter of dentinal tubules compared to permanent teeth (Sumikawa et al., 1999). The orientation of dentinal tubules also varies between both dentitions. In permanent dentition, dentinal tubules present a curve sigmoid configuration (S-shaped) caused by crowding of the odontoblasts as they move from the EDJ toward the pulp during dentinogenesis (Chowdhary and Reddy, 2010). In comparison, primary tooth dentine showed less curved configuration (Chowdhary and Reddy, 2010). This difference could be a result of two factors; the reduced numerical tubule densities and the wider pulp: tooth-ratio in primary teeth which allow less crowding around the pulp chamber during the movement of

odontoblasts and consequently a straighter course of the tubules (Chowdhary and Reddy, 2010; Sumikawa et al., 1999). The diameter and orientation of dentinal tubules in primary teeth increase its permeability compared to permanent teeth.

Clinically, these variations in dentine features may explain the faster rate of caries progression in primary teeth compared to permanent ones.

1.1.5 Other features

Posterior primary teeth exhibit broader and more flattened contact areas. In non-spaced dentitions, this may complicate the detection of approximal carious lesions, particularly initial enamel lesions (Ribeiro et al., 2015). This feature may explain the reported decreased sensitivity with visual examination alone, compared to a combined visual and radiographic examination, in diagnosing approximal caries in children (Chawla et al., 2012; Novaes et al., 2009; Pitts and Rimmer, 1992). It may also account for the variation between the probabilities of cavitation in previous in-vivo studies in primary teeth (Coutinho and daRocha, 2014; De Araujo et al., 1996; Nielsen et al., 1996; Pitts and Rimmer, 1992).

1.2 Mechanism of caries process

It has previously been proposed that caries is a transmittable and infectious bacterial disease caused by a single organism (Fitzgerald and Keyes, 1960). This proposal does not seem to be true as several studies showed that dental caries is a complex multifactorial process. Numerous factors contribute to the development of caries such as diet, tooth morphology, fluoride exposure, oral microflora, salivary content and flow rate, oral hygiene, and other factors that remain under investigation (Cummins, 2013; Fejerskov, 2004; Punitha et al., 2015). Although the effect of each factor may vary on an individual basis, caries always occurs as a net result of the dissolution of dental tissue caused by the acidic by-products released by cariogenic bacteria (Kidd and Fejerskov, 2004).

Dental caries is a dynamic process of demineralisation and remineralisation of dental hard tissue (Featherstone and Domejean, 2012). With the presence of bacterial biofilm and fermentable carbohydrates, cariogenic bacteria produce acidic by-products in direct contact with the enamel surface. These by-products modify the oral cavity environment and reduce pH to a critical level leading to demineralisation and increased porosity of enamel. These chemical and morphological changes present early stages of dental caries at the outermost layer of enamel and are not detectable with the traditional diagnostic methods (Fejerskov and Kidd, 2009). The continuous presence of demineralisation causative factors without an appropriate intervention will cause continuous loss of enamel minerals and progression of the carious lesion into deeper levels. In addition, persistent demineralisation will increase enamel porosity to the extent that makes the lesion clinically detectable, which is known in the literature as white-spot or early enamel lesion. The lesion usually appears in the plaque retentive areas including occlusal pits and fissures and approximal smooth surfaces.

In posterior primary teeth, an approximal carious lesions develop between the contact area and the gingival margin giving the lesion a kidney-shaped appearance. Some of these lesions may also extend lingually or buccally parallel to the gingival margin which makes them easily detected by visual examination.

Although white-spot lesions demonstrate detectable morphological changes, in most of the lesions the enamel maintains its surface integrity (non-cavitated lesions). Different probabilities of cavitation in association with enamel lesions in the primary dentition have been reported in various studies, with a maximum being 14% (Nielsen et al., 1996).

The probability of surface cavitation increases when the lesion progresses into dentine, this will be discussed further in the next section. Some studies suggested a median time of 2.5 years for the lesion to progress through the enamel (Pitts, 1983). A recent cohort study examined 1969 tooth surfaces in 469 children aged 12-59 months reported that 6-14% of occlusal surfaces exhibited progression of early non-cavitated enamel lesions to dentine cavitated lesions after two years. In comparison, approximal and smooth surface

demonstrated a lower risk of early lesion progression over the same period (Guedes et al., 2016). This period provides an excellent opportunity to intervene with preventive therapies which help to reverse or arrest the lesion.

During the demineralisation stage, calcium and phosphate diffuse out from hydroxyapatite, however, if the acidic environment is buffered the process reverses to enhance uptake of these minerals back into the hydroxyapatite matrix and promote remineralisation (Kidd and Fejerskov, 2004). The process needs four main components to start (Cawson and Odell, 2008):

1. A vulnerable tooth surface.
2. Cariogenic bacteria: Lactobacillus and *Streptococcus mutans (S. mutans)* have been considered the major aetiological agent of dental caries (Soames and Southam, 2005). However, it is now recognised that a more complex community of bacterial species may be involved in the process and these species change with the lesion progression and differ between primary and permanent dentitions (Aas et al., 2008; Takahashi and Nyvad, 2011). In early carious lesions, *S. mutans* forms about 2% of the bacterial population and Actinomyces in combination with non-mutans Streptococci, such as *S. sanguinis, S. salivarius, S. oralis, S. mitis*, form the major bacterial group (Aas et al., 2008; Takahashi and Nyvad, 2011) in both dentitions. However, in these lesions, Corynebacterium *species* and *Actinomyces gerencseriae* were found at high levels in primary dentition whereas a high level of Leptotrichia species, *Campylobacter gracilis,* and Selenomonas species were detected in the permanent dentition (Aas et al., 2008; Takahashi and Nyvad, 2011). In dentinal lesions, the microflora was dominated by *S. mutans,* Lactobacilli, Propionibacterium species and Atopobium genomospecies in both dentitions in addition to Bifidobacterium species in the primary dentition (Aas et al., 2008; Takahashi and Nyvad, 2011). The bacteria survive in a complex microbial matrix (known as a biofilm) consisting of polymers of bacterial and salivary origin. This biofilm attaches

to the tooth surface which is, unlike the epithelium, non-shedding so provides an ideal place for colonisation. These species are able to proliferate and survive in the oral cavity by fermenting carbohydrates and sugars to produce acidic by-products which cause demineralisation (Soames and Southam, 2005).

3. Substrate: different fermentable carbohydrates and sugars, each have different cariogenic potential, are metabolised by bacteria in the dental biofilm. Compared to other sugars, lactose is less cariogenic and sucrose has the ability to support *Streptococcus mutans* to produce extra-cellular glucans that help bacterial accumulation in plaque (Kidd and Fejerskov, 2004). Some synthetic sweeteners (saccharin, aspartame Xylitol and sorbitol) and natural sweeteners (Stevia) are non-cariogenic and can be used as alternatives to cariogenic sugars (Maguire and Rugg-Gunn, 2003; Nayak et al., 2014; Kishta-Derani et al., 2016).
4. Time: for the carious process to start, the biofilm needs a time-span to develop and remain undisturbed.

1.3 The Enamel Lesion

Dental caries can affect the enamel of the tooth at any surface. The most susceptible surfaces are the occlusal fissures and approximal areas which are the least accessible for mechanical removal of plaque. The caries process is not self-limiting and if it continues without intervention will cause mineral loss at nano-proportions or destruction level. Under masticatory force and stress toward the lesion, the demineralised enamel may collapse leading to surface cavitation (Bjorndal, 2002).

Enamel caries can be:

- Rapid/acute: this is characterised by a generalised spread of caries and can be seen, for example, in infants, toddlers or elderly (Curzon and Preston, 2004).
- Slow/chronic: occurs over a long period of time, allowing the pulp to respond by stimulating tertiary dentine formation.

- Arrested: caries is in a static status with no further development.

1.3.1 The sub-clinical lesion

The enamel of primary teeth develops partly during gestation and continues its development after birth. During eruption, the enamel shows high porosity exhibited by the Striae of Retzius and the perikymata grooves which provide diffusion pathways. Once erupting in the oral cavity, the enamel continuously exchanges minerals during demineralisation and remineralisation processes. This exchange starts at the outer surface of the enamel and, if demineralisation predominates, there may be a net loss of minerals from the outer surface of the enamel. This loss increases the enamel porosity leading to further diffusion of bacterial acidic by-products and consequently further dissolution of enamel. After the initial surface reaction, demineralisation extends to the subsurface enamel crystal leaving the outer 20-50 micrometres relatively intact (Ekstrand et al., 1988). This protected surface zone is formed because of the dynamic remineralisation from calcium, phosphate and fluoride from the oral fluid adjacent to the lesion. During remineralisation, and with the presence of fluoride, hydroxyapatite may be replaced by fluorapatite which dissolves at a lower pH level compared to hydroxyapatite; thus, decreases the demineralisation rate (McCann, 1968; Ten Cate and Featherstone, 1991).

1.3.2 The Early Clinical Lesion

The early clinical carious lesion is often described as a cone-shaped white-spot lesion. Following the direction of enamel rods, the apex of the lesion extends towards the EDJ in approximal and smooth surfaces and towards the surface of the enamel in occlusal lesions (Soames and Southam, 2005). The lesion exhibits a series of zones reflecting the level of demineralisation: the surface zone, the body of the lesion, the dark zone and the translucent zone.

1.3.2.1 The surface zone

This zone forms the outermost layer of the enamel lesion with a thickness of 40-50 μm and a pore volume of less than 5% (Pitts, 2016). Although this zone remains relatively normal with visual inspection, it presents surface changes, as described in the subclinical lesion, leading to a rough surface texture detectable by a probe (Pitts, 2016). Being in direct contact with minerals in the oral fluid, this zone is more mineralised than the other lesion zones. It shows a reduction of 9.9% in mineral contents and, compared to other zones, exhibits an unchanged level of magnesium (Pitts, 2016).

1.3.2.2 The body of the lesion

This zone (also known as the subsurface layer) is the second outermost with a thickness of 30μm and shows an increased level of both pore volume, of 10-25%, and demineralisation, of 24% (Pitts, 2016). The Striae of Retzius are more prominent and show a pattern of cross-striation in addition to laminated well-mineralised bands across the body of the lesion (Pitts, 2016). In this zone, magnesium levels reduce to 20% (Pitts, 2016).

1.3.2.3 The dark zone

This zone occurs in 85-90% of the carious lesions. It presents a pore volume of 2-4%, a mineral loss of 6% and a magnesium reduction of 12%. The dark pigmentation was explained to be a result of the arrest of microorganisms which are found within the demineralised enamel (Pitts, 2016).

Caries progression rate appears to be associated with the width of this zone. A chronic caries lesion provides more time for remineralisation compared to a rapidly progressing lesion, therefore, accounting for a wider dark zone (Silverstone, 1973).

1.3.2.4 The translucent zone

This zone forms the innermost layer of the lesion with a width of 5-100μm, it is not always present in the carious lesion. It has a more porous structure, with 1% pore volume, compared to the pore volume of 0.1% in normal enamel (Pitts, 2016). It also presents a

reduced level of magnesium and carbonate and an increased level of fluoride compared to adjacent enamel (Pitts, 2016).

1.4 The Dentine lesion

Untreated enamel caries will eventually progress into deeper layers of enamel and then dentine which will increase the risk of enamel cavitation. In contrast, if preventive interventions are undertaken before cavitation remineralisation of the enamel can occur (Deery, 2013). Dentine appears not be infected until the enamel surface has cavitated (Bjorndal and Kidd, 2005).

The dentine-pulp complex responds to the enamel changes by forming translucent dentine before the lesion reaching the EDJ; however, demineralisation of dentine may not occur until the lesion reaches the EDJ. When the lesion progresses to superficial dentinal layers, there may be a painful pulpal response caused by the odontoblasts being affected by the acidogenic changes. Repeated acid attacks result in a continuous mineral loss, consequently leading to caries progression into deeper layers of dentine. The site of active carious lesions is often found in the periphery along the EDJ.

The dentinal lesion shows four zones arranged in a cone-shaped appearance.

1.4.1 The translucent zone

This zone is the innermost area of the lesion and is referred to as the zone of sclerosis by some authors (Pitts, 2016). It is invariably present and generally has a broader base compared to the sides of the lesion. It was suggested that this zone forms due to a defence reaction from the odontoblasts. Two types of mineralisation have been hypothesised to occur in this zone, both lead to an increased mineral content:

- Acceleration of peritubular mineralisation which plugs the dentinal tubules; thus, slows down the acidic attacks.
- Mineralisation of the odontoblastic processes which occludes the tubules.

Radiographically, this zone shows a radiopaque area below the carious lesion. The lateral deposits have been described as Type 1 Sclerosis and caused by passive deposits. These deposits appear as narrow radiopaque bands of a 100μm width bordering the body of the lesion. Type 2 Sclerosis extends from the body of the lesion to the pulp chamber and presents a greater radiodensity compared to type 1 Sclerosis (Levine, 1974).

1.4.2 Zone of demineralisation

The influx of bacterial acids down the dentinal tubules affects the dentine prior to the microbial invasion. This zone shows dentine demineralisation, yet is not infected. A reduced content by percentage weight of calcium (23.06) and phosphate (11.9) compared to a content of 39.1 and 18.2 respectively in normal dentine was reported (Arnold et al., 2001). This reduction appears to affect intra-tubular dentine more than inter-tubular dentine. The level of mineral content in intra-tubular dentine does not alter the mechanical properties of dentine as this is determined by the inter-tubular structure (Marshall et al., 2001).

1.4.3 Zone of bacterial invasion

Some dentinal tubules become invaded by bacteria, mostly Gram-positive (Frank, 1990). The bacteria multiply within the tubules and may totally occlude them; some may remain vacant. The bacterial attack occurs in stages, and the acidogenic bacteria initially extend to involve both peri-tubular and inter-tubular dentine. Following the acidogenic bacterial attack, further bacterial species invade and multiply within the tubules. These species include proteolytic microorganisms which are accountable for the matrix degradation following demineralisation. Due to the bacterial proliferation and softening of dentine, the tubules expand and form multiple liquefaction foci (Frank, 1990).

1.4.4 Zone of destruction

The increased number and size of the liquefaction foci form transverse clefts perpendicular to the dentinal tubules facilitate the bacterial invasion of the inter-tubular dentine (Frank,

1990). The bacteria may also extend into the lateral branches of the dentinal tubules before infiltrating the inter-tubular dentine. The net result will be a destruction of the normal structure of the tooth and cavitation of the enamel surface (Frank, 1990).

In the rapidly progressing caries, the dentine appears soft and yellowish; however, in the slowly progressing lesions, the dentine shows a brown-black discolouration and a leathery texture.

Similar findings have been found in primary teeth where there are a translucent zone, penetration zone and a zone of destruction; however, mineralisation can take different forms (Johnson et al., 1969). Type I is like normal peritubular dentine, however, sometimes the matrix may contain acid mucopolysaccharides, but the crystals are similar in each. In type II mineralisation, large leaf-shaped crystals, which may be octacalcium phosphate, are formed. These crystals may occlude the dentinal tubules. Type III mineralisation form whitlockite crystals, which is an unusual form of calcium phosphate. These crystals are isodiametric rhombohedral and often combined with bacterial remnants. They partially occlude the tubules and are believed to be formed by the re-precipitation of hydroxyapatite. Type IV is a variant of the three types and may also occlude the tubule to prevent further transmission of acids and proteolytic enzymes.

In both dentitions, dentinal lesions show two distinctly different layers, an infected outer layer and an inner affected layer. The outer layer is highly infected with bacteria, irreversibly denatured and unable to remineralise. In comparison, the inner layer exhibits maintained collagen structure and a potential ability to repair under proper circumstances (Shimizu et al., 1981). Recognising these layers is of potential importance in a clinical setting to avoid over-preparation of cavities. Also, certain restorative techniques require removal of infected dentine only, and this will be explained further in Section 1.7.2. Moreover, adhesive materials depend on dentine hydrophilicity which is provided by the dentinal tubules. Demineralisation and subsequent denaturation of dentine reduce the

number and diameter of dentinal tubules, therefore, minimise the strength of the adhesive bonds between dental materials and dentine (Schilke et al., 2000).

The clinical difference between the affected and infected layers is caused mainly by the amount of collagen denaturation in each layer. Different methods; such as autofluorescence (Banerjee and Watson, 2000) and fixative solutions (Rajan, 2011; Smith et al., 2000) were used to differentiate between histological infected and affected dentine in previous studies. Caries-detection agents such as protein dyes have been used in some studies to distinguish between the two tissues (Dyes, 2000). Yet, evidence has established that these dyes stain the organic matrix of less mineralised dentine, including normal circumpulpal dentine and sound dentine, instead of staining bacteria; therefore, they are unreliable in detecting infected dentine (Dyes, 2000). A considerable body of evidence shows that in the clinical setting conventional visual and tactile assessment provides a satisfactory reflection of dentine status during cavity preparation (Dyes, 2000).

Discrimination between affected and infected dentine layers can be achieved using the sensitive tactile feedback felt by dental excavators (Banerjee and Watson, 2000; Celiberti et al., 2006). The infected dentine is usually soft and deforms easily under light pressure; therefore, it tends to be easily removed with sharp dental excavators (Banerjee and Watson, 2000; Innes et al., 2016). Affected dentine, however, shows some resistance to hand excavation and more pressure needs to be applied to remove it (Banerjee and Watson, 2000; Innes et al., 2016). A thin layer of partially demineralised dentine which forms a transition between infected and affected dentine can be found between the two layers. This dentine is also partially infected and may provide a bacterial passage to the underlying tissue and pulp (Langeland, 1987), therefore, it requires to be removed with a slightly higher pressure than with soft dentine.

1.4.5 Degradation of organic matrix

Dentine matrix is predominantly composed of collagen type 1. Some collagen type V and non-collagenous components, such as proteoglycans, phosphorylated proteins, sialoprotein, osteocalcin, osteonectin and lipids, can also be found in the matrix. Collagen is formed of a triple helix of polypeptide chains with hydroxyproline stabilising its structure. The helices are edged by short, non-helical ends with a length of 300 nm which form fibrils with characteristic banding. These fibrils adhere together to form fibres (van der Rest and Bruckner, 1993). Dentinal collagen can be degraded by proteolytic enzymes such as trypsin at neutral pH (Carmichael et al., 1977).

It was found that dentine should be demineralised before the matrix components could be degraded (Klont and Ten Cate, 1991). Also, the presence of proteolytic activity at a lesion surface enhances the underlying demineralisation process (Kleter et al., 1994).

Dentinal caries can be arrested if the lesion can be accessed for cleaning, fluoride application and self-cleansing with oral fluids (Santamaria et al., 2015).

1.5 Prevalence of dental caries in children

Recent health surveys have shown a general reduction in dental caries affecting permanent teeth in children; however, the disease continues to affect large proportions of children.

A recent health survey in England, Wales and Northern Ireland (Health and Social Care Information Centre, 2015) reported that 31% and 46% of five and eight-year-olds, respectively, experienced obvious dental caries in 2013. The prevalence of untreated dentinal caries in primary teeth was 28% of five-year-olds and 39% of eight-year-olds. This prevalence was lower in permanent teeth and decreased from 2003. The survey also showed that nearly 35% of the parents of fifteen-year-olds experienced an adverse impact on family life and 23% had to take time off work due to their children's oral health. In England, caries experience was also reported in an oral health survey of three-year-olds

children (Public Health, 2014). The survey reported that 12% of children in this age group had obvious dental caries, with an overall prevalence of 4% of Early Childhood Caries (ECC). The survey included only children who attended nurseries, nursery classes attached to schools and playgroups so these results could be biased.

Other international health surveys have reported different prevalence. For example, in the United States (Dye et al., 2015), nearly 37% of children aged 2–8 years had shown dental caries in primary teeth in 2011–2012. Children aged 6–8 years showed a higher prevalence of 56% compared with 23% among those aged 2–5 years. Higher prevalence was reported in some of the developing countries such as Qatar, Nigeria and Saudi Arabia (Al-Darwish et al., 2014; Farooqi et al., 2015; Sofola et al., 2014).

1.6 Diagnosis of dental caries

Caries diagnosis is a three-step process which starts with the lesion detection followed by an assessment of the lesion severity and finally an evaluation of the lesion activity (Ekstrand et al., 2001).

Several methods have been used to detect caries, some of which include: visual/tactile examination with/without teeth separation, conventional radiographic assessment, digital radiography, including the DIAGNOdent, Digora image plate system, digital imaging Fiber-optic Transillumination, Electrical Conductive Fixed Frequency, Light-emitting diode and caries-detector dyes.

Diagnosis of the clinical status of demineralised lesions remains a challenging clinical situation, especially in approximal tooth surfaces.

The performance of any diagnostic method is assessed based on its validity and reliability in detecting a disease (Kyriacou, 2001). Validity is determined by the ability to correctly detect the presence of a disease, which is known as sensitivity, and ability to correctly exclude the presence of the disease, or specificity. Reliability is a measure of consistency

which is evaluated by the ability of the test to reproduce similar results each time it is conducted. The performance of different methods in diagnosing dental caries has been investigated in several studies. Visual examination alone has reported the highest validity in diagnosing early caries involving occlusal surfaces of primary molars (Attrill and Ashley, 2001a; da Silva et al., 2010). In comparison, in approximal carious lesions the highest sensitivity and specificity has been recorded when a combined visual and radiographic assessment was used (Chawla et al., 2012; Novaes et al., 2009; Pitts and Rimmer, 1992).

In deep dentinal caries, diagnosis of different dental tissue length and width is important to provide the appropriate management. Measuring tooth tissue needs some additional diagnostic methods in combination with visual assessment. The radiographic image is considered the most practical adjunct method for this purpose (Wenzel, 2014). For example, the radiographic image has been used to determine the proximity of the lesion from the pulp and to determine the working length in endodontics.

Despite the newer diagnostic methods in dental practice, the routine visual examination and radiographic assessment remain the most accurate, most accessible and least costly tools in diagnosing carious lesions in primary molars (Wenzel, 2014).

1.6.1 Visual examination

Clinical visual assessment is usually the first approach used for caries detection. More than two decades ago concerns were raised about how clinical and epidemiological caries data could be recorded using a valid assessment of the disease status (Pitts, 1993). Different caries detection and assessment systems have since been designed with an aim to provide a standardised visual quantitative method for measuring and scoring dental caries. Table 1.2 summarises the most commonly used systems, their classification, strengths and limitations.

Table 1-2 Summary of the most common visual assessment systems of dental caries (Fisher et al., 2012)

Diagnostic system	Classification	Strengths	Limitations
G.V. Black system	Describes cavitated lesions based on the type of carious tooth (anterior or posterior) and the location of the lesion (occlusal, lingual, buccal).	• Accepted worldwide. • Simple and practical.	• Does not record non-cavitated carious lesions. • Underestimates caries experience.
World Health Organization (WHO) based on the DMF/DMFT index	Describes the tooth as carious if there is unmistakable cavity, undermined enamel, soft surface, temporary filling or sealant with recurrent caries.	• Accepted worldwide. • Simple and practical. • Evidence-based. • Allows for comparison of caries experience between different populations.	• Does not record non-cavitated lesions. • Underestimates caries experience.
International Caries Detection and Assessment System (ICDAS II)	Classifies the entire range of caries from early non-cavitated lesions to severely extensive lesions. Describes coronal caries, root caries and caries associated with restoration/sealant.	• Evidence based. • Valid and reliable in permanent and primary dentition. • Provide a full range of classification based on lesion progression.	• Needs special education and training.
American Dental Association Caries Classification System (CCS)	Classifies the entire range of caries as a process and describes its effect on patient's care.	• Easy to use in clinical practice.	• Has not been validated yet. • Limited data available.
Mount-Hume Classification System	Describes the extent and complexity of a lesion and recommend a conservative restorative approach to preserve tooth structure.	• Simple to use in clinical practice.	• Does not assess lesion activity. • Limited data available.
Site-Stage (SI/STA) Classification System	It classifies lesion according to the site ("SI") and stage ("STA") of the lesion. Suggest some guidance on the choice of restorative approach.	• Simple to use in clinical practice.	• Does not assess lesion activity. • Limited data available.
The Caries Assessment Spectrum and Treatment (CAST) Index	Provides a comprehensive hierarchical assessment index describing the stages of caries progression.	• Allows for easy communication between health professionals. • Was designed based on the strength of the previous indices (ICDAS II, DMF).	• Used only in epidemiologic surveys. • Limited data available.

Other caries detection systems, such as Specific Caries Index, PUFA (pulp-ulcer-fistula-abscess) index, Nyvad system and Nytun system have been designed but remain under investigation (Mehta, 2012; Gimenez et al., 2015).

Some authors suggest teeth separation to aid diagnosis of approximal caries and to reduce the need for radiographic assessment in non-spaced posterior teeth (Coutinho and daRocha, 2014; De Araujo et al., 1996; Pitts and Rimmer, 1992). Although this approach may enhance caries detection, it does not always provide thorough direct visualisation of approximal surfaces; thus, some carious lesions can still be missed. In addition, a higher level of discomfort associated with this approach has been reported in young patients (Novaes et al., 2012; Subka, 2015) which presents a barrier to its use in this group of patients. The most important fact is that even if this approach provides a complete direct visual assessment to identify the presence or absence of cavitation, it does not provide information on the depth of the lesion. Therefore, the radiographic image remains the best adjunct method to diagnose the presence and the depth of approximal lesions.

If early enamel lesions are left untreated, they may extend to the underlying layers of dentine which increases the risk of cavitation. At this stage, the choice of intervention approach requires interpretation of different factors including pulpal status of the tooth. The diagnosis of pulpal status should be based on an integration of several factors such as a history of the lesion, clinical examination, pulp sensibility tests and radiographic assessment. In addition, patient-related factors such as developmental stage of the dentition caries risk assessment, oral hygiene, the patient's cooperation and parent's compliance should also be considered (Rodd et al., 2006). Pulp tests and history of the lesion are subjective indicators for pulp status, especially in very young patients and in paediatric dentistry the diagnosis is mostly based on clinical and radiographic findings (Fuks et al., 2010). In carious primary molars, clinical features such as marginal ridge breakdown and the degree of intercuspal distance involvement are more objective indicators of pulpal status (Duggal et al., 2002).

Remaining Dentine Thickness (RDT) below deep caries is an important indicator that can aid dentists in assessing pulpal status. In primary molars, pulp inflammation was found to be likely with an RDT of less than 1.8mm (Rayner and Southarn, 1979) and the severity of pulp inflammation increases as caries progresses deeper towards the pulp (Kassa et al., 2009). Also, it has been reported that a cavity preparation with an RDT less than 0.25 mm significantly increases pulp inflammatory reaction in the presence of bacteria and significantly reduces the number of odontoblasts which are responsible for reparative dentine formation (Fuks et al., 2010). However, it is still not possible to predict if the inflammation seen is reversible or not based on the RDT.

Measuring RDT cannot be performed by visual assessment and requires additional diagnostic tools. Although technologies, such as ultrasonic micrometry have shown promising results in measuring the RDT clinically (Hatton et al., 1994), the radiographic image remains the most practical diagnostic method to diagnose deep caries in the primary dentition (Rodd et al., 2006; Espelid et al., 2003).

1.6.2 Radiographic assessment

As mentioned earlier, a combined visual examination and radiographic assessment remains the most practical diagnostic method for caries detection in paediatric dentistry, particularly in non-spaced posterior primary teeth. Bitewings are considered as the most appropriate radiographic image for caries detection in posterior teeth (Kidd and Pitts, 1990) however, at least 30-40% mineral loss is needed to enable radiographic detection of the lesion (Whaites and Drage, 2013).

1.6.2.1 Film-based radiography

Before the last decade, film-based dental radiography was mainly used to diagnose dental caries. A non-exposed film contains green silver halide emulsion. Upon exposure, the silver halide crystals become sensitised and form an invisible latent image which needs to be processed to convert into the visible black and white radiographic image (Whaites and Drage, 2013). Film processing starts with converting the sensitised silver ions to black

metallic silver granules by immersing the exposed film in the developer solution (Whaites and Drage, 2013). This is followed by immersing the film in a fixing solution which helps to dissolve and remove unsensitised silver halide crystals to reveal the white part of the image and to harden the emulsion (Whaites and Drage, 2013).

Film-based radiography, however, shows some shortcomings. Image quality can be influenced by light sources in the darkroom, depletion or contamination of developer or fixer solutions, insufficient washing of developer or fixer solutions and many other reasons (Whaites and Drage, 2013).

1.6.2.2 Digital radiography

Digital dental radiography has been used increasingly by dental professionals. In this technique, the conventional film is replaced by a sensor to pick up the image. To date, there are two main types of sensors; solid-state detectors and PhotoStimulable Phosphor storage plates (PSP) (Whaites and Drage, 2013). In the solid-state system, also known as the charge-coupled device, the sensor is connected to the computer by a cable and the image can be viewed immediately on a computer after exposure (Wenzel, 1998). In comparison, PSP is not connected by a cable to a computer, therefore, requires an intermediary stage to read the plate. The time required to read the plate depends on the system being used but typically ranges from 5-100 seconds (Whaites and Drage, 2013). However, PSP are less bulky than solid-state detectors and can be found in standard intraoral sizes; hence, are used more in dental practices. Upon exposure, the phosphor layer stores the X-ray energy that has not been absorbed by the patient (Whaites and Drage, 2013). The plate is then read by scanning with a laser beam which releases the stored energy as visible blue light. This light is then detected by a photomultiplier tube and converted by the computer into a digital image. Finally, the plate is erased to allow reuse.

Digital radiography has a number of advantages compared to film-based radiography. These include (Wenzel, 1998):

- The diagnostic quality of the digital image can be enhanced using the manipulation tools; thus, reduce the number of retakes.
- With the digital image, the processing using chemical solutions is avoided which reduces the time needed to view the image, the environmental problems and the cost of chemicals.
- In digital radiography, the diagnostic accuracy can be achieved with lower radiation doses, up to 50% less, compared to film-based radiography. With this lower dose, radiation exposure from dental diagnosis is reduced further with the consequence that the X-ray tube will last longer.
- The digital image can be saved and retrieved more easily than with film-based images.

Regarding the ability to diagnose dental caries, no statistically significant difference was found between film-based and digital radiography in caries detection both in enamel or dentine (Abesi et al., 2012; Alomari et al., 2015; Nielsen et al., 1996). However, the ability to diagnose caries with the digital image can be enhanced by the type of monitor used for viewing the images. Svanaes et al. 2000 showed that after image enhancement, the use of storage phosphor images improved caries assessment depths in the outer half of enamel compared to film-based images (Svanaes et al., 2000).

Due to its advantages in radiation dose reduction, fast image processing and image quality enhancement, digital radiography has been used widely in dental practices (Mestriner et al., 2005).

1.6.2.3 Measurement of carious lesion

The reliability of the radiographic images in reflecting the real status of demineralised dental tissue has been a question of interest to many researchers. It has been suggested that radiographic images underestimate the actual depth of approximal caries (Jacobsen et al., 2004; Jessee et al., 1998; Kooistra et al., 2005; Syriopoulos et al., 2000). However, a recent review article advised that radiographs can fairly accurately estimate lesion depth when compared to the histological depth of the lesion (Wenzel, 2014).

In general, it was concluded that bitewing radiography detects dentinal lesions better than enamel lesions in both approximal and occlusal surfaces (Attrill and Ashley, 2001a; Braga et al., 2009b; Chawla et al., 2012; da Silva et al., 2010; Nielsen et al., 1996; Pitts and Rimmer, 1992). It has been agreed for decades that when caries extends radiographically beyond the enamel-dentine junction (EDJ), the enamel loses its support from the dentine and collapses under masticatory force. However, recent studies continue to prove that this is inconsistent as dentinal lesions are not necessarily cavitated. With modern preventive dentistry, non-cavitated enamel and dentinal lesions can be successfully managed which preserve the dental structure and reduce the need for surgical treatment (Holmgren et al., 2014; Kielbassa et al., 2009; Martignon et al., 2010). Detection of non-cavitated lesions can be, however, difficult particularly in approximal lesions present in a non-spaced dentition which necessitate the use of bitewings.

The ability of bitewings to provide information about surface status has been investigated previously. The relationship between the radiographic extent of the lesion and cavitation threshold is poorly described in the primary dentition. Previous studies have correlated the clinical cavitation of the lesion to its radiographic progression in enamel and dentine (Table 1.3).

These studies were conducted with an aim to validate different visual scoring systems and assess their abilities of caries detection. For this purpose, these studies used different radiographic scoring systems adapted from Ekstrand's radiographic classification system. The original system has classified lesion depth based on its extension; 0: describes no lesion, 1: lesions into outer half of the enamel, 2: lesions into inner half of the enamel, 3: lesions into outer half of the dentine and 4: lesions into inner half of the dentine (Ekstrand et al., 2007). Some studies used the system without modifications and others collapsed some scores to describe lesions either into enamel or dentine. Most of the studies in this field performed in-vivo investigations (Coutinho and daRocha, 2014; De Araujo et al., 1996; Pitts and Rimmer, 1992) and the results were not validated by an in-vitro assessment which

could underestimate the reported probability of cavitation. Only one in-vitro investigation of extracted primary teeth has been performed to correlate cavitation to the radiographic depth of the lesion using Digora system (Nielsen et al., 1996).

Although the pattern of outcomes among these studies is somewhat different, there is a general agreement that the probability of cavitation is low when the lesion extends into the enamel and increases as the lesions progress into dentine. The studies also agreed that not all dentinal lesions are cavitated. These findings are of diagnostic value, but a better characterisation of radiographic and clinical findings is needed to ensure appropriate treatment is delivered. In the permanent dentition, a more precise description of the relationship between radiographic appearance and clinical status of approximal carious lesions was demonstrated by some authors (Ratledge et al., 2000). A study in permanent posterior teeth examined the probability of cavitation according to the lesion extent from the EDJ (Ratledge et al., 2001). The study found that the probability of cavitation with dentinal lesions extending radiographically > 0.5mm beyond EDJ is significantly higher (93%) than lesions extending < 0.5mm (64%) (Ratledge et al., 2001).

Table 1-3 Summary of previous studies outcomes (cavitation probability with radiographic caries extension)

Study/method of assessment	Lesion (N)	Teeth/Surface	Probability of cavitation
(Pitts and Rimmer, 1992)/ (In-vivo)	380	Primary posterior/ Approximal	• 2% of the lesions in outer half of enamel. • 2.9% of the lesions in inner half of enamel. • 28.4% of the lesions in outer half of dentine. • 95.5% of the lesions in inner half of dentine (50% of any dentine lesions).
(De Araujo et al., 1996)/ (In-vivo)	72	Primary posterior/ Approximal	• None of the lesions in inner half of enamel. • 6% of the lesions in outer half of enamel. • 84% of the lesions in dentine.
(Nielsen et al., 1996)/ (in-vitro)	43	Primary posterior/ Approximal	• 11% of the lesions in outer half of enamel. • 14% of the lesions in inner half of enamel. • 63% of lesions in dentine.
(Coutinho and daRocha, 2014)/ (in-vivo)	335	Primary posterior/ Approximal	• 5.3% of all enamel lesions of the enamel lesions are cavitated. • 30% of the lesions in outer third of dentine. • 34.3% of the lesions in middle third of dentine. • 68.4% of the lesions in inner third dentine were cavitated.

1.6.2.4 Measurement of sound tooth-tissue

The reliability of radiographic images in reflecting the real thickness of sound dental tissue has been studied before. A study in permanent molars showed that both conventional and digital radiographic images overestimate the remaining dentine thickness below deep carious lesion (Lancaster et al., 2011). Further studies demonstrated that radiographic image overestimates tooth wall thickness by approximately 0.38mm in primary and 0.2-0.36mm in permanent posterior teeth (Arastoo and Azadani, 2015; Raiden et al., 2001; Souza et al., 2008).

1.7 Management of dental caries

1.7.1 Management of early non-cavitated carious lesion

With the advancement in caries diagnostic methods and caries preventive strategies, the philosophy of treating early carious lesions has changed to a less surgically orientated approach. The effectiveness of such preventive strategies has been demonstrated in a systematic review (Holmgren et al., 2014).

The presence or absence of clinical cavitation is now the main criteria for the choice of a preventive or preventive and surgical approach. Preventive approaches are beneficial in preserving the tooth surface integrity but require early detection of non-cavitated carious lesions. In the primary dentition, preventive approaches such as tooth-brushing with fluoride toothpaste, dietary advice, application of remineralising agents (such as professional topical fluoride and CPP-ACP) have been found to enhance remineralisation of early non-cavitated lesions (Frencken et al., 2012; Holmgren et al., 2014).

Fissure sealant is another preventive technique which can be applied to seal early occlusal caries in place and prevent its progression into deeper dental layers (Deery, 2013; Uribe, 2006). A higher effectiveness in caries reduction, compared to fluoride varnish, was

reported with fissure sealant in occlusal surfaces (Beauchamp et al., 2008; Hiiri et al., 2010).

Recently, micro-invasive approaches such as caries infiltration and sealing have been introduced clinically to help managing approximal non-cavitated caries lesions in primary and permanent teeth. These techniques aim to seal or infiltrate the lesion hence preventing progression. The benefits of these interventions have been tested in approximal carious lesions in the primary dentition and showed a significant preventive effect compared to non-invasive techniques such as oral hygiene habits and remineralisation agents (Dorri et al., 2015; Martignon et al., 2012).

Once cavitation occurs, other intervention modalities should be considered. Great care should be taken with the diagnosis as misdiagnosing a lesion as cavitated will lead to an unnecessary and irreversible destruction of the tooth tissue.

1.7.2 Management of cavitated carious lesion

Management of cavitated carious lesions, especially those with close association to the pulp, presents a dilemma with respect to the type of restorative intervention that should be used. Historically, operative intervention (e.g., “drilling and filling”) was the only treatment option available to treat cavitated caries regardless of the site or depth of the carious lesions. Complete caries removal may weaken the tooth structure as a result of the additional removal of affected dentine as well as risk the exposure of the pulp chamber. Recently, biological approaches with no or partial caries removal have found wide acceptance with dental practitioners and young patients. These approaches aim to alter the environment surrounding the carious lesion and thus, arrest the lesion. Providing that the tooth is asymptomatic and vital, these approaches were found to be more conservative to the tooth tissues, less traumatic to the pulp and reduce the need for local anaesthetics (Innes and Evans, 2013; Innes et al., 2011; Kidd and Bjorndal, 2015). The success of the biological approaches in primary molars depends on the healing ability of pulp tissue. In the

presence of any sign or symptom of irreversible pulp inflammation or pulp necrosis, more comprehensive approaches such as pulp therapy, pulpectomy or extraction are likely to be the most appropriate choice of treatment. Therefore, the proximity of caries to the pulp chamber and the pulpal status of the tooth must be carefully diagnosed to identify the most appropriate treatment option and to minimise the risk and consequences of treatment failure.

When considering these approaches along the traditional approach, there are five main management strategies to be used in cavitated primary teeth (Innes et al., 2016):

- Complete caries removal (non-selective removal of hard dentine) followed by restoration.
- Partial caries removal followed by restoration. Partial caries removal can be performed using selective removal of soft dentine or stepwise removal of dentine. Stepwise excavation starts selective removal to soft dentine and followed by selective removal to firm dentine 6-12 months later.
- No caries removal, seal the lesion in place with restoration, for example, Hall technique.
- No caries removal, prevention with/without making the lesion cleansable. The tooth may not be restorable. This approach reported good results in arresting caries and high acceptability in young children (Santamaria et al., 2015).
- Extraction.

As described earlier, RDT can influence treatment options and outcomes in different aspects. For example:

- The Hall technique is a biological approach used to arrest caries in cavitated and non-cavitated primary molars. The caries is left in situ and the tooth is restored with a preformed stainless steel crown. This procedure requires vital pulp with no signs or symptoms (clinically or radiographically) of irreversible pulpitis (Innes and Evans, 2014). As described in Section 1.6.1 RDT plays a protective role as an RDT below

0.25mm results in a significant reduction in the number of odontoblasts with subsequent reduction in tertiary dentine formation and increased risk of pulpal irritation and inflammation (Fuks et al., 2010).

- Partial caries removal is used in primary and permanent teeth with deep carious lesions to reduce the risk of pulpal exposure. RDT should be assessed carefully before considering this technique for many reasons. Inappropriate estimation of RDT may lead to pulp exposure during caries excavation. Also, the reduced RDT increases the possibility of pulpal inflammation by excavation trauma and chemical activity of restorative materials (Murray et al., 2003). Also, a reduced dentine thickness reduces dentine permeability; thus, reduces the strength of the adhesive bonds between dental materials and dentine which affect the long-term outcome of the permanent restoration (Schilke et al., 2000).

1.8 Summary of the literature and overall aim

In the literature, studies in the primary dentition have tested the accuracy of the radiographic image in diagnosis of early demineralised lesions (Attrill and Ashley, 2001a; Braga et al., 2009b; Chawla et al., 2012; da Silva et al., 2010; Nielsen et al., 1996; Pitts and Rimmer, 1992). The literature recognises that the probability of cavitation increases when carious lesions extend into the inner enamel and outer dentine, however, there is no clear, evidence-based criteria to assist clinicians when planning treatment for early carious primary molars based on the radiographic appearance of the lesion. A better characterisation was reported in the permanent dentition based on the extension of caries from the EDJ.

In deep carious lesions, the importance of assessing RDT during diagnosis and treatment plan has been described thoroughly (Fuks et al., 2010; Kassa et al., 2009; Rayner and Southarn, 1979). This importance has been concluded from histological studies of RDT. Clinically, the only available practical way to assess RDT is to use a radiographic image.

However, the ability of the radiographic image to estimate RDT under deep carious lesions has not been investigated in primary molars. In the permanent dentition, Lancaster et al., 2011 reported that the radiographic image tended to overestimate the real RDT (Lancaster et al., 2011).

It is inappropriate, however, to assume radiographic and clinical findings from the permanent dentition are applicable to the primary dentition owing to the different anatomy and physiology between both dentitions.

Therefore, this work carried out two studies with an aim to:

- Investigate the relationship between the radiographic appearance of early approximal carious lesions and cavitation threshold in primary molar teeth (Study A).
- Investigate the correlation between radiographic and histological assessment of the Remaining Dentine Thickness (RDT) beneath deep carious lesions in primary molar teeth (Study B).

The purpose of these investigations is to describe how carious lesions and sound tooth structure behave radiographically thus aiding clinicians in diagnosing carious teeth and deciding the appropriate treatment plan.

1.9 Study objectives

The objectives of this study are:

- **Study A**: To describe the relationship between radiographic appearance and clinical status of early interproximal carious lesions in primary teeth.
- **Study B:** To examine the agreement between the histological Remaining Dentine Thickness (RDT) beneath deep caries and its radiographic appearance in primary posterior teeth.

1.10 Null Hypotheses

- **Study A**: For primary molars with early approximal caries, there is no association between radiographic appearance and clinical status of the tooth surface (presence/absence of clinical cavitation).
- **Study B**: For primary molars with deep carious lesions, there is no difference between the radiographic measurements and the histological RDT.

Chapter 2 Materials and Methods

2.1 Ethical considerations

2.1.1 Ethical approval

Ethical (14/ES/1110) and research approvals were successfully achieved in January 2015. Appendices 1-3 provide further details.

2.1.2 Recruitment and consent process

Following referral to LDI, patients attended a consultant clinic for clinical and radiographic examination. For some children following discussions with their parents, a decision was made to provide dental care under general anaesthetic. These children were then placed on a waiting list for care. About 2-3 weeks prior to their general anaesthetic, a confirmation letter of their operation date was sent out to the family. For children meeting the study inclusion criteria, the operation letter included the parent and child information sheets (Appendices 4-6). On the day of the general anaesthetic, children and their parents/legal guardian/carer were approached by the chief investigator (AW). Families who showed interest in participating were given the opportunity to ask further questions. Parents/legal guardian/carer who agreed to participate were consented for the study by signing a "parental consent form" (Appendix 7). Children aged 10 years old and above, were asked for their own consent (Appendix 8), while children aged six but less than ten years were encouraged to give assent (Appendix 9) to participate in the study.

2.2 Study design

This cross-sectional study involved clinical, radiographic and laboratory investigation of extracted carious primary teeth.

2.3 Experimental material

2.3.1 Study population

Primary molars were collected from children undergoing routine dental extractions under general anaesthesia if they met the inclusion criteria. The sample consisted of primary molars with approximal or occlusal carious lesions extending radiographically into enamel and dentine. Figure 2.1 below shows the operating theatre where the teeth were extracted.

Figure 2-1 Operating theatre at the LDI

2.3.2 Inclusion criteria

2.3.2.1 Patient-related criteria

- Children aged between 3-12 years.
- Parents/guardian should give informed written consent before participation.
- Children aged ten years old and older must give consent to participate.
- Children between the age of six-years-old and ten-years-old must give assent to participate.

2.3.2.2 Tooth-related criteria

- Carious primary molars being extracted under general anaesthesia.
- Teeth must have in-vivo digital bitewing radiographs.
- Teeth should be restorable but have not been restored.
- Teeth should show no evidence of radiographic or clinical infection.
- For study A:
    - Radiographic approximal lesions extending into enamel (radiographic ICDAS score 1 and 2) or into the outer or middle third of dentine (radiographic ICDAS score 3 and 4).
    - Clinical evidence of approximal early non-cavitated lesion and cavitated carious lesions, based on in-vitro examination of teeth to be extracted.
- For study B:
    - Radiographic occlusal or approximal lesions extending into the middle or inner third of dentine (radiographic ICDAS score 4 and 5).
    - Clinical evidence of cavitated occlusal or approximal carious lesions.

2.3.3 Exclusion Criteria

Children/teeth not fulfilling the criteria detailed in section 2.3.2.

2.4 Assessment of eligibility

Before recruitment, clinical and radiographic dental records were checked to assess if the tooth and the patient met the inclusion criteria in section 2.3.2. Each eligible tooth was given a study number and a data collection sheet (Appendix 10) was completed for each tooth using the unique study number.

2.5 Methodology

2.5.1 Clinical assessment

For both studies, clinical assessment was carried out before (in-vivo) and after (in-vitro) extraction. Once the child was anaesthetised and before dental extractions, teeth were examined visually. For study A, teeth were scored using clinical ICDAS II scoring system (Table 2.1). This system was used for clinical assessment due to its higher validity and reliability, compared to other caries assessment systems, for approximal carious lesions in primary dentition (Braga, M. et al., 2009; Braga, M.M. et al., 2009; Chawla et al., 2012; Ekstrand, K. et al., 2011; Qudeimat et al., 2016; Shoaib et al., 2009). Before the examination, teeth were cleaned and dried using sterilised gauze swabs. Clinical examination was carried out by using a dental mirror and a ball-ended CPITN C-94 probe (Ash/Dentsply, Weybridge, UK) without applying pressure to detect surface texture. Following extraction, teeth were cleaned, dried using compressed air and visually examined again using a ball-ended CPITN C-94 probe. Data were recorded on data collection sheet (Appendix 12).

For study B, teeth were also examined to assess the presence/absence of signs of infection. In addition, patient's dental records were checked for history of dental pain associated with the tooth to be collected. Where this information was not available in the records, history of pain was taken on the day of the extractions. Pain history, if any, was recorded on data collection sheet (Appendix 10).

2.5.2 Radiographic assessment

Digital bitewing radiographs were retrieved from the patient's dental records which were taken as part of their clinical assessment and diagnosis.

The aim of this study was to use in-vivo radiographs to measure caries extension (study A) and RDT (for study B) as this reflects the real clinical practice. However, it was important to

perform a pilot study to assess if the in-vivo radiographs were consistent with radiographs taken in-vitro which are not subject to patient cooperation and other factors that can affect clinical radiographic images. For this purpose, a second in-vitro digital radiograph was taken for 20% of the teeth (10% for each study) within 3-4 hours following extraction to assess the level of agreement between in-vivo and in-vitro radiographic measurements of both lesion extension (Figure 2.2) and RDT (Figure 2.3). Each tooth was stabilised on a size 2 Vistascan image plate (Dürr Dental, Bietigheim-Bissingen, Germany) using vinyl polysiloxane impression material (Imprep AC, UnoDent, Essex, U.K) and the radiographic cone was oriented perpendicular to the long axis of the tooth (Figure 2.4). The teeth were radiographed using Planmeca Prostyle Intra (Planmeca OY, Helsinki, Finland) at a fixed settings of 60 kVp, 7 mA, 0.125 s and the plates were processed with the VistaScan image plate scanner (Dürr Dental, Bietigheim-Bissingen, Germany). Final radiographic assessment for all samples was performed on in-vivo radiographs based on this pilot work. Measurements for both studies were performed using digital radiography software, Infinitt (INFINITT PACS; INFINITT Healthcare Co. Ltd).

For study A, enamel lesions were scored using ICDAS radiographic scoring system (Table 2.1) which has been described by the ICDAS foundation (Pitts and Ekstrand, 2013). For dentinal lesions, caries progression was assessed according to two radiographic criteria: (i) ICDAS radiographic scoring system and (ii) the extent of the lesion from the enamel-dentine junction (EDJ) into dentine in mm. Dentine lesions were then classified as extending less or more than 0.5 mm from EDJ. This classification was used by Ratledge et al. to characterise the relationship between radiographic caries extension and clinical status of the tooth surface in permanent dentition (Ratledge et al., 2001). Data were recorded on data collection sheet (Appendix 11).

For study B, radiographic RDT was measured in millimetres, from the deepest point of the lesion to the outermost border of the pulp chamber.

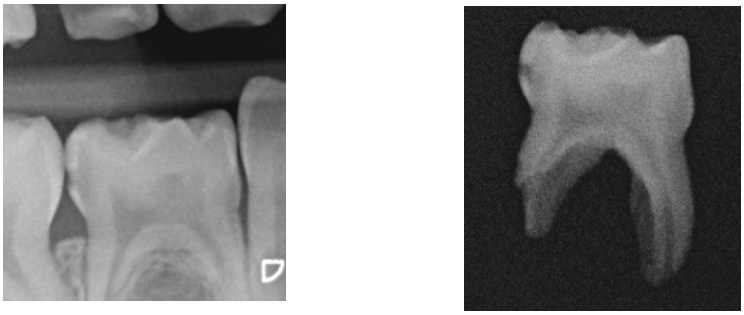


Figure 2-2 In-vivo (Left) & in-vitro (Right) radiograph showing early dental caries

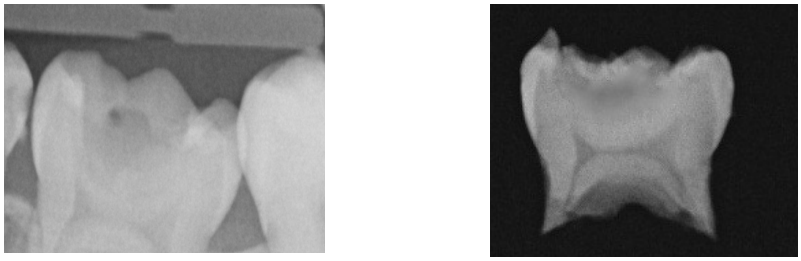


Figure 2-3 In-vivo (Left) & in-vitro (Right) radiograph showing RDT

Figure 2-4 Extracted tooth on image plate. Right: Prostyle Intra machine

Table 2-1 Description of scores used for clinical and radiographic assessment

Score	Visual criteria (ICDAS II) (Pitts et al., 2014)	Radiographic criteria (ICDAS) (Pitts et al., 2014)
0	Sound tooth surface, no evidence of caries after drying.	No radiolucency.
1	Enamel opacity/discolouration, visible after drying.	Radiolucency in outer ½ of the enamel.
2	Distinct enamel changes, visible when wet.	Radiolucency in inner ½ of the enamel.
3	Localised enamel breakdown (e.g cavitation).	Radiolucency in outer 1/3 of the dentine.
4	Underlying dark shadow from dentine with/ without enamel breakdown.	Radiolucency in middle 1/3 of the dentine.
5	Distinct cavity with visible dentine.	Radiolucency in inner 1/3 of the dentine.
6	Extensive distinct cavity (>1/2 the surface) with visible dentine.	Radiolucency into the pulp.

2.5.3 Histological assessment

In this study, radiographic findings were correlated to histological findings to identify how bitewing image reflects the true status of demineralised (study A) and sound (study B) dental tissue.

Immediately following extraction, the buccal surface of each tooth was marked using a nail polish to aid identification of tooth surfaces and was placed in a 20ml plastic container

(Sterilin UK) with distilled water. Each container was labelled by the relevant study number and was kept in a refrigerator at 6°C. Teeth were then transported (in three protective containers) from Leeds Dental Institute to the Oral Biology Laboratory at the St. James's University Hospital. Each tooth was stabilised with wax on the mocrotome block and sectioned into several thin sections in the mesiodistal plane within 24 hours after extraction. Sectioning was performed using a microtome (Accutom-50, Struers, Denmark) at an interval of 0.1 mm (Figure 2.5 & 2.6) and the section with the deepest caries was used for histological measurements. Histological measurements (in mm) were performed using a digital micrometer (Mestra, Bilbao, Spain) (Figure 2.7).

For study A, caries progression was measured in millimetres from the EDJ to the deepest point of the lesion.

For study B, soft/leathery caries was excavated before sectioning using small and medium excavators (Henry Schein, Kent, UK) to the point of firm dentine. A similar method was used in the study in permanent dentition (Lancaster et al., 2011). The section with the deepest lesion (lowest RDT) was used for histological measurements. Histological RDT was measured in millimetres from the deepest point of the lesion to the outermost border of the pulp chamber.

For both studies, histological findings were recorded on data collection sheet (Appendix 13). These findings were set as the gold standard for comparison with radiographic caries extension from EDJ and RDT measurements.

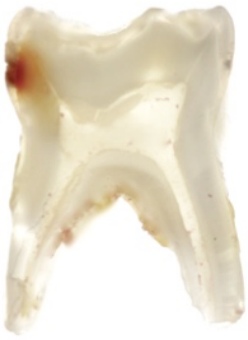


Figure 2-5 Histological section shows early caries extending into dentine

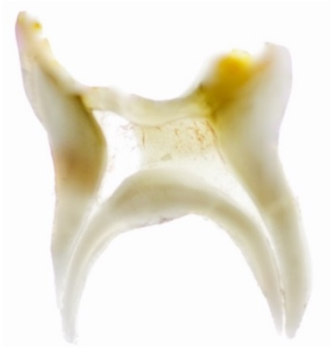


Figure 2-6 Histological section showing RDT

Figure 2-7 Digital micrometer

2.5.4 Training, calibration and reproducibility

The Chief Investigator (AW) performed the clinical, radiographic and histological assessment in addition to the laboratory work. Before the commencement of data collection, training and calibration for the investigator were undertaken in the ICDAS recording, dental radiology and histological sectioning. This is explained further in each section below.

2.5.4.1 Clinical ICDAS II training

The Chief Investigator (AW) watched the 90-minute online ICDAS II E-Learning programme which was developed by the ICDAS Foundation to explain clinical examination protocol and review the coding system. (Topping GVA, Hally J, Bonner B, Pitts NB (2008) International Caries Detection and Assessment System (ICDAS) e-learning Package Interactive CD ROM and Web-based software. Smile-on, London). This e-learning training was followed by three training sessions on both extracted and photographed clinical teeth by my supervisor (DG) who is an expert in ICDAS training and calibration. Due to ethical considerations, clinical in-vivo training was not possible to be performed on real patients. Instead, training was conducted using in-vivo clinical pictures of approximal and occlusal lesions with different clinical presentation (ICDAS II codes 0-6). This method of training was used in similar studies and is considered to be more representative of clinical situations compared to extracted teeth (Nogueira et al., 2017).

One week following training, the chief investigator (AW) had scored ten photographed clinical and ten extracted teeth by the ICDAS II and was calibrated against the supervisor (DG). A month later, the chief investigator (AW) rescored the same samples to assess intra-examiner reproducibility.

2.5.4.2 Radiographic assessment

Training was provided on taking in-vitro radiographs and using the software to take the measurements. Radiographic measurement in mm was performed on ten teeth (five for each study) by the chief investigator and a Specialist Registrar in Dental & Maxillofacial

Radiology. This included measuring caries extension from EDJ for study A and RDT for study B to evaluate the inter-examiner agreement of measuring these variables.

Radiographic ICDAS training was provided by the supervisor (DG). One week following training, the chief investigator (AW) assessed ten teeth using the ICDAS radiographic scoring system and was calibrated against the supervisor (DG). A month later, the chief investigator (AW) rescored the same samples to assess intra-examiner reproducibility.

2.5.4.3 Histological assessment

The Chief Investigator (AW) received training on teeth sectioning using a microtome (Accutom-50, Struers, Denmark). The chief investigator assessed 10 sectioned teeth for the relevant measurements in mm (five teeth for each study). A month later, the same sections were reassessed by the same investigator to evaluate intra-examiner reproducibility.

2.6 Statistical Considerations

2.6.1 Sample size calculations

No previous studies in the primary dentition used assessment criteria similar to those chosen for this study. Therefore, sample size calculation was not possible based on results from previous studies. Statistical advice was sought and it was advised to collect 50 teeth for each study. However, during the period of teeth collection, more teeth were available to be used for study A (72 teeth).

2.6.2 Statistical analysis

All data, except for weighted Kappa, were analysed using SPSS software (Statistical Package for Social Sciences) v22.0 (SPSS Inc. Chicago, USA). Linear weighted Kappa was calculated using a free online calculator (Lowry, 2004).

Graphs and charts were produced using SPSS 22 or Microsoft Excel v15.31 (Microsoft Corporation, USA). Data were assessed for normality before statistical analysis.

2.6.3 Statistical tests

2.6.3.1 Demographic data

Descriptive statistics were used to analyse distribution and frequency of demographic data.

2.6.3.2 Assessment of agreement and reproducibility

2.6.3.2.1 Continuous variables

Intraclass Correlation Coefficient (ICC) was calculated (Koo and Li, 2016) to assess reproducibility and agreement between continuous measurements.

Level of agreement was determined as (Portney and Watkins, 2000)

- ICC < 0.5 indicates poor agreement.
- ICC 0.5-0.75 indicates moderate agreement.
- ICC 0.75-0.9 indicates good agreement.
- ICC > 0.90 indicates excellent agreement.

In addition, Bland-Altman plots were generated to show agreement level between continuous tested variables graphically (Altman and Bland, 1983).

2.6.3.2.2 Categorical variables

Linear weighted Kappa (Cicchetti and Allison, 1971) was used to assess reproducibility and agreement between categorical variables :

Level of agreement was determined as (Fleiss, 2003):

- Kw < 0 indicates poor agreement.
- $0 \leq Kw \leq 0.2$ indicates slight agreement.
- $0.2 < Kw \leq 0.4$ indicates fair agreement.
- $0.4 < Kw \leq 0.6$ indicates moderate agreement.
- $0.6 < Kw \leq 0.8$ indicates substantial agreement.

- 0.8 < Kw ≤ 1.0 indicates excellent agreement.

2.6.3.3 Analysis related to study A

2.6.3.3.1 Correlation between cavitation and radiographic caries extension from EDJ

A chi-square test for association was conducted between the radiographic extension of the lesion from EDJ (as > or < 0.5 mm) and surface status (as cavitated or non-cavitated). A p value of less than 0.05 indicates significance association between both variables (Petrie and Sabin, 2013).

2.6.3.3.2 Correlation between cavitation and radiographic caries extension from EDJ according to tooth type, lesion site and arch

Cochran-Mantel-Haenszel test was performed to assess is the relationship between cavitation and extension of caries from EDJ (as > or < 0.5 mm) is affected by tooth type, site of the lesion and arch.

2.6.3.3.3 Correlation between cavitation and radiographic ICDAS

A Cochran-Armitage test of trend was run to determine whether a linear trend exists between the radiographic ICDAS scores and the proportion of clinical cavitation. A p-value < 0.05 supports that there is an increasing trend in binomial proportions across all levels of the independent variable (Agresti, 2013)

2.6.3.3.4 Sensitivity and specificity of diagnostic methods

Sensitivity and specificity were calculated using the following formula:

Sensitivity (%) = True positive values/ (True positive values + False negative values).

Specificity (%)= True negative values/ (False positive values + True negative values)

2.6.3.4 Analysis related to study B:

2.6.3.4.1 Consistency of the difference between radiographic and histological RDT

Mann-Whitney U test was run to assess the consistency of the difference between radiographic and histological RDT among maxillary and mandibular teeth, first and second molars and occlusal and approximal lesions. A p-value of more than 0.05 indicates no

statistically significantly different between both groups (Agresti, 2013; Petrie and Sabin, 2013).

2.6.3.4.2 Relationship between pain history and radiographic RDT

A binomial logistic regression was performed to assess the relationship between the history of dental pain and the radiographic extension of the lesion. A p-value of less than 0.05 indicates significance association between both variables.

Chapter 3 RESULTS

3.1 Study A

3.1.1 Experimental material

3.1.1.1 Patient-related variables

Teeth were collected from 31 patients, 18 females and 13 males aged 5-12 years. Age was non-normally distributed (Shapiro-Wilk, $p<0.05$) with a median of 8.6 and interquartile range (IQR) of 6.9-9.7.

3.1.1.2 Tooth-related variables

Experimental material was collected over ten months between January 2016 and February 2017. The sample consisted of 72 primary molars with approximal carious lesions extending into enamel and dentine. Teeth were a mixture of first and second primary molars (30 and 42 respectively), maxillary and mandibular teeth (36 teeth each), and mesial and distal lesions (34 and 38 respectively). The distribution of teeth type and arch is shown in Table 3.1.

Table 3-1 Teeth distribution

Tooth	Lesion site		Total
	Mesial	Distal	
Maxillary First molar (UD)	3	10	13
Maxillary Second molar (UE)	15	8	23
Mandibular First molar (LD)	3	14	17
Mandibular Second molar (LE)	13	6	19
Total	34	38	72

Out of the 72 teeth, 50 teeth showed dentine caries and 22 showed enamel caries. Teeth with dentine lesions demonstrated a radiographic extension from EDJ into dentine ranging from 0.25 to 1.15 mm and a histological extension ranged from 0.55 to 1.45 mm. Both variables showed non-parametric distribution (Shapiro-Wilk, $p<0.05$) with a median of 0.5 (IQR 0.4-0.8) for radiographic extension and a median of 0.85 (IQR 0.7-1.0) for histological extension (Figure 3.1).

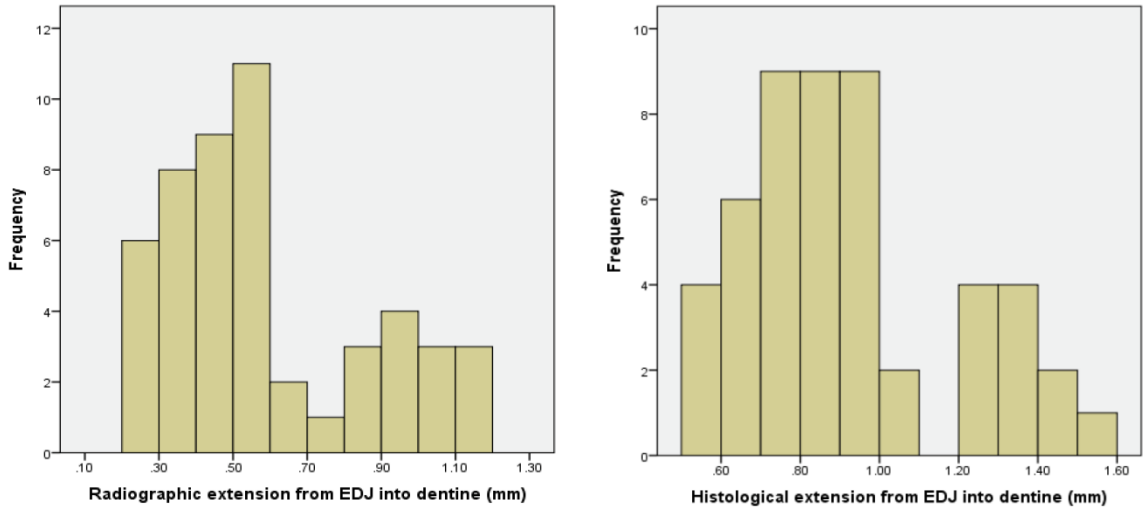


Figure 3-1 Distribution of radiographic (left) and histological (right) extension from EDJ into dentine (n=50)

3.1.2 Assessment of agreement and reproducibility

3.1.2.1 Continuous measurements

Assessment of both inter-examiner agreements of measuring radiographic caries extension from EDJ and intra-examiner agreement of measuring histological caries extension from EDJ was carried out using 10% of the samples. Also, the agreement between in-vivo and in-vitro radiographic caries extension from EDJ was assessed using 10% of the samples.

The difference between the compared variables was normally distributed (Shapiro-Wilk, p> 0.05). A summary of the mean differences of these variables is presented in Table 3.2.

Table 3-2 Mean differences between the continuous variables

Variable	Mean difference	SD	95% CI	
			Lower	Upper
Inter-examiner measurements of radiographic caries extension (mm)	-0.05	0.26	0.006	0.188
Intra-examiner measurements of histological caries extension (mm)	0.02	0.11	0.007	0.235
In-vivo and in-vitro radiographic caries extension (mm)	-0.054	0.14	-0.224	0.116

The level of agreement between these variables was assessed using ICC. The test (Table 3.3) showed a good degree of inter-examiner agreement of measuring radiographic caries extension and an excellent degree of intra-examiner agreement of measuring histological caries extension. An excellent level of agreement was also found between in-vivo and in-vitro radiographic caries extension from EDJ.

Table 3-3 ICC outcomes for continuous variables

Variable	ICC	95% CI	
		Lower	Upper
Inter-examiner agreement of measuring radiographic caries extension	0.856	0.595	0.985
Intra-examiner agreement of measuring histological caries extension	0.973	0.751	0.997
Agreement between in-vivo and in-vitro radiographic caries extension	0.965	0.739	0.996

3.1.2.2 Categorical measurements

Before commencing the study, intra- and inter-examiner agreement of clinical and radiographic ICDAS scoring systems was assessed using linear weighted Kappa. Table 3.4 summarises the results of weighted Kappa for all scoring systems. The table shows an excellent level of intra- an inter-agreement for clinical scoring and a substantial level of intra- and inter-examiner agreement for radiographic scoring.

Table 3-4 Linear weighted Kappa of intra and inter-examiner agreement for clinical ICDAS II scoring and radiographic ICDAS scoring

Variable	Weighted Kappa	Standard error	95% CI	
			Lower limit	Upper limit
Intra-examiner (clinical photographs)	0.927	0.07	0.789	1.0
Inter-examiner (clinical photographs)	0.893	0.06	0.768	1.0
Intra-examiner (extracted teeth)	0.947	0.05	0.854	1.0
Intra-examiner (extracted teeth)	0.859	0.09	0.678	1.0
Intra-examiner (radiographic ICDAS)	0.726	0.13	0.476	0.976
Inter-examiner (radiographic ICDAS)	0.792	0.091	0.614	0.969

3.1.3 Main outcomes

3.1.3.1 Correlation between cavitation and radiographic caries extension from EDJ

Fifty teeth with dentine lesions were included in this part of the analysis. The overall distribution of cavitation (based on in-vitro clinical examination) according to the radiographic lesion extension is illustrated in Table 3.5 and Figure 3.2.

Table 3-5 Distribution of surface status (in-vitro) and radiographic lesion extension (< or > 0.5 mm) from EDJ into dentine

		Surface status		Total
		Cavitated	Non-cavitated	
Radiographic extension from EDJ into dentine	**< 0.5 mm**	7	17	24
	> 0.5 mm	24	2	28
Total		31	19	50

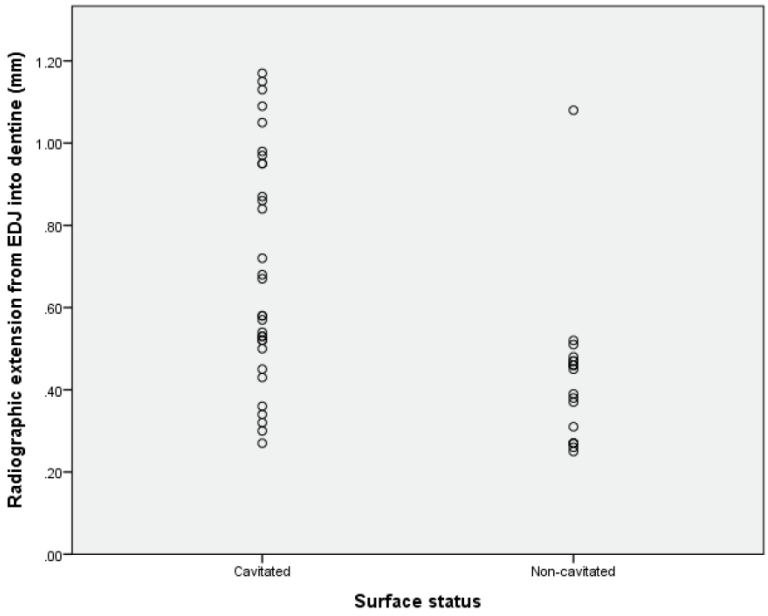


Figure 3-2 Distribution of surface status and radiographic lesion extension (in mm) from EDJ into dentine

A chi-square test for association was conducted between the extension of the lesion from EDJ (as > or < 0.5 mm) and surface status (as cavitated or non-cavitated). There was a statistically significant association between the extension of the lesion from EDJ and cavitation status ($p < 0.05$, Chi-square).

For lesions extending less than 0.5 mm radiographically 29% of lesions were cavitated while for lesions extending more than 0.5 mm 92% were cavitated.

3.1.3.2 Correlation between cavitation and radiographic caries extension from EDJ according to tooth type, lesion site and arch

Further analysis was performed to predict the effect of tooth type, lesion site and arch on the relationship between cavitation and radiographic caries extension from EDJ. Only teeth with dentine lesions, 50 teeth, were included in this part of the analysis. The distribution of cavitation and radiographic caries extension from EDJ according to these variables is shown in Table 3.6.

Cochran-Mantel-Haenszel test was performed to assess the relationship between cavitation and extension of caries from EDJ based on tooth type, site of the lesion and arch (Table 3.7). The test suggested that the association between cavitation and caries extension from EDJ remains significant regardless of the tooth type, site of the lesion and arch.

Table 3-6 Distribution of cavitation and radiographic caries extension according to tooth type, lesion site and arch

Variable		Extension from EDJ	Surface status		Total
			Cavitated	Non-cavitated	
Tooth type	**First primary molar**	**Less than 0.5mm**	4	8	12
		More than 0.5mm	11	1	12
	Second primary molar	**Less than 0.5mm**	3	9	12
		More than 0.5mm	13	1	14
	Total		31	19	50
Site of lesion	**Mesial**	**Less than 0.5mm**	3	5	8
		More than 0.5mm	12	1	13
	Distal	**Less than 0.5mm**	4	12	16
		More than 0.5mm	12	1	13
	Total		31	19	50
Arch	**Maxillary**	**Less than 0.5mm**	5	5	10
		More than 0.5mm	18	1	19
	Mandibular	**Less than 0.5mm**	2	12	14
		More than 0.5mm	6	1	7
	Total		31	19	50

Table 3-7 Cochrane-Mantel-Haenszel test between cavitation and extension of caries from EDJ based on tooth type, lesion site and arch

Variable	OR	Significance (p value)	
		Test of homogeneity	Test of conditional independence
Tooth type	0.35	0.741	<0.05
Arch	0.41	0.699	<0.05
Lesion site	0.036	0.735	<0.05

3.1.3.3 Correlation between cavitation and radiographic ICDAS

All of the 72 teeth which were collected for study A were included in this part of the analysis. Radiographic ICDAS scoring was compared to the clinical status of the tooth (as cavitated or non-cavitated) based on in-vivo and in-vitro clinical assessment.

Distribution of cavitation (based on in-vivo and in-vitro clinical examination) according to the radiographic ICDAS scoring is shown in Table 3.8.

Table 3-8 Cross tabulation of radiographic ICDAS scoring and surface status (based on in-vivo and in-vitro clinical examination)

		Surface status			
		Cavitated		Non-cavitated	
		In-vivo	In-vitro	In-vivo	In-vitro
Radiographic ICDAS score	0	0	0	6	6
	1	0	0	7	7
	2	0	1	9	8
	3	10	13	19	16
	4	13	18	8	3
Total		23	32	49	40

In-vivo clinical examination showed no cavitation associated with radiographic scores 0, 1 and 2. However, for scores 3 and 4 the percentages of cavitation were 34.5% and 62% respectively.

Similarly, in-vitro clinical examination showed no cavitation associated with radiographic scores 0 and 1. However, for scores 2, 3 and 4 the percentages of cavitation were 11%, 45% and 86%, respectively which are higher than that reported by in-vivo clinical assessment.

In addition, a Cochran-Armitage test for trend was calculated to determine whether a linear trend exists between the radiographic ICDAS scores and the proportion of clinical cavitation (based on in-vitro clinical assessment). The test showed a statistically significant linear trend, $p<0.05$, with higher radiographic ICDAS scores associated with a higher proportion of cavitation.

3.1.4 Additional outcomes

3.1.4.1 Performance of visual and radiographic assessment in detecting approximal carious lesions (as present/absent)

In this study, it was of interest to assess the validity of in-vivo visual and radiographic assessment in detecting approximal carious lesions in primary molars (as present/absent). This was assessed at different lesion thresholds (non-cavitated and cavitated) using in-vitro visual assessment as a reference standard. In addition, the validity of in-vivo visual assessment in diagnosing the real status of the surface (as cavitated/non-cavitated) was evaluated.

Validity of visual assessment:

Distribution of carious lesions based on in-vivo and in-vitro visual assessment is summarized in Table 3.9. When assessing the validity of visual assessment in detecting the

presence/absence of the lesion the table shows that, compared to in-vitro visual assessment:

- Overall, ten out of the 72 approximal lesions were missed when the teeth were examined in-vivo.
- At non-cavitated level, nine out of the 40 non-cavitated lesions were missed when they were examined in-vivo.
- At cavitation threshold, one out of the 32 lesions was missed when the teeth were examined in-vivo.

Table 3-9 Cross tabulation of in-vivo visual and in-vitro visual assessment

Diagnostic method		Carious status (in-vitro visual assessment)			Total
		Sound	Non-cavitated	cavitated	
In-vivo visual assessment	**Sound**	0	9	1	10
	Non-cavitated	0	31	8	39
	Cavitated	0	0	23	23
	Total	0	40	32	72

Validity of radiographic assessment:

Distribution of carious lesions based on radiographic and in-vitro visual assessment is summarized in Table 3.10. When assessing the validity of radiographic assessment in detecting the presence/absence of the lesion the table shows that, compared to in-vitro visual assessment:

- Overall, six out of the 72 approximal lesions were missed when the teeth were examined in-vivo.

- At non-cavitated level, six out of the 40 non-cavitated lesions were missed when they were examined in-vivo.
- At cavitation threshold, none of the lesions were missed radiographically.

Table 3-10 Cross tabulation of radiographic and in-vitro visual assessment

Diagnostic method		Carious status (in-vitro visual assessment)			Total
		Sound	Non-cavitated	cavitated	
Radiographic assessment	**Sound**	0	6	0	6
	Radiolucent	0	34	32	66
	Total	0	40	32	72

Validity is usually evaluated by reporting both sensitivity (ability to correctly detect a disease) along with specificity (ability to correctly exclude a disease).

The sensitivity of both visual and radiographic assessment in detecting approximal caries lesions is summarised in Table 3.11. The table shows overall sensitivity in addition to sensitivity at different lesion thresholds (Non-cavitated and cavitated lesions).

As described earlier, specificity evaluates the ability of a diagnostic method to correctly exclude the disease. To apply this here, the disease is the presence of a carious lesion. Since all surfaces are carious (based on in-vitro assessment) it would be unreliable to evaluate specificity.

Table 3-11 Sensitivity of in-vivo visual assessment and radiographic assessment at different lesion threshold

Diagnostic method		Sensitivity (%)
Visual assessment	Overall	86
	Non-cavitated lesions	78
	Cavitated lesions	97
Radiographic assessment	Overall	92
	Non-cavitated lesions	85
	Cavitated lesions	100

Table 3.11 shows that:

- The sensitivity of radiographic assessment is higher than that of in-vivo visual assessment at all lesion thresholds.
- The overall sensitivity of radiographic image (92%) was higher compared to in-vivo visual assessment (86%).
- At early non-cavitation threshold, radiographic assessment showed higher sensitivity than in-vivo visual (85 and 78% respectively).
- At cavitated lesion threshold, radiographic and in-vivo visual assessment showed approximately similar sensitivity (100 and 97% respectively).

3.1.4.2 Performance of visual assessment in diagnosing approximal carious lesions (as cavitated/non-cavitated)

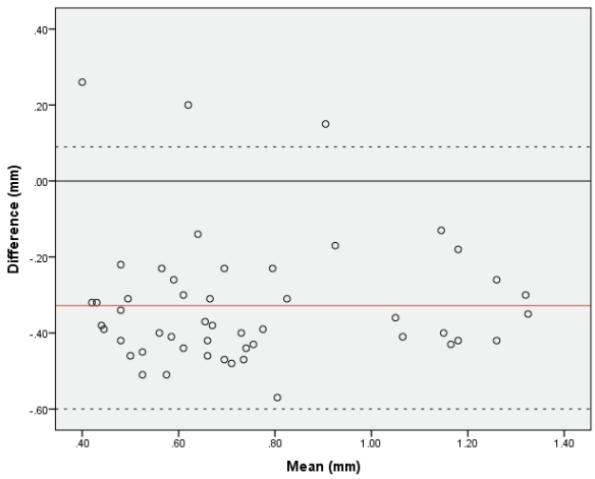
In addition, the validity of in-vivo clinical assessment in assessing surface status (as cavitated or non-cavitated) was evaluated. This was performed by calculating both specificity (ability to correctly detect cavitation) and sensitivity (ability to correctly exclude cavitation). Table 3.9 shows that:

- All in-vitro non-cavitated lesions were recorded sound or non-cavitated when they were examined in-vivo.
- Out of the 32 cavitated lesions, nine were recorded as non-cavitated or sound in-vivo.
- The sensitivity of in-vivo visual assessment in diagnosing cavitated lesions was 72% and specificity was 100%.

3.1.4.3 Reliability of radiographic image in measuring dentinal caries extension in mm

ICC and Bland-Altman plots were utilised to assess the agreement between in-vivo radiographic and histological caries extension from the EDJ for the 50 samples of dentinal lesions. The difference between in-vivo radiographic and histological caries extension from EDJ was normally distributed (Shapiro-Wilk, p=0.09). Radiographic caries extension was less than histological extension by a mean of -0.33 ± SD 0.17 mm.

A poor agreement was found between the two measurements with an ICC of 0.490, a 95% CI from -0.087 to 0.807 ($p<0.05$). Bland-Altman plot (Figure 3.3) showed that the difference 0.15 mm, 0.2 mm and 0.26 mm fall outside the limits of agreement. All other values lie within limits of agreement and below the zero line.



Solid red line: the bias line at -0.33 mm.

Solid black line: Zero difference.

Dashed lines: limits of agreements

Figure 3-3 Bland-Altman plot representing the agreement between radiographic and histological caries extension (mm). N=50

3.2 Study B

3.2.1 Experimental material

3.2.1.1 Patient-related variables

Teeth were collected from 22 patients, ten females and 12 males aged 5-12 years. Age was non-normally distributed (Shapiro-Wilk, $p<0.05$) with a median of 7.7 and IQR of 6.5-9.7.

3.2.1.2 Tooth-related variables

Experimental material was collected over ten months between January 2016 and February 2017. The sample consisted of 50 primary molars with approximal and occlusal carious lesions extending into dentine. Teeth were a mixture of 21 first and 29 second primary molars of which 23 were maxillary and 27 were mandibular teeth with approximal and occlusal lesions (28 and 22 respectively). The distribution of teeth type, arch and lesion site is illustrated in Table 3.12.

Table 3-12 Teeth distribution (numbers)

Tooth	Lesion site		Total
	Occlusal	Approximal	
Maxillary first molar (UD)	2	9	11
Maxillary second molar (UE)	5	7	12
Mandibular first molar (LD)	5	5	10
Mandibular second molar (LE)	10	7	17
Total	22	28	50

The 50 teeth showed a radiographic RDT from 0.4 to 1.6 mm and histological RDT from 0.2 to 1.2 mm. Both variables showed non-parametric distribution (Shapiro-Wilk, $p<0.05$) with a median of 0.9 (IQR 0.8-1.2) for radiographic RDT and a median of 0.5 (IQR 0.4-0.8) for histological RDT (Figure 3.4).

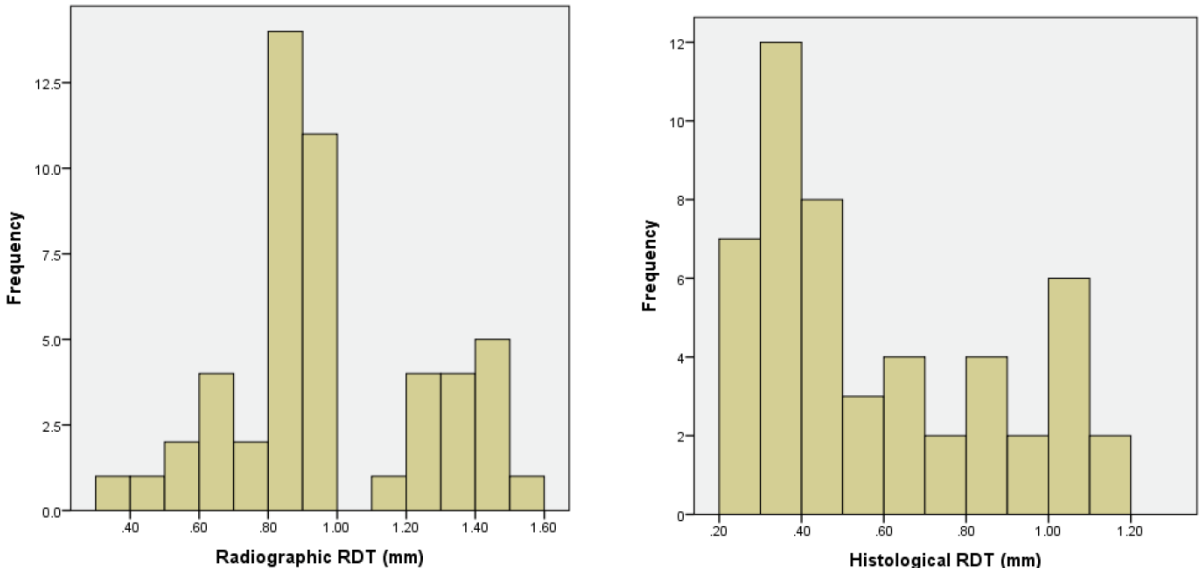


Figure 3-4 Distribution of radiographic (left) and histological (right) RDT (n=50)

3.2.2 Assessment of reproducibility

Assessment of inter-examiner agreement of measuring radiographic RDT, intra-examiner agreement of measuring histological RDT and agreement between in-vivo and in-vitro radiographic RDT was undertaken using 10% of the samples. The difference between the compared variables was normally distributed (Shapiro-Wilk, $p > 0.05$). A summary of the mean differences of these variables is presented in table 3.13.

Table 3-13 Mean differences of variables

Difference	Mean difference	SD	95% CI	
			Lower	Upper
Inter-examiner measurements of radiographic RDT (mm)	-0.142	0.18	-0.366	0.082
Intra-examiner measurements of histological RDT (mm)	0.03	0.19	-0.210	0.270
In-vivo and in-vitro radiographic RDT (mm)	-0.064	0.125	-0.219	0.1

The level of agreement between these variables was assessed using ICC. The results (Table 3.14) showed a good degree of inter-examiner agreement of measuring radiographic RDT and an excellent degree of intra-examiner agreement of measuring histological RDT. An excellent level of agreement was also found between in-vivo and in-vitro radiographic RDT.

Table 3-14 ICC outcomes of variables

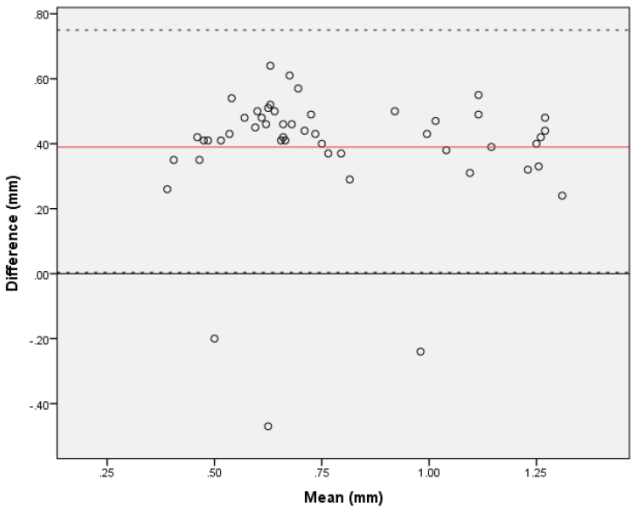
Agreement	ICC	95% CI	
		Lower	Upper
Inter-examiner measuring radiographic RDT	0.718	-0.466	0.968
Intra-examiner measuring histological RDT	0.904	-0.038	0.990
In-vivo and in-vitro radiographic RDT	0.914	0.384	0.991

3.2.3 Main outcomes

3.2.3.1 Agreement between radiographic and histological RDT

This agreement was assessed for all the 50 teeth that were included in study B. The difference between in-vivo radiographic and histological measurements of RDT was normally distributed (Shapiro-Wilk, p> 0.05) with a mean of 0.39 ± SD 0.197 mm.

ICC reported poor agreement with the single measures ICC of 0.405 and a 95% CI from -.087 to 0.749. Figure 3.5 shows that most values (47 out of 50) lie above zero and the values -0.2, -0.24, -0.47 lie below zero line and outside the limits of agreement.



Solid red line: the bias line at 0.39 mm.

Solid black line: Zero difference.

Dashed lines: limits of agreements.

Figure 3-5 Bland-Altman of the agreement between radiographic and histological RDT (mm). N=50

3.2.3.2 Consistency of the difference between radiographic and histological RDT

Mann-Whitney U test was calculated to assess the consistency of the difference between radiographic and histological RDT among maxillary and mandibular teeth, first and second molars and occlusal and approximal lesions. Table 3.15 summarises the outcome of Mann-Whitney U test for all tested variables. The results show that the median difference between

radiographic and histologic RDT was not statistically significantly different between the tested groups (p> 0.05).

Table 3-15 Mann-Whitney U test of the difference between radiographic and histological RDT according to tooth type, lesion site and arch

Variable		N	P
Tooth type	First primary molar	21	0.431
	Second primary molar	29	
Site of lesion	Approximal	28	0.930
	Occlusal	22	
Arch	Maxillary	23	0.202
	Mandibular	27	

3.2.3.3 Relationship between pain history and radiographic RDT

The distribution of pain history according to the tooth type, lesion site and arch is illustrated in Table 3.16. A binomial logistic regression was performed to assess the relationship between radiographic caries extension and dental pain history. There was no significant association between radiographic caries extension and history of pain (OR= 1.33, 95% CI= 0.299-47.397, P=0.3).

Table 3-16 Distribution of pain history according to tooth type, lesion site and arch

Variable		Pain history		Total
		Yes	No	
Tooth type	**First primary molar**	3	18	21
	Second primary molar	6	23	29
	Total	9	41	50
Site of lesion	**Occlusal**	4	18	22
	Approximal	5	23	28
	Total	9	41	50
Arch	**Maxillary**	3	20	23
	Mandibular	6	21	27
	Total	9	41	50

Chapter 4 DISCUSSION

4.1 Study Design

4.1.1 Recruitment and consent

This study was conducted following the recommendations guiding ethical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 48th General Assembly, Somerset West Republic of South Africa, October 1996.

A provisional opinion was given by the East of Scotland Research Ethics Committee in December 2014 with some recommendations regarding the Parent Information Sheet (PIS) and consent process which need to be considered prior to obtaining approval (please refer to Appendix 1 for further details). Based on these recommendations, the adult PIS was redesigned to provide more information and the children PISs were redesigned to provide age-appropriate information about the study. The consent process was discussed with a Research Ethics Senior Training & Development Officer at the University of Leeds who suggested that children from 10 years up should be asked for their own consent and from 6 years and over can be asked to give assent. In addition, she advised that a child decision not to participate would override the provision of parental consent and if the child wanted to take part and the parents did not consent the parents' wishes would need to be upheld. Therefore, age-appropriate consent and assent forms were designed. After applying these changes, a favourable ethical opinion was given.

For children who met the inclusion criteria, PIS (for parents and children) were attached with the appointment letter for GA and were sent to patients by post. This was normally sent out 2-3 weeks prior to their general anaesthetic.

Children and their parents/legal guardians who attended the clinic for further appointments prior to their dental treatment under GA consented at this earlier appointment. This was attempted to reduce the number of participants who are consented on the day of treatment. Where this was not possible the standard recruitment and consent procedure which was used in a number of similar studies (Reference number: 06/Q1205/236, 10/H1306/91, 06/Q1205/235) and for the School of Dentistry, University of Leeds tooth and tissue bank was followed.

Parents and their children were advised of their right to withdraw from the study at any time for any reason. Fortunately, none of the participants withdrew from the study.

4.1.2 Sample Collection

The aim of the study was to collect one tooth from each patient to avoid the confounding effect of personal factors such as exposure to preventive therapy or having systemic diseases or medication in early childhood which may alter tooth mineralisation. Before the start of this study, it was explained in the ethical application that the initial aim was to collect one tooth from each patient and where this was not feasible an alternative plan would be to collect more than one tooth from the same patient.

Over the first four months it was clear that this initial aim was not possible; therefore, it was decided to follow the alternative plan of collecting more than one tooth from the same patient. A maximum of 3 teeth was collected per patient. At the end of the study, 72 teeth for study A and 50 teeth for study B were collected. For each tooth, no more than one lesion could only be present on either the approximal or occlusal surface, not both.

4.2 Methodology

4.2.1 Clinical Assessment

Studies in the primary dentition have reported promising results using different diagnostic tools, such as laser fluorescence, DIAGNO-dent and thermal imaging. Nonetheless, a combined visual and radiographic inspection remains the most practical method to diagnose caries in the primary dentition (Lussi et al., 2006; Sanden et al., 2003; Wenzel, 2000).

In study A, The International Caries Detection and Assessment System II (ICDAS II) (Ismail et al., 2007) was used to visually score primary teeth with approximal carious lesions. This system has been utilised as a diagnostic tool in different studies in primary teeth. This evidence-based system has shown higher validity in caries detection (both early and cavitated lesions) in primary and permanent teeth over other clinical diagnostic systems (Braga et al., 2009a; Braga et al., 2009b; Chawla et al., 2012; Ekstrand et al., 2011; Qudeimat et al., 2016; Shoaib et al., 2009).

The in-vivo clinical examination was performed by one examiner (WA) to ensure adherence to ethical considerations and safety of patients under GA and to ensure consistency and reproducibility of examination. Therefore, it was imperative to plan an adequate training in ICDAS scoring and to evaluate both intra- and inter-examiner agreement of scoring before the commencement of the study.

Prior to the study, the chief investigator received theoretical and practical training on using the clinical ICDAS II scoring system. The training was conducted using online E-Learning sources which are provided by the ICDAS Foundation; moreover, personal training was provided by the supervisor (DG) who is an expert in ICDAS and a current member of the ICDAS committee. These training sessions were followed by an assessment of both clinical photographic images of teeth and extracted teeth with a variety of ICDAS II scores from score 0 to 6. The assessment was validated by the supervisor (DG). A month later, the chief investigator (AW) reassessed the same samples. The weighted Kappa values for the intra-

(Kw> 0.9) and inter-examiner (Kw> 0.8) agreement were excellent. This same level of agreement was reported in primary and permanent dentitions by previous groups (Ekstrand et al., 2011; Ismail et al., 2007; Jablonski-Momeni et al., 2008; Shoaib et al., 2009; Qudeimat et al., 2016).

In the operating theatre, compressed air for drying was not available; therefore, teeth were cleaned and dried using sterilised gauze swabs. This is an approved modification which has been suggested by the ICDAS Foundation to be used where compressed air is not feasible (Pitts and Ekstrand, 2013). According to the ICDAS committee advice on clinical examination, a ball-ended probe was used to examine surface texture while applying light pressure to maintain surface integrity (Pitts and Ekstrand, 2013).

For study B, each tooth showed a clinical cavitation (occlusal or approximal) without signs of clinical infection (such as a presence of abscess, fistula, increased unexplained mobility, gingival swelling or exposed pulp).

4.2.2 Radiographic Assessment

The study used digital bitewing images to measure the study outcomes. The decision to use this imaging technique was supported by two main facts. First, radiographic conventional imaging has been replaced currently by digital imaging in many dental practices including Leeds Dental Institute. Second, a bitewing radiograph is the most practical and effective radiographic image for diagnosing dental caries in the primary dentition (Attrill and Ashley, 2001b; Bradley, 2014; Chawla et al., 2012; Dias da Silva et al., 2010; Newman et al., 2009; Nielsen et al., 1996; Pitts and Rimmer, 1992).

The aim of this study was to use in-vivo radiographs for all measurements to ensure that the results replicate the real clinical practice. However, a pilot study was performed to assess if the in-vivo radiographs would be ideal to carry out the measurements. For this purpose, a second in-vitro digital radiograph, based on the recommended imaging geometry, was taken for 10% of the teeth within 3-4 hours following extraction to assess the level of agreement

between in-vivo and in-vitro radiographic measurements. This was performed using the paralleling technique; the long axis of the film was placed in parallel with the long axis of the tooth and the x-ray beam was aligned perpendicular to both. This technique ensures a correct source-to-object-distance; hence, reduces magnification and tooth distortion. In clinical settings, this can be achieved by using a film-holder and a beam-aiming device (Carmichael, 2005).

Agreement between in-vivo and in-vitro radiographic measurements was excellent for ICDAS scoring (Kw = 0.872), caries extension measurements (ICC= 0.965) and RDT measurements (ICC= 0.914). This high level of agreement indicates that taking an in-vitro image for each tooth is not necessary, therefore, it was decided to use the available in-vivo bitewings for radiographic assessment for all the samples. In the radiology department at the LDI, a sensor-holder with a beam-aiming device is always used when taking radiographic images; this may explain the high level of agreement between in-vitro and in-vivo images reported in our study.

For all radiographic assessment methods, radiographs were viewed using Infinit software and no magnification or image enhancement was used. Images were viewed under normal conditions of lighting to ensure measurements replicated those available in the clinical setting.

The radiographs were analysed using the ICDAS 6-point scoring system and reproducibility for this system has not been reported before. In this study, both intra-examiner (Kw= 0.726) and inter-examiner (Kw= 0.792) reproducibility of the radiographic scores were lower than that for the clinical ICDAS II but substantial. Previous studies reported slightly different levels of inter- and intra-examiner reproducibility (ranged from 0.6-0.8) of radiographic scoring (Ekstrand et al., 2011; Ismail et al., 2007; Jablonski-Momeni et al., 2008; Shoaib et al., 2009; Qudeimat et al., 2016). However, comparing our results to theirs is not possible as they either used a collapsed radiographic scoring system or assessed the level of agreement using unweighted Kappa or ICC.

For continuous measurements (caries extension and RDT), inter-examiner agreement was good (ICC= 0.7-0.8) and intra-examiner agreement was excellent (ICC> 0.9).

4.2.3 Histologic Assessment

Sectioning was performed in the oral biology laboratory at the Welcome Trust Brenner Building (WTBB), St. James's University Hospital. Before the commencement of histological training and sectioning, the chief investigator received an induction on laboratory health and safety organised by a senior research fellow in the department of oral biology.

For study B, each tooth was excavated after extraction using small and medium spoon excavators to a clinically acceptable dentine; only soft and leathery dentine was removed until visual and tactile firm dentine was observed. This excavation technique was used in a similar study in the permanent dentition (Lancaster et al., 2011) and was shown to be the most effective method for removing softened dentine with sensitive tactile feedback in clinical settings (Banerjee and Watson, 2000; Innes et al., 2016). Spoon excavators are one of the most difficult instruments to sharpen as sharpening would only improve cutting ability of the rounded outside surface of the spoon (Heymann et al., 2014). Therefore, blunt excavators were disposed and replaced by new sharp ones. Before each excavation, the sharpness of the excavator was tested by lightly sliding it over a hard-plastic surface. Smooth sliding, rather than digging in, indicates that the excavator is blunt and needs to be replaced (Heymann et al., 2014).

All teeth, for study A and B, were then stored as described in section 2.4 and prepared for transportation to WTBB.

Arrangements for transfer of samples were discussed and agreed with the research supervisor (PD), senior laboratory manager (Mrs Jackie Hudson), HTA lead 2008-2016 (Mrs Claire Godfrey). As the Trust's specimen transportation was not feasible to transfer the samples within the time required for this study, the chief investigator used her car which is covered by business insurance for transportation as advised by Mrs Godfrey.

Prior to transportation from LDI to WTBB, samples were placed in secure labelled, sealed, containers, in sealed plastic bags and then placed in a Human Tissue Medical transport bag (Versapak International Ltd) for transportation to provide three protective containers. Samples were sectioned immediately upon arrival at WTBB.

Teeth were sectioned into 0.1 mm sections using a microtome (Accutom-50, Struers, Denmark). Each section was examined on both sides to detect the section with maximum lesion extension (for study A) and the minimum RDT (for study B). During sectioning, some of the sections were lost or damaged from six teeth, two from Study A and four from study B samples. The relevant tooth was then excluded from the study and a further tooth was collected. This complication was anticipated before commencing the study; hence, the additional numbers of teeth were included in the ethics application.

Intra-examiner agreement of measuring both histological caries extension and RDT (in mm) was excellent (ICC> 0.9). Assessment of inter-examiner agreement for histological measurements was not possible as having two examiners available to assess the freshly sectioned teeth was virtually infeasible.

4.3 Null hypothesis

Even with the presence of limitations in both studies, it still possible to address the null hypotheses as below:

1. The hypothesis that there is no association between the radiographic appearance and the clinical status of early carious lesions in primary molars was rejected. It was found that a lesion extension of more than 0.5mm from EDJ is significantly associated with cavitation of approximal surfaces.

2. The hypothesis that there is no difference between the radiographic and the histological RDT was also rejected as it was found that radiographic image significantly overestimates RDT.

4.4 Discussion of key outcomes

4.4.1 Study A outcomes

4.4.1.1 Main outcome: Correlation between cavitation and radiographic presentation of approximal lesion

Evidence-based preventive caries approaches, such as fluoride application and resin infiltrate, can be used effectively to manage approximal carious in primary teeth (Dorri et al., 2015; Martignon et al., 2012). The principal prerequisite for using these techniques is the absence of clinical cavitation. As described earlier in Section 1.6, clinical examination usually needs to be combined with bitewings to diagnose approximal caries in posterior primary teeth when direct assessment of the surface is not possible. Although a radiographic image helps to diagnose the presence and extension of caries, it does not give a clear picture of the surface status of the tooth, e.g., whether it is cavitated or not. This is a key diagnostic decision as it influences whether a preventative or restorative approach is adopted.

The aim of the present study was to investigate only approximal lesions exhibiting radiolucency in enamel, outer third and middle third of dentine. These lesions represent a dilemma with respect to both clinical diagnosis and the type of intervention that should be used (Coutinho and daRocha, 2014; De Araujo et al., 1996; Nielsen et al., 1996; Pitts and Rimmer, 1992; Wenzel, 2014).

Enamel lesions

Previous studies in the primary dentition investigated cavitation threshold in relation to the radiographic lesion extension into outer and inner half of enamel. The current study carried out a similar investigation based on both in-vivo and in-vitro clinical assessment. A comparison between findings from this study and previous studies is shown in Table 4.1.

Table 4-1 A summary of probability of cavitation associated with radiographic enamel lesions

Study	Enamel lesion (N)	Teeth/Surface	Probability of cavitation
(Pitts and Rimmer, 1992), in-vivo study	180	Primary posterior/ Approximal	• 2% of the lesions in outer half of enamel. • 2.9% of the lesions in inner half of enamel.
(De Araujo et al., 1996), in-vivo study	41	Primary posterior/ Approximal	• 6% of the lesions in outer half of enamel. • None of the lesions in inner half of enamel.
(Nielsen et al., 1996), in-vitro study	37	Primary posterior/ Approximal	• 11% of the lesions in outer half of enamel. • 14% of the lesions in inner half of enamel.
(Coutinho and daRocha, 2014), in-vivo study	114	Primary posterior/ Approximal	• 5.3% of all enamel lesions of the enamel lesions are cavitated.
The current study, in-vivo & in-vitro study	22	Primary posterior/ Approximal	In-vivo: • None of the enamel lesions. In-vitro: • None of the lesions in outer half of enamel. • 11% of the lesions in inner half of enamel.

Our investigation, based on in-vivo clinical examination, reported no cavitation with enamel lesions. However, previous in-vivo studies reported a different probability of cavitation, based on clinical examination after mechanical separation of teeth, with radiographic enamel

lesions (Coutinho and daRocha, 2014; De Araujo et al., 1996; Pitts and Rimmer, 1992). When the teeth were examined directly in-vitro, our study reported a higher proportion of cavitation (11%) with lesions presented in the inner half of enamel. The clinical reality of examining approximal surfaces post separation is frequently challenging especially with a young child with limited co-operation. It may explain the lower prevalence of cavitation using this methodology rather than the gold standard where the extracted tooth is available.

An in-vitro study by Nielsen et al. (1996) showed a probability of cavitation of 14% with the lesions in the inner half of enamel (Nielsen et al., 1996). In the current study, results of inner enamel lesions are in good accordance with theirs. This finding supports the fact that mechanical separation may aid in the clinical diagnosis of approximal surfaces, but it does not always provide thorough direct visualisation of the surfaces; thus, some cavitated lesions can still be missed. The proportion of cavitated outer enamel lesions, however, was much higher (11%) in Nielsen et al. (1996) study compared to ours (none). This could be a result of the difference in sample size used in each study. The earlier study investigated a sample of eight inner enamel lesions which is approximate to our sample size (ten lesions); however, that study included 29 outer enamel lesions which is much higher than the number of lesions (five) in ours. Moreover, in their study, Nielsen et al. used the Digora system at 70 kV, 15 mA but the current study used the Planmeca Prostyle Intra at 60 kVp, 7 mA. They also used processing facilities in the system programme to enhance image quality while in our study; none of the images were manipulated. These differences in exposure settings, imaging system and electronic manipulation could result in some discrepancy in image quality and possibly participate in the discrepancy in the obtained results (Ghom, 2017; Li et al., 2008; Syriopoulos et al., 2000; Wenzel et al., 2002; Svanaes et al., 2000).

Dentinal lesions

It has been agreed for decades that when caries extends radiographically beyond the EDJ, the weakened enamel collapses under masticatory force. However, this is not true as recent

studies continue to prove that dentinal lesions are not necessarily cavitated (Pitts, 2016; Pitts and Ekstrand, 2013).

Previous reports in primary dentition provided an overall picture of the lesion behaviour by investigating cavitation threshold in accordance with radiographic lesion extension into inner or outer half of dentine (Table 2.1). However, investigations continue to show that lesions extending into the outer third of the dentine manifest less cavitation compared to lesions extending into the middle and inner third of dentine (Pitts and Ekstrand, 2013; Wenzel, 2014); thus, the classification of outer and inner half of dentine is of no significant help. This fact has been adopted by some researchers who suggested a threshold for operative treatment decision when a lesion extends radiographically beyond the outer third of dentine (Pitts and Ekstrand, 2013; Wenzel, 2014). This suggestion was based on results from investigations in the permanent dentition. A recent in-vivo study in primary teeth reported cavitation in relation to caries extension into the outer, middle and inner third of dentine (Coutinho and daRocha, 2014). Our study used the same radiographic classification combined with different clinical and histological approaches. A comparison between findings from our study and previous studies is shown in Table 4.2.

Table 4-2 A summary of probability of cavitation associated with radiographic dentine lesions

Study	Dentine lesion (N)	Teeth/Surface	Probability of cavitation
(Pitts and Rimmer, 1992), in-vivo study	200	Primary posterior/ Approximal	• 28.4% of the lesions in outer ½ of dentine. • 95.5% of the lesions in inner ½ of dentine. • 50% of any dentine lesions.
(De Araujo et al., 1996), in-vivo study	31	Primary posterior/ Approximal	• 84% of the lesions in dentine.
(Nielsen et al., 1996), in-vitro study	6	Primary posterior/ Approximal	• 63% of lesions in dentine.
(Coutinho and daRocha, 2014), in-vivo study	132	Primary posterior/ Approximal	• 30% of the lesions in outer 1/3 of dentine. • 34.3% of the lesions in middle 1/3 of dentine. • 68.4% of the lesions in inner 1/3 dentine were cavitated.
The current study, in-vivo & in-vitro study	50	Primary posterior/ Approximal	In-vivo: • 34.5% of the lesions in outer 1/3 of dentine. • 62% of the lesions in middle 1/3 of dentine. In-vitro: • 45% of the lesions in outer 1/3 of dentine. • 86% of the lesions in middle 1/3 of dentine.

These studies reported a different probability of cavitation ranged between 50-84% of any dentine lesions (Coutinho and daRocha, 2014; De Araujo et al., 1996; Nielsen et al., 1996; Pitts and Rimmer, 1992). Despite the exclusion of lesions into inner third of dentine in our study, the probability of cavitation reported for all dentinal lesions was 62% which falls within the range reported before.

Only one study investigated cavitation (based on in-vivo assessment) in association with the same radiographic scoring system that has been used in this study. The study concluded that cavitation was evident in 30% of the lesions in the outer third of dentine and 34.3% of the lesions in the middle third of dentine (Coutinho and daRocha, 2014). Based on our findings from in-vivo assessment, cavitation was noticed in 34.5% of the outer dentine and 62% in the inner dentine lesions. These probabilities increased further (45% and 86% respectively) when teeth were examined in-vitro after extraction. The differences in the results of both studies could be a result of the difference in sample size used in each study, the approach of clinical examination, underlying mineralisation defects or exposure to preventive therapy.

Direct comparison of our results with the results from other studies in primary teeth cannot be made owing to the use of different radiographic classification of dentinal lesion depth. When compared to the scoring system that has been used in this study, these studies collapsed dentinal lesions into two radiographic codes, outer or inner half of dentine (De Araujo et al., 1996; Nielsen et al., 1996; Pitts and Rimmer, 1992). Our study, however, supports their conclusion that the probability of cavitation significantly increases when caries extends into deeper layers of dentine (Cochran-Armitage test, $p<0.05$).

Ratledge et al. investigated the relationship between cavitation of approximal lesions and their bitewing radiographic depth in 54 permanent molars (Ratledge et al., 2001). According to their results, 93% of the lesions extending > 0.5 mm were cavitated. The current study

used approximately similar sample size (50 primary teeth) and reported a similar probability of cavitation (92%) for lesions extending > 0.5 mm beyond EDJ.

However, for lesions extending less than 0.5 mm, the current study reported a lower probability of cavitation (29%) compared to the study in permanent dentition (64%). These findings support the previous suggestions that permanent teeth cavitate at an earlier stage of demineralization than primary teeth (Nielsen et al., 1996).

In the present study, Cochran-Mantel-Haenszel test suggested that the association between cavitation and caries extension from EDJ remains significant regardless of the tooth type, site of the lesion and arch. However, this association exhibits slightly lower homogeneity between maxillary and mandibular teeth (Table 3.7) compared to other tested groups.

Looking to the results from our study and previous studies, the probability of cavitation according to the radiographic dentinal caries depth in different layers of dentine (as in the outer or middle third) remains unclear. This is particularly evident with the radiographic lesions in the outer third of dentine which reported a probability of cavitation of 30-45%. This could explain why clinicians are still uncertain whether to manage these lesions with a preventive or restorative approach based on the radiographic assessment. This becomes even more challenging in a non-spaced dentition when clinicians cannot achieve direct visual examination of approximal surfaces to assess surface status.

In this study, results showed better characterisation of cavitation in relation to radiographic caries extension from EDJ (Figure 4.1) rather than in outer/middle third of dentine. When the lesion extends < 0.5mm beyond the EDJ the probability of cavitation was 29% compared to 92% in lesions extending > 0.5mm.

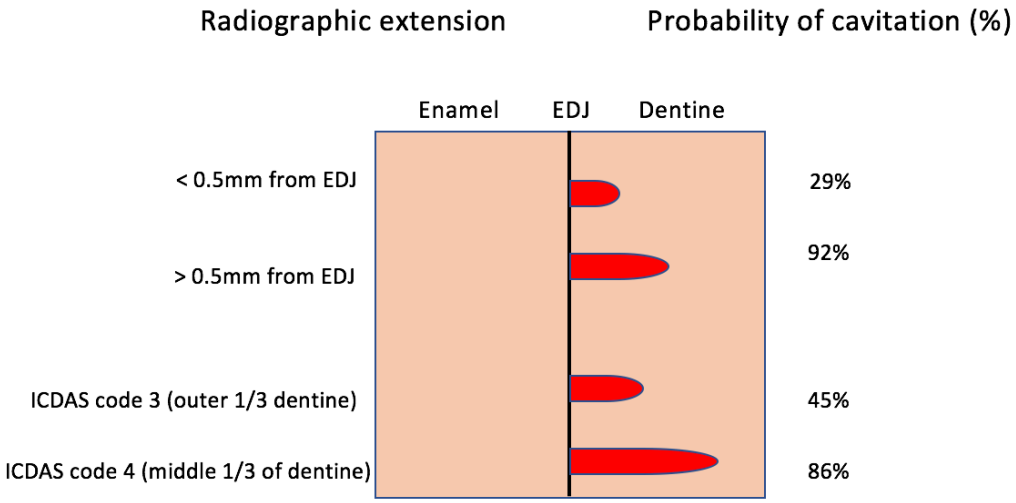


Figure 4-1 comparison between probability of clinical cavitation according to radiographic caries extension from EDJ (> or < 0.5mm) and radiographic ICDAS scoring

With the absence of direct visual assessment, the significant low probability of cavitation (29%) associated with radiographic lesions extending < 0.5mm from EDJ would be more satisfying for clinicians to perform preventive measurements. In addition, the significant high probability of cavitation with radiographic lesions extending > 0.5mm beyond EDJ would make it more reasonable to consider restorative approaches to manage these lesions. With the presence of digital radiography and software programs, measuring radiographic extension from the EDJ would be even easier than assessing radiographic extension into different layers of dentine (for example into the outer or middle third of dentine).

These findings have an important effect on clinical-decision making, thus, should be investigated using a larger sample size to identify if it should replace the current classification of dentinal lesions as being only in the outer/middle third of dentine.

4.4.1.2 Performance of visual and radiographic assessment in detecting approximal carious lesions

The main aim of this assessment was to assess the ability of visual and radiographic assessment to detect approximal carious lesions. This assessment would allow evaluating the need for the radiographic image when diagnosing approximal carious lesions in primary molars. Therefore, in-vivo visual and radiographic assessments were compared to direct in-vitro visual assessment (as present/absent) which was set as a reference standard.

Earlier studies have reported a different sensitivity of visual and radiographic assessment in detecting approximal lesions in primary teeth. Table 4.3 summarises the findings from previous studies and the current study.

Table 4-3 A summary of sensitivity of visual assessment (VA) and radiographic assessment (RA) reported by different studies in primary dentition

Study	Diagnostic method	Sensitivity (%)		
		Overall	Non-cavitated lesions	Cavitated lesions
(Novaes et al., 2009)	VA	20-21	20-21	30
(in-vivo)	RA	21-23	16-23	65-70
(Ekstrand et al., 2011)	VA	100	100	100
(in-vitro)	RA	95	93	98
(Braga et al., 2009b)	VA	-	67-85	59-66
(in-vitro)	RA	-	52	44-47
(Coutinho and daRocha, 2014)	VA	14	-	-
(In-vivo)	RA	80	-	-
(Freitas et al., 2016)	VA	55	-	-
(In-vivo)	RA	49	-	-
The current study	VA	86	78	97
	RA	92	85	100

From the table, it can be seen that studies reported different sensitivity with in-vivo clinical and radiographic assessment in detecting approximal carious lesions in primary teeth. In-vivo studies generally (Freitas et al., 2016; Novaes et al., 2009) reported lower sensitivity with both diagnostic methods compared to in-vitro studies (including current study). These studies used in-vivo investigation post separation which could underestimate their findings.

An earlier in-vitro study in primary molars with approximal lesions reported a higher sensitivity with visual assessment (67-85%) than with radiographic assessment (52%) at

early non-cavitated threshold (Braga et al., 2009a). At the same lesion threshold, the current study reported an approximately similar sensitivity of visual assessment (78%) but a higher sensitivity with radiographic assessment (85%). At advanced lesions, the same study reported a sensitivity of 44-47% with radiographic image and 59-66% with visual assessment. This is much lower than what we reported in both radiographic and visual assessment in this study (100% and 97%, respectively). In the earlier study, both visual and radiographic assessment were performed by newly graduated students who were not trained or calibrated to carry out the assessment which could explain the discrepancy between both studies.

Ekstrand et al. 2011 investigated the sensitivity of radiographic and in-vivo clinical assessment using the similar approach that has been used in this study (Ekstrand et al., 2011). In the present study, the reported sensitivity with radiographic assessment is in accordance with their results except for visual assessment of early non-cavitated lesions. The reported sensitivity of visual assessment in detecting early lesions was much higher (100%) than ours (78%) which could be explained by two factors. First, the earlier study used histological assessment with a stereomicroscope (5X magnification) as a reference standard rather than in-vitro visual examination, which may reveal early sub-clinical demineralisation before surface changes occur. Second, in this study, compressed air for drying was not available during in-vivo visual assessment, which may underestimate the presence of early lesions.

4.4.1.3 Performance of visual assessment in diagnosing approximal carious lesions (as cavitated/non-cavitated)

Although previous studies showed that sensitivity and specificity of visual assessment in detecting approximal lesions improved with direct assessment of the surface (either after separation or in-vitro), no study specifies how many cavitated surfaces were assessed as non-cavitated and vice versa.

This study showed that all in-vitro non-cavitated lesions were recorded as sound or non-cavitated when they were examined in-vivo. Out of the 32 cavitated lesions, nine were recorded as non-cavitated or sound in-vivo. The sensitivity of in-vivo visual assessment in diagnosing cavitated lesions was 72% and specificity was 100%.

This low sensitivity of in-vivo visual assessment in diagnosing the surface status (as cavitated/non-cavitated) of approximal carious surfaces support the need for further studies to help characterise the relationship between surface status and radiographic presentation of these lesions.

4.4.1.4 Reliability of radiographic image in measuring dentinal caries extension

Radiographic findings were compared to the gold standard histological findings to assess its reliability in measuring caries extension from EDJ.

It has been accepted for decades that radiographic images underestimate the actual depth of approximal caries (Jacobsen et al., 2004; Jessee et al., 1998; Kooistra et al., 2005; Syriopoulos et al., 2000). However, a recent review article advised that digital radiographs can accurately estimate lesion depth when compared to the histological depth of the lesion (Wenzel, 2014).

Our findings support the earlier reports in that radiographic image underestimates the extension of dentinal approximal carious lesions (extending into the outer and middle third of dentine). There was a statistically significant disagreement between radiographic and histological caries extension from EDJ into dentine (ICC of 0.490, a 95% CI from -0.087 to 0.807, $p<0.05$). None of the paired measurements recorded by both diagnostic methods were equal. In the majority of the samples (47/50) the radiographic image underestimates caries extension by a mean of 0.33 ± 0.17 mm when compared to the histological extension. Bland-Altman plot (Figure 3.3) shows that in three of the teeth (presented as outliers), the radiographic image overestimated caries extension from the EDJ. This deviation could be a result of a confounding effect such as an underlying mineralisation defect, exposure to

preventive therapy which may have altered the mineralisation content of the teeth, human measurement errors or radiographic exposure errors.

4.4.1.5 Clinical implications

Although direct comparison of this work with previous reports is complex because of variations in sample size and analytical methodology, some conclusive comments can still be made.

- The null hypothesis that there is no association between the radiographic appearance and the clinical status of early carious lesions in primary molars was rejected.
- Our findings, combined with previous reports in the primary dentition, continue to prove that the probability of cavitation associated with radiographic enamel lesions is low. This probability increases as caries progress towards the enamel-dentine junction (De Araujo et al., 1996; Nielsen et al., 1996; Pitts and Rimmer, 1992).
- This study supports that visual assessment alone has lower sensitivity in diagnosing approximal carious lesions compared to a combined visual and radiographic assessment.

  Moreover, the visual assessment reported a sensitivity of 72% in assessing caries status (as cavitated or non-cavitated). Therefore, the radiographic image should be used not only to assess the presence/absence of caries but also to assess caries status as cavitated/non-cavitated. This is particularly important in dentinal lesions which present challenges in clinical decision-making. Although previous efforts were made to characterise the relationship between cavitation and radiographic dentinal lesions, this relationship remains controversial and unclear. This study along with a study in permanent dentition found a better characterisation by correlating cavitation to radiographic caries extension from EDJ in mm. The very high probability of cavitation with lesions extending >0.5mm and lower probability in lesions extending < 0.5mm makes it easier for clinicians to decide whether to use preventive or restorative intervention.

- The bitewing image tends to underestimate the extension of approximal carious lesions by approximately 0.33 ± 0.17 mm. This is an important fact to consider while diagnosing and planning treatment for these lesions.

4.4.2 Study B outcomes

4.4.2.1 Reliability of radiographic image in measuring RDT

ICC reported poor agreement between radiographic and histological RDT (ICC= 0.405, 95% CI -.087 to 0.749).

Figure 3.9 shows that in 94% of the teeth included in this study, radiographic values of RDT are greater than histological values indicating a trend for the radiographic image to overestimate the RDT. The difference between both measurements showed a mean of 0.39mm and a wide limit of agreement up to 0.78mm. Bland-Altman plot (Figure 3.5) shows that in three of the teeth (presented as outliers), the radiographic image underestimated RDT (overestimated caries extension). As explained in study A, this deviation from other findings could be a result of a confounding effect such as an underlying mineralisation defect, exposure to preventive therapy which may have altered the mineralisation content of the teeth, human measurement errors or radiographic exposure errors.

This finding is in line with previous reports in the primary and permanent dentition. The study conducted by Lancaster et al. reported approximately similar radiographic overestimation (0.3 mm) of RDT below deep carious lesions in the permanent dentition (Lancaster et al., 2011). Similarly, other studies in primary and permanent dentitions demonstrated approximately similar radiographic overestimation of tooth wall thickness (Arastoo and Azadani, 2015; Raiden et al., 2001; Souza et al., 2008).

According to the tooth location in the arch, overestimation in maxillary molars appears to be higher than that found with mandibular molars (p=0.2, Mann Whitney U); however, the difference was not statistically significant. This finding can be investigated in future using a larger sample size to assess if there is a significant difference between maxillary and

mandibular molars. Other group comparison showed no statistically significant difference in radiographic overestimation of RDT between the tested groups (Table 3.16).

4.4.2.2 Relationship between pain history and radiographic RDT

This study reported that 9 out of 50 teeth had a history of pain reported either by the child or the parent/carer. The reported history of pain was not significantly associated with the radiographic dentine thickness below the carious lesion (OR= 1.33, 95% CI= 0.299-47.397, P=0.3).

In carious primary teeth, dental pain is primarily caused by pulpal inflammation (Dummer et al., 1980). Kassa et al. reported that when approximal caries involves at least half of the dentine thickness, inflammation of pulpal tissue significantly increases in primary teeth (Kassa et al., 2009). In addition, Gopinath and Anwar stated that teeth present with occlusal or approximal lesions progressing to involve 2/3 of the dentine would exhibit pulpal inflammation (Gopinath and Anwar, 2014). Our findings, however, are inconsistent with these reports. This could be explained by the fact that young children do not possess the required communication skills or memories to recall previous pain history (Rathnam et al., 2010; Zonneveld et al., 1997) or potentially they may deny pain to avoid dental treatment. In addition, parents may not have recognised when the child is in pain or may fail to recall pain history.

4.4.2.3 Clinical importance of study B

- In deep carious lesions, clinical investigation and history of the lesion may provide an insight into the pulpal status. However, in the absence of signs and symptoms of pulpal inflammation clinicians rely only on radiographic presentation to diagnose pulpal health.
- Remaining dentine thickness (RDT) was identified as an important indicator of pulpal status in primary dentition (Gopinath and Anwar, 2014; Kassa et al., 2009; Rayner and Southarn, 1979). It was defined as the thinnest dentine from the base of the lesion and the outermost surface of pulp chamber (Smith et al., 2000). This thickness

cannot be assessed clinically and requires additional diagnostic method, usually radiographs.

- In this study, the null hypothesis that there is no association between the radiographic measurements and the histological RDT was rejected. The study findings demonstrated that digital bitewings overestimate RDT by a mean of 0.39 ± 0.197 mm with a wide limit of agreement up to 0.8mm which means that for some teeth, RDT is overestimated by 0.8mm. This finding must be considered carefully as this thickness could be much reduced clinically. Failure to do so could result in an inappropriate diagnosis, treatment plan and consequently treatment outcome. This is particularly important in the absence of signs and symptoms of pulpal inflammation where clinicians rely on radiographic presentation to diagnose pulpal status.
- From a clinical point of view, this finding is of particular importance when considering the new biological approaches, such as the “Hall technique” or indirect pulp treatment, for treating deep carious primary molars. In these approaches, the caries is sealed (after none or partial caries removal), thus preventing further caries progression. However, the success of these approaches depends highly on the proximity of the lesion to the pulp and healing ability of the pulp. Unlike the traditional surgical approach of complete caries removal, there is no opportunity to evaluate the proximity of the lesion to the pulp or assessment of pulpal status. Therefore, indications for the Hall technique or indirect pulp treatment rely on an accurate diagnosis of the pulpal health based on the clinical and radiographic presentation. This study helps to support the clinical indications for the Hall technique or indirect pulp treatment which identify the need for a clear band of dentine visible on the radiograph between the base of the lesion and pulp chamber. The findings from this study show the radiographic band (RDT) is overestimated radiographically by up to 0.8mm. Therefore, this study would support the clinical anecdote of requiring at least 1mm of RDT before proceeding with a Hall crown or indirect pulpotomy.

- Other approaches, such as partial caries removal, require at least 0.25mm to avoid pulpal irritation during excavation and restoration. The discrepancy between radiographic and real RDT must not be neglected as it may lead to incorrect diagnosis of the proximity of the lesion from the pulp and it would be possible to irritate the pulp or accidentally expose it during caries excavation.
- In conclusion, this study suggests that the treatment plan for teeth with deep caries should not be based solely on findings from radiographic image and clinicians must always integrate radiographic presentation of RDT along with other diagnostic factors prior to treatment planning. In addition, it encourages the need for further investigation using a larger sample size while considering factors that may affect the radiographic presentation of RDT.
- The study also showed that history of dental pain is not a reliable indicator of pulpal status in children with deep carious lesions.

4.5 Study Strengths and Limitations

4.5.1 Strengths

- This study is the first to investigate the relationship between radiographic caries extension and clinical surface status of approximal primary molars based on findings from both in-vivo and in-vitro clinical examination.
- In addition, it is the first to investigate the relationship between cavitation and radiographic caries extension from EDJ.
- Moreover, it is the first study to investigate the reliability of the radiographic image in measuring RDT below deep carious lesions in primary molars.
- The chief investigator in this study received a thorough training in the use of ICDAS which was provided and validated by an expert in ICDAS training and calibration,

whereas some of the previous researchers were conducted by examiners who are inexperienced in the use of ICDAS.

4.5.2 Limitations

- The study collected a relatively smaller sample compared to older studies and this may limit the confidence in the study findings. This is particularly true with the radiographic enamel lesions which did not allow a fair comparison with other studies.
- Examining teeth in the mouth under GA is close to the real-world situation, however, it may provide limited access and may not be the same as examining teeth in a fully conscious patient which could affect our results. Comparison between in-vivo and in-vitro clinical examination revealed some discrepancies, especially with early enamel lesions. The presence of approximal contact between posterior teeth could reduce the ability to detect initial lesions on the proximal surfaces (Ekstrand et al., 2011; Ribeiro et al., 2015). Therefore, it would be of interest to investigate if this discrepancy was consistent between spaced and crowded posterior teeth.
- The in-vitro radiographic imaging could be made more consistent by using a device to maintain a fixed distance between the teeth and the radiographic plates (Souza et al., 2008) and to try to imitate the soft tissue by using plasticine, (Naoum et al., 2003), wax or acrylic (Schropp et al., 2012). Although the findings from this study found very little difference between in-vitro and in-vivo radiographs when such precautions were not undertaken.
- In this study, radiographic measurements were performed using images which were taken 3-4 months before the general anaesthetic and carious lesion may have progressed further before the tooth was extracted. However, studies showed that radiographic caries progression occurs over a longer period of time in primary teeth (Guedes et al., 2016; Pitts, 1983; Solanki and Sheiham, 1992; Tinanoff and Douglass, 2001).

- Regarding histological assessment, teeth were sectioned into 0.1 mm and were checked carefully for any missing sections, nonetheless, some tooth tissue was destroyed during sectioning. Although this amount of tissue is negligible, it may involve the area representing the deepest part of the lesion. One may argue that visual measurement of the RDT after sectioning could be validated using autofluorescence, fixative solution or dyes. Although these techniques were used in different studies with different aims, their use in paediatric dentistry is limited due to impracticability especially in very young children with a limited level of co-operation. Therefore, the main aim of this study was to use findings which replicate real clinical practice.
- It would be interesting to assess inter-examiner agreement for histological measurements but having two examiners available to assess the freshly sectioned teeth was virtually infeasible.

4.6 Conclusions

With the limitations of the current study, this study has given an additional insight into the radiographic interpretation in the primary dentition. From the above analysis, the following conclusions may be made:

- The radiographic bitewing image can be of value as an adjunct method for diagnosis of approximal caries in the primary dentition.
- Despite the underestimation of actual lesion depth, radiographic depth of approximal lesions may be used as an additional diagnostic indicator of the surface status.
- This study showed a better characterisation of the relationship between clinical cavitation and radiographic extension of approximal dentinal lesions from the EDJ (Figure 4.1) compared to the radiographic extension as in the outer/middle third of dentine. When the lesion extends < 0.5mm beyond the EDJ the probability of cavitation was 29% compared to 92% in lesions extending > 0.5mm.

- Although caries progression rate is faster in primary molars, our study combined with previous studies suggest that cavitation occurs at later stages in primary molars compared to permanent molars.
- Digital bitewings overestimate the actual dental tissue thickness. This fact should be considered while managing primary molars with occlusal and approximal deep carious lesions. This is particularly important when considering biological approaches where direct assessment of lesion proximity to the pulp and clinical assessment of pulpal status is not feasible.

4.7 Future research

Study A

Evidence continues to highlight the effectiveness of preventive measurements in managing non-cavitated approximal surfaces. However, approximal surfaces are sometimes difficult to be clinically assessed which necessitate the use of the radiographic image. There are few research studies which have examined this relationship between cavitation and radiographic caries extension in approximal surfaces of primary molars. Therefore, more studies with larger sample size are required to characterise this relationship.

While investigating this relationship, future studies may consider investigating the possible underlying causes of the discrepancy between the published reports in this area. For example, systemic disease and medication during early childhood may alter mineral content of enamel; thus, affect its ability to remineralise. In addition, lesions which are exposed to preventive therapy exhibit higher ability to remineralise, therefore, may exhibit different clinical behaviour than the teeth with no preventive exposure.

Study B

RDT is an important factor which should be integrated with other patient-related and clinical-related factors during diagnosis and treatment plan for deep carious lesions. This thickness cannot be examined clinically and needs additional diagnostic tools, usually radiographs.

Limited data, however, describe the ability of the radiographic image to estimate RDT and more studies with larger sample size are required to characterise this relationship. In addition, the radiographic image reflects the mineral content of the tooth structure. This content could be affected, as described above, by systemic or environmental factors which may influence the presentation of the tooth structure radiographically. The effect of these factors on the radiographic presentation of RDT should be investigated by considering a large sample size including children with a different previous history of exposure to these factors. In addition, up to date, there is no study to assess pulpal status (as reversibly or irreversibly inflamed) based on the radiographic presentation of the lesion extension. It would be interesting and of clinical importance to assess this according to the RDT in deep carious primary molars.

Appendices

Appendix 1: The Provisional Ethical Opinion

NHS SCOTLAND

East of Scotland Research Ethics Service *(EoSRES)* **Research Ethics Service**

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Mrs Wafa Almutairi
Professional Doctorate in Paediatric Dentistry
University of Leeds
Department of Paediatric dentistry, School of Dentistry
Level 6, Worsley Building,
Clarendon Way,
Leeds, W.Yorkshire
LS2 9LU

Date: 10 December 2014
Your Ref:
Our Ref: LR/**14/ES/1110**
Enquiries to: Mrs Lorraine Reilly
Direct Line: 01382 383878
Email: eosres.tayside@nhs.net

Dear Mrs Almutairi

Study title: **The association between radiographic and clinical findings in primary molar teeth**
REC reference: **14/ES/1110**
IRAS project ID: **158017**

The Proportionate Review Sub-Committee of the East of Scotland Research Ethics Service REC 1 reviewed the above application on 08 December 2014. Advice was also received from an external referee.

Provisional opinion

The Sub-Committee would be content to give a favourable ethical opinion of the research, subject to clarification of the following issues and/or the following changes being made to the documentation for study participants:

1. Regarding the application form:

- The Committee asked that the children (particularly the older children) would have a chance to speak to the dentist or dental nurse without parents/carers present as they felt it may be a good idea to avoid coercion especially if the parents/carers had given consent but the children did not want to donate their teeth.
- The Committee asked for reassurance that the parents/carer would not know that a child had not given/withdrawn consent.
- The Committee asked for clarification regarding what would happen if there was a clash in consent between the child and parents/carer.
- The Committee requested further information why the study would not be registered on a public database.

2. Regarding the Participant Information Sheet (PIS):

- The Information Sheets and Consent form should be printed on appropriate departmental/University headed paper.
- The Committee felt the PIS for parents/carers was a bit confusing and should explain the purpose of the study and lay out what happened in a different order. It should also include indemnity statements and procedures for anyone who may wish to make a complaint.
- The Committee suggested that the adult information sheet was very complicated for 11 year olds to understand and suggested the information sheet would be more appropriate for children 14 years old and upwards.
- The Committee confirmed that the child information sheet was good, but more suitable for children of 10 years old upwards and suggested a simpler information sheet be developed for the younger children with a picture story which they could use very effectively if it also had written words on it.
- The Committee suggested for guidance on composing participant information sheets the researchers could view the sample participant information sheets on the HRA website http://www.hra-decisiontools.org.uk/consent/content.html

When submitting a response to the Sub-Committee, the requested information should be electronically submitted from IRAS. A step-by-step guide on submitting your response to the REC provisional opinion is available on the HRA website using the following link: http://www.hra.nhs.uk/nhs-research-ethics-committee-rec-submitting-response-provisional-opinion/

Please submit revised documentation where appropriate underlining or otherwise highlighting the changes which have been made and giving revised version numbers and dates. You do not have to make any changes to the REC application form unless you have been specifically requested to do so by the REC.

Authority to consider your response and to confirm the final opinion on behalf of the Committee has been delegated to the Chair.

Please contact Mrs Lorraine Reilly, Senior Co-ordinator (details at top of letter) if you need any further clarification or would find it helpful to discuss the changes required with the lead reviewer.

The Committee will confirm the final ethical opinion within 7 days of receiving a full response. A response should be submitted by no later than 09 January 2015.

Documents reviewed

The documents reviewed were:

Document	Version	Date
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		17 September 2014
IRAS Checklist XML [Checklist_01122014]		01 December 2014
Other [PIS children]	1.1	20 July 2014
Other [Assent form for children]	1.1	20 July 2014

Other [PIS Children's Story]		
Participant consent form	1.1	20 July 2014
Participant information sheet (PIS)	1.1	20 July 2014
REC Application Form [REC_Form_28112014]		28 November 2014
Research protocol or project proposal	1.1	20 July 2014
Summary CV for Chief Investigator (CI)		10 October 2014
Summary CV for supervisor (student research)		12 March 2013

Membership of the Committee

The members of the Committee who were present at the meeting are listed on the attached sheet.

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.

14/ES/1110	**Please quote this number on all correspondence**

Yours sincerely

L Reilly

pp
Dr Carol Macmillan
Chair

Email: eosres.tayside@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Anne Gowing, Leeds Trust/Acute organisation

East of Scotland Research Ethics Service REC 1

Attendance at PRS Sub-Committee of the REC meeting on 08 December 2014

Committee Members:

Name	Profession	Present	Notes
Dr Carol Macmillan	Consultant Anaesthetist	Yes	Chair
Dr Gary Lyon	Retired	Yes	
Dr Astrid Schloerscheidt	Lecturer	Yes	

Also in attendance:

Name	Position (or reason for attending)
Mrs Lorraine Reilly	Senior Co-ordinator

Written comments received from:

Name	Position
Dr Carol Macmillan	Consultant Anaesthetist, Chair
Dr Gary Lyon	Retired
Dr Astrid Schloerscheidt	Lecturer

Appendix 2: The Favourable Ethical Opinion

NHS SCOTLAND
Research Ethics Service

East of Scotland Research Ethics Service *(EoSRES)*

TAyside medical Science Centre
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Mrs Wafa Almutairi
Professional Doctorate in Paediatric Dentistry
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Date: 20 January 2015
Your Ref:
Our Ref: LR/14/ES/1110
Enquiries to: Mrs Lorraine Reilly
Direct Line: 01382 383878
Email: eosres.tayside@nhs.net

Dear Mrs Almutairi

Study title: **The association between radiographic and clinical findings in primary molar teeth**
REC reference: **14/ES/1110**
IRAS project ID: **158017**

Thank you for your letter of 08 January 2015, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Co-ordinator Mrs Lorraine Reilly, eosres.tayside@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

POSITIVE ABOUT DISABLED PEOPLE

The Committee suggested that you register the study on a public database.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.
There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

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" above).

Approved documents

The documents reviewed and approved by the Committee are:

Document	Version	Date
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		17 September 2014

IRAS Checklist XML [Checklist_12012015]		12 January 2015
Other [PIS children]	1.1	20 July 2014
Other [PIS Children's Story]		
Other [PIS for children 10 years and upwards]	1.2	19 December 2014
Other [Consent Form for Children 10 years and older]	1.2	19 December 2014
Other [PIS for children 3 years and upwards]	1.2	19 December 2014
Other [Assent form for children 6 years and older]	1.2	19 December 2014
Other [Response to request for further information]		08 January 2015
Participant consent form [Parent/Guardian]	1.2	19 December 2014
Participant information sheet (PIS) [Parent/Guardian]	1.2	19 December 2014
REC Application Form [REC_Form_12012015]		12 January 2015
Research protocol or project proposal	1.2	19 December 2014
Summary CV for Chief Investigator (CI)		10 October 2014
Summary CV for supervisor (student research)		12 March 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

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 HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

14/ES/1110	Please quote this number on all correspondence

Yours sincerely

pp
Dr Carol Macmillan
Chair

Email: eosres.tayside@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Anne Gowing, Leeds Trust/Acute organisation

Appendix 3: R&D approval

The Leeds Teaching Hospitals NHS
NHS Trust

Amanda Burd

Research & Innovation

13/03/2015

Leeds Teaching Hospitals NHS Trust
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Tel: 0113 392 0162
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r&d@leedsth.nhs.uk
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Mrs Wafa Almutairi
Department of Paediatric Dentistry
School of Dentistry
Level 6, Worsley building
Clarendon way , Leeds
LS2 9LU

Dear Mrs Wafa Almutairi

Re: NHS Permission at LTHT for: The association between radiographic and clinical findings in primary molar teeth
LTHT R&I Number: DT14/11394:
REC: 14/ES/1110

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&I Department.

Permission is granted on the understanding that the study is conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable) and NHS Trust policies and procedures available at http://www.leedsth.nhs.uk/research/

This permission is granted only on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet Conditions of Approval.

If you have any queries about this approval please do not hesitate to contact the R&I Department on telephone 0113 392 0162.

Indemnity Arrangements

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority "Clinical Negligence Scheme for NHS Trusts" for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity that has been managerially approved by the R&I Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as investigator and the researchers listed on the Site Specific Information form. Should there be any changes to the research team please ensure that you

inform the R&I Department and that s/he obtains an appropriate contract, or letter of access, with the Trust if required.

Yours sincerely

Dr D R Norfolk
Associate Director of R&I

Approved documents
The documents reviewed and approved are listed as follows:-

Document	*Version*	*Date of document*
NHS R&D Form	3.5	05/03/2015
SSI Form	3.5	11/02/2015
Directorate Approval		27/01/2015
PIS; Children 10 years and upwards	1.2	19/12/2014
IFC: Children 10 years upwards	1.2	19/12/2014
PIS: Children 3 years and upwards	1.2	19/12/2014
Assent Form: 6 years and upwards	1.2	19/12/2014
PIS: Parents / guardian	1.2	19/12/2014
ICF: Consent form	1.2	19/12/2014
Protocol	1.2	19/12/2014
Evidence of Insurance		17/09/2014
REC Letter confirming favourable opinion		20/01/2015

Cc: Peter Day

Appendix 4: Parent information sheet

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Version number 1.2

Date 19/12/2014

INFORMATION SHEET FOR PARENT/GUARDIAN

Title of the study: How to detect decay in baby molar teeth.

Your child is scheduled to have his or her tooth/teeth extracted here at Leeds Dental Institute as part of their routine dental care. It is our hospital policy that extracted teeth cannot be given back to the patient and should be discarded in clinical waste. However, these teeth could be used in scientific research to help us diagnose dental decay in baby teeth. We are therefore asking you and your child to donate their decayed extracted tooth/teeth for use in research. ***Please note that participation is entirely up to you and your child.***

Before you decide to participate we would like you to read more about this study and what it means to you and your child below.

Part 1 *tells you the purpose of the study and what will happen if you and your child decide to take part.*

Part 2 *gives you more detailed information about the study.*

Please read the following information carefully and ask the dentist who is taking care of your child to clarify any issues that you are unsure about.

Part 1

Why we doing this research?

In this research, we are going to use extracted baby teeth to study how much dental radiographs help us in diagnosing dental decay. The results of this research will help us to provide the appropriate treatment that may benefit children in the future.

Why has my child been chosen?

We are looking for participants who are 2-14 years of age and require extraction of decayed baby teeth.

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Head of Department of Paediatric Dentistry

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Does my child have to take part?

Participation in this study is entirely voluntary. If you and your child decide to participate, you will be asked to complete and sign a consent form to show that you agree to take part in the study. If you decide you would not like to take part you are free to do so and this will not affect your child's treatment.

What happens if I change my mind?

If you or your child wish to withdraw your consent to your child's extracted tooth being used in our research you are free to do so, at any time, up to the point of extraction. After extraction the teeth will not be labelled with any of your child's details and we would not be able to identify your child's tooth in order to withdraw it from the study.

What will happen to my child if decide to take part?

If you and your child decide to take part in this study the investigator, Wafa Almutairi, will examine the tooth/teeth before extraction on the same day of treatment. This examination will take approximately 5 minutes and will not affect the treatment decision. No further intervention is required.

As part of this research, the investigator will need to check your child's dental record and radiographs to compare the diagnosis made in the clinic with what is found in the laboratory tests.

What will happen to the tooth/teeth I give?

As part of this research, the extracted tooth/teeth will be investigated into the laboratory using special techniques looking at the depth of the dental decay. These techniques are destructive and irreversible. At the end of this study the teeth will be discarded in clinical waste according to the hospital policy.

Will my child benefit directly by donating teeth to the study?

Donation of extracted teeth is completely voluntary and declining to take part will not affect your child's current or future treatment in any way. If your child does choose to donate their tooth/teeth, they do so as a gift to scientific research.

If you and your child choose to donate their tooth, you will not be contacted again unless you have specifically requested information about the results of the study.

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How will my child's information be kept confidential?

If your child donates their extracted tooth, it will be given a code number to ensure that their identity and personal details are no longer linked to your child and that your child's information will remain confidential. Only age, pain history and radiographic and clinical appearance of the tooth/teeth concerned will be recorded.

We would like to invite you and your child to donate the extracted tooth or teeth to be used in our research.

Part 2

What will happen to the results of this study?

The results of this study will be a part of a thesis for a higher degree. The results may be published in an international journal and presented at both national and international meetings. Your child's personal information will not be identified in any report. If you like to receive the results of this study please feel free to inform the investigator whose contact details are below.

Who funds the research?

Our research is supported by the University of Leeds.

Teeth are donated on the basis of an unconditional gift. It is not possible for us to accept teeth where the donor wishes to restrict their use in research.

Who has reviewed this study?

This study has been reviewed and approved by:

Dental Research Ethics Committee, University Of Leeds (DREC)

East of Scotland Research Ethics Service (EoSRES) Reference: 14/ES/1110

What should I do if I have a complaint?

In the first instance, if you have a concern about any aspect of this study, you should ask to speak to the researcher, Wafa Almutairi, who will do her best to answer your questions [contact details below]. Alternatively, you may contact Dr Peter Day [address as below, email: p.f.day@leeds.ac.uk].

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the Leeds Dental Institute.

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In the event that something does go wrong and you are harmed during the study, and this is due to someone's negligence, then you may ask for compensation against the University of Leeds, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Further information and contact details

Wafa Almutairi
Department of Paediatric Dentistry
School of Dentistry, University of Leeds.
Level 6, Worsley Building, Clarendon Way, Leeds, W. Yorkshire,
LS2 9LU.
dnwa@leeds.ac.uk
Telephone +447448793098

Dr. Peter Day
Associate Professor And Consultant in Paediatric Dentistry
Department of Paediatric Dentistry
School of Dentistry, University of Leeds.
Level 6, Worsley Building, Clarendon Way, Leeds, W. Yorkshire,
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Thank you for taking time to read this sheet.

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Appendix 5: Children PIS (10 years and older)

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PATIENT INFORMATION SHEET FOR CHILDREN

(Age: 10 years upwards)

Title: How to detect decay in baby molar teeth.

Version number 1.2 Date 19/12/2014

The dentist will be taking out your tooth because it has gone bad or maybe because he or she is trying to make more room for your other teeth. If you want to, you can give the tooth away to our scientists to help them in their work. If you agree, your tooth will be taken away to a special place where a scientist can study it further. Your Mum, Dad or Carer have a lot more information about this and they will help you decide whether you want to give us your tooth.

What is research?

Research is a way of finding out an answer to an important question. We use teeth that have been taken out to answer: Why do teeth go bad? How can we stop teeth from going bad? This will help us to find new ways of repairing teeth.

Will anything different happen to me if I give away my tooth?

No. Your tooth will be taken out in the same way whether you choose to give it to us or not.

What will happen to my tooth after it has been taken out?

After your tooth has been taken out, it will be given a special number, taken to a lab where scientists will examine it.

Will giving my tooth for research help me?

No. Giving us your tooth will not help you in any way but we hope that we may help children in the future by using your tooth to answer important questions.

Will anyone else know I'm doing this?

Your details will be kept very private. No one will know which tooth is yours. The only person that will know will be the dentist speaking to you.

Can I get my tooth back after I have given it away?

It would be very hard for us to be able to give you back your tooth later because it will have been used in our work.

What happens if I don't want to give away my tooth?

If you don't want to give away your tooth after it has been taken out, just tell your parents, dentist or nurse. Nobody will be cross with you.

Thank you for reading this and please ask any questions if you need to.

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Appendix 6: Children PIS (younger than 10 years)

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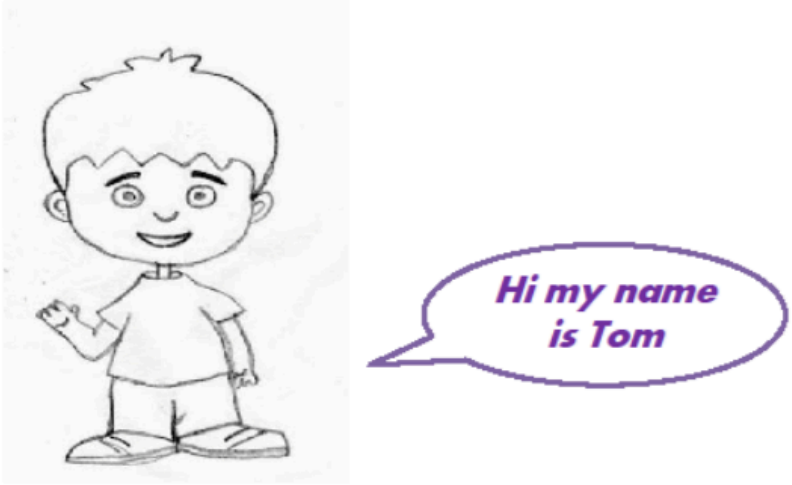
PATIENT INFORMATION SHEET FOR CHILDREN

(Age: 3 years and upwards)

Title: How to detect decay in baby molar teeth.

Version number 1.2
Date 19/12/2014



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After the dentist takes my tooth out, they will swap the bad tooth for an envelope for me to give to the tooth fairy instead

The dentist will ask me if I can give the tooth for an investigation about tooth decay

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Don't worry Tom, it is ok to say yes or no. Tom is welcome to ask any other questions he has.

If you say yes the only thing we need to do is to check the tooth again before the dentist wobbles it out.. You will still get an envelope for the tooth fairy

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Appendix 7: Parent's Consent Form

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Parental Consent Form

Title: How to detect decay in baby molar teeth.

Version number 1.2

Date 19/12/2014

Name of Researchers: Mrs, Wafa Almutairi, Dr Peter Day

Please initial box

1. I confirm that I have read and understand the information sheet dated 19/12/2014 (Version 1.2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my child's participation is voluntary and that I or my child are free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my child's medical notes and data collected during the study, may be looked at by individuals from Leeds Dental Institute, from regulatory authorities or from the NHS Trust, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child's records. ☐

4. I agree for my child to take part in the above study. ☐

Name of parent/guardian……Date……Signature……

Name of the researcher……Date……Signature……

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

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Appendix 8: Children's Consent Form (10 years and older)

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Version number 1.2 Date 19/12/2014 Centre Number: 1

Consent Form for Children

(10 years old and older)

Title of Project: **How to detect decay in baby molar teeth.**
Name of Researcher: Mrs, Wafa Almutairi, Dr Peter Day

To be completed by the child

Please tick the boxes to show that you agree with what is written.

1) Have you read about our study? Or has somebody else explained it to you?	Yes ☐ No ☐
2) Do you understand why we are asking you to give us your bad tooth and what will happen to it after you give it to us?	Yes ☐ No ☐
3) Have you asked all the questions you want?	Yes ☐ No ☐
4) Have you had your questions answered in a way you understand??	Yes ☐ No ☐
5) Do you understand that it is OK to say no to giving us your bad tooth if you don't want to?	Yes ☐ No ☐
6) Are you happy to give us your bad tooth to use it in this study?	Yes ☐ No ☐

Sign your name below only if you answered YES to all of the questions above. If you don't want to give us your tooth, don't sign your name!

Name of Child: .....Date: .....Sign: .....

Name of the researcher: .....Date: .....Sign: .....

Thank you for your help

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Appendix 9: Assent Form for children (6 years and older)

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Version number 1.2 Date 19/12/2014 Centre Number: 1

Assent Form for Children

(6 years old and older)

Title of Project: **How to detect decay in baby molar teeth.**
Name of Researcher: Mrs, Wafa Almutairi, Dr Peter Day

To be completed by the child and their Parent/Guardian

Please tick the boxes to show that you agree with what is written. If unable, parent/guardian can tick the boxes on the child's behalf

1) Have you read about our study? Or has somebody else explained it to you?	Yes ☐ No ☐
2) Do you understand why we are asking you to give us your bad tooth and what will happen to it after you give it to us?	Yes ☐ No ☐
3) Have you asked all the questions you want?	Yes ☐ No ☐
4) Have you had your questions answered in a way you understand??	Yes ☐ No ☐
5) Do you understand that it is OK to say no to giving us your bad tooth if you don't want to?	Yes ☐ No ☐
6) Are you happy to give us your bad tooth to use it in this study?	Yes ☐ No ☐

Sign your name below only if you answered YES to all of the questions above. If you don't want to give us your tooth, don't sign your name!

Name of Child: .....Date: .....Sign: ..... Your parent/guardian must write their name here too if they are happy for you to give us your tooth.

Print Name: ..... Date: .....Sign: .....

Name of the researcher: .....Date: .....Sign: .....

Thank you for your help

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Appendix 10: Data Collection Sheet

Study title: The association between radiographic and clinical findings in primary molar teeth

REC reference: 14/ES/1110

IRAS project ID: 158017

Study Number:

Date of GA: --------/--------/--------- (AM / PM)

Consultant:

Before extraction:

- ☐ Photograph
- ☐ BW Radiographs (R/L)

Recruitment:

- ☐ Parental PIS given
- ☐ Parental consent form signed
- ☐ Children PIS (☐ 3-9 yrs / ☐ 10 yrs and older)
- ☐ Children (assent / consent) form signed

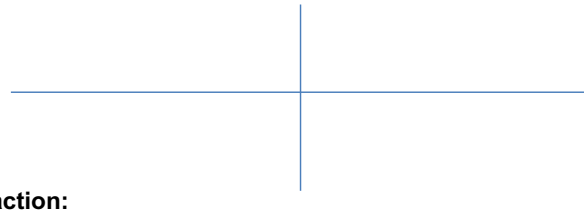
Pain history, if any: ----------

Eligibility

Based on the inclusion and exclusion criteria for this study, is the subject eligible to participate? ☐ Yes ☐ No

Tooth suitable for study (Study A/Study B)

R **L**



After extraction:

- ☐ Photograph
- ☐ Radiograph

Appendix 11: Radiograph Data Collection Sheet

Study title: The association between radiographic and clinical findings in primary molar teeth

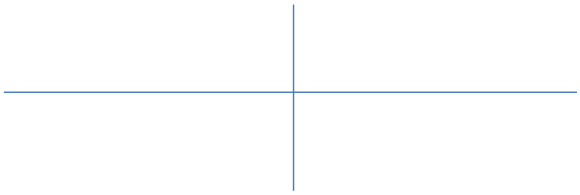
REC reference: 14/ES/1110

IRAS project ID: 158017

Study Number:

STUDY A / B

Tooth used for study

R  **L**

Before extraction:

Radiographic ICDAS:

Study A: Extension from EDJ in mm (dentine lesion) =

Study B: RDT in mm =

After extraction:

Radiographic ICDAS

Study A: Extension from EDJ in mm (dentine lesion) =

Study B: RDT in mm =

Appendix 12: Clinical data collection sheet

Study title: The association between radiographic and clinical findings in primary molar teeth

REC reference: 14/ES/1110

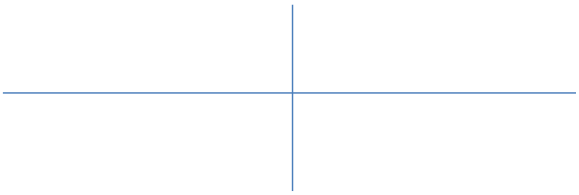
IRAS project ID: 158017

Study Number:

STUDY A / B

Tooth used for study

R **L**



Clinical ICDAS before extraction

Clinical ICDAS after extraction

Appendix 13: Histology data collection sheet

Study title: The association between radiographic and clinical findings in primary molar teeth

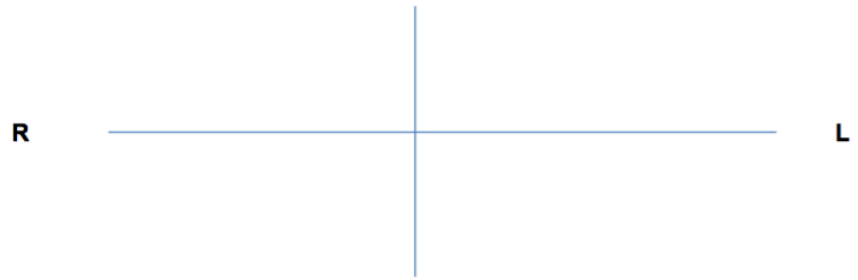
REC reference: 14/ES/1110

IRAS project ID: 158017

Study Number:

STUDY A / B

Tooth / teeth used for study



Study A: Extension from EDJ in mm (dentine lesion) =

Study B: RDT in mm =

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