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Radiographic and laser fluorescence caries detection methods in mixed dentition



**Author**
Lin, Doris T.

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# RADIOGRAPHIC AND LASER FLUORESCENCE CARIES DETECTION METHODS IN MIXED DENTITION

by

DORIS T. LIN, D.D.S.

THESIS

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# RADIOGRAPHIC AND LASER FLUORESCENCE CARIES DETECTION METHODS IN MIXED DENTITION

DORIS T. LIN, D.D.S.

2003

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# ABSTRACT

**Purpose:** An in vitro study aimed to evaluate the diagnostic performance of scanned radiography (flatbed scanning at 474 dpi), charged-couple device direct digital radiography (MPDx, Dental Medical Diagnostics), and laser fluorescence (DIAGNOdent, Kavo) for proximal and occlusal caries detection compared to conventional radiography (Insight F speed films, Kodak) in the mixed dentition.

**Methods:** Thirty quadrant blocks (primary canine, primary first and second molars, and permanent molar) were used to mimic the mixed dentition. Blocks and method sequence were randomly ordered among 7 clinicians evaluating all radiographs for caries presence and extent. Proximal caries was compared among conventional (C), scanned (S), and direct digital (D) radiography; occlusal caries added laser-fluorescence (L). Stereomicroscopic and polarized light microscopic histology represented the gold standard. Generalized estimating equations proportional odds models assessed validity versus histology.

**Results:** For primary and permanent proximal surfaces, all radiographic methods underestimated caries extent compared to the histology gold standard with odds ratios (OR) significantly > 1 and there was no difference in diagnostic accuracy between C, S, and D. For primary and permanent occlusal surfaces, all radiographic methods underestimated caries extent compared to histology except C and D, which equaled histology in detecting primary occlusal caries. Compared to C, there was no difference between S and D in detecting permanent occlusal caries, but both S and D underestimated primary occlusal caries. On the other hand, compared to histology, L underestimated occlusal caries extent both primary and permanent occlusal surfaces, but L

underestimated primary occlusal surfaces significantly more than permanent occlusal surfaces. Compared to C, L underestimated primary occlusal caries extent and overestimated permanent occlusal caries such that it detected more caries extent compared to C. ROC analysis of L revealed that L performed better at detecting dentinal caries than inner enamel caries. L cut-off limits were similar to other in vitro studies: no caries (0-5), outer enamel caries (6-8), inner enamel caries (8-11), and dentin caries (>11). The inter-rater kappa scores for radiographic caries presence were 0.321 (C), 0.337 (S), and 0.320 (D) and those for radiographic caries extent were 0.334 (C), 0.343 (S), and 0.320 (D). Although S had slightly higher kappa values across caries presence and extent, there were no significant differences among C, S, and D confirmed with Z-score. The intra-rater reliability of L was assessed by Lin's concordance, which yielded excellent reliability of 0.96.

**Conclusions:** Inter-rater reliability of radiography showed that dentists in general agreed only fairly in detecting caries with radiography, but the intra-rater reliability of L showed excellent reproducibility enabling it to monitor caries activity and assess outcome of preventive interventions. S and D were not significantly different from C for detecting primary and permanent proximal caries and permanent occlusal caries, but both S and D significantly underestimated primary occlusal caries compared to C. L is superior to C in detecting permanent occlusal caries, but L is inferior to C in primary occlusal caries using Kavo's cut-off points. Therefore, caution should be used in diagnosing primary occlusal caries when using S and D radiographic methods and further research is needed to redefine L cut-off points in primary teeth.

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# A. INTRODUCTION AND SPECIFIC AIMS

## A.1. INTRODUCTION

The most common disease in children is dental caries (White 1984; Milgrom and Reisine 2000; Anderson 2002). Dental caries possesses the characteristics of being chronic, infectious, and slowly progressive (Angmar-Mansson, Al-Khateeb et al. 1998). They are seldom self-limiting and sometimes progress to total tooth destruction in the absence of intervention (Angmar-Mansson, Al-Khateeb et al. 1998). As one of the human conditions, dental caries has existed ever since humans evolved (Anderson, Bales et al. 1993). Over the past 50 years, the nature of dental caries in Western countries has dramatically changed due to the addition of fluoride into community water supplies and oral hygiene products (Stookey, Jackson et al. 1999; Udin 1999; Milgrom and Reisine 2000). The universal availability of fluoride lowers progression and rates of caries or even arrests smaller lesions. With this change in dental caries, there has been a shift in treatment philosophy from the original GV Black surgical approach of "extension for prevention" to the modern nonsurgical approach of "early caries intervention". There are several emerging preventive interventions for non-cavitated tooth structure; i.e. pit and fissure sealants; topical fluorides in the forms of varnish, gel and rinse; antimicrobial chlorohexidine therapy in the forms of varnishes, gels, and rinses; and the saliva enhancement by sucrose-free and xylitol-containing chewing gum (RTI 2001). However, the modern nonsurgical approach is only effective if caries can be diagnosed at an early stage and its progression monitored. Thus, the changes in caries prevalence and progression call for early caries diagnosis in modern dental offices.

Traditionally, occlusal and proximal caries have been diagnosed by visual-tactile (with sharp explorer) and radiographic examinations. For detecting occlusal caries, the tactile sign of resistance while withdrawing the explorer has been considered diagnostic for caries. However, several studies questioned the value of the sharp explorer due to its extremely low sensitivity values when attempting to detect occlusal caries (Lussi 1991; Penning, van Amerongen et al. 1992; Lussi 1993). Many European schools believe that it is unethical to use an explorer because it was shown in 1969 in Sweden (Bergman and Linde 1969) that iatrogenic damage could readily be produced, particularly on initial caries within occlusal fissures, and favor subsequent enamel demineralization (van Dorp, Exterkate et al. 1988). Also, Lussi (Lussi 1996) suggested that there is no diagnostic benefit from the combination of visual and tactile methods over the visual-only method meaning that the use of the sharp explorer for coronal caries diagnosis should be discontinued. Radiographic examination alone is more sensitive and reliable than visual-tactile examination for detecting occlusal dentin caries (Wenzel, Larsen et al. 1991; Ketley and Holt 1993; Lussi 1993). However, at the non-cavitated enamel level, clinical exam performed better than radiographic exam particularly on occlusal surfaces (Machiulskiene, Nyvad et al. 1999).

Furthermore, proximal caries is generally detected by bitewing radiography with either D-speed or E-speed films, which disclosed more than twice as many carious lesions in posterior teeth than did clinical inspection alone (Hintze 1993). Nonetheless, in a study of dentinal caries detection, both conventional radiography film types D- and E-speed have low sensitivity (8-22%) to disclose proximal caries (Ricketts, Whaites et al. 1997). Therefore, with traditional caries detection methods such as visual/tactile and radiographic methods, incipient occlusal and proximal caries can remain undetected until it is too late for non-surgical therapeutic intervention.

Clearly, the development of more refined caries detection methods that are sensitive and specific enough for current presentations of clinical caries is necessary to allow for detection at its earliest stages and for monitoring pathologic changes from early demineralization to cavitation (Angmar-Mansson, Al-Khateeb et al. 1998; Murdoch-Kinch 1999; Stookey, Jackson et al. 1999). Technology has improved over the last two decades to provide more accurate diagnostic tools and to lower radiation exposure to minimal to zero levels of radiation in some techniques. In August 2000, Kodak (Eastman Kodak Company, Rochester, NY) introduced its newest high speed intraoral film- "Insight" which is an F speed film. Several studies have shown that F speed films are as diagnostic as E speed films in permanent teeth (Ludlow, Abreu et al. 2001; Nair and Nair 2001). Digital radiography and laser fluorescence are now available for detecting proximal and occlusal caries. Many studies of digital radiography and laser fluorescence have reported that these techniques have equal or superior diagnostic ability as compared to conventional means of visual/tactile and conventional radiography (Wenzel, Larsen et al. 1991; Hintze et al. 1994; Dagenais and Clark 1995; Wenzel 1995; Nielsen, Hoernoe et al. 1996; Svanaes, Moystad et al. 1996; Uprichard, Potter et al. 1999; Attrill and Ashley 2001; El-Housseiny and Jamjoum 2001; Lussi, Megert et al. 2001; Anttonen, Seppa et al. 2003; Lussi and Francescut 2003). However, most F speed films, digital radiography, and laser fluorescence studies have focused on permanent dentition and very few research studies have been done on primary dentition (Bader, Shugars et al. 2001; NIH 2001). If digital radiography and laser fluorescence are better caries detection tools than those used currently and they can detect early caries with minimal or no radiation, then they should be preferable over the explorer and conventional radiography. Early caries detection will especially benefit pediatric patients by indicating early intervention, thereby preventing gross caries, dental pain, extraction, emotional stress, or eruptive problems.

## A.2. PURPOSE OF STUDY

The purpose of this study was to evaluate the detection of caries in proximal and occlusal surfaces by three new caries detection methods (indirect digital radiography, direct digital radiography, and laser fluorescence) in comparison to conventional radiography. A flatbed scanner with transparency adaptor (Epson Expression 1600) was chosen to produce the indirect digital radiography. The charged-couple device-based sensor (MPDx) from Dental Medical Diagnostics was selected for the direct digital radiography. The laser fluorescence device was DIAGNOdent (Kavo, Germany). Insight F-speed films (Kodak, Rochester, NY) represented conventional radiography.

## A.3. NULL HYPOTHESES

For Proximal Caries Detection:

- There is no difference in detecting proximal caries in the mixed dentition between indirect digital images (flatbed scanner, Epson Expression 1600) and direct digital images (charged-couple-device, MPDx) from conventional radiography (F-speed film, Kodak Insight).

For Occlusal Caries Detection:

- There is no difference in detecting overall occlusal caries between indirect digital images (flatbed scanner, Epson Expression 1600) and direct digital images (charged-couple-device, MPDx) from conventional radiography (F-speed film, Kodak Insight), and laser fluorescence (DIAGNOdent.) when using histology as the gold standard.

- When using laser fluorescence (DIAGNOdent), there is no difference in detecting occlusal caries in different moisture conditions of 100% humidity, 1 drop of water with a three second air blast, and 10 minutes air drying.
- There is no difference in detecting occlusal caries with the DIAGNOdent using different calibration techniques of calibration disc only vs. using the calibration disc followed by zeroing after subtraction of natural enamel fluorescence.
- Gamma irradiation sterilization of tooth samples does not significantly alter the laser fluorescence readings.

### A.4. LONG TERM OBJECTIVES

If the aforementioned 3 new caries detection technologies are equal or better than conventional radiography based upon an in vitro study, then it will be necessary to investigate their clinical effectiveness and the possibility of monitoring the success of early inhibition of incipient occlusal and proximal caries with these methods rather than treating by more invasive means.

## B. BACKGROUND AND REVIEW OF THE LITERATURE

### B.1. STATEMENT OF THE PROBLEM

In past, almost all children had large carious lesions with frank cavitation and the task of caries detection and diagnosis was straightforward. However, with availability of new early caries interventions, dental caries can be prevented and progression reduced, or even arrested. Clearly, with traditional caries detection methods, incipient occlusal and proximal caries can remain undetected until it is too late for non-surgical therapeutic intervention. Thus, the development of more refined caries detection methods that are sensitive and specific enough for current presentations of clinical caries is necessary to allow for detection at its earliest stages and for monitoring pathologic changes from early demineralization to cavitation (Angmar-Mansson, Al-Khateeb et al. 1998; Murdoch-Kinch 1999; Stookey, Jackson et al. 1999).

## B.2. DENTAL CARIES

### B.2.1. MICROBIOLOGY OF DENTAL CARIES

As an infectious disease, dental caries is caused by acidogenic and aciduric bacteria living in the dental plaque, including mutans streptococci, lactobacilli, Sanguinis streptococci, S. salivarius, and certain actinomyces (NIH 2001). Mutans streptococci colonize the host only after the first teeth erupt and their preferential colonization site is the tooth (NIH 2001). Their abundance in the plaque is the highest and increases level as sucrose consumption increases. However, they are recovered on cultivation of initial and established carious lesion sites. Their virulence is strongly associated with consumption of carbohydrates, especially sucrose (NIH 2001). On the other hand, lactobacilli preferentially colonize the dorsum of the tongue and are carried into saliva by sloughing of the tongue's epithelium (NIH 2001). They are often cultured from established carious lesions. Their cariogenicity is dependent upon consumption of carbohydrate-rich-diets. Furthermore, other non-mutans streptococci of several types, including the sanguinis group of organisms and S. salivarius, are extremely abundant in the mouth; some are tooth surface colonizers, while some are mucosal colonizers (NIH 2001). There is minimal evidence of their virulence in experimental animals. Lastly, actinomyces are abundant in the human mouth and induce root surface caries in hamsters and gnotobiotic animals (NIH 2001). However, their acidgenicity or acid-tolerance is low compared to mutans streptococci and lactobacilli.

Mutans streptococci and the lactobacilli are most commonly isolated in human dental caries (van Houte 1994; van Palenstein Helderman, Mattee et al. 1996). Using modern molecular and genetic methods to trace the source of transmission of infection by cariogenic bacteria, the mutans streptococci group is generally considered to be largely responsible (NIH 2001). Thus, the consensus document from the National Institute of Health conference (NIH 2001) summarizes that the mutans group of streptococci has a central role in initiation of caries of smooth surfaces and fissures of the crowns of the teeth. It is still unclear whether other streptococci groups and actinomyces as prominent etiological agents of dental caries in human.

B.2.2. MECHANISM OF DENTAL CARIES

It is important to understand the dynamic mechanism of the caries process in order to know how to diagnose and treat early dental caries (Featherstone 1996; Featherstone 1999). As these bacteria metabolize dietary fermentable carbohydrates, they produce acids, which diffuse rapidly into the underlying enamel or dentin and begin dissolving the mineral. The calcium and phosphate then diffuses out of the tooth, leading to subsurface demineralization. If the demineralization process continues, this eventually leads to cavitation. However, the demineralization process can be arrested or reversed by a remineralization process via the protective factors of saliva and fluoride. During remineralization, minerals like calcium, phosphate, and fluoride can diffuse back into the tooth. Thus, caries progression or reversal is a balance between pathologic factors (bacteria, carbohydrates, and salivary dysfunction) and protective factors (fluoride, saliva, calcium, and phosphate) (Featherstone 1996; Featherstone 1999).

B.2.3. SITE SPECIFIC

Dental caries is also site specific as each tooth and each site have different susceptibilities because of their specific anatomical, physiologic, and environmental characteristics. The occlusal surfaces of posterior teeth have invaginations called pits and fissures, whereas the facial, lingual, and proximal aspects of tooth are smooth. These anatomical variations provide different environmental niches that permit very different forms of plaque to flourish (Zero 1999).

B.2.4. PRIMARY TEETH VERSUS PERMANENT TEETH

Although both primary and permanent teeth have the similar bacteria and mechanism for dental caries, there are differences in morphology and composition that make primary teeth more susceptible to demineralization. Morphologically, the enamel and dentin layers of the primary dentition are known to be much thinner than those of their permanent successors (Hunter, Westb et al. 2000). Compositionally, primary teeth demonstrate less calcium and phosphate ions in dentin (Nor, Feigal et al. 1996), a lower degree of minerals (Wilson and Beynon 1989), and a higher degree of enamel porosity (Shellis 1984) than permanent teeth.

Furthermore, Shellis (1984) has shown that primary teeth are more susceptible to caries-like acid attack than permanent teeth in vitro. These artificial caries-like lesions in primary teeth were 75% deeper than in permanent tissue. Thus, primary enamel is less acid-resistant than permanent enamel and caries progression is faster in primary than permanent teeth

Knowing the morphological and compositional differences between primary and permanent teeth, it is important to make sure that new caries diagnostic techniques are equally suitable for the detection and quantification of caries in both the primary and the permanent dentition.

## B.3. CONVENTIONAL RADIOGRAPHY

### B.3.1. USE OF RADIOGRAPHY AND DIAGNOSTIC ACCURACY OF D & E SPEED FILMS

Radiographic examination in dentistry is a valuable diagnostic tool to help the practitioner make a diagnosis and accurately plan treatment of dental diseases. With dental caries as the most common disease in children (White 1984), early detection of dental caries, infection, and developmental conditions such as missing teeth, supernumerary teeth, ectopic eruption, delayed root resorption of primary teeth, and deflected eruptive paths of permanent teeth are very important to achieve the optimal development of the child's dentition. Over the past few decades, the frequency of bitewing radiography among children has been reduced due to an enormous decline in caries prevalence resulting in the "benefits" of radiography being gradually outweighed by the "costs" (Roeters, Verdonschot et al. 1994). Efforts to use risk factors as caries predictors have further reduced recommended exposure to children. However, in spite of reduced usage, radiographs remain a common and necessary diagnostic tool in dentistry.

Clinical study data showed that radiography reveals two to three times more proximal and occlusal carious lesions than do visual examinations alone (Hintze 1993). In one study involving young adult air force recruits, only one-third of occlusal dentinal lesions were diagnosed visually, whereas two-thirds were discovered on bitewing radiographs (Richardson and McIntyre 1996). Another study reported that bitewing radiographs revealed obvious lesions into the dentin in 15% of apparently sound occlusal surfaces (Weerheijm, Gruythuysen et al. 1992). On the contrary, in a study comparing clinical and radiographic caries diagnoses in posterior teeth of 12-year-old Lithuanian children, Machiulskiene et al (1999) concluded that the diagnostic yield of bite-wing radiography is higher for proximal than for occlusal surfaces and clinical exam performed better than radiographic exam at the non-cavitated enamel level on occlusal surfaces when the clinical caries diagnostic criteria include non-cavitated diagnoses.

Both Proximal and occlusal caries can present in a spectrum of forms from incipient enamel demineralization to large cavity formation. Van Amerongen et al (1992) evaluated dentinal caries involvement in 60 extracted molars containing small but visible occlusal cavities. They found all teeth had dentin caries involvement with 25% that reaching the dentino-enamel junction and 75% extending far into the dentin. Thus, the presence of visible cavitation of the enamel surface most likely indicates dentin involvement. When definite cavitation is present, the carious process has probably penetrated far into the dentin.

In an in vitro study, Lussi evaluated cavitated occlusal lesions and compared the results with his previous study of non-cavitated occlusal lesions with the same evaluators (Lussi 1996). The investigator showed that bitewing radiography had higher sensitivity than visual inspection with or without magnification for both cavitated and non-cavitated occlusal lesions (Table 1). Also, the sensitivity and specificity for cavitated lesions in all diagnostic methods were higher than those for non-cavitated lesions. Thus, the status of proximal or occlusal surfaces (demineralized vs. cavitated) has a direct impact on the diagnostic accuracy

| Diagnostic methods | Cavitated Occlusal Sensitivity (%) | Non-cavitated Occlusal Sensitivity (%) |
|---|---|---|
| Visual inspection | 62 | 12 |
| Visual inspection with magnification | 75 | 20 |
| Bitewing radiograph | 79 | 45 |
| Visual inspection with bitewing | 90 | 49 |
| Visual inspection with explorer | 82 | 14 |

Table 1. Illustration of Sensitivity of 5 different diagnostic methods in cavitated and non-cavitated occlusal lesions per (Lussi 1996).

Similarly, an in vitro study evaluating radiographic accuracy of small proximal and occlusal lesion in a low prevalence sample (4% cavitations both proximal and occlusal surfaces), Ricketts and co-workers found that both D and E speed films detected dentin caries with low sensitivity (proximal 8-22%; occlusal 0-30%) and high specificity (proximal 98-100%; occlusal 79-100%) (Ricketts, Whaites et al. 1997). They explained that the overall low sensitivity finding in both proximal and occlusal caries reflected the difficulty of diagnosing demineralized tooth structure when x-rays have to pass through intact buccal and lingual enamel. The specificity of occlusal caries was lower than that of proximal caries, which reflected the uncertainty of occlusal caries diagnosis. Also, their findings agree with other studies using a histological validation technique to show lesions were actually larger than they appeared radiographically (Ricketts, Whaites et al. 1997). Thus, they reported that using radiographs, all examiners apparently under-diagnosed the number and extent of the lesions identified using a histological gold standard (Ricketts, Whaites et al. 1997).

Similar results were found in an in vitro study of occlusal dentin caries in the second primary molars and the first permanent molars without cavitation, visual inspection sensitivity and specificity were 31% and 98% respectively, whereas radiographic sensitivity and specificity were 67% and 92% respectively (Ketley and Holt 1993). Combining the two (visual and radiographic) examinations improved sensitivity to 75% and maintained specificity at 90% (Ketley and Holt 1993).

In summary, conventional radiography (mainly E-speed films) can detect dentin caries better than demineralized enamel caries and can better detect in proximal surfaces than occlusal surfaces. Diagnostic ability is improved when clinical examination is combined with radiographs.

### B.3.2. RADIATION CONCERNS OF RADIOGRAPHY

To put dental x-ray exposure in perspective, individuals in the general population of the United States receive approximately 3.6 mSv exposure to ionizing radiation annually (White 1992). On average, 82% of radiation exposure is received from natural sources (radon, cosmic, terrestrial, and internal radiations) and 18% received from man-made sources. Medical and dental procedures account for the vast majority of man-made radiation sources, mostly for diagnostic rather than therapeutic purposes. Medical diagnostic radiation accounts for only about 11% of all exposures. Only about 1% of this 11%, or about 0.1% (0.1% x 3.6 mSv = 3.6 x $10^{-3}$ mSv) of the total exposure, results from dental radiography (White 1992).

Three authoritative committees have assessed the radiobiological risks for low-dose radiation: the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the International Commission on Radiological Protection (ICRP), and the National Academy of Sciences of the USA, Committee on the Biological Effects of Ionizing Radiations (BEIR III). All three committees estimated the risks of low-dose ionizing radiation by extrapolating from high dose exposures where the effects are measurable and obvious. These exposures were documented from people such as survivors of the Hiroshima and Nagasaki atom bombs, the Three Mile Island incident, and the meltdown at the Chernobyl nuclear plant in the Soviet Union (Gibbs 1982; Underhill, Kimura et al. 1988; Farman 1991). Estimation of risk from groups of exposed individuals requires use of mathematical models that fit the epidemiological data. However, inconsistent data have resulted for low dose and low dose-rate conditions across these three different committee studies.

Utilizing the findings of the committees as a base, a number of authors have estimated risk from various dental radiographic procedures. When an individual is exposed to ionizing radiation from a dental x-ray machine, the postulated risk from this procedure is the induction of damage to either the somatic or genetic tissues. The principal types of damage induced are cancers, mutations, and congenital abnormalities (Gibbs 1982; Sikorski and Taylor 1984; Preston-Martin, Thomas et al. 1988; Preston-Martin and White 1990; Farman 1991; Wood, Harris et al. 1991; White 1992; Thunthy 1993; Bricker and Kasle 1994). Critical organs or tissues (with associated tissue damage) include skin (cancer), thyroid (cancer), eye (cataracts), hematopoietic system (leukemia), and gonads (mutation, infertility, fetal malformations).

Over the years, various authors have presented their estimation on risks from dental radiographic procedures. For example, Gibbs (1982) estimated the total cancer risk from dental radiographic procedures as one in a million for the average dental radiographic examination meaning one radiation-induced cancer at some time in the life span of one million individuals that receive an average, but unspecified, dental radiographic examination. Gibbs (1982) also stated that gonadal dose to patients from full mouth intraoral dental radiographic examinations ranges from 2 to 20 μGy with a very small risk of mutation, about one in a billion, depending on beam energy (kVp) and type of collimator. Comparatively, Gibbs tabulated that a person has a one in a million risk of dying from such events as an accident in an airplane traveling for 1,000 miles or cirrhosis by drinking 500 cc of wine.

Moreover, in 1984, White stated that "For all but a few types of cancer there is no evidence of a threshold limit and no dose is so low that it does not have some potential for carcinogenesis." White (1984) also believed that the induction rate of leukemia is higher in children and the elderly than for young adults. For benign thyroid lesions, the induction rate is four times higher for children under ten than for individuals over twenty. Thus, White concluded that clinicians needed to reduce radiation by optimizing types and frequency of radiography selection, using appropriate radiographic accessories to aid dose reduction. However, Bricker (Bricker and Kasle 1994) described that carcinogenesis and malformations of somatic tissues have a threshold response, but mutations of genetic tissues have no threshold limit. The threshold doses of critical organ doses were compared from a full mouth radiographic survey and a panoramic survey and showed that the dosages are quite low in relation to the various thresholds. Different articles concerning effects of low dose radiation are frequently contradictory due to different statistical methods.

While the harmful long-term effects of low dose radiation are not fully known, an action was called to minimize unnecessary radiation exposure. In 1977, the International Commission on Radiological Protection (ICRP) of dose limitation required radiation exposures to be kept "as low as reasonably achievable" (ALARA) (Fleishman, Notley et al. 1983). In 1988, the United States Food and Drug Administration issued guidelines to help dental practitioners reduce the amount of x-ray exposure to patients without reducing the quality of care (Table 1) (AAPD 1998). This guideline stated that the decision to take radiographs is based on a thorough clinical evaluation and examination of the patient. Selection criteria when deciding on the radiographic examination are (1) the stage of dentition development and (2) the risk of dental caries. A clinical examination prior to x-ray exposure is required to evaluate the history of caries, oral hygiene, and related factors to determine type and frequency of radiographic examination. Other radiographs should be obtained when there are clinical signs or symptoms suggesting disease or other abnormal conditions. Thus, to minimize unnecessary radiation exposure, radiographic examination should not be routine and obtained only when necessary. Furthermore, Atchinson (Atchison, White et al. 1995) assessed the efficacy of the FDA guideline for ordering dental radiographs and concluded that dentists can reduce a patient's exposure to x-rays by using these guidelines with a low level of missed radiographic findings, most of which would have no effect on the patient's treatment.

Minimizing the potential radiobiological risks to young patients is a priority because tissues and organ systems are in continuous growth phases compared to mature adults. It is appropriate that the clinician understand the potential these patients have in developing radiation effects. The harmful effects of low doses of x-radiation normally associated with radiographic procedures cannot be proven scientifically as a directly observable discrete change in a specific individual. The higher the dose, the higher the cancer rate, and the easier it is to demonstrate a correlation between irradiation (the cause) and the effect. Therefore, at very low doses, it becomes increasingly difficult to demonstrate the cause/effect relationships (especially dealing with human being as subjects) and it becomes highly dependent on statistical extrapolations. As a result, the literature concerning effects of low-dose radiation tends to be highly confusing and frequently contradictory.

It is the clinician's responsibility to reduce radiation dose to patients. It is possible that genetic change will be expressed in a much more subtle fashion than a clinically detectable malformation. Thus, the relative lack of knowledge of the genetic effects of radiation in humans severely compromises attempts to quantify the extent of this type of risk. Nonetheless, it is necessary to realize that dental radiographic exposure is much less than the natural variation of background exposure (0.1% of all sources of radiation annually in United States). However, since dental caries is the most common disease in children and diagnostic radiographs are useful aids, clinicians should err on the safer side.

B.3.3. WAYS TO MINIMIZE RADIATION

Radiation reduction in dental radiography continues to be a clinical and ethical concern. The goal is to maximize visualization of diagnostic information (benefit) with minimal radiation dose (risk) to the patient, or ALARA (as low as reasonably achievable). Thus, besides prescribing radiographs to patients who really need it, there are many ways to minimize patients' radiation exposure. First, unnecessary radiation exposure can be avoid by obtaining quality radiographs and avoiding retakes. Second, systematic practices to assure the proper operation of x-ray equipment (darkroom, x-ray unit, radiographs, and office radiation safety policies) will reduce patient and operator exposure improve the quality of radiographs, and save professional time (White 1984).

Finally, using appropriate radiographic accessories can aid dose reduction as well. For example, radiation dose can be decreased by (1) use of lead apron (White 1984; Kircos and Angin 1987; Preece 1988; Wood, Harris et al. 1991; Bricker and Kasle 1994), (2) lead thyroid collar (Sikorski and Taylor 1984; Kircos and Angin 1987; Preece 1988; Wood, Harris et al. 1991; Bricker and Kasle 1994), (3) positioning devices (White 1984; Kircos and Angin 1987; Preece 1988; Bricker and Kasle 1994), (4) long rectangular collimation (Kircos and Angin 1987; Preece 1988; Bricker and Kasle 1994), (5) higher kilovolt peak (White 1984; Kircos and Angin 1987; Preece 1988; Bricker and Kasle 1994), and (6) faster speed films (White 1984; Kircos and Angin 1987; Preece 1988; Bricker and Kasle 1994; Price 2001). The use of lead apron and thyroid collar is helpful in protecting the patient from scatter radiation by about 98% to the gonads and 50% to the thyroid (Preece 1988). Positioning devices provide stability of the film during radiographic procedures to eliminate technical errors (White 1984; Preece 1988; Bricker and Kasle 1994). Long rectangular collimation is preferable to a circular field of radiation since it limits the size and shape of the useful radiation to the patient. It does not reduce radiation dose, but it can improve the image quality by reducing excessive scatter radiation and thus increase subject contrast (Preece 1988). The use of higher kVp results in short exposure time (White 1984; Preece 1988; Bricker and Kasle 1994). In 2000, Kodak introduced an F-speed film, "Insight", as its newest high speed intraoral films. Insight (F-speed film) is 20% faster than Kodak Ektaspeed Plus (E-speed films), and 60% faster than Kodak Ultraspeed film (D-speed films). Thus, radiation exposure to the patient can be significantly reduced by conversion from D-speed film to F-speed film alone (Price 2001).

Over time, dental radiology has decreased exposure to patients with the advent of new technology and materials. In 1976, the common x-ray machine output was 600 milliampere seconds (mAs) per exposure (Antoku, Kihara et al. 1976). Today, a common setting with F-speed films yields 70mAs and E-speed film yields 90 mAs per exposure.

B.3.4. DIAGNOSTIC ACCURACY OF F-SPEED FILMS

Several studies have investigated the sensitometric and diagnostic qualities of F-speed films. When comparing the speed and contrast characteristics of F-speed with E-speed film, Geist (2001) found that F-speed films not only provide 20-24% exposure reduction when compared to E-speed films, but also had similar contrast over several density ranges and was less resistant to decreases in speed when processed in used chemicals. Price (2001) also reported that F-speed film had almost identical film contrasts as E-speed except that F-speed film had slightly greater contrast in the higher density range. Also both E- and F-speed films resolved 10 line-pairs per millimeter. Additionally, Ludlow (2001) found E- and F-speed are capable of resolving at least 20 line-pairs per millimeter and F-speed films provide more stable contrast in depleted processing solutions. When comparing diagnostic qualities of F-speed films, many studies have reported that Insight's performance does not appear to be different from E-speed or D-speed for caries detection (Geist and Brand 2001; Ludlow, Abreu et al. 2001; Ludlow, Platin et al. 2001). Ludlow (Ludlow, Abreu et al. 2001) also reported that Kodak's F-speed film is not statistically different from E or D speed films for proximal caries detection. Thus, compared with other existing films, F-speed film provides the advantages of less radiation and equal or better image quality over other films.

## B.4. DIGITAL RADIOGRAPHY

Digital radiography can be direct or indirect. For direct digital radiography, there are two types for caries diagnosis: (1) Charge-coupled-devices (CCD) and (2) storage-phosphors (SP) (Anderson, Bales et al. 1993; Lackey 1998). The CCD systems use a mouth sensor and a cord connected to a computer to display a digital x-ray image on the monitor. The SP system is cordless, using a storage phosphor transfer image plate, which after laser scanning integrates digitally into a computer and displays the image on a monitor. Both systems can create digital images from dramatically less radiation and eliminate the need for film. On the other hand, indirect digital radiography can be obtained by digitizing conventional radiography through CCD camera, rotating drum, a laser scanner, or a flatbed scanner with a transparency adaptor (Chen and Hollender 1995). Thus, although indirect digital radiography does not reduce radiation, the digitized radiographic images can be enhanced and transmitted electronically.

### B.4.1. DIRECT DIGITAL

#### B.4.1.1. Radiation Concern of Direct Digital Radiography

Intraoral direct digital systems require an average of about 53% less radiation than current film based images (E Speed film) and 75% to 80% less radiation than D speed film (Lim, Loh et al. 1996; AAPD 1998; Tyndall, Ludlow et al. 1998; Yoshiura, Kawazu et al. 1999). Direct digital systems seem to require less radiation than F speed films.

B.4.1.2. Advantages and Disadvantages of Direct Digital Radiography

Unlike film and chemical processing, digital image quality can be enhanced by the computer system after the exposure is made. Digital contrast enhancement, digital subtraction, high-speed image acquisition, environmental conservation (elimination of chemical waste), easy image transfer and organization, and image quality manipulation are major advantages of digital radiography (Wenzel 1993; Wenzel 1995; Murdoch-Kinch 1999; Stookey, Jackson et al. 1999). This, in conjunction with the low doses of radiation, makes digital radiography a powerful tool over traditional film radiographs in detecting caries .

There are some potential disadvantages to digital imaging. Obviously, one major disadvantage would be the initial financial investment in hardware and software that must be overcome with individual office planning and priority (Wenzel 1995). Besides the monetary disadvantage, one study indicated that digital radiographs were not as discriminatory between natural versus artificial lesions, thus indicating reduced diagnostic performance for digital radiography in comparison to E-speed film (Kang, Farman et al. 1996). Digital images are much larger (magnification) than conventional radiographs, which require more training and experience for those accustomed for films (Versteeg, Sanderink et al. 1997). Furthermore, the area of the sensor is smaller than that of comparably sized conventional film, thus increasing the possible need for additional exposures to obtain adequate anatomical coverage (Vandre and Webber 1995; Wenzel 1998). Moreover, the sensor is rigid and may cause more discomfort to the patient compared to flexible film. A wire extends from the sensor to the computer possibly becoming cumbersome to the operator. However, the instant images may be an amusement to pediatric patients.

B.4.1.3. Diagnostic Accuracy of Direct Digital Radiography

A diagnostic digital image must have adequate gray-level (contrast) and spatial resolution (Van der Stelt 2000). A typical digital image comprises a range of 256 gray levels (values 0 to 255, 8 bits per pixel) while the human eye can only distinguish approximately 100 gray levels. Thus, 256 different gray levels are more than sufficient. Theoretically, higher spatial resolution (greater number of line pairs per millimeter, lp/mm) is better, although the human eye is limited in the number of line pairs it can distinguish without magnification to about 6-10 lp/mm (Sanderink and Miles 2000). Dental film is generally thought to have more than 15 lp/mm (Preston 1998). Many current charge-coupled devices have 8-12 lp/mm (Preston 1998). Thus, for digital radiography, as opposed to film-based radiography, the diagnostic accuracy is based more on contrast rather than spatial resolution. Even though all sensor-based systems can resolve greater than 8 lp/mm, they depend more on contrast resolution capability to compensate for the lower spatial resolution compared to X-ray film. The new charged-couple devised-based sensor (MPDx) from Dental Medical Diagnostics claimed to have thin sensor thickness of 3.2mm and a superior resolution of 22 lp/mm (Miles, Langlais et al. 1999). Thus, it was chosen in the present study, to represent the direct digital radiography.

When the first direct digital CCD system was introduced, it had a higher sensitivity of 70% compared to conventional radiograph and digitized films of 45% and no increase in false positives in detecting deep dentinal lesions of non-cavitated occlusal surfaces (Wenzel, Larsen et al. 1991). In permanent teeth, several others reported no differences in proximal and occlusal caries diagnostic accuracy between conventional radiography and direct digital systems (Wenzel, Larsen et al. 1991; Hintze, A et al. 1994; Wenzel 1995; Svanaes, Moystad et al. 1996) while others have shown greater accuracy with conventional film (Dagenais and Clark 1995). Price et al also found CCD systems to be less accurate than film for both natural and artificial proximal caries (Price and Ergul 1997).

In primary teeth, the PSP system did not perform differently from E speed film for the detection of cavitated and non-cavitated proximal surfaces (Nielsen, Hoernoe et al. 1996). The majority of lesions diagnosed radiographically to be in dentin were cavitated. They reported no significant difference in the proportion of lesions in outer enamel, inner enamel and dentin detected by the two methods. However, in a similar in vitro study, comparing CCD and conventional radiography (D and E speed films) in the mixed dentition, Uprichard et al (1999) concluded that CCD based direct digital radiography was not as accurate as conventional film images for diagnosing proximal surfaces.

Currently, there are two studies comparing diagnostic accuracy of Kodak's F-speed films with digital radiography. Nair (Nair and Nair 2001) found that F-speed film is as good as the E-speed film and the Schick CMOS-APS digital sensor in diagnosing natural proximal caries in an in vitro study. The inter- and intra-observer agreements were 0.42 and 0.66 respectively, fair to good. There were a significant difference with respect to caries depth and observers. Recently, in an in vitro study, Hintze et al (2002) evaluated the influence of validation methods on the diagnostic accuracy of 6 CCD systems (MPDx, Dixi, Sidexis, RVG old, RVG new, Visualix) and 2 film systems (E and F speed). For both proximal and occlusal caries, the 8 radiographic systems all had significantly higher areas under the receiver operating characteristic curve using the radiographic prediction versus histologic validation with $p<0.001$. For proximal caries detection using histological validation, Dixi was comparable to E speed, which was significantly more accurate than MPDx, all other CCDs and F speed film. When using radiographic validation, E speed was more accurate than Dixi, which was significantly more accurate than MPDx and all other CCD systems. For occlusal caries detection using histological validation, the E and F speed films, and 2 other CCD systems (Dixi, Sidexis) were significantly more accurate than MPDx. Using radiographic validation for occlusal caries, MPDx was found to be significantly less accurate than RVG old and RVG new. They concluded that the diagnostic efficacies for caries of 8 radiographic systems were strongly influenced by the validation method.

### B.4.2. INDIRECT DIGITAL RADIOGRAPHY

#### B.4.2.1. Advantages and Disadvantages of Indirect Digital Radiography

Digitizing conventional radiographs is becoming an essential feature of paperless (digital) dental offices during the transition period between the use of conventional radiographs and the routine adoption of digital imaging. The advantages of obtaining an indirect digital radiograph are as following: (1) providing an inexpensive method to incorporate digital radiography into dental practice (Lavine 2001), (2) allowing digital image processing (Chen and Hollender 1995; Lavine 2001), (3) improving image archiving (Chen and Hollender 1995; Lavine 2001), and (4) improving communication with patient, dental professionals, and insurance companies (Chen and Hollender 1995;

Lavine 2001). Disadvantages of indirect digital radiographs are as follows: (1) no reduction of radiation, (2) image is only as good as the original film scanned (Miles, Langlais et al. 1999), and (3) multiple steps involved including taking a conventional radiograph, scanning radiograph with a scanner with a transparency adaptor, using integrated software to organize and manipulating images (Lavine 2001).

#### B.4.2.2. Diagnostic Accuracy of Indirect Digital Radiography

There are few studies investigating scanning resolutions of radiographs for different tasks in dentistry. Many focused on interproximal bone loss and not dental caries (Hildebolt, Vannier et al. 1990; Eickholz, Riess et al. 1999), or using a scanner with a CCD digital camera (Hildebolt, Vannier et al. 1990; Malek, Izumo et al. 1997), or using a 35mm slide scanner (Shrout, Potter et al. 1993; Shrout, Potter et al. 1993). As a less expensive alternative becoming available, flatbed scanners with a transparency adaptor can also be used to digitize radiographs. However, there are only two studies focused on flatbed scanner and dental caries.

Resolutions of 150, 300, 600, or 900 dpi are typical for scanned radiographs since diagnostic details are preserved and higher resolutions do not produce better quality images since silver grains of x-ray film become evident (Van der Stelt 2000). Chen et al (1995) evaluated the reproducibility and performance of a flatbed scanner (ArtiScan) at 200 dpi with different scanning time settings. Optical densities were measured by a densitometer and were compared to the original films. As a result, the ArtiScan flatbed scanner had a narrower dynamic range than the images on the x-ray film and an increased scanning time widened the pixel value range and increased the number of steps in the middle pixel value range of the image, but the steps in the lighter and darker part of the image were not identifiable. The investigators discussed that the narrow dynamic range of this digitizing system can affect the diagnostic performance of the digitized images mainly in the darker or lighter part of the images; thus diagnosing enamel caries would be especially impaired. Reproducible scanning of images was possible when the images were scanned over the same area of the scanning field without changing the scanning settings. However, scanned images were not reproducible when the image was scanned at different locations or when the scanner was turned off between scanning. Thus, investigators recommended standardizing scanning settings and film positions before each scan in order to use the flatbed scanner to digitize dental x-ray film for diagnostic purposes.

In an in vitro study, Janhom et al (2001) evaluated the different resolutions (150, 300, and 600 dpi) of digitizing x-ray film with a flatbed scanner in diagnosing proximal caries. Ten observers assessed proximal caries presence and extent and severity scores were validated with a histology gold standard. They found that lesion extent had a significant effect on confidence of lesion recognition. The confidence increased as the resolution increased but no significant difference was found between 300 and 600 dpi. Similar scores for caries extent estimation were obtained at the 300 dpi scanning resolution, but the digital file size was smaller and thus the storage requirement was also lower. Thus, the scanning resolution of this present study is chosen to be 474 dpi, which is between 300 and 600 dpi and the same resolution as the 100% actual pixel view of MPDx (1:1 ratio sensor pixel: monitor pixel).

Enhanced digitized radiography for occlusal caries is strongly correlated to the quantitative measures of occlusal caries depth compared to clinical and conventional radiographic assessment (Wenzel, Fejerskov et al. 1990). However, another study concluded that there was no significant difference in diagnostic accuracy detected in proximal dental caries between the non-enhanced digitized images and conventional film (Dove and McDavid 1992). Thus, in an effort to minimize subjective variables for this current study, evaluators viewed digital images (both direct and indirect) without enhancement or filtering.

## B.5. LASER FLUORESCENCE

DIAGNOdent is a novel optical caries detector. It consists of a laser diode light source, which produces 655nm wavelength light and is coupled into an optical fiber and transmitted to the tooth (Hibst R, Paulus R et al. 2001). The excitation fiber is surrounded by a bundle of 9 thin central fibers, which gather fluorescence of a chromospheres as well as backscattered light and guide it to the detection unit. The fluorescence of the backscattered light is discriminated via the laser diode modulation (Hibst R, Paulus R et al. 2001). The fluorescence of the chromospheres (bacterial porphyrins) is a marker that supposedly correlates with the caries extent. The intensity of the fluorescence is then reflected back through the hand piece to the detector, where it is translated into a number (0-99). The instructions for the DIAGNOdent system suggest optimal cut-off limits as: 0-14 (no caries), 15-20 (outer enamel caries), 21-99 (dentinal caries) (DIAGNOdent user's manual, KaVo, Biberach, Germany).

### B.5.1. ADVANTAGES & DISADVANTAGES OF DIAGNODENT

Like any other diagnostic method, DIAGNOdent has both advantages and disadvantages. Ross (1999, 2000) suggested seven possible advantages of DIAGNOdent as follows: (1) quantitative, not subjective: DIAGNOdent gives a number from 0-99 which can be compared at a later exam; (2) know when to treat and when to watch: DIAGNOdent is very accurate (>90%) in diagnosing the severity of a lesion; (3) know amount of caries to treat: allows mapping caries in a tooth; (4) increased patient confidence: as the patients hear the audio sound and sees the digital readout, patients can readily accept the dentist's treatment plan; (5) ability to monitor the effect of preventive measures; (6) more accurate record keeping; and (7) ability to check a tooth for any caries before a sealant is placed. However, many research studies are still investigating the above-mentioned advantages.

The main disadvantage of DIAGNOdent is due to false positives occurring from presence of (1) plaque, (2) calculus in fissures, (3) discoloration, (4) tartar, (5) food, (6) composite resin, (7) sealants, and (8) ceramic restorations (KaVo, Biberach, Germany, Ross 1999, Ross 2000).

B.5.2. DIAGNOSTIC ACCURACY OF DIAGNODENT

There are several in vitro and a few in vivo studies comparing diagnostic accuracy of DIAGNOdent from new and conventional diagnostic methods. Lussi et al (Lussi, Imwinkelried et al. 1999) evaluated the DIAGNOdent system in vitro with extracted non-cavitated molars. DIAGNOdent was compared with a fixed –frequency type of electrical detection system (ECM). The sensitivity and specificity of DIAGNOdent were 0.76 to 0.87 and 0.72 to 0.87 respectively from D2 (inner enamel) to D3 (outer dentin). Those of ECM were 0.87 to 0.92 (sensitivity) and 0.64 to 0.78 (specificity). Also, DIAGNOdent had high intra-examiner kappa scores of 0.88 (D2) and 0.90 (D3). The inter-examiner reproducibility was 0.65 (D2) and 0.73 (D3). They also reported optimal (yet different) cut-off limits for DIAGNOdent in permanent teeth as 0-4 (no caries), 4.01-10 (enamel caries), 10.01-18 (outer dentin caries), >18.01 (inner dentin caries). However, the investigators suggested using caution in extrapolating their results to clinical situations. They concluded that overall DIAGNOdent had a higher diagnostic validity than the ECM and DIAGNOdent measurements were highly reproducible making it a valuable tool for the longitudinal monitoring of caries and for assessing the outcome of preventive interventions.

In similar in vitro studies, DIAGNOdent was compared to conventional diagnostic methods. El-Housseiny et al (2001) reported DIAGNOdent was superior to visual inspection or explorer. Shi et al (2000) compared DIAGNOdent to conventional radiographs in extracted non-cavitated premolars and molars using ROC analysis. They showed that DIAGNOdent was significantly superior to radiographs for detecting enamel and dentin caries in occlusal surfaces ($p \leq 0.001$). Radiographic and DIAGNOdent had statistically significantly higher diagnostic accuracy in the detection of dentinal caries than enamel caries ($p \leq 0.001$ for radiography and $p=0.029$ for DIAGNOdent). Also, they reported the humidity of the teeth did influence the measurements from DIAGNOdent ($p<0.001$) such that dry conditions led to higher cut-off points. However, it did not seem to affect the diagnostic performance. Therefore, the investigators recommended that maintaining a constant physical environment was important.

Moreover, Sheehy et al (2001) evaluated the DIAGNOdent system in an in vivo study by comparing it with visual inspection. Since there was no gold standard, the investigators tried to match DIAGNOdent readings with visual inspection. The agreement of the DIAGNOdent and visual inspection was better matched when using the cut-off limits recommended by the manufacturer than those based on laboratory research with histological validations by Lussi et al, 1999. As a result, since there is no true histology validation, the investigators suggested that either DIAGNOdent was over-scoring some lesions or the visual inspection underscoring them. They recommended in using DIAGNOdent as an adjunct to a clinical exam and when using it in the clinical setting, the cut-off limits recommended by the manufacturers should be used to interpret the results.

Another in vivo study by Lussi et al (2001) compared DIAGNOdent with visual inspection and conventional radiographs with operative intervention as the gold standard. Prior professional cleaning of tooth surfaces was not carried out, but if needed, an explorer carefully removed plaque from the fissures without applying apically directed force. They showed DIAGNOdent had statistically significant higher sensitivity ($\geq$ 92%) than visual inspection and conventional radiographs (31-63%). Based on their results, the optimal cut-off limits were similar to those recommended by the manufacturer: 0-13 (no caries); 14-20 (enamel caries); >20 (dentin caries). Investigators suggested that in vitro results should not be transferred automatically to the in vivo situation due to (1) unidentified fluoropheres could change characteristics as a consequence of the storage media of the extracted teeth and (2) histological exam of test teeth in vitro could identify minute changes in dentin.

There are only three studies focused on DIAGNOdent detection in occlusal caries of primary teeth. In an in vitro study, Attrill et al (Attrill and Ashley 2001) found that DIAGNOdent gave the highest sensitivity while offset by a lower specificity than visual inspection and radiographic methods. DIAGNOdent had the highest values of kappa for inter-examiner repeatability (0.7) while radiography had the lowest (0.56). Intra-examiner repeatability for DIAGNOdent also had the highest kappa value (0.78) except examiner 2 gave the best repeatability for visual inspection (0.77). Their cut-off limits were: 0-9 (no caries); 10-17 (enamel caries); and 18-99 (dentin caries.) The investigators concluded that DIAGNOdent was the most accurate system for the detection of occlusal dentin caries in primary teeth; moreover, visual inspection produced the highest combination of sensitivity and specificity at the pre-cavitation level (opacity or discoloration visible with or without air drying), but there was no statistical significance between visual inspection and DIAGNOdent at the pre-cavitation level.

Lussi et al (2003) also compared DIAGNOdent with conventional methods (visual inspection, visual inspection with magnification, visual inspection combined with light pressure probing, and bitewing radiography) in non-cavitated occlusal surfaces of primary molars. They found that DIAGNOdent was significantly better than all conventional methods in detecting dentin caries in occlusal surfaces of primary teeth with $p < 0.05$ (D3 or D4) and radiography performed significantly better than visual inspection and visual inspection combined with light pressure probing at D3. At the D2 level, DIAGNOdent performed significantly better than conventional methods with $p < 0.05$ except visual inspection with magnification. Intra-examiner reproducibility at D2 and D3 were good to excellent agreement (kappa values $\geq 0.76$). The optimal cut-off limits for DIAGNOdent based on sum of sensitivity and specificity were as follows: 0-4 (no caries and outer enamel caries; D0, D1); 5-12 (inner enamel caries; D2); >12 (dentin caries; D3, D4). They discussed that the difference of macro- and micromorphological characteristics of primary and permanent teeth may subsequently affect their physical and optical property. Primary teeth have greater enamel porosity (more light scattering leads to less fluorescence) and thinner enamel (less masking of dentin fluorescence lead to more fluorescence). They suggested that due to the canceling effects of these two optical properties of primary teeth, there was a small overall change of the fluorescence signal of primary teeth compared to the permanent teeth. Thus, the investigators concluded that DIAGNOdent had an overall performance in primary teeth similar to that obtained in previous studies on permanent teeth and its good reproducibility should allow DIAGNOdent to be used for longitudinal monitoring of the caries process.

Lastly, the only in vivo study of both primary and permanent teeth was performed by Anttonen et al (2003) who evaluated DIAGNOdent and conventional methods (visual inspection and radiographic method). Teeth were not cleaned professionally before examination and the gold standard for visually determined carious teeth was achieved by visual exam upon operative drilling. Investigators discussed that due to the study design the three diagnostic methods could not be directly compared. However, visual exam had high specificity such that all teeth opened with a drill had dentinal caries. Partly validated by operative intervention in visually determined carious teeth, the radiographic method was the least accurate of the methods in both enamel and dentin caries. DIAGNOdent performed best in detecting dentinal caries at a value of about 30 with both visual score and observed clinical lesion depth for validation. The mean DIAGNOdent values showed a steady gradient across the categories for visual inspection with a clear difference between the values for inactive and active enamel caries. The distribution of DIAGNOdent values for permanent and primary molars differed slightly such that the primary teeth had significantly lower mean DIAGNOdent values than permanent teeth in visually sound teeth. However, there was no difference between the values for inactive and active enamel caries. The investigators also reported that clear fissure sealants on permanent molars did not affect DIAGNOdent measurements, which is in agreement with an in vitro study performed by Takamori et al (2001). Thus, they concluded that DIAGNOdent appeared to be useful as an adjunct to visual examination in routine dental exams for children.

# C. EXPERIMENTAL DESIGN AND METHODS

## C.1 OUTLINE OF EXPERIMENTAL DESIGN

- 90 primary posterior teeth and 30 permanent posterior teeth were obtained and mounted in 30 quadrant blocks.
- Proximal caries was detected and compared by conventional, indirect digital (scanned), and direct digital radiography.
- Occlusal caries was detected and compared by conventional, indirect digital (scanned), direct digital radiography, and laser fluorescence using the Kavo DIAGNOdent.
- Six pediatric dentists and one general dentist with emphasis in oral radiology evaluated all radiographic and digital images to rank presence and extent of caries.
- One evaluator (D. Lin) measured occlusal caries using the Laser fluorescence DIAGNOdent device.
- All teeth were then sectioned with a hard tissue microtome and evaluated under stereomicroscopy and polarized light microscopy.
- A generalized estimating equation (GEE) regression model approach as described in (Williamson, Manatunga et al. 2000) was used to analyze data.

## C.2. PILOT STUDY

A pilot study was designed to ensure an effective and efficient study design for the main study and to do a preliminary test of the hypothesis before the full research protocol was utilized.

### C.2.1. TOOTH SAMPLES FOR PILOT STUDY

#### C.2.1.1. Collection

A total of 9 primary (primary canines, primary 1st and 2nd molars) and 3 permanent molars were obtained following routine extractions from various pediatric dental offices and oral surgery offices in San Francisco and Oakland, California. Teeth were stored immediately in 0.1% thymol aqueous solution to serve as an anti-fungal and anti-bacterial agent. The teeth were classified as exempt under the rules of the Committee of Human Research (CHR) at UCSF because the teeth had been extracted for other purposes. The patients were unknown to the investigator and were not identified with the teeth in any way. CHR approved the exempt status and human subject consent was not required.

#### C.2.1.2. Gamma Irradiation

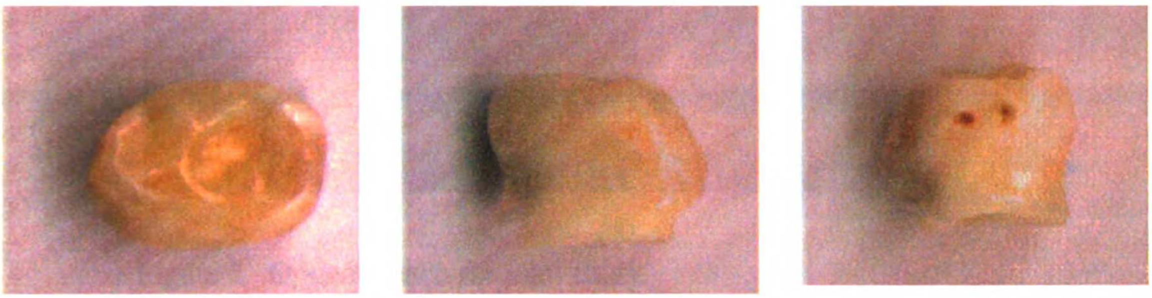
The teeth were then sterilized by overnight gamma irradiation using the protocol described by White and co-workers (1994).

#### C.2.1.3. Inclusion and Exclusion Criteria

Visual inspection of occlusal and proximal surfaces was conducted in order to provide a mix of caries-free teeth and those with proximal decalcification/stain to frank cavitation. Teeth with caries on buccal or lingual surfaces and teeth with existing restorations were excluded.

C.2.1.4. Photography

All proximal surfaces and occlusal surfaces were scanned with an AcerScan 320U at 600 dpi (flatbed scanner). All photographs were printed for future reference. Typical views are shown in Figure 1.



Occlusal View Mesial View Distal View

Figure 1. Typical scanned views of a test tooth from this study.

C.2.1.5. Sorting and Mounting

Sterilized teeth were sorted to produce 3 quadrants of teeth, arranged from the primary canine to the permanent molar. Each quadrant was mounted in dental stone (plaster of Paris), closely approximating normal anatomic positions, and each phantom was identified with a unique number.

C.2.2. RADIOGRAPHY SETUP FOR PILOT STUDY

The optical bench was constructed to provide consistent and ideal alignment between the x-ray beam, object, and film/sensor. The radiation source was a General Electric X-ray machine equipped with a long round cone as illustrated in Figure 2.

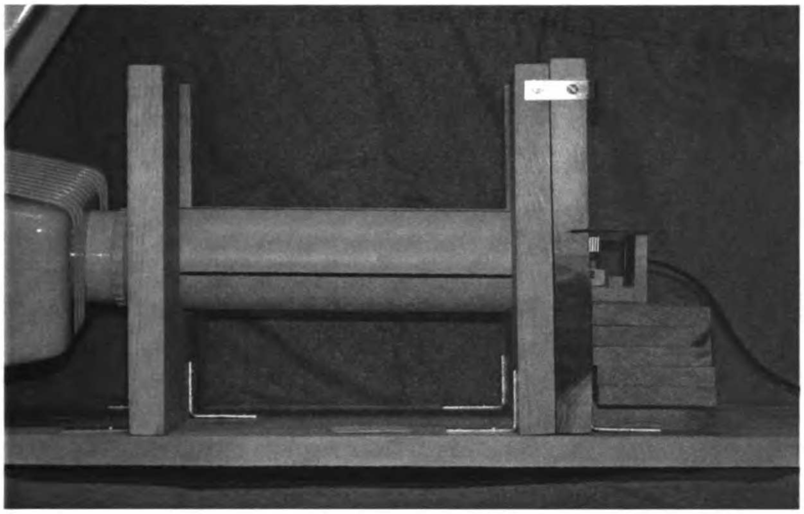


Figure 2. Optical bench for pilot study.

To ensure a standard exposure, a preliminary trial with a 10-step aluminum step wedge was used to verify clinically acceptable film density (with densitometer) for conventional films. A densitometer (X-Rite model 301 Black and White Transmission Densitometer) was calibrated annually, was provided by UCSF Medical Radiology. The primary and permanent dentin radiographic densities were best correlated with the 7$^{th}$ and 3$^{rd}$ steps respectively of the 10-step aluminum step wedge. To find the standard exposure, the densitometer readings of sound primary and permanent dentin were approximated to one. The standardized setting for conventional E-speed film (Ektaspeed Plus from Eastman Kodak, Rochester, NY, USA) was established at 15mA, 75kVp, 12 Imp. The conventional radiography was obtained as illustrated in figure 4.1. All films were developed in an automatic roller processor with developer temperature at 25°C and fixer temperature at 23°C.

The conventional E-speed films were then scanned using a flatbed scanner, Epson Expression 1600 (Epson America, Long Beach, CA, USA), which was equipped with a transparency adapter (TUP positive film, 800 dpi) as illustrated in Figure 3. The resolution of 800 dpi was arbitrarily chosen as the literature indicated that a minimum dpi of 150 and a file size of 800 dpi were manageable. A cardboard holder was made to the size of the scanner with an open square in the center to seat the x-ray film ensured a consistency in scanning position The scanned images were saved in TIFF (Tag Image File Format). All the scanned images were first auto contrasted with the direct digital DMD program as the color of the scanned images was different from the direct digital images. Then the scanned images were cropped (to rid the imprint of the corner dot from the conventional films) and displayed with the Power Point Presentation program (Microsoft, Redmond, WA).

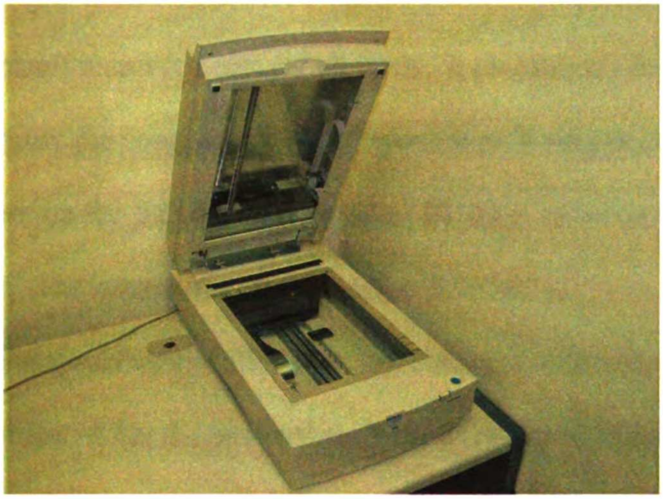


Figure 3. Flatbed scanner (Epson Expression 1600).

The standardized setting for direct digital radiography, MPDx (Dental/Medical Diagnostic Systems Inc., Woodland Hills, CA, USA) was chosen to generate a subjectively acceptable density at 15mA, 75 kVp, 6 Imp. Direct digital images were obtained as illustrated in Figure 4.2. All the digital images were cropped and displayed with the Power Point Presentation program.

Figure 4.1. Conventional radiography

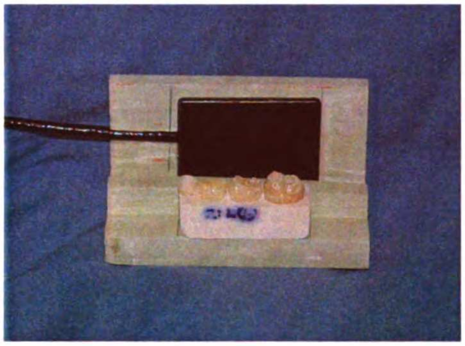


Figure 4.2. Direct digital radiography

C.2.3. LASER FLUORESCENCE FOR PILOT STUDY

One operator (D. Lin) performed all laser fluorescence (DIAGNOdent; KaVo, Biberach, Germany) measurements for all teeth. A preliminary reproducibility test was performed to ensure the consistency of the operator on 8 measurements of 3 teeth repeated 10 times on the first day and repeated 10 times again on the same pits and teeth one month later. The intraclass correlation was 0.9556.

The occlusal surfaces of all molars were cleaned with a slow-speed prophy angle and flour of pumice. After the occlusal surfaces were rinsed and dried, they were measured at room temperature. The DIAGNOdent device was calibrated for each tooth. A conical probe was used to measure specific pits of each occlusal surface.

C.2.4. IMAGE INTERPRETATION FOR PILOT STUDY

Six residents in the pediatric dentistry specialty program at UCSF evaluated and diagnosed the conventional E-speed radiographs, scanned images (at 800dpi), and direct digital images in random order at three different times (at least 2 weeks apart.) Selecting

1 in 3 folded paper created the random order of the type of images viewed. The image orders within each type of film or digital image of the 3 blocks were also displayed in random order of (1,2,3), (1,3,2), or (3,2,1). These 6 evaluators rated the presence of occlusal and interproximal caries on a 5-point scale (1 = definitely not present, 2 = probably not present, 3 = unsure, 4 = probably present, 5 = definitely present). The six evaluators also rated the extent of caries on a 5-point scale (1 = no caries, 2 = < ½ way to DEJ, 3 = ≥ ½ way to DEJ but not to DEJ, 4 = DEJ or < ½ way to pulp, 5 = ≥ ½ way to pulp).

Film was viewed on a viewing box without magnification, scanned images and direct digital images were viewed on a lap top computer screen without any manipulation.

C.2.5. CARIES VALIDATION FOR PILOT STUDY

All teeth were sectioned mesio-distally with a hard tissue microtome to verify the presence and extent of interproximal and occlusal caries. The tooth was first aligned with the diamond blade prior to cutting to ensure sections included all interproximal and occlusal pits. Then, all the sections were marked in the photograph of the tooth to indicate which section had which proximal caries and/or occlusal caries score. After the cutting, all the actual sections were compared with the marking of the photographs to identify all caries and any missing data from tissue loss. The gold standards of occlusal and proximal caries were obtained by evaluating histologic sections with polarized light microscopy and stereomicroscopy at 20X and/or 50X magnifications. Gold standard was rated on 5-point scale (1 = no caries, 2 = Outer Enamel caries -- <½ way to DEJ, 3 =

Inner Enamel caries-- ≥½ way to DEJ, 4 = Outer Dentin caries-- <½ way to pulp, 5 = Inner Dentin caries-- ≥½ way to pulp).

C.2.6. STATISTICS FOR PILOT STUDY

Receivers operating characteristic curves (ROC; ROCKIT program; Metz et al, 1978; Department of Radiology, The University of Chicago, Ill., USA) were plotted for all the methods including conventional, indirect, and direct radiography examination by the 6 observers and the DIAGNOdent measurements. This program does not account for correlated measures (teeth) within blocks, but was deemed adequate for the pilot study.

**C.3. GAMMA IRRADIATION EFFECT STUDY**

A side study was performed using the DIAGNOdent device to evaluate (1) gamma irradiation effects on natural fluorescence of teeth and (2) effect of standardized calibration with and without subtraction of natural fluorescence of teeth. Two null hypotheses were proposed as follows:

- Gamma irradiation does not significantly alter the laser fluorescence readings.
- There is no difference in detecting occlusal caries between zeroing for natural fluorescence vs. calibration standardization only.

C.3.1. TOOTH SAMPLES

C.3.1.1. Collection (as described in C.2.1.1)

A total of 18 primary molars (primary 1st and 2nd molars) were obtained following routine extractions from various pediatric dental offices and oral surgery offices in San Francisco and Oakland, California. Teeth were stored immediately in 0.1% thymol aqueous solution to serve as an anti-fungal and anti-bacterial agent.

C.3.1.2. Inclusion and Exclusion Criteria

Teeth were sorted by visual inspection into 3 groups: from cavitation free, small cavitation (< 1 mm diameter), and large cavitation (≥ 1 mm in diameter.) Teeth with huge cavitations, such that the teeth were without any caries free buccal or lingual wall were excluded, as it would be impossible to subtract natural enamel fluorescence without a caries free smooth surface on which to work.

C.3.1.3. Mounting

Teeth were then mounted in petri dishes in groups of cavitation free, small cavitation (< 1 mm diameter), and large cavitation (≥ 1 mm in diameter.)

C.3.1.4. Photography

All teeth were scanned with an AcerScan 320U at 600 dpi (flatbed scanner) to capture the images of occlusal surfaces as they were mounted in petri dishes. All photographs were printed for future reference.

C.3.2. LASER FLUORESCENCE BEFORE GAMMA IRRADIATION

The occlusal surfaces of all molars were cleaned with a slow-speed prophy angle and flour of pumice. After the occlusal surfaces were rinsed and dried, they were stored in a 100% moisture environment (moist 0.1% thymol towel without touching any teeth inside of sealed specimen cups).

One operator (D Lin) performed all laser fluorescence (DIAGNOdent; KaVo, Biberach, Germany) measurements for all teeth. A conical probe was used to measure one occlusal pit in each tooth. The DIAGNOdent device was calibrated for each tooth and the natural fluorescence of a caries free smooth surface was subtracted from the measurements as well, according to the manufacturer's instructions. Laser fluorescence measurements were obtained on the teeth after storage in controlled moisture conditions: 100% moisture. Laser fluorescence measurements were also obtained to see if there was any difference in measurements when calibration was used (1) with and (2) without subtraction of natural enamel fluorescence.

C.3.3. GAMMA IRRADIATION

The teeth were then placed in 0.1 % thymol aqueous solution and sterilized by overnight gamma irradiation using the protocol described by White el al (1994).

C.3.4. LASER FLUORESCENCE AFTER GAMMA IRRADIATION

All petri dishes were removed from 0.1% thymol aqueous solution. All the occlusal surfaces were rinsed, dried, and stored in 100% moist environment (moist 0.1% thymol towel without touching any teeth inside of sealed specimen cups).

Laser fluorescence measurements were obtained in the same occlusal pit by using the reference photograph. Laser fluorescence measurements were obtained to see if there was any difference in measurements when calibration was used (1) with and (2) without subtraction of natural enamel fluorescence.

C.3.5. STATISTICS

All data were analyzed with a paired t-test and Wilcoxon signed rank test with statistical significance defined as $p \leq 0.05$.

## C.4. MATERIALS AND METHODS – MAIN STUDY

With the findings from the pilot study, the design of final study was refined with the improvements as following: (1) F speed film instead of E speed film with appropriate radiation setting, (2) scan radiographs at 474 dpi instead of at 800 dpi, (3) addition of rectangular collimator to optical bench with acrylic supports, (4) evaluators viewed conventional radiographs with 2X magnification device, and (5) scanned and direct digital images displayed with Polyview image management program instead of Microsoft PowerPoint.

C.4.1. TOOTH SAMPLES

C.4.1.1. Collection (as described in C.2.1.1)

A total of 90 primary (primary canines, primary 1st and 2nd molars) and 30 permanent molars were obtained following routine extractions from various pediatric dental offices and oral surgery offices in San Francisco and Oakland, California.

C.4.1.2. Gamma Irradiation

As described in C.2.1.2.

C.4.1.3. Inclusion and Exclusion Criteria

As described in C.2.1.3.

C.4.1.4. Photography

As described in C. 2.1.4.

C.4.1.5. Sorting and Mounting

As described in C. 2.1.5. sterilized teeth were sorted to produce 30 quadrants of teeth, arranged from the primary canine to the permanent molar.

C.4.2. RADIOGRAPHY SETUP

As described in C. 2.2 except that a clip-on rectangular collimator (Universal Collimator model 54-0853 Densply Rinn, Rinn Corporation Elgin IL, USA) was added to the optical bench. Custom sized acrylic bars were also added to provide extra support of the long round cone as illustrated in Figure 5.

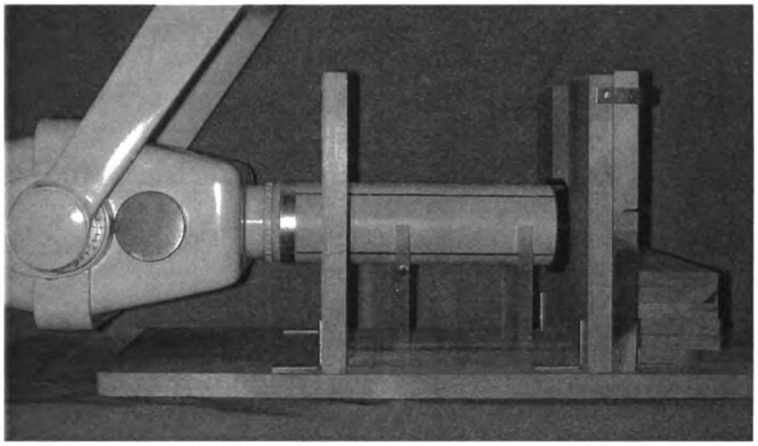


Figure 5. Modified optical bench with collimator and extra acrylic support.

Due to advancement of Kodak conventional film series, an F-speed film (Insight from Eastman Kodak, Rochester, NY, USA) was used for the study instead of the E-speed (Ektaspeed Plus), which was used for the pilot study. To ensure a standard exposure, a preliminary trial with a 10-step aluminum step wedge was used to verify clinically acceptable film density (with densitometer) for F-speed films. A densitometer (X-Rite model 301 Black and White Transmission Densitometer) was provided by UCSF medical radiology and was calibrated annually. The primary and permanent dentin radiographic densities were best correlated with the $7^{th}$ and $3^{rd}$ steps of the 10-step aluminum step wedge. To find the standard exposure, the densitometer readings of sound primary and permanent dentin were approximated to one. The standardized setting for conventional F-speed film was established at 15mA, 70kVp, 12 Imp. All films were developed in an automatic roller processor with developer temperature at 25°C and fixer temperature at 23°C.

The conventional F-speed films were scanned by a flatbed scanner, Epson Expression 1600, which was equipped with transparency adapter (TUP positive film, 474 dpi). The resolution of 474 dpi was chosen to provide the scanned images with the same resolution as the 100% actual pixel view of the MPDx (1:1 ratio of sensor pixel: monitor pixel). All the scanned images were first auto-contrasted with the direct digital DMD program as the color of scanned images was different from the direct digital images. Then, scanned images were saved as TIFF (Tag Image File Format). All the scanned images were cropped with the image editing program, PhotoImpact (Ulead Systems, Inc., Torrance, CA, USA) to rid the imprint of the corner dot from the conventional films. Then scanned images were displayed with the image management program, Polyview program (PolyBites, Inc., Cedar Rapids, Iowa, USA.).

The standardized setting for direct digital radiography, MPDx (Dental/Medical Diagnostic Systems Inc., Woodland Hills, CA, USA) was chosen so as to generate a subjectively acceptable density at 15mA, 70 kVp, 12 Imp. All the digital images were cropped with PhotoImpact to ensure the same image dimensions as the cropped scanned images. Then the digital images were displayed with PolyView.

C.4.3. LASER FLUORESCENCE

One operator performed all laser fluorescence (DIAGNOdent, Kavo, Germany) measurements for all teeth. The occlusal surfaces of all molars were cleaned with a slow-speed prophy angle and flour of pumice. After the occlusal surfaces were copiously rinsed and dried, they were stored in a 100% moisture environment created by moistened paper towel with 0.1% Thymol solution inside (but not in direct contact with the teeth/block) closed test tubes. A conical probe on the DIAGNOdent device was used. The tip of the probe was placed on the pit or fissure and rotated around a vertical axis until the highest fluorescence reading was found, according to the manufacturer's instructions. To test the DIAGNOdent in different moisture conditions, laser fluorescence was measured on teeth stored in (1) 100% moisture condition (fresh out of test tube within 1-2 minutes) in all 30 blocks of teeth, (2) place one drop of water and brief air-drying for 3 sec with air-water syringe in all 30 blocks of teeth, and (3) natural air dry for more than 10 minutes in 3 blocks of teeth. Calibration of the DIAGNOdent was performed for each tooth by the provided calibration disc and zeroed-in by subtracting each tooth's natural fluorescence on a sound lingual surface. At room temperature and moisture condition created by 1 drop of water followed by 3 seconds of air blast, the operator carefully measured all suspicious pits and fissures of all molars of 30 blocks and the specific occlusal pit for each molar.

C.4.4. IMAGE INTERPRETATION

Six pediatric dentists and 1 general dentist with emphasis in oral radiology evaluated and diagnosed the conventional F-speed radiographs, scanned images, and direct digital images in random order at three different times (at least 2 weeks apart.) The image orders within each type of film or digital images were also displayed in random order created by the Research Randomizer program (www.randomizer.org). Evaluators rated the presence of proximal caries, overall occlusal, and specific occlusal pit on a 5-point scale:

1 = definitely not present,
2 = probably not present,
3 = unsure,
4 = probably present,
5 = definitely present

All evaluators also rated the extent of caries on a 5-point scale:

1 = no caries,
2 = < ½ way to DEJ,
3 = ≥ ½ way to DEJ but not to DEJ,
4 = DEJ or < ½ way to pulp,
5 = ≥ ½ way to pulp

Film was viewed on a viewing box with a 2X magnification device, scanned images and direct digital images were viewed on a lap top computer screen without any image manipulation.

C.4.5. CARIES VALIDATION

All teeth were sectioned mesio-distally with a hard tissue microtome to verify the presence and extent of proximal and occlusal caries. The tooth was first aligned with the diamond blade prior to cutting to ensure sections included all proximal and occlusal pits. Then, all the sections were marked in the photograph of the tooth to indicate which section had which proximal caries and/or occlusal caries. After the cutting, all the actual sections were compared with the marking of the photographs to identify all caries and any missing data from tissue loss. The gold standards of occlusal and proximal caries were obtained by evaluating histologic sections with polarized light microscopy and stereomicroscopy at 20X and/or 50X magnifications.

Gold standards were ranked on a 5-point scale:

1 = no caries,

2 = Outer Enamel caries (< ½ way to DEJ),

3 = Inner Enamel caries (≥ ½ way to DEJ),

4 = Outer Dentin caries (< ½ way to pulp),

5 = Inner Dentin caries( ≥ ½ way to pulp).

C.4.6. STATISTICS

A generalized estimating equation (GEE) regression model approach as described by Williamson et al (2000) but extended to another level of nesting (surfaces within quadrants/blocks) was used to analyze data. Lin's concordance correlation was used to assess reliability of repeat measurements (i.e. departure from the 2 measures being the same as opposed to Pearson or Spearmen correlation incorrectly measuring linear association).

Thus, for all radiographic methods, the precision (inter-rater reliability and between methods reliability) were assessed by GEE approach to estimating Kappa statistics and Z-score accounting for correlation across methods, whereas their diagnostic accuracies were assessed by GEE proportional odds model in 2 different analyses (modal vote and silver standards vs. histology). For DIAGNOdent, the precision (intra-rater reliability) was assessed by Lin's concordance correlations, whereas its diagnostic accuracy was assessed by GEE proportional odds model with exchangeable correlation, binary variance and logit link to generate ROC curves for various diagnostic cut-off levels. Gamma irradiation effect and calibration methods affecting the performance of DIAGNOdent were also assessed by paired t-test and Wilcoxon signed ranked test. Lastly, moisture effects on DIAGNOdent readings were evaluated by mixed effects linear model with fixed moisture effect and random tooth within block effect and exchangeable (compound symmetric) correlation structure.

## D. RESULTS AND INTERPRETATIONS

Accuracy and precision are both fundamentally important in assessing the diagnostic performance of a caries detection method. As a trial run of the study setup, the pilot study did not calculate all diagnostic performances or repeatability of all diagnostic methods. For example, the diagnostic accuracies of radiographic methods were evaluated by the ROC analysis, but the precision was not calculated; also, the precision of DIAGNOdent was assessed by intra-class correlations, but the accuracy was not calculated. Nonetheless, statistical analyses were performed to assess both accuracy and precision of all diagnostic methods in the main study.

In the main study, the diagnostic accuracies of all radiographic methods were assessed by GEE proportional odds model in 2 different analyses (modal vote and silver standards vs. histology), whereas the precisions (inter-rater reliability and between methods reliability) were assessed by GEE approach to estimating Kappa statistics and Z-score. On the other hand, the accuracy of DIAGNOdent was accessed by GEE proportional odds model, ROC analysis, and sensitivity and specificity for different cut-off levels. The precision (intra-rater reliability) of DIAGNOdent was assessed by Lin's concordance correlations. Variables such as gamma irradiation, calibration methods, and moisture conditions, which may affect the performance of the DIAGNOdent, were also assessed.

## D.1 PILOT STUDY

### D.1.1. OCCURENCE OF CARIES IN SAMPLE

Each of the three quadrant blocks had a primary canine, a primary 1st molar, a primary 2nd molar, and a permanent molar. The pilot study evaluated 15 primary proximal surfaces, six permanent proximal surfaces, 20 primary occlusal pits, and 16 permanent occlusal pits. Percent of caries in the sample is illustrated in Table 2.

Table 2. Occurrence of caries in pilot study sample.

| Caries Prevalence in Pilot Sample | No Caries (%) | Enamel Caries (%) | Dentin Caries (%) | Total surfaces/pits evaluated |
|---|---|---|---|---|
| Primary Proximal Surfaces | 13 | 67 | 20 | 15 |
| Permanent Proximal Surfaces | 33 | 67 | 0 | 6 |
| Primary Occlusal Pits | 0 | 95 | 5 | 20 |
| Permanent Occlusal Pits | 0 | 56 | 44 | 16 |

### D.1.2. INTRA-EVALUATER RELIABILITY OF DIAGNODENT EVALUATOR

Lin's concordance correlation verified intra-evaluator reliability of the DIAGNOdent duplicating 10 DIAGNOdent readings one week apart on 5 primary occlusal pits and 3 permanent occlusal pits. Lin's concordance correlation yielded 0.96 showing excellent reproducibility.

### D.1.3. RECEIVER OPERATING CHARACTERISTIC CURVE (ROC)

The ROC analysis is used to evaluate the viewer's performance for detecting caries' presence and extent using the three different image receptor types (E-speed film, Scanned digital images, and Direct digital images). It compares the test result with the actual presence or absence of disease. The results of the analysis may be presented as a curve or as a number that represents the area under the curve (Az/AUC) (Kantor, Zeichner et al. 1989). Az is the probability that one diseased surface and one nondiseased surface are both correctly classified. The ROC curve is a plot of the true-positive fraction (sensitivity) against the false-positive fraction (1-specificity) (Kantor, Zeichner et al. 1989).

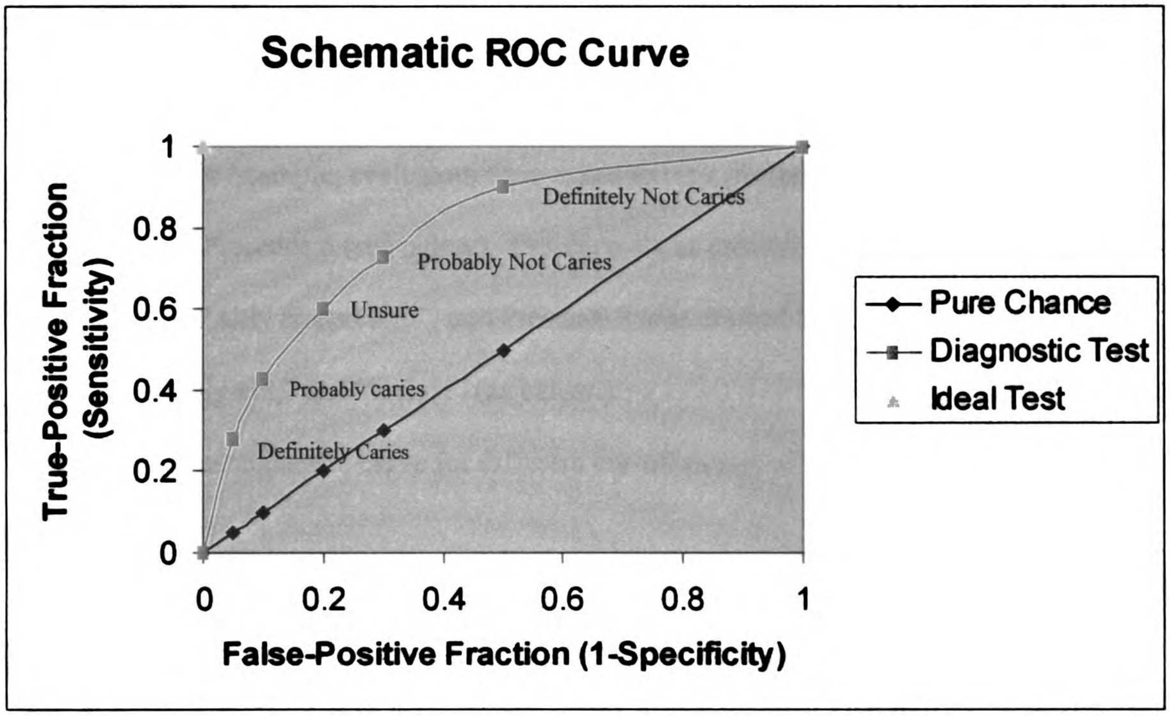



Figure 6. Schematic ROC curve.

As illustrated in the schematic ROC curve in Figure 6: The straight diagonal line (Pure Chance) represents a diagnostic test that is no better than chance alone. A perfect ideal test with sensitivity (true-positive fraction) equal to 1.0 and specificity equal to 1.0 (1 - false positive fraction=0) is plotted as a point in the upper left corner or as a "curve" that follows the left and upper borders of the graph. The curve for an actual test would lie between the two curves Pure Chance and Ideal Test, and will have an area between 0.5 (pure chance) and 1.0 (perfect accuracy). The evaluation of differences in Az was completed using analysis of variance with the level of significance established at $\alpha=0.05$.

When compiling the results for ROC analysis, raw data demonstrated that pilot study evaluators ranked most frequently on "definitely not present" and "definitely present" instead of the 3 middle categories ("probably not present, unsure, or probably present") on the ordinal scale. Therefore, a decision was made to combine different categories to see if the study results changed when different cut-off points were evaluated. For example, evaluators were asked to rank presence of caries in five different categories as Presence 5 (see below). Presence 4 was created by combining "definitely not" and "probably not present"; and Presence 3 was created by combining "definitely not", "probably not," and "unsure" (as below.)

Table 3. Areas under the curve for different cut-off points of caries presence in pilot study.

| Presence | 5 | 4 | 3 |
|---|---|---|---|
| | 1 (Definitely Not) | 1 & 2 (Definitely Not & Probably Not) | 1, 2, & 3 (Definitely Not, Probably Not, & Unsure) |
| | 2 (Probably Not) | 3 (Unsure) | 4 (Probably Present) |
| | 3 (Unsure) | 4 (Probably Present) | 5 (Definitely Present) |
| | 4 (Probably Present) | 5 (Definitely Present) | |
| | 5 (Definitely Present) | | |
| Conventional | Az = 0.6293 | Az = 0.6204 | Az = 0.7680 |
| Scanned Digital | Az = 0.6750 | Az = 0.6822** | Az = 0.8044** |
| Direct Digital | Az = 0.5222** | Az = 0.6295 | Az = 0.7650 |
| Significance | Digital significantly different from Conv & Scan w/ $p \leq 0.05$ | Scan significantly different from Conv & Digital w/ $p \leq 0.05$ | Scan significantly different from Digital w/ $p \leq 0.05$ |

** Statistical significance with $p \leq 0.05$

For detecting the presence of caries, the above results indicated that the pilot study radiographic evaluators were more comfortable diagnosing caries with scanned images as shown by higher values of Az of scanned digital. Even though this result was not always statistically significant across the 3 different cut-off points at Presence 5, Presence 4, and Presence 3, the areas under the ROC curve of scanned digital were consistently higher than conventional and direct digital results. A question arose as to why the scanned digital images of the conventional film had higher correspondence to the true histology when compared with the original conventional film. That is, how could a copy of the original be a better diagnostic tool than the original? Discrepancy of magnification may be the source of problem such that the original conventional film is physically small when evaluated compared to the scanned digital image when displayed on the much larger computer screen. Both scanned digital and direct digital were approximately twice the physical size of the conventional films when displayed on the computer screen. Thus, it was decided for the main study to use a lens with 2X magnification when evaluating conventional films.

Another concern was that scanned digital images were displayed at an arbitrarily chosen resolution of 800 dpi compared to direct digital images which were displayed at 474 dpi. So, direct digital images displayed in 1:1 ratio of sensor pixel: monitor pixel would occupy 474 pixels on the monitor. On the other hand, scanned digital images at 800 dpi would normally occupy 800 pixels on the monitor. Therefore, evaluators might recognize digital images. In the pilot study, the Power Point program was used to crop and display scanned and direct digital images. Scanned digital images were shrunk to the size of direct digital images. However, even though both scanned and direct digital images can be displayed in the same dimensions of 474 monitor pixels for viewers to evaluate in Power Point, each monitor pixel contained more combined information when displaying the scanned digital images and not at a 1:1 ratio as the direct digital images. Also, in the digital world, scanned digital images are most effective if they are as diagnostic as conventional radiographs with a file size is as small as possible. Thus, for the main study we chose a scanning resolution of 474 dpi to provide the scanned images with the same resolution as the 100% actual pixel view of the direct digital images (1:1 ratio of sensor pixel: monitor pixel). Scanned digital and direct digital images had the same image resolution of 474 dpi, pixel ratio of 1:1 (image pixel: monitor pixel), PhotoImpact image editing program, PolyView image presenting program, and TIFF (Tag Image File Format) image storage format. Thus, the only experimental variable was the method of obtaining the digital image whether from a scanner or a sensor.

Also, the area under the curve increases as more categories combine, i.e. Az of Presence 3 is higher than Presence 5. Thus, as more categories were combined, the evaluators performed better in diagnosing caries presence in all receptor types.

Similarly in evaluating extent of caries, pilot study evaluators ranked most frequently on "no caries" instead of "outer enamel caries," "inner enamel caries," "outer dentin caries," and "inner dentin caries". Therefore, by combining different categories, Extent 4 and Extent 3 were created at different cut-off points.

Table 4. Areas under the curve for different cut-off points of caries extent in pilot study.

| Extent | 5 | 4 | 3*** |
|---|---|---|---|
| | 1 (No Caries) | 1 & 2 (No Caries & Outer Enamel) | 1, 2,& 3 (No Caries, Outer, & Inner Enamel ) |
| | 2 (Outer Enamel) | 3 (Inner Enamel) | 4 (Outer Dentin) |
| | 3 (Inner Enamel) | 4 (Outer Dentin) | 5 (Inner Dentin) |
| | 4 (Outer Dentin) | 5 (Inner Dentin) | |
| | 5 (Inner Dentin) | | |
| Conventional | Az = 0.8166 | Az = 0.6036 | Az = 0.8098 |
| Scanned Digital | Az = 0.6739 | Az = 0.6348 ** | Az = 0.8223 ** |
| Direct Digital | Az = 0.5189** | Az = 0.5945 | Az = 0.7916 |
| Significance | Digital significantly different from Conv & Scan w/ p≤0.05 | Scan significantly different from Digital w/ p≤0.05 | Scan significantly different from Digital w/ p≤0.05 |

** Statistically significance with p≤0.05
*** Extent category with the highest Azs

Without combining any categories in Extent 5, conventional radiographs performed best, then scanned digital images and lastly direct digital. Direct digital was statistically different from conventional and scanned digital with p≤0.05. Note that direct digital had Az=0.5189 comparing Pure Chance with Az=0.5. There was no statistically significant difference between conventional E-speed film and scanned digital at Extent 5, Extent 4, and Extent 3. However, as categories were combined, scanned digital was statistically different from direct digital with p≤0.05. These results showed that scanned digital images performed similar to conventional radiographs, but scanned digital performed significantly better than direct digital images. However, the above-mentioned problems of magnification, scanning resolution, and display: pixel ratio may also have affected the caries extent results.

This pilot study was designed to be similar to many dental radiography studies in terms of optical bench setup and statistical analysis. ROC analysis is an important technique for measuring radiographic efficacy commonly used by many studies. However, there were some concerns about using ROCKIT for ROC analysis in this context. All evaluation data were pooled and calculated to the ROC curve and Az. (1) Az was dependent on the ROC curve created from 5 points or less (if categories were combined) as illustrated in Figure 6. The ROC curve fitting software (ROCKIT) also assumed the underlying variables as continuous binomial distributions. Since the evaluation used 5 ordinal categories, the assumptions of continuous binomial distributions to create a smooth curve may not be justified. (2) The sample size used for calculation included all tooth surfaces instead of number of quadrant blocks. (3) The available ROC software does not account for within-block or within-tooth correlation. Thus, although many dental radiography studies used ROC curves to analyze performance of different diagnostic tests, Generalized Estimating Equation (GEE) analysis for the main study would not be subject to the limitations of ROCKIT for ROC analysis. ROC results for the main study used GEE models but did not calculate Az. ROC results of the pilot study are illustrated in the following 6 sets of ROC curves.

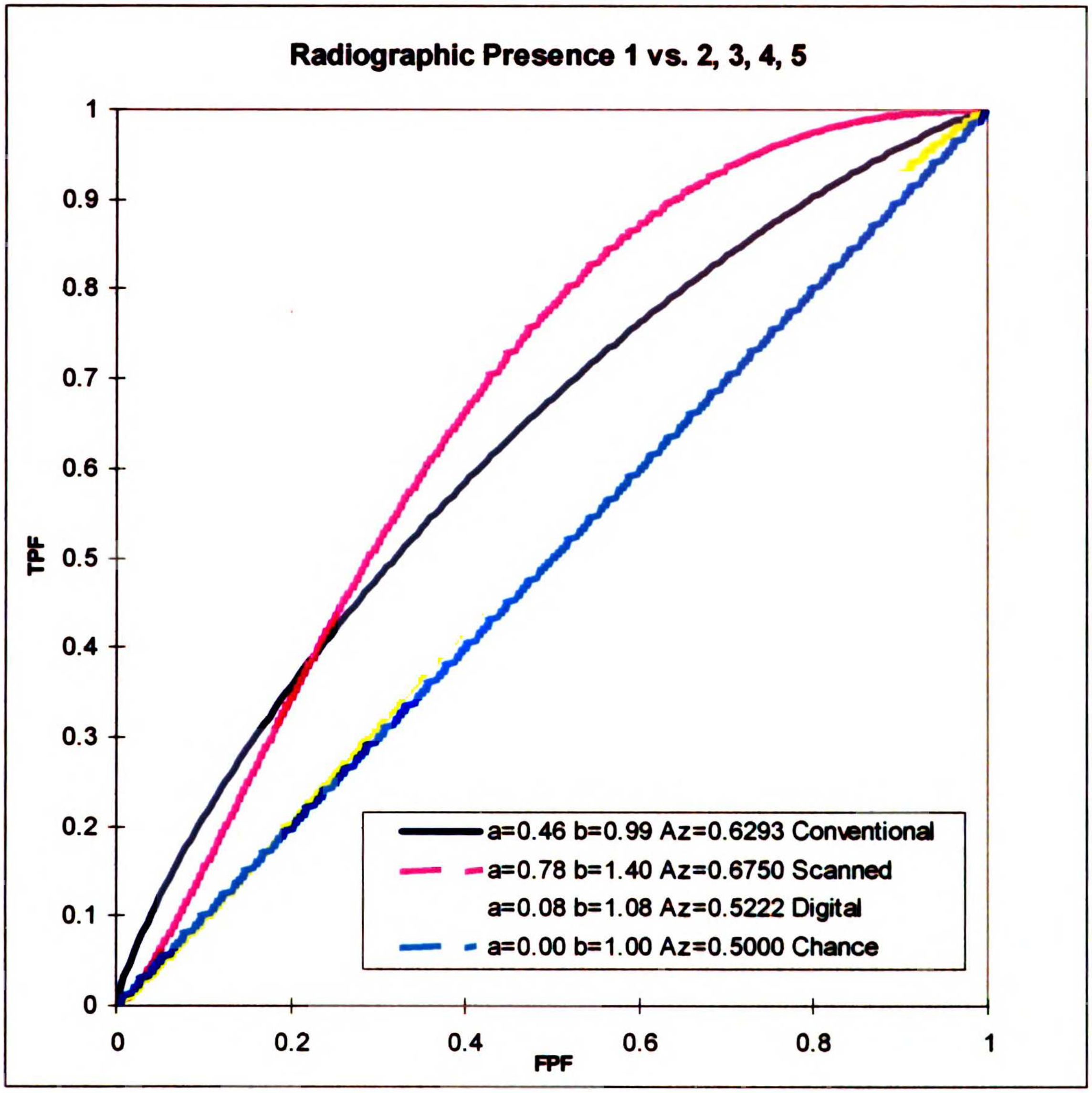



Figure 7. Pilot study: ROC curve of radiographic methods for detecting any caries presence.

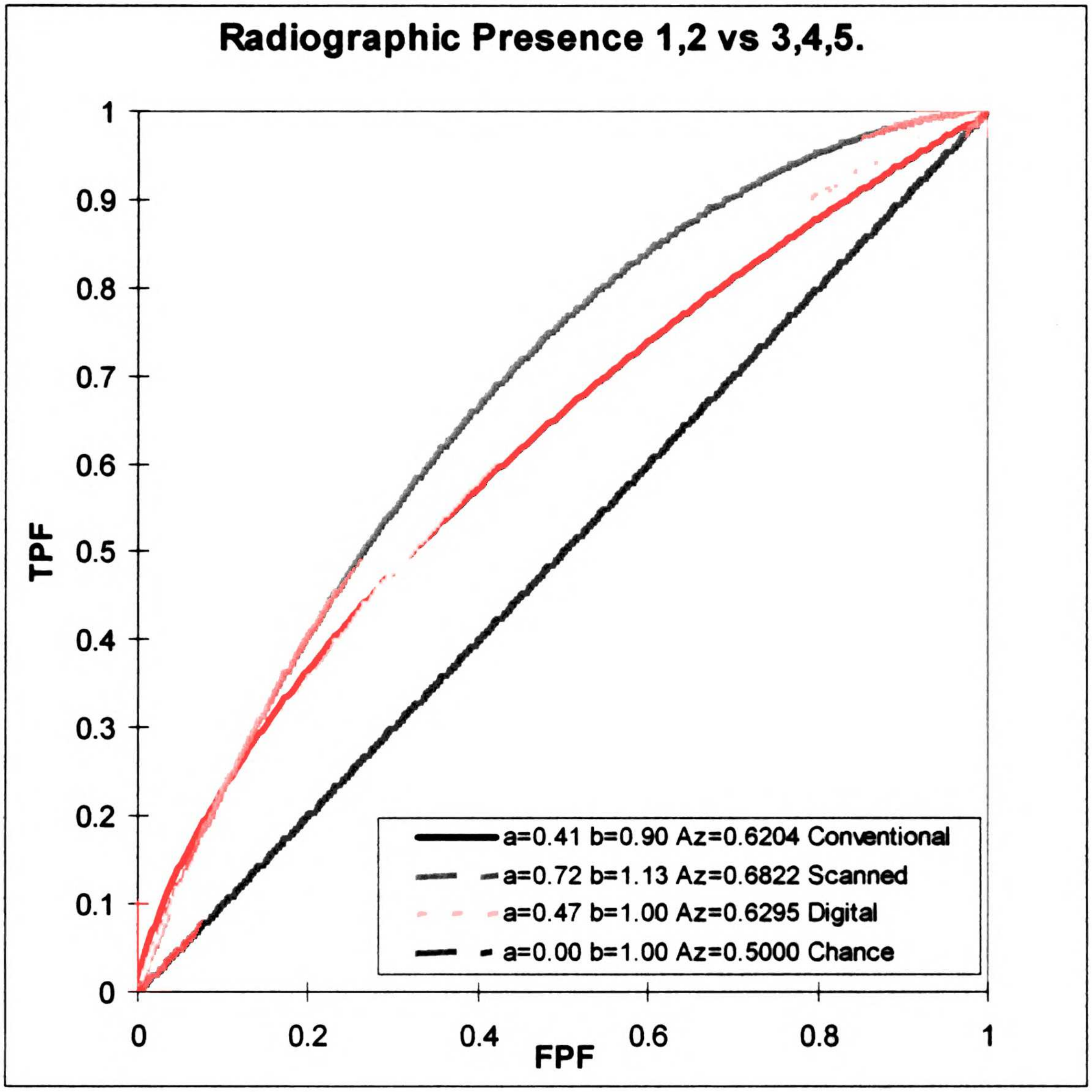



Figure 8. Pilot study: ROC curve of radiographic methods for detecting inner enamel caries presence.

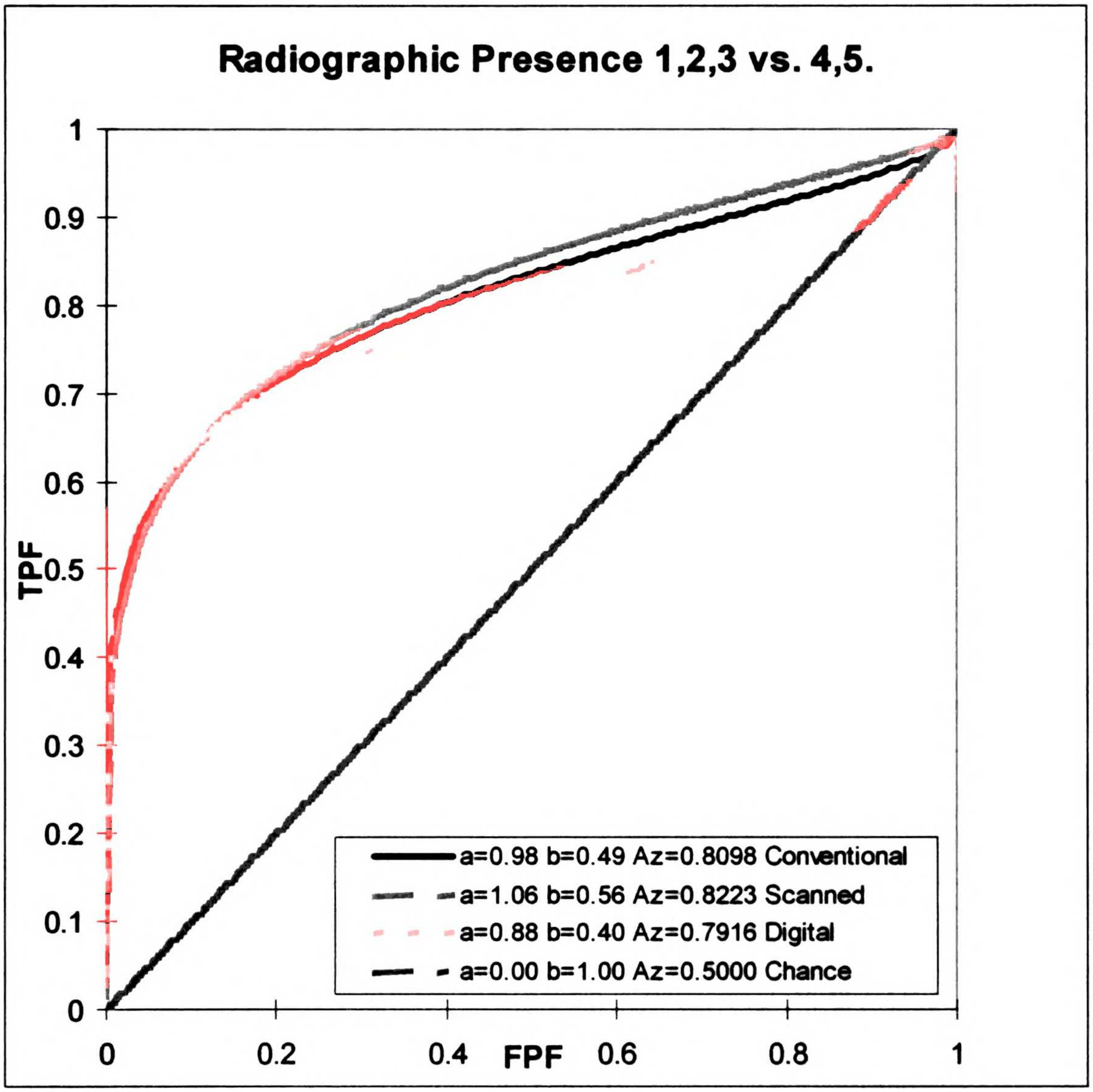



Figure 9. Pilot study: ROC curve of radiographic methods for detecting dentinal caries presence.

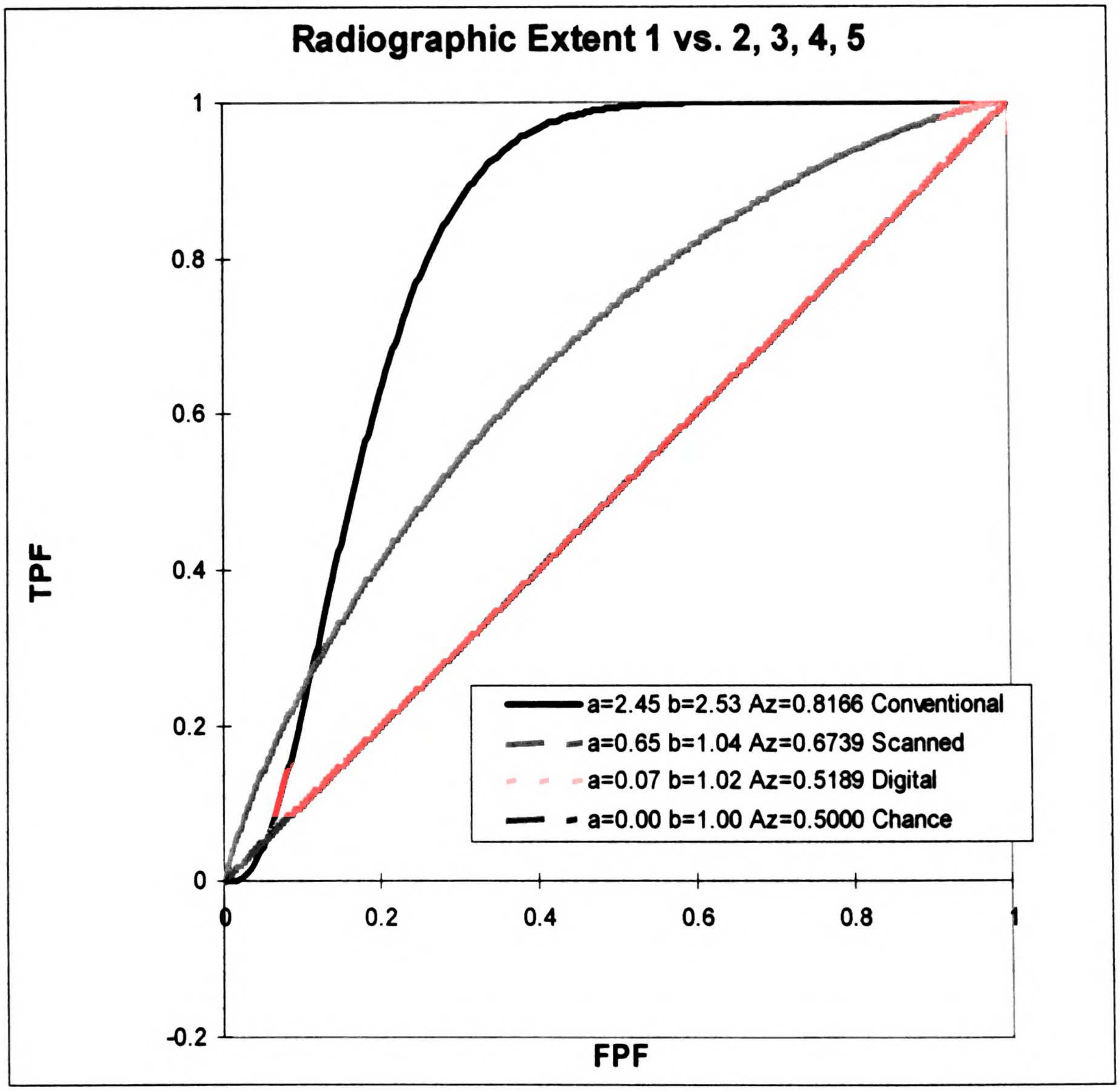



Figure 10. Pilot study: ROC curve of radiographic methods for detecting any caries extent.

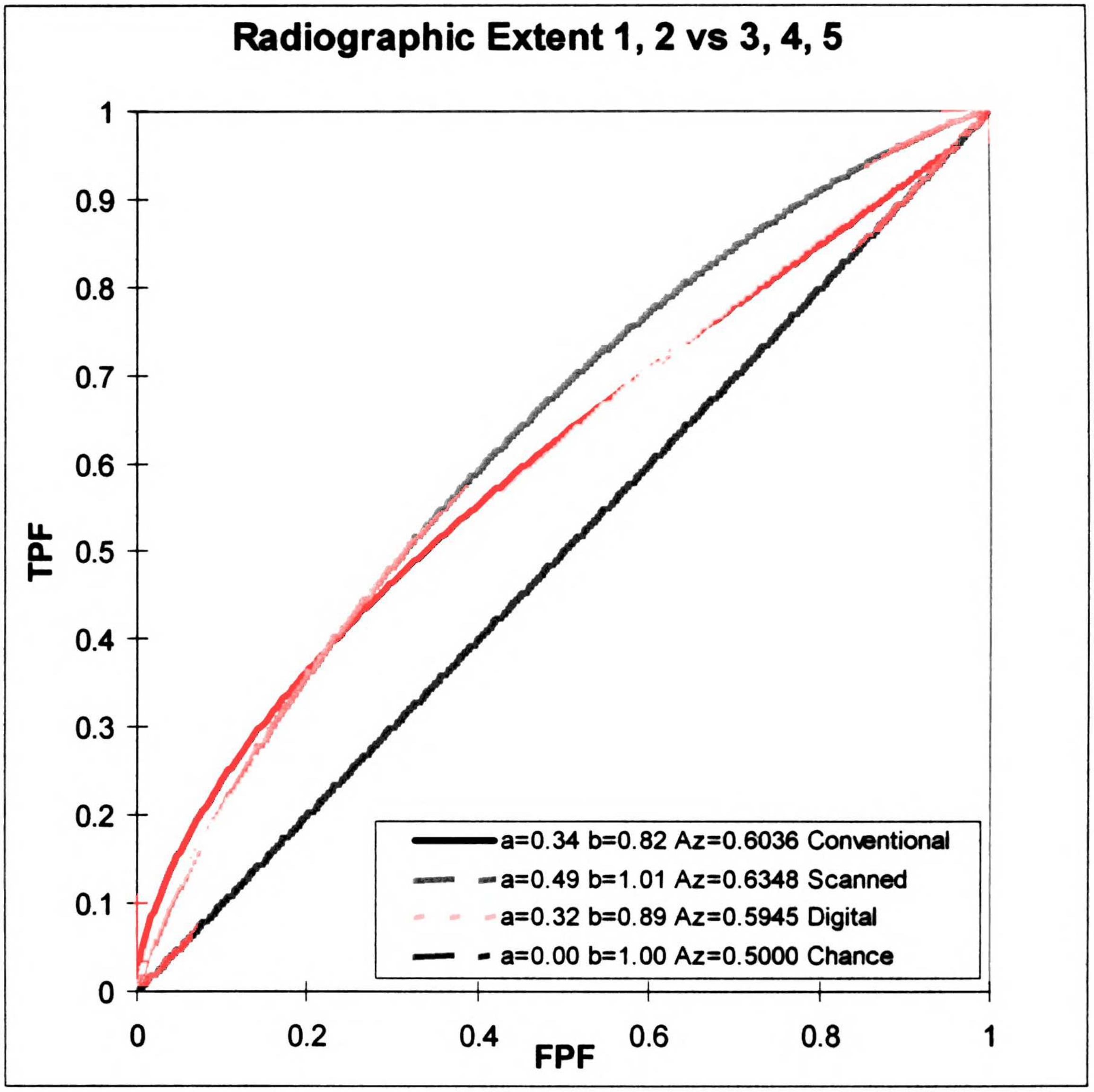



Figure 11. Pilot study: ROC curve of radiographic methods for detecting inner enamel caries extent.

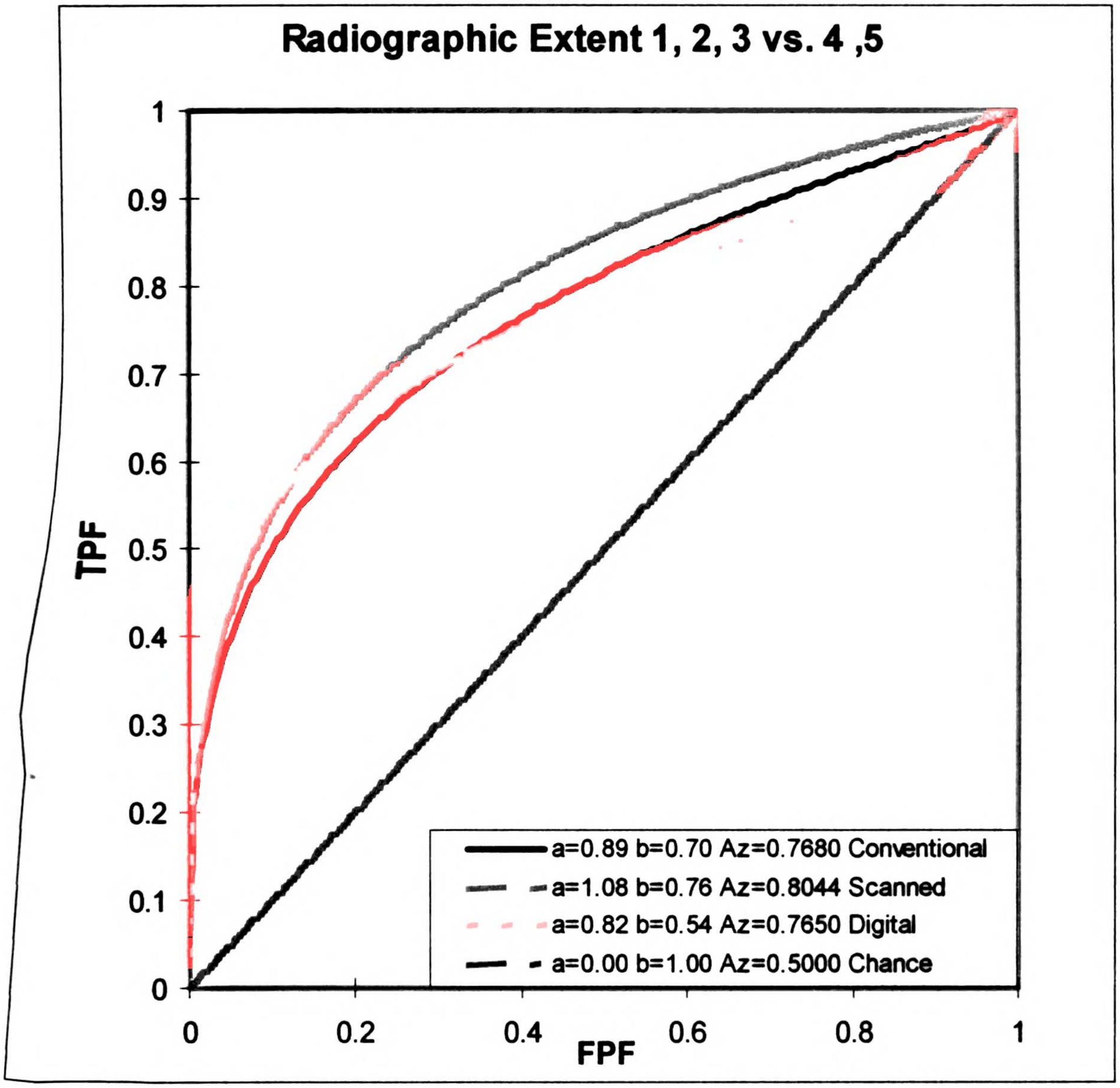



Figure 12. Pilot study: ROC curve of radiographic methods for detecting dentinal caries extent.

## D.2. GAMMA IRRADIATION EFFECT STUDY

### D.2.1. PAIRED T-TEST AND WILCOXON SIGNED RANK TEST

| Gamma Irradiation Effect w/ | Calibration disc only | Calibration Disc & ZeroIn (Subtract Enamel Natural Fluorescence) |
|---|---|---|
| Paired t-test (P-values) | 0.2035 | 0.2985 |
| Wilcoxon Signed Rank test (p-values) | 0.2327 | 0.013 ** |
| Interpretation | Gamma irradiation was not statistical significant | Gamma irradiation was significantly different for a calibration disc with zero-in technique. |

** Statistical significance with $p \leq 0.05$

Table 5. Gamma irradiation effect on DIAGNOdent readings.

The paired t-test revealed that gamma irradiation does not significantly alter the laser fluorescence measurements for either calibration technique. The Wilcoxon signed ranked test also found no statistical significance when using the calibration disc only; however, laser fluorescence readings were statistically significant (p=0.013) when using the calibration disc versus the zero-in technique as recommended in the DIAGNOdent's user manual. Wilcoxon signed ranked test (nonparametric test) is less efficient (able to detect an effect if it exists) than the parried t-test if data are normally distributed (i.e. more likely for t-test to be statistically significant than Wilcoxon signed ranked test if data are normal). However, if data are not normally distributed, Wilcoxon signed ranked test is more efficient than the paired t-test. So, the data are probably not normally distributed that gamma irradiation with ceramic calibration disc only differs from gamma irradiation with calibration and zero-in technique. Thus, gamma irradiation may have some effect on the fluorescence of the teeth and may affect the laser fluorescence readings. Since sterilization is a must for laboratory handling biological specimens, gamma irradiation sterilization was utilized for the main study. The statistical analysis performed for the main study used measurements with calibration via calibration disc instead.

### D.3. MOISTURE EFFECT ON DIAGNODENT

The effect of moisture on DIAGNOdent readings was evaluated in 3 different moisture conditions: (1) moist 100 (100% moisture), (2) moist water/air (1 drop of water followed up with 3 seconds of air blast dry), and (3) moist air (10 minutes air dry). The controlled factors included gamma irradiation sterilization, calibration via ceramic standardization disc only, and analysis combining both primary and permanent teeth. A mixed effects linear model with fixed moisture effect and random tooth within block effect and exchangeable (compound symmetric) correlation structure were calculated. Results are shown in Table 6.

Table 6. Moisture effect on DIAGNOdent readings.

| Moisture Effect | Moist 100 Vs. Moist Water/air | Moist 100 Vs. Moist Air | Moist Water/air Vs. Moist Air |
|---|---|---|---|
| Mean difference | -1.25 | -5.91 | -4.02 |
| Statistic Test (p-value) | 0.0001 ** | 0.0016 ** | 0.004 ** |
| 95% Confidence Interval | -1.86 to -0.64 | -8.83 to -2.98 | -6.35 to -1.69 |
| Interpretation | Moist 100 was statistically different from moist water/air. | Moist 100 was statistically different from moist air. | Moist water/air was statistically different from moist air. |

** Statistical significance with $p \leq 0.05$

All 3 moisture conditions were statistically different from each other. As a result, the DIAGNOdent values increased as teeth were exposed to dryer conditions. Moist air had the highest mean values, then moist water/air and moist 100. Moist 100 were statistically significantly different from moist water/air and moist air with mean difference of DIAGNOdent readings of 1.25 value lower and 5.91 value lower respectively. Furthermore, moist water/air was statistically significantly different from moist air with mean difference of 4.02 lower. So, the moisture condition does affect DIAGNOdent readings in vitro. To put clinical relevance in perspective, a mean difference of 1.25 out of 100 possible values between moist 100 and moist water/air is not very large in percentage and would not have any clinical significance. The KaVo DIAGNOdent user's manual recommends, "In the event of a deviation of greater than +/- 3 from the reference value of the ceramic standard, a new calibration must be performed." Although the statement was not referring to moisture condition, it suggested that DIAGNOdent could have readings deviating +/- 3. Using moist 100 simulating the actual oral moisture condition, moist air would be a clinically relevant different from moist 100, but moist water/air would not. Thus, even though all 3 moisture conditions were statistically different from each other, it was suggested that moist 100 and moist water/air differences from each other were not clinically relevant, but moist air was clinically different from moist 100 and moist water/air. Nonetheless, the statistical analysis performed for the main study used measurements with moist water/air condition (1 drop of water and 3 second of air blast dry) instead.

## D.4. MAIN STUDY

### D.4.1. OCCURANCE OF CARIES IN SAMPLE

Each of the 30 quadrant blocks had a primary canine, a primary 1st molar, a primary 2nd molar, and a permanent molar. Of a total of 521 histologic sections made, 23 histologic sections were omitted from data input since 7 sections had occlusal pits that had calculus and 16 sections were lost during preparation (either broken or accidentally dropped in drainage). Each histologic section may contain more than 1 occlusal pit. Nonetheless, it is unlikely those omitted histologic sections affected the study results. Thus, the study evaluated 148 primary proximal surfaces (lost 2 primary canine sections due to breakage), 60 permanent proximal surfaces, 301 primary occlusal pits, and 284 permanent occlusal pits. The distribution of caries determined histologically in the study samples is illustrated in Table 7.

Table 7. Occurrence of caries in main study sample.

| Caries Prevalence in Main Study | No Caries (%) | Enamel Caries (%) | Dentinal Caries (%) | Total surfaces/pits evaluated |
|---|---|---|---|---|
| Primary Proximal Surfaces | 7 | 58 | 35 | 148 |
| Permanent Proximal Surfaces | 6 | 90 | 4 | 60 |
| Primary Occlusal Pits | 0 | 56 | 44 | 301 |
| Permanent Occlusal Pits | 0 | 37 | 63 | 284 |

### D.4.2. INTER-RATER RELIABILITY AMONG 7 RADIOGRAPHIC EVALUATORS

A generalized estimating equation (GEE) approach was performed to estimate Kappa statistics for reliability among raters accounting for correlation within blocks and tooth-surfaces (Williamson, Manatunga et al. 2000). Z-scores were determined to compare Kappa among diagnostic methods. The z-score indicates how far and in what direction the diagnostic methods deviate from its distribution's mean, expressed in units of its distribution's standard deviation. The z-score distribution always has a mean of zero and a standard deviation of one with p=0.05 corresponds to $z = \pm 1.96$. Kappa statistics and Z-scores among the 7 raters for caries presence across all tooth surfaces in the 30 blocks were as follows:

Table 8. Inter-rater reliability with kappa statistics for caries presence among 7 radiographic evaluators.

| | Categories | | |
|---|---|---|---|
| Presence Categories | Presence 5 | 3 *** | 3b |
| | 1 (Definitely Not) | 1 & 2<br>(Definitely Not & Probably Not) | 1<br>(Definitely Not) |
| | 2 (Probably Not) | 3<br>(Unsure) | 2, 3, & 4<br>(Probably Not, Unsure, & Probably) |
| | 3 (Unsure) | 4 & 5<br>(Probably & Definitely Present) | 5<br>(Definitely Present) |
| | 4 (Probably Present) | | |
| | 5 (Definitely Present) | | |
| Kappa Values | | | |
| Conventional | 0.180 | 0.321 | 0.203 |
| Scanned | 0.228 | 0.337 | 0.249 |
| Direct Digital | 0.185 | 0.320 | 0.207 |
| Z Score | | | |
| C vs. S | -3.327** | -0.670 | -2.841** |
| C vs. D | -0.408 | 0.115 | -0.366 |
| S vs. D | 2.459** | 0.665 | 2.254** |

** Statistical significance with $p \leq 0.05$

*** Category with highest kappa value

For detecting presence of caries in the main study, there were 3 findings. (1) Kappa values for all diagnostic methods in 3 different cut-off points were from slight to fair in agreement strength per Landis and Koch (Landis and Koch 1977). Adequate kappa values for inter-rater reliability are considered at least 0.8. Thus, this study shows that dentists in general agree only fairly (not adequately) in detecting caries with radiography. (2) Although with relatively low kappa values, evaluators seemed to be more reliable in assessing caries with the scanned digital images rather than conventional and direct digital images across different cut-off points. (3) Z-scores showed scanned digital images were significantly better than conventional and direct digital images, but conventional radiographs were not significantly different from direct digital images. However, for presence with 3 categories (1&2, 3, 4&5), conventional radiographs, scanned digital, and direct digital images were not statistically significantly different.

Table 9. Inter-rater reliability with kappa statistics for caries extent among 7 radiographic evaluators.

| | Categories | | | | | |
|---|---|---|---|---|---|---|
| Extent Categories | Extent 5 | 3*** | 3b | 4 | 4b | 4c |
| | 1 (No Caries) | 1 & 2 (No Caries & Outer Enamel) | 1 (No Caries) | 1 (No Caries) | 1 (No Caries) | 1 (No Caries) |
| | 2 (Outer Enamel) | 3 (Inner Enamel) | 2 & 3 (Outer & Inner Enamel) | 2 & 3 (Outer & Inner Enamel) | 2 (Outer Enamel) | 2 (Outer Enamel) |
| | 3 (Inner Enamel) | 4 & 5 (Outer & Inner Dentin) | 4 & 5 (Outer & Inner Dentin) | 4 (Outer Dentin) | 3 & 4 (Inner Enamel & Outer Dentin) | 3 (Inner Enamel) |
| | 4 (Outer Dentin) | | | 5 (Inner Dentin) | 5 (Inner Dentin) | 4 & 5 (Outer & Inner Dentin) |
| | 5 (Inner Dentin) | | | | | |
| Kappa Values | | | | | | |
| Conventional | 0.203 | 0.334 | 0.202 | 0.227 | 0.227 | 0.175 |
| Scanned | 0.237 | 0.343 | 0.243 | 0.260 | 0.251 | 0.219 |
| Direct Digital | 0.207 | 0.320 | 0.211 | 0.231 | 0.220 | 0.184 |
| Z Score | | | | | | |
| C vs. S | -2.238** | -0.459 | -2.864** | -1.894 | -1.349 | -3.37** |
| C vs. D | -0.243 | 0.875 | -0.832 | -0.257 | 0.384 | -0.804 |
| S vs. D | 1.556 | 1.191 | 1.732 | 1.280 | 1.395 | 2.249** |

** Statistically significance with p≤0.05

*** Category with highest kappa value

For detecting caries extent in the study, there were 3 findings. (1) Kappa values for all six cut-off points were low with Extent 3 (1&2, 3, 4&5) having the highest kappa values. (2) Overall, scanned digital images had higher kappa values than conventional radiographs and direct digital images across all 6 cut-off points, although not always to a statistically significant level. (3) Z-scores of conventional radiographs were statistically different from scanned digital images for Extent 5, Extent 3b, and Extent 4c, but scanned digital and direct digital were not statistically different from each other for Extent 5 and Extent 3b. Also, direct digital images were not statistically different from conventional radiographs across 6 cut-off points.

D.4.3. INTRA-RATER RELIABILITY OF DIAGNODENT EVALUATOR

Since only one evaluator (D Lin) performed all DIAGNOdent measurement, the intra-rater reliability was assessed. All measurements were performed with the controlled conditions of gamma irradiation, calibration with ceramic standardized disc only, and 100% moisture condition. Lin's concordance correlation assessed intra-examiner reliability of the DIAGNOdent evaluator. By duplicating measurements of DIAGNOdent readings in 5 different quadrant blocks (a total of 51 primary occlusal pits and 53 permanent occlusal pits), Lin's concordance correlation yielded 0.96, which was excellent for intra-examiner reliability. Specifically, Lin's concordance correlation for primary molars was 0.943 and for permanent molars was 0.986.

## D.4.4. GEE PROPORTIONAL ODDS MODELS

### D.4.4.1. Modal "Majority" Vote Radiographic Results Compared to Histology Gold Standard

Among the 7 pediatric dental faculty members, the modal or the majority vote of radiograph readings was used to compare to the gold standard histology findings. Proximal (mesial and distal) and occlusal surfaces were evaluated separately, since only occlusal surfaces had DIAGNOdent readings. Proximal surface caries of primary teeth was rated on a 5-point scale (no caries, outer enamel, inner enamel, outer dentin, and inner dentin); however, the proximal surface caries of permanent teeth was rated on a 4-point scale (no caries, outer enamel, inner enamel, outer dentin + inner dentin) due to few ratings extending into pulp. For occlusal surfaces, the 5-point scale was condensed to a 3-point scale (no caries + outer enamel, inner enamel, outer dentin + inner dentin) corresponding to DIAGNOdent cutoffs recommended in the manufacturer's manual. All DIAGNOdent measurements were performed with the controlled conditions of gamma irradiation, calibration with ceramic standardized disc only, and moisture condition with 1 drop of water and 3 seconds of air blast. GEE equation proportional odds models with multinomial variance, logit link and independence working correlation were fitted. The odds ratios (OR) were calculated. An OR corresponds to the odds of a method being 1 extent category lower than the histology rating. Thus, an OR of 1.0 corresponds to a diagnostic test equaling histology. An OR of greater than 1.0 corresponds to the diagnostic test underestimating caries extent and an OR of less than 1.0 corresponds to the diagnostic test overestimating caries extent compared to histology. As an indicator of significance if the 95% confidence interval includes value of 1.0, then the diagnostic test is not statistically different from histology. However, if the 95% confidence interval excludes the value of 1.0, then the diagnostic test is statistically significantly different from histology.

Table 10. OR and 95% confidence interval for primary proximal surfaces.

Primary Proximal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 3.29 | 2.53 - 4.31* | ----- |
| Scanned | 3.41 | 2.64 - 4.39* | 0.696 |
| Direct Digital | 3.37 | 2.62 - 4.34* | 0.784 |

* Statistically significant from histology $p \leq 0.05$

For primary proximal surfaces, all radiographic methods significantly underestimated caries extent compared to histology. When compared to conventional radiographs, there was no statistically significant difference among conventional radiographs, scanned digital images, and direct digital images.

Table 11. OR and 95% confidence interval for permanent proximal surfaces.

Permanent Proximal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 204 | 24.7 – 1683* | ----- |
| Scanned | 98.1 | 20.4 – 471* | 0.157 |
| Direct Digital | 98.1 | 21.6 – 446* | 0.157 |

* Statistically significant from histology $p \leq 0.05$

For permanent proximal surfaces, all radiographic methods significantly underestimated caries extent compared to histology. Also, compared to conventional radiographs, there was no statistical difference among conventional radiographs, scanned digital images, and direct digital images. The interaction between dentition and method appeared to be one of degree/magnitude rather than one of direction such that all radiographic methods detected significantly lower caries in primary and even lower in permanent teeth. Also, distal surfaces had significantly lower caries extent than mesial surfaces (OR=3.0) with P<0.001. Furthermore, it was noted that the caries prevalence of primary proximal surfaces was 58% enamel caries and 35% dentin caries while that of permanent proximal surfaces was 90% enamel caries and 4% dentin caries. This may help explain such high OR ratios for permanent proximal surfaces compared to primary proximal surfaces.

Table 12. OR and 95% confidence interval for primary occlusal surfaces.

Primary Occlusal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 0.94 | 0.55 - 1.63 | ----- |
| Scanned | 2.12 | 1.30 - 3.44* | < 0.001** |
| Direct Digital | 1.44 | 0.83 - 2.49 | 0.045** |
| DIAGNOdent | 3.02 | 1.89 - 4.81* | 0.004** |

** Statistical significance vs. conventional radiographs with $p \leq 0.05$
* Statistically significant from histology $p \leq 0.05$

For primary occlusal surfaces, conventional radiographs and direct digital images were not statistically different from histology, but scanned and DIAGNOdent significantly underestimated caries extent compared to histology. Compared to conventional radiographs, DIAGNOdent significantly underestimated caries. Therefore, even though the 95% confidence intervals of all three radiographic methods overlapped, there were statistically significant differences between scanned digital and direct digital compared to conventional radiographs after positive correlations were accounted (all step-down Bonferroni p<0.001 for scanned, and p<0.045 for direct digital). Thus, scanned, direct digital, and DIAGNOdent significantly underestimated caries extent compared to conventional radiographs in detecting primary occlusal caries.

Table 13. OR and 95% confidence interval for permanent occlusal surfaces.

Permanent Occlusal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 9.88 | 3.39 - 28.8* | ----- |
| Scanned | 11.70 | 4.53 - 30.0* | 0.712 |
| Direct Digital | 22.60 | 5.81 - 88.2* | 0.103 |
| DIAGNOdent | 2.66 | 1.49 - 4.75* | 0.015** |

** Statistical significance vs. conventional radiographs with $p \leq 0.05$
* Statistically significant from histology $p \leq 0.05$

For permanent occlusal surfaces, all diagnostic methods significantly underestimated caries extent compared to histology with DIAGNOdent closest to histology. Even though the 95% confidence intervals of all four diagnostic methods overlapped, there was a statistically significant difference between DIAGNOdent and conventional radiographs after positive correlations were accounted for (p=0.015; step-down Bonferroni multiple comparison adjusted p=0.045). Thus, DIAGNOdent significantly overestimated caries extent compared to conventional radiographs while scanned and direct digital images were not statistically different from conventional radiographs.

In summary, using the majority vote among the 7 dental faculty members, compared to the histology gold standard, all radiographic methods detected significantly lower caries extent in primary proximal surfaces and even lower in permanent proximal surfaces. There were no statistically significant differences among the 3 radiographic methods for proximal surfaces. However, for primary occlusal surfaces, conventional radiographs and direct digital images were not significantly different from histology, but scanned and DIAGNOdent detected significantly lower caries extent compared to histology. Compared to conventional radiographs, scanned, direct digital images, and DIAGNOdent all significantly underestimated caries extent in primary occlusal surfaces. For permanent occlusal surfaces, all methods detected significantly lower caries extent than histology; scanned and digital did not differ from conventional, but DIAGNOdent (with a lower OR) detected significantly more caries than conventional radiographs.

D.4.4.2. Radiographic "Silver Standard" Compared to Histology Gold Standard

The dental radiology instructor was used as the "silver standard" evaluator to compare radiographic readings to histology findings. Proximal (mesial and distal) and occlusal surfaces were evaluated separately, since only occlusal surfaces had DIAGNOdent readings. Proximal surface caries extent was rated on a 5-point scale (no caries, outer enamel, inner enamel, outer dentin, and inner dentin). For occlusal surfaces, the 5-point scale was condensed to a 3-point scale (no caries + outer enamel, inner enamel, outer dentin + inner dentin) corresponding to DIAGNOdent cutoffs recommended in the manufacturer's manual. All DIAGNOdent measurements were performed with the controlled conditions of gamma irradiation, calibration with ceramic standardized disc only, and moisture condition with 1 drop of water and 3 seconds of air blast. GEE equation proportional odds models with multinomial variance, logit link and independence working correlation were fitted. As described in section D 4.4.1, Odds ratio (OR) corresponds to the odds of a method being 1 extent category lower than the histology rating.

Table 14. OR and 95% confidence interval for primary proximal surfaces.

Primary Proximal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 2.35 | 1.82 - 3.04* | ----- |
| Scanned | 1.38 | 1.16 - 1.64* | <0.001** |
| Direct Digital | 2.73 | 2.02 - 3.69* | 0.322 |

** Statistical significance vs. conventional radiographs with $p \leq 0.001$
* Statistically significant from histology $p \leq 0.05$

For primary proximal surfaces, all radiographic methods detected significantly lower caries extent compared to histology. When comparing with conventional radiographs, scanned digital images were significantly different from conventional radiographs ($p<0.001$), but direct digital images were not different from conventional radiographs. Interestingly, at the same resolution of 474 dpi and ensuring a display ratio of 1:1 of sensor/image pixel: monitor pixel, scanned digital images (Epson Expression 1600) still detected significantly more caries extent than the direct digital images (MPDx). Thus, scanning by a flatbed scanner detects more primary proximal caries extent than the direct digital sensor. Moreover, after attempting to adjust the magnification discrepancy by using a 2X magnification device for viewing conventional radiographs, scanned digital images can still detect more caries extent than conventional radiographs when displaying scanned images via the Polyview. This demonstrates that in the silver standard study, caries extent can be detected better when displayed in a larger dimension such as a computer monitor than in the conventional radiograph dimension with a 2X magnification device.

Table 15. OR and 95% confidence interval for permanent proximal surfaces.

Permanent Proximal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 50.1 | 14.5 - 179.1* | ----- |
| Scanned | 18.1 | 6.11 - 53.6* | 0.048** |
| Direct Digital | 169.5 | 24.4 – 1180* | 0.046** |

** Statistical significance vs. conventional radiographs with $p \leq 0.05$
* Statistically significant from histology $p \leq 0.05$

For permanent proximal surfaces, all radiographic methods detected significantly lower caries extent compared to histology. Even though the 95% confidence intervals of all three radiographic methods overlapped, there were statistically significant differences between scanned digital and direct digital compared to conventional radiographs after positive correlations were taken into account (all step-down Bonferroni $p<0.048$ for scanned, $p<0.046$ for direct digital). Scanned digital images were significantly better than conventional radiographs, which were significantly better than direct digital images. Thus, scanned was better than direct digital images. Moreover, the interaction between dentition and method appeared to be one of degree/magnitude rather than one of direction such that all radiographic methods detected significantly lower caries extent in primary teeth, but even lower in permanent teeth. Furthermore, carious surfaces of primary proximal surfaces were 58% enamel caries and 35% dentin caries while those of permanent proximal surfaces were 90% enamel caries and 4% dentin caries. This may explain the high OR ratios for permanent proximal surfaces compared to primary proximal surfaces.

Table 16. OR and 95% confidence interval for primary occlusal surfaces.

Primary Occlusal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 0.20 | 0.10 - 0.41* | ----- |
| Scanned | 0.02 | 0.01 - 0.07* | 0.003** |
| Direct Digital | 0.45 | 0.24 - 0.85* | 0.069 |
| DIAGNOdent | 2.35 | 1.62 - 3.42* | <0.001** |

** Statistical significance vs. conventional radiographs with $p \leq 0.05$
* Statistically significant from histology $p \leq 0.05$

For primary occlusal surfaces, all diagnostic methods indicated significantly different caries extent compared to histology. Conventional, scanned, and direct digital images overestimated significantly caries extent than histology while DIAGNOdent underestimated significantly. When compared to conventional radiographs, direct digital was not statistically different from conventional radiographs. However, scanned digital images seemed to significantly overestimate primary occlusal caries extent while DIAGNOdent tended to significantly underestimate caries extent compared to conventional radiographs. The caries prevalence of primary occlusal surfaces was 56% enamel caries and 44% dentin caries. Even though slightly more than 50% enamel caries presented, primary teeth have thinner enamel than permanent teeth, which allowed more x-ray to penetrate through as compared to permanent enamel. Thus, radiographic methods overestimated dental caries extent in primary occlusal surfaces in general.

Table 17. OR and 95% confidence interval for permanent occlusal surfaces.

Permanent Occlusal Surfaces

| Method | Odds Ratio (OR) | 95% Confidence Interval | P-Value vs. Conventional |
|---|---|---|---|
| Conventional | 1.45 | 0.65 - 3.21 | ----- |
| Scanned | 0.35 | 0.14 - 0.87* | 0.009** |
| Direct Digital | 4.32 | 2.08 - 8.97* | 0.012** |
| DIAGNOdent | 2.94 | 1.71 - 5.06* | 0.140 |

** Statistical significance vs. conventional radiographs with $p \leq 0.05$
* Statistically significant from histology $p \leq 0.05$

For permanent occlusal surfaces, all methods other than conventional radiographs detected significantly different extent compared to histology. Compared to histology, scanned digital images statistically overestimated caries extent while direct digital images and DIAGNOdent statistically underestimated caries extent. In comparison to conventional radiographs, DIAGNOdent was not statistically different in detecting permanent occlusal caries. However, even though the 95% confidence intervals of diagnostic methods overlapped, there were statistically significant differences between scanned digital and direct digital compared to conventional radiographs after correlations were taken into account (all step-down Bonferroni $p<0.009$ for scanned and $p<0.012$ for direct digital). Thus, scanned significantly overestimates, but direct digital significantly underestimated caries extent compared to conventional radiographs. Furthermore, it was noted that the caries prevalence of permanent occlusal surfaces was 37% enamel caries and 63% dentin caries. This demonstrates that with a sample of predominately dentin caries, OR was lower and closer to 1(histology.)

In summary, using one evaluator (dental radiology instructor) as a silver standard compared to the histology gold standard, all radiographic methods detected significantly lower caries extent in primary and permanent proximal caries. Compared to conventional radiographs, scanned digital images detected more primary and permanent proximal caries than conventional radiographs. On the other hand, direct digital images found no difference from conventional radiographs in detecting primary proximal caries, but significantly underestimated caries extent than conventional radiographs in permanent proximal surfaces. When detecting primary occlusal surfaces, all radiographic methods overestimated caries extent compared to histology; while in permanent occlusal surfaces, conventional radiographs found no difference from histology, but scanned overestimated and direct digital underestimated compared to histology. DIAGNOdent underestimated dental caries extent in primary and permanent teeth, but it detected significantly less than radiographic methods in primary teeth but not in permanent teeth.

<u>D.4.4.3. DIAGNOdent readings Compared to Histology Gold Standard</u>

The DIAGNOdent readings of all permanent and primary occlusal surfaces were compared to histology findings. All measurements were performed with the controlled conditions of gamma irradiation, calibration with ceramic standardized disc only, and 100% moisture condition. The 5-point scale was condensed to a 3-point scale (no caries + outer enamel, inner enamel, outer dentin + inner dentin) corresponding to DIAGNOdent cutoffs recommended in the manufacturer's manual. GEE proportional odds models with multinomial variance, logit link and independence working correlation were fitted. Primary and permanent teeth had to be analyzed separately since there were significant method x dentition interactions. The odds ratios (OR)s were calculated. An OR corresponds to the odds of a method being 1 extent category lower than the histology rating.

Table 18. OR and 95% confidence interval for DIAGNOdent readings vs. histology.

| DIAGNOdent Readings | Odds Ratio (OR) | 95% Confidence Interval | P-Value |
|---|---|---|---|
| Primary Occlusal Surfaces | 8.69 | 5.37-14.08 | <0.0001** |
| Permanent Occlusal Surfaces | 2.78 | 1.64-4.72 | 0.0002** |

** Statistical significance vs. conventional radiographs with $p \leq 0.05$

Although permanent teeth had a somewhat smaller OR than primary teeth, DIAGNOdent significantly underestimated caries extent in both primary and permanent teeth compared to the histology gold standard. Furthermore, since 95% confidence intervals of primary teeth did not overlap that of permanent teeth, DIAGNOdent significantly underestimated caries extent in primary occlusal surfaces compared to permanent occlusal surfaces.

D.4.5. ROC ANALYSIS OF DIAGNODENT

The ROC analysis was used to evaluate the DIAGNOdent's performance for detecting caries' extent in both primary and permanent teeth using the three different caries levels (any caries, inner enamel caries, and dentinal caries). It compared the test result with the actual presence or absence of disease at different caries levels. The controlled factors included gamma irradiation sterilization, calibration with standardized disc only, moisture condition of 1 drop of water followed up with 3 seconds of air blast dry, and evaluated the combined result of both primary and permanent occlusal pits. The

ROC curve is a plot of the true-positive fraction (sensitivity) against the false-positive fraction (1-specificity) as in Figure 14.

The ROC curve below demonstrated that DIAGNOdent readings had the largest area under the curve with dentinal caries, then inner enamel caries, and followed by any caries. Thus, DIAGNOdent performed best when detecting dentinal caries, followed by inner enamel caries and any caries.

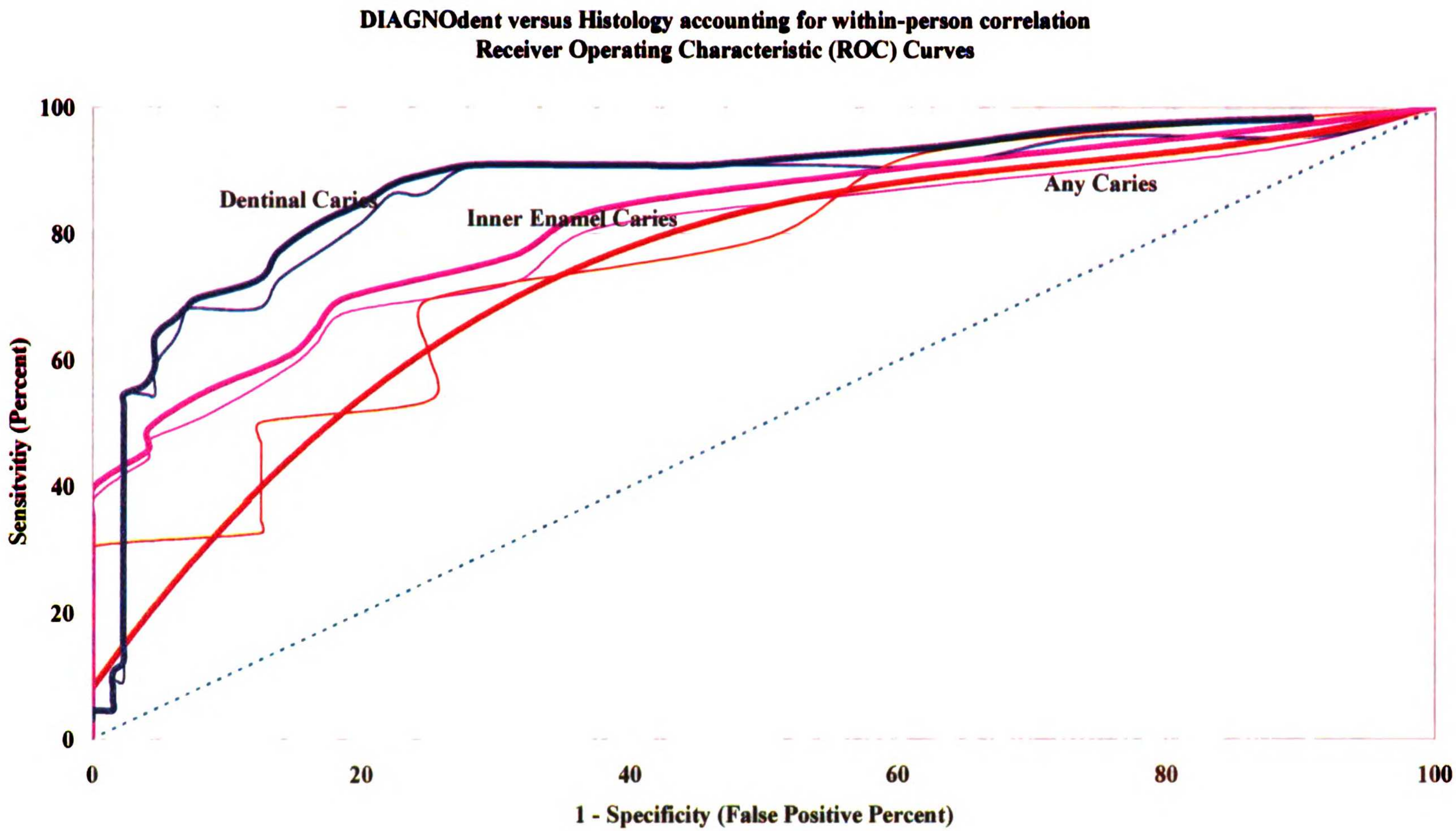



Figure 13. ROC curve of DIAGNOdent vs. Histology at different cut-offs.

## D.4.6. SENSITIVITY AND SPECIFICITY OF DIAGNODENT

The sensitivity equals the percentage of actual lesions detected (true positives /(true positives and false negatives) (Kantor, Zeichner et al. 1989). The specificity equals the percentage of actual no lesions (true negatives/(false positives and true negatives). The positive predictive value is the likelihood that lesions are actually present when the test result is positive and the negative predictive value is the likelihood that the lesions are actually absent when the test result is negative. The ideal test has a sensitivity, specificity, positive predictive value, and negative predictive value of 100% (Kantor, Zeichner et al. 1989). However, all actual tests have such measurements that are less than 100%. Also, there is a trade off relationship between sensitivity and specificity (Kantor, Zeichner et al. 1989). Moreover, the predictive values are influenced by the prevalence of the disease in the population (Kantor, Zeichner et al. 1989). The sensitivity and specificity of DIAGNOdent readings were calculated at three different caries levels (any caries, inner enamel caries, and dentinal caries). The controlled factors included gamma irradiation sterilization, calibration with standardized disc only, moisture condition of 1 drop of water followed up with 3 seconds of air blast dry, and evaluated both primary and permanent occlusal pits. As shown in Figures 15-17, the sensitivity and specificity curves are plotted against the DIAGNOdent readings from 1 to 99 in 3 different caries levels (any caries, inner enamel caries, and dentinal caries.)

In Figures 14-16, as the DIAGNOdent values increased from 1 to 99, the sensitivity decreased and specificity increased as expected. The DIAGNOdent cut-off limit of each caries level was determined at the point of the highest sensitivity curve crossing the highest specificity curve. Based on the results, the following readings on the

DIAGNOdent were associated with the following states of caries: Values 1-5: no caries (sensitivity 79%, specificity 50%); values 6-8: outer enamel caries (sensitivity 69%, specificity 75%); values 8-11: inner enamel caries (sensitivity 67%, specificity 81%); and values >11: dentinal caries (sensitivity 91%, specificity 92%).

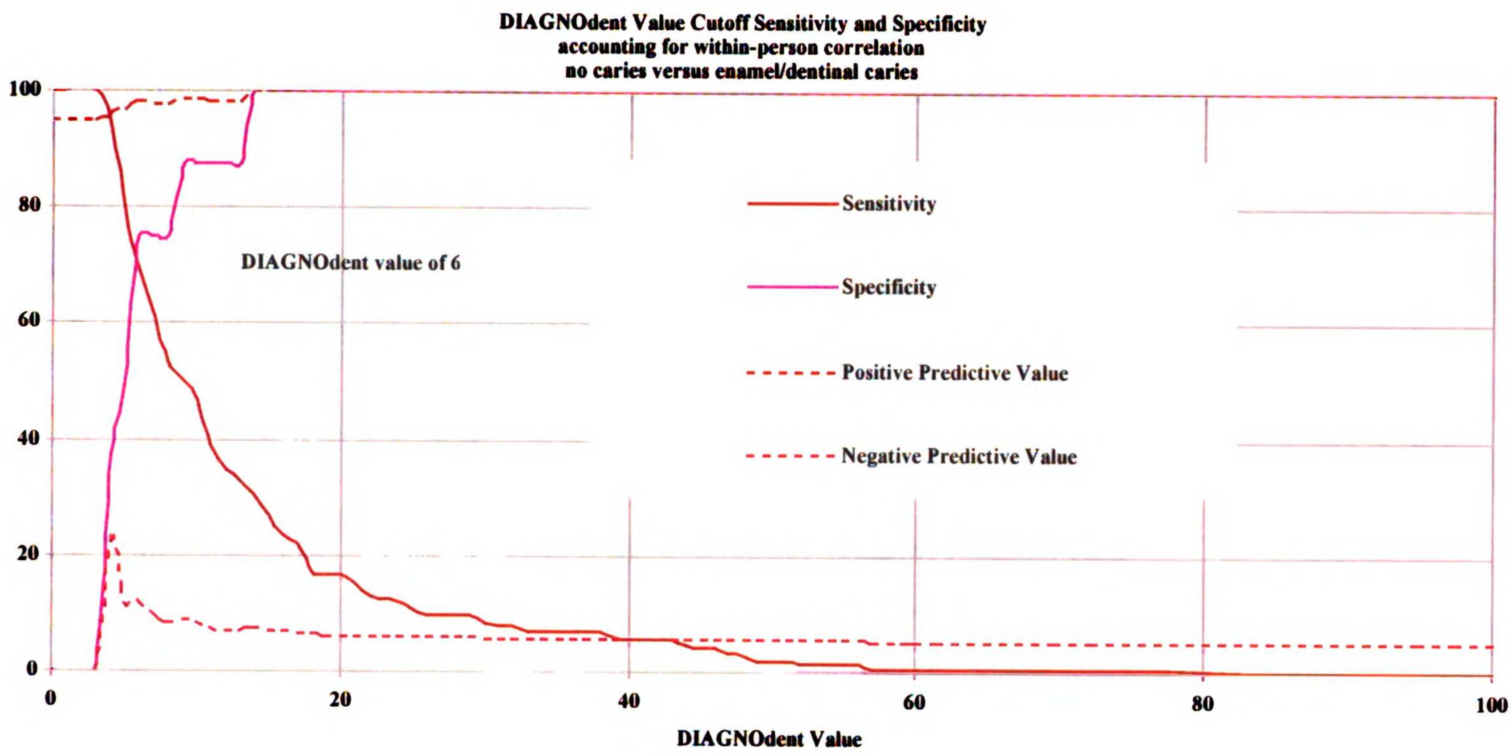



Figure 14. Sensitivity and specificity vs. DIAGNOdent values at any caries level.

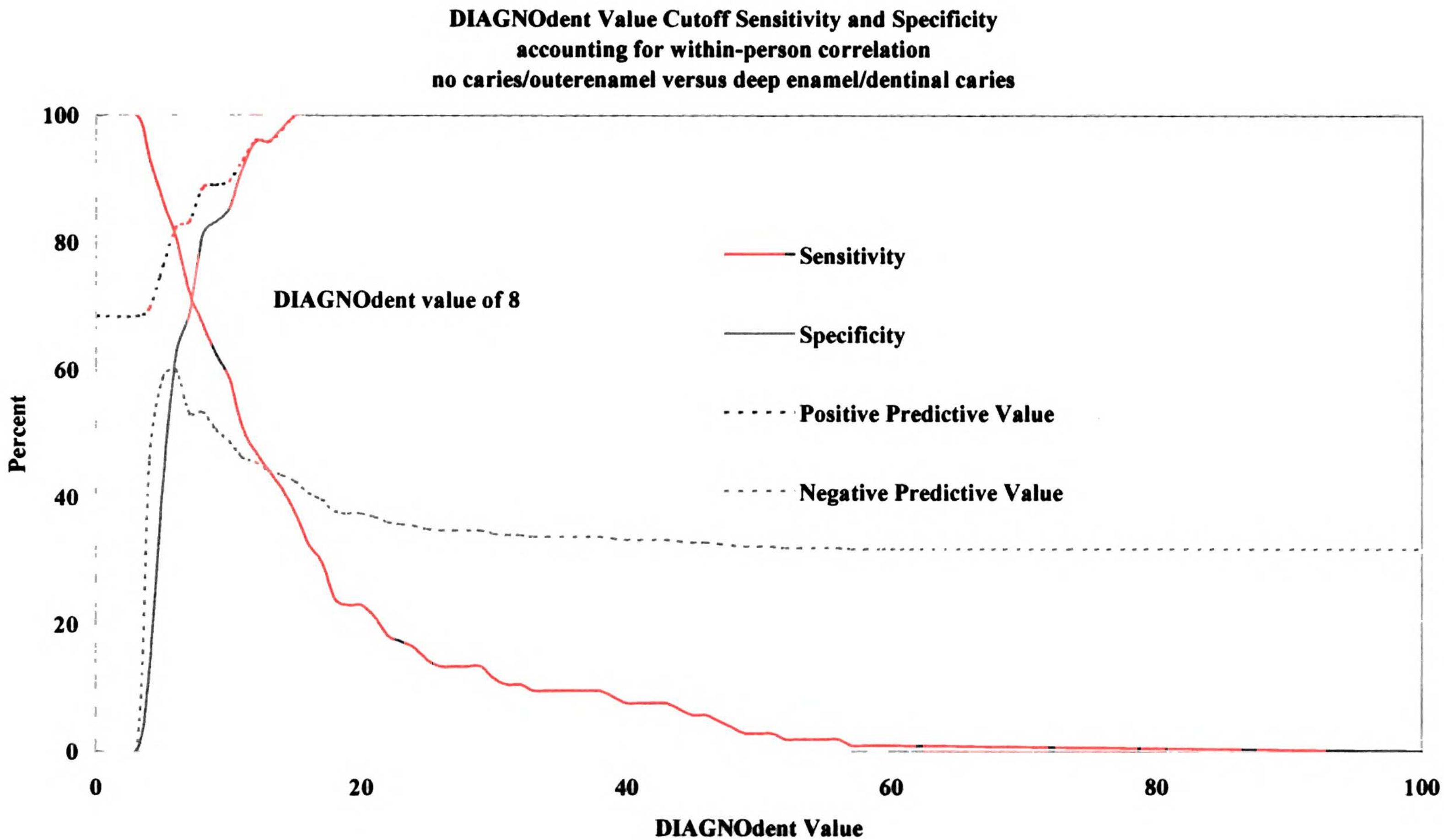



Figure 15. Sensitivity and specificity vs. DIAGNOdent values at inner enamel and dentinal caries level.

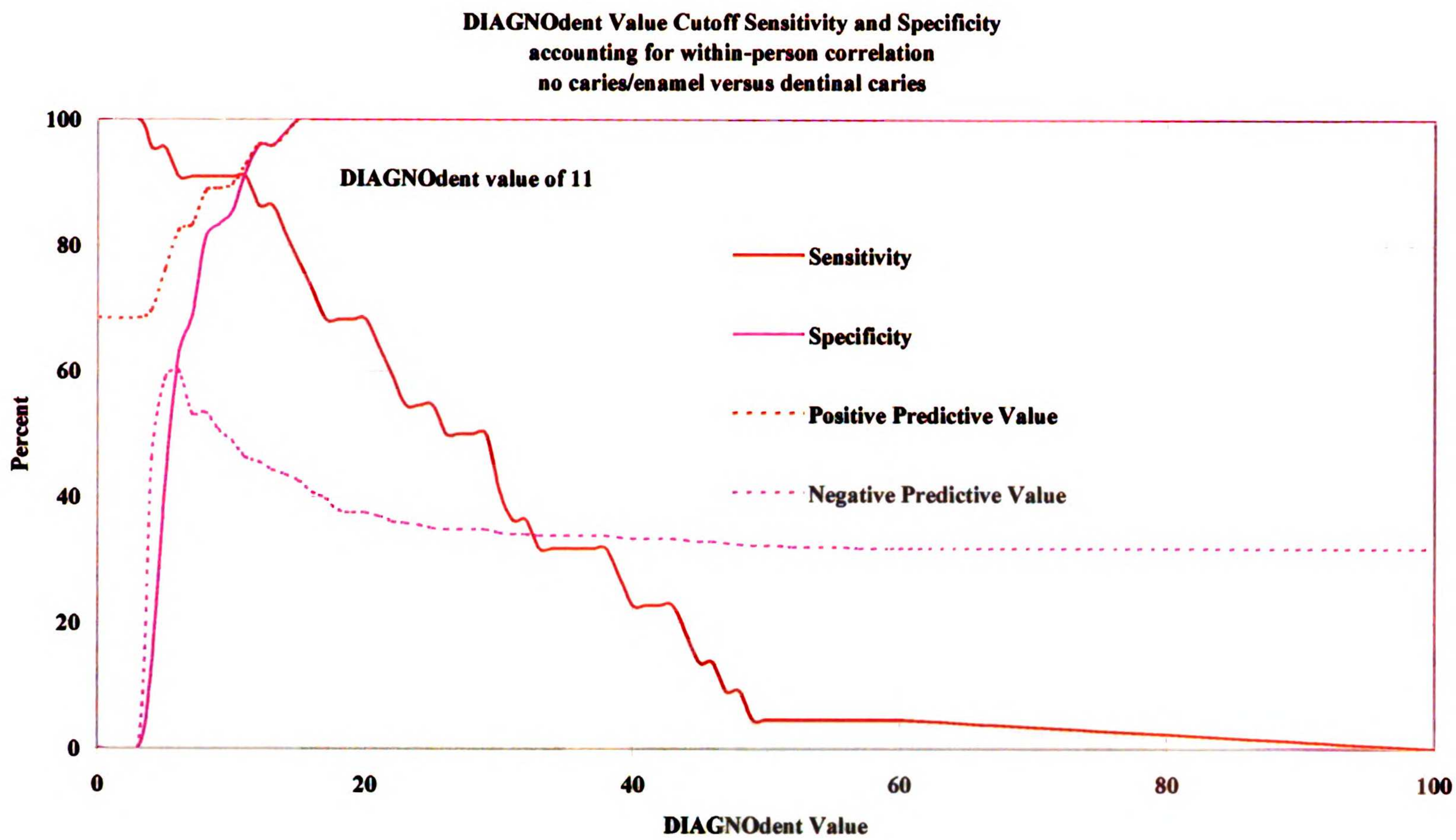
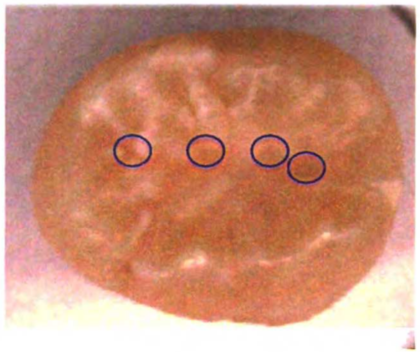



Figure 16. Sensitivity and specificity vs. DIAGNOdent values at dentinal caries level.

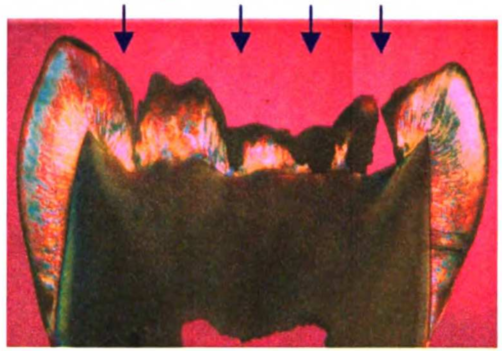
## D.4.7. HISTOLOGY MICROSCOPY

### D.4.7.1. Permanent Molar (Figure 17)

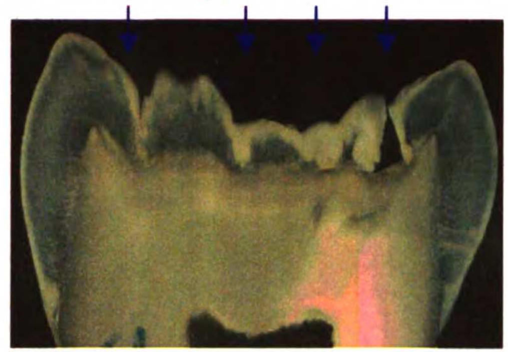
Gross Image



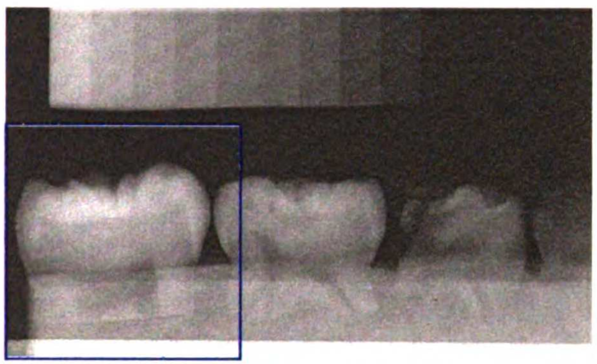
Polarized Light Microscopy



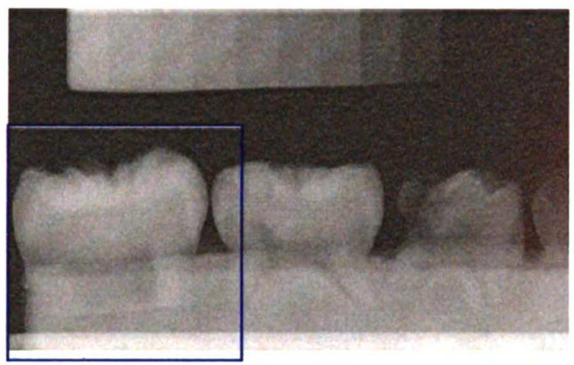
Stereomicroscopy



| | | | | |
|---|---|---|---|---|
| DIAGNOdent Reading | 6 | 8 | 11 | 11 |
| Histology Validation | D2 | D3 | D4 | D4 |



Scanned Digital Image (Flatbed Scanner 474 dpi)



Direct Digital Image (MPDx)

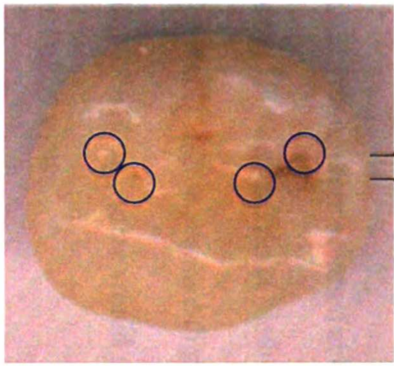
D2 = outer enamel caries

D3 = inner enamel caries

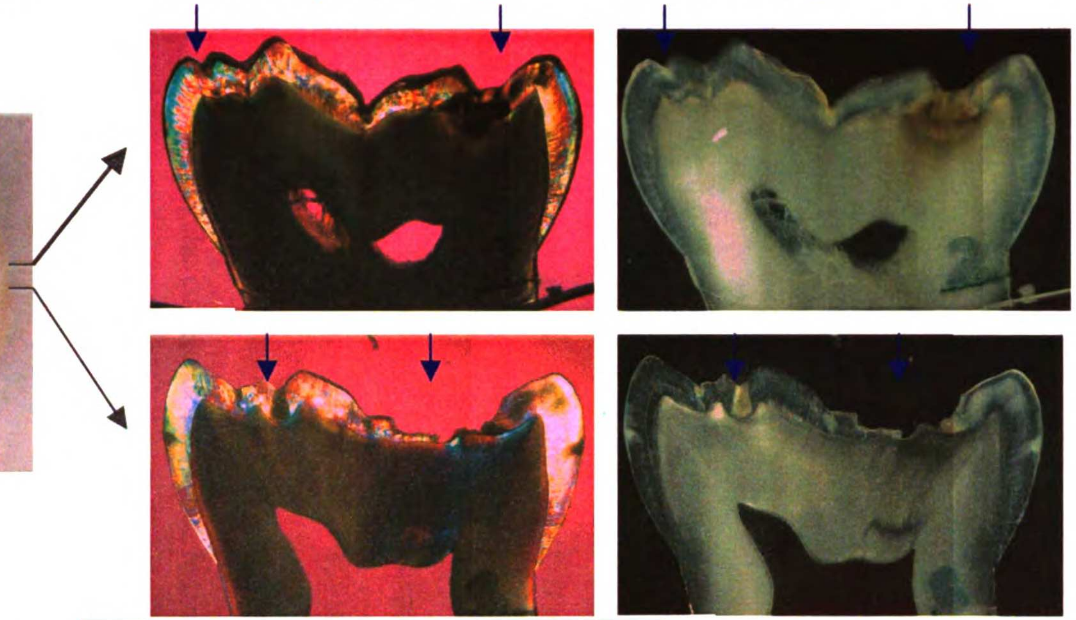
D4 = outer dentin caries

D.4.7.2. Primary Second Molar (Figure 18)

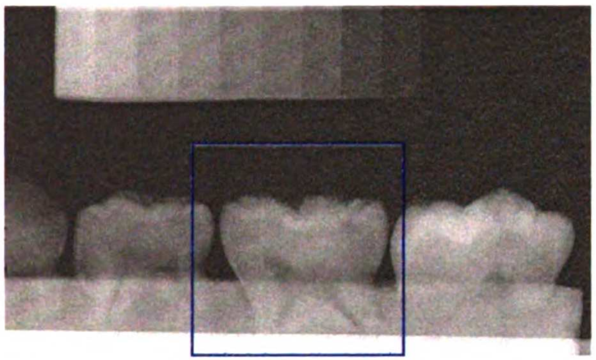
Gross Image



Polarized Light Microscopy Stereomicroscopy



DIAGNOdent Reading 6 13 25 90
Histology Validation D2 D3 D4 D4



Scanned Digital Image (Flatbed Scanner 474 dpi )

Direct Digital Image (MPDx)

D2 = outer enamel caries

D3 = inner enamel caries

D4 = outer dentin caries

# E. DISCUSSIONS & CONCLUSIONS

## E.1. PILOT STUDY

With the findings from pilot study, the design of the main study was refined with the improvements as following:

(1) F speed film was used for the main study with appropriate radiation settings instead of E speed film. The International Commission on Radiological Protection (ICRP) system of dose limitation requires radiation exposures to be kept "as low as reasonably achievable" (ALARA) (Fleishman, Notley et al. 1983). Several studies have reported that F speed films are faster and yet as diagnostic as E speed films (Geist and Brand 2001; Ludlow, Abreu et al. 2001; Ludlow, Platin et al. 2001; Price 2001). So, F speed films were chosen to represent conventional radiographs in the main study.

(2) A rectangular collimator was added to the optical bench with acrylic supports. Rectangular collimator can improve the image quality by reducing excessive scatter radiation and thus increase subject contrast (Preece 1988).

(3) Radiographs were scanned at 474 dpi instead of at 800 dpi to ensure the same resolution as the 100% actual pixel view of the direct digital images (1:1 ratio of sensor pixel: monitor pixel). Since scanned digital and direct digital images were acquired at the same resolution of 474 dpi, there would not be a size discrepancy as they were displayed at 100% actual pixel views.

(4) Evaluators viewed conventional radiographs with 2X magnification device in an attempt to minimize magnification problems, as digital images are physically larger than conventional radiographs when displayed on a laptop monitor.

(5) A different image management program was used, namely the Polyview program instead of Microsoft PowerPoint, to display scanned digital and direct digital images. The Microsoft Power Point automatically saved image files in Joint Photographic Experts Groups (JPEG) by lossy data compression, which loses image quality. Lossy compression removed gray values or spatial frequencies that occurred less frequently (Van der Stelt 2000). So, the lossy compression was achieved by reducing the number of bits allocated for presenting image data, thereby irreversibly changing the image. On the contrary, the Polyview program offers the option to save images in Tag Image File Format (TIFF) by lossless data compression. Although some studies have shown that JPEG files at certain compression levels do not alter the diagnostic quality of dental digital radiographs (Wenzel, Gotfredsen et al. 1996; Janhom, Van der Stelt et al. 1999; Janhom, van der Stelt et al. 2000), TIFF storage format would be best to control image resolution.

(6) Extensions of the General Estimating Equation (GEE) approach as described by (Williamson, Manatunga et al. 2000) were used for statistical calculations along with GEE derived Receiver Operating Characteristic Curve (ROC) instead of the ROCKIT program for the main study.

## E.2. MAIN STUDY

The results of the study demonstrate several findings of laboratory and clinical importance in the use of scanned digital, direct digital radiography, and laser fluorescence caries detection methods in mixed dentitions.

### E.2.1. RADIOGRAPHY PERFORMANCE

#### E.2.1.1. Diagnostic Precision of Radiography

The kappa statistics have been applied to the evaluation of reliability in radiographic methods (Landis and Koch 1977; Langlais, Skoczylas et al. 1987; Naitoh, Yuasa et al. 1998). As described by Landis and Koch (1977), the strength of agreement correlates with kappa value as shown in Table 19.

| Kappa Value | Strength of agreement |
|---|---|
| < 0.00 | Poor |
| 0.0 – 0.20 | Slight |
| 0.21 – 0.40 | Fair |
| 0.41 – 0.60 | Moderate |
| 0.61 – 0.80 | Substantial |
| 0.81 – 1.00 | Almost perfect |

Table 19. Strength of agreement according to kappa value.

For the overall caries presence across all categories in this study, the inter-rater kappa values ranged from 0.180 (conventional radiograph of Presence 5) to 0.337 (scanned digital images of Presence 3) as shown in Table 8 with agreement strength of slight to fair. For detecting overall caries extent in all categories, the inter-rater kappa values ranged from 0.175 (conventional radiograph of Extent 4c) to 0.343 (scanned digital images of Extent 3) as shown in Table 9 also with agreement strength of slight to fair. This demonstrated that dentists in this study agreed only slightly to fairly in detecting the presence or extent of caries with radiography methods.

Specifically, inter-rater kappa values of scanned digital radiographs in detecting caries presence and extent were consistently higher than conventional radiographs and direct digital images in the ranges of 0.219-0.343 (fair agreement strength), although not always to a statistically significant level. This finding was similar to a study performed by Wenzel and co-workers (1990) in which the kappa values were higher for the digitized images than the conventional radiographs in detecting proximal caries. However, their overall digitized kappa value (0.5) is higher than the present study. The discrepancy may be the result of differences (1) in the calculations of kappa, which included both proximal and occlusal caries in both primary and permanent teeth and (2) in the study sample, which included a high proportion of enamel caries (ranging 56-90% enamel caries). Studies have shown that occlusal caries are hard to detect in radiographs (Kidd, Ricketts et al. 1993; Machiulskiene, Nyvad et al. 1999) and the inter-rater reliability was lower in the evaluation of caries limited to the enamel than in that of caries beyond the enamel (Espelid and Tveit 1986; Langlais, Skoczylas et al. 1987; Naitoh, Yuasa et al. 1998).

In this study, the inter-rater reliability kappa values of direct digital in detecting caries presence and extent were not significantly different from conventional radiograph in the ranges of 0.184-0.320 (slight to fair agreement strength). This finding was similar to a study performed by Naitoh et al (Naitoh, Yuasa et al. 1998) who reported the overall kappa values for inter-observer agreement of direct digital images (0.439) did not significantly improve compared to those of conventional radiographs (0.424). Again, their kappa values were higher than this study. Similar reasons mentioned above also applied here that (1) the inter-rater kappa calculations included both proximal and occlusal caries in both primary and permanent teeth (Kidd, Ricketts et al. 1993;

Machiulskiene, Nyvad et al. 1999), and (2) this study sample included a high proportion of enamel caries (ranging 56-90%).

In summary, the overall inter-rater reliability of this study had slight-fair agreement strength in detecting a sample of mainly enamel caries. Evaluators of this study seemed to rate scanned digital images more consistently than conventional radiographs and direct digital images; moreover, the inter-rater reliabilities of conventional radiographs and direct digital images were not statistically different from each other.

E.2.1.2. Proximal Caries Detection

To assess the diagnostic accuracy of radiographic methods in proximal surfaces of primary and permanent teeth, two different analyses were performed (1) modal vote of all 7 raters vs. histology validations and (2) silver standard vs. histology. These analyses resulted in different findings as summarized in Table 20.

| Teeth type and Surface<br>Enamel caries (%) | Modal Vote (Majority)<br>OR value | Silver Standard<br>OR value |
|---|---|---|
| Primary Proximal Caries<br>58 | **Hist** < Conv= Digital= Scan<br>3.29 3.37 3.41 | **Hist** < Scan< Conv=Digital<br>1.38 2.35 2.73 |
| Permanent Proximal Caries<br>90 | **Hist** < Scan= Digital= Conv<br>98.1 98.1 204 | **Hist** < Scan< Conv< Digital<br>18.1 50.1 169.5 |

Table 20. Summary of modal vote and silver standard analyses.

- **Hist**: histology gold standard; Conv: conventional radiographs; Scan: scanned digital images; Digital: direct digital images
- Under histological validation, all radiographic methods compared to conventional radiographs only.
- <: In the context of X < Conv < Y, means that Y underestimated caries extent compared to conventional radiographs but X overestimates compared to conventional radiographs.
- =: In the context of X = Conv = Y, means that X and Y are not significantly different from conventional radiographs.

In the modal (majority) vote analysis, there was no statistically significant difference in detecting primary and permanent proximal caries between scanned and direct digital images from conventional radiography with $p>0.05$. However, in the silver standard analysis, different radiographic results were found in primary and permanent proximal caries detection. In permanent proximal caries detection, direct digital images significantly underestimated, but scanned digital images overestimated caries extent compared to conventional radiographs; however, in primary proximal caries detection, direct digital images were not statistically different from conventional radiographs, but scanned digital images detected more caries and overestimated compared to conventional radiographs.

Both the modal (majority) vote and silver standard analyses found all radiographic methods underestimated primary and permanent proximal caries extent compared to histology validation. This is similar to several studies that have been completed previously that histological validation showed lesions were actually larger than they appeared radiographically (Kleier, Hicks et al. 1987; Kidd, Ricketts et al. 1993; Ricketts, Whaites et al. 1997; Syriopoulos, Sanderink et al. 2000; Hintze and Wenzel 2002). Another study by Hintze et al (2002) evaluated the influence of validation method on the diagnostic accuracy for proximal and occlusal caries. They reported that the diagnostic performance of conventional and direct digital radiography was significantly lower versus histological validation. Thus, radiographs considerably underestimated lesion number and size compared to the histology gold standard.

The second similar finding in comparing the modal and silver standard analyses was that all radiographic methods underestimated caries extent in primary teeth (odds ratio > 1), but underestimated permanent teeth even more (much higher odds ratios). The occurrence of enamel caries in primary proximal surfaces (58%) vs. permanent proximal surfaces (90%) may explain the radiographic diagnostic difference in different dentitions. Studies have shown that (1) currently used E speed films are of almost no value in the detection of small (histologically within the enamel) caries lesions in both occlusal and proximal surfaces (Wenzel, Larsen et al. 1991; Hintze, A et al. 1994), and (2) lower actual caries prevalence gives rise to a lower positive predictive value, specificity, and inter-rater reliability of the diagnostic method (Hans-Goran 1979; Naitoh, Yuasa et al. 1998; Wenzel 1998; Nair and Nair 2001).

It was interesting to note the overall odds ratio values were lower (closer to 1) in the silver standard analysis than those of the modal vote analysis when compared to the histology gold standard. This suggests that the nominal radiology expert (radiology instructor) in the silver standard analysis seemed to detect more caries compared to the combined result of 7 dental faculty members (6 pediatric faculty with 2 to 30 years of experience and 1 radiology faculty member). The study of Syriopoulos (Syriopoulos, Sanderink et al. 2000) also found that the radiologist performed significantly better than general practitioners in caries diagnosis with conventional radiographs, CCD, and SP digital images. They reported that the observer's ability to recognize caries correctly is the main factor contributing to variation in radiographic diagnosis. So, radiographic accuracy is observer to be dependent.

Also, in the silver standard analysis, the radiology expert detected significantly more caries with the scanned digital images than conventional radiographs and direct digital images while in the modal analysis, the 7 dental faculty members found no difference in proximal caries detection in scanned, direct digital images, and conventional radiographs. This finding demonstrated an interesting issue of magnification and observer performance. The radiology expert could detect proximal caries better when scanned and displayed digital image in a larger dimension such as a laptop monitor than in the conventional radiograph dimension with a 2X magnification device. So, in the silver standard analysis, magnification improved the diagnostic performance of the radiology expert. However, the magnification did not improve the combined diagnostic performance of the 7 dental faculty members since more observer variables were introduced and made the magnification issue a subjective one. A similar effect was found in another study (Moystad, Svanaes et al. 1995) that digital image magnification has a significant influence on observer performance in the detection of proximal caries. They also reported that magnification did not necessarily improve diagnostic performance of observers and that there was evidence that there is an upper limit of magnification beyond which diagnostic accuracy may be reduced.

Lastly, both modal vote and silver standard analyses found similar performance results for direct digital images. In comparing to the histology gold standard, both analyses found direct digital images to underestimate caries extent in primary and permanent proximal surfaces. As mentioned before, the histological validation techniques could reveal larger lesions and more numerous lesions than radiography methods (Kleier, Hicks et al. 1987; Kidd, Ricketts et al. 1993; Ricketts, Whaites et al. 1997; Syriopoulos, Sanderink et al. 2000; Hintze and Wenzel 2002). In comparing to conventional radiographs, the modal vote analysis found no difference in detecting permanent proximal caries between conventional radiographs and direct digital images, but the silver standard analysis found the caries extent underestimated compared to conventional radiographs in permanent proximal surfaces. The modal analysis result was supported by many previous studies (Hintze, A et al. 1994; Wenzel 1995; Svanaes, Moystad et al. 1996) and a very recent study (Hintze and Wenzel 2002), which found no difference in proximal caries detection between F speed films and MPDx. However, the silver standard finding was also confirmed by the result from a study by Price and Ergul (Price and Ergul 1997). Also, when compared to conventional radiographs, both analyses found direct digital images to be equal conventional radiographs in primary proximal caries detection, which is supported by one study (Nielsen, Hoernoe et al. 1996) but opposed by another (Uprichard, Potter et al. 1999). Nielsen's study had a smaller study sample (N=72 proximal surfaces) compared to Uprichard's study (N=270 proximal surfaces). Due to small sample size, Nielsen's and coworker's study has had a lower power resulted no statistically significant difference between direct digital images and conventional radiographs.

To summarize this present study, the diagnostic accuracy of radiography in detecting proximal caries was validation method dependent, observer dependent, and caries lesion depth dependent. Even though the silver standard analysis showed an overall better radiographic performance in detecting proximal caries in both dentitions than the modal vote analysis, the modal vote analysis was probably better in representing the true diagnostic performance for the general dental practitioners. Thus, using histology validation, all three radiographic methods underestimated caries extent and there was no difference in diagnostic accuracy between scanned digital images (flatbed scanner at 474 dpi resolution), direct digital images (CCD based, MPDx), and conventional radiography (F speed films) in detecting primary and permanent proximal caries primarily enamel lesions.

E.2.1.3. Occlusal Caries Detection

| Teeth type and Surface<br>Enamel caries (%) | Modal Vote (Majority)<br>OR value | Silver Standard<br>OR value |
|---|---|---|
| Primary Occlusal Caries<br>56 | Conv = **Hist**≤ Digital< Scan<br>0.94 1.44 2.12 | Scan < Conv=Digital< **Hist**<br>0.02 0.20 0.45 |
| Permanent Occlusal Caries<br>63 | **Hist** < Conv= Scan= Digital<br>9.88 11.7 22.6 | Scan < **Hist**=Conv < Digital<br>0.35 1.45 4.32 |

Table 21. Occlusal caries detection findings in modal vote and silver standard analyses.

- **Hist**: histology gold standard; Conv: conventional radiographs; Scan: scanned digital images; Digital: direct digital images; DD: direct digital images
- Under histological validation, all radiographic and laser fluorescence methods were compared to conventional radiographs only.
- <: In the context of X < Conv < Y, means that Y underestimated caries extent compared to conventional radiographs but X overestimates compared to conventional radiographs.
- ≤: In the context of Conv=X≤Y, means that Y equals X, but Y underestimated caries extent compared to conventional radiographs.
- =: In the context of X = Conv = Y, means that X and Y are not significantly different from conventional radiographs.

The modal vote and silver standard analysis showed different radiographic and laser fluorescence performances in primary and permanent occlusal caries detection. In the modal vote analysis, all radiographic methods underestimated occlusal caries extent in primary and permanent occlusal surfaces compared to histology except conventional radiographs and direct digital images, which had no difference from histology in detecting primary occlusal caries. However, in the silver standard analysis, different radiographic results were found in primary and permanent occlusal caries detection. In primary occlusal caries detection, all radiographic methods overestimated caries extent compare to histology. However, in permanent occlusal caries detection, conventional radiographs showed no difference from histology while scanned digital overestimated and direct digital underestimated caries extent. This demonstrated that occlusal caries are difficult to diagnose and observers do not agree very much. Researchers have reported that enamel occlusal caries are also very difficult to diagnose because they are generally not visible and early dentinal occlusal lesions also have low agreement with radiographs due to superimposition of buccal and lingual enamel (Kidd, Ricketts et al. 1993; Machiulskiene, Nyvad et al. 1999).

The first interesting finding in comparing the modal vote and silver standard analyses was that the silver standard evaluator had an overall lower OR than the modal vote evaluators, but not necessarily more diagnostic accuracy especially for primary occlusal caries detection. It seems that the radiology instructor diagnosed permanent occlusal caries extent closest to histology, but overestimated caries extent in primary molars. On the other hand, the modal vote evaluators of 6 pediatric dental faculty members and 1 radiology instructor seemed to diagnose primary occlusal caries extent closest to histology but underestimated caries extent in permanent molars.

Observer variability is a possible explanation to this difference such that (1) the radiology instructor was more comfortable in diagnosing occlusal caries in permanent molars and tended to overestimated occlusal caries in primary molars, or (2) pediatric dental faculty members were more comfortable in diagnosing primary occlusal caries and tended to underestimate permanent occlusal caries.

Another explanation would be the density of radiograph/digital images. Although this factor did not affect the proximal caries detection in this study, it seems to be a relevant factor to mention for detecting occlusal caries. Skodje et al (1998) demonstrated that density of radiographs influenced the diagnostic outcome in small occlusal caries lesion in the outer third of dentin. Specifically, occlusal caries are diagnosed best from darker density radiographs. The specificity was higher with light density radiographs, but sensitivity increased with density. So, underdiagnosis is more frequent with light radiographs, while overdiagnosis occurs more often with dark ones especially at the dentino-enamel junction. Thus, it was possible that the optimal radiographic setting established in this study may still have posed density discrepancies significant enough to affect the diagnostic outcomes in primary and permanent occlusal caries. The standard exposure was established by approximating the densitometer readings of sound primary and permanent dentin as value of one. Specifically, the permanent dentin had a densitometer value of 0.7 and primary dentin had 1.25. Thus, permanent molars in the radiograph may be slightly underexposed while primary molars may be slightly overexposed resulting in underdiagnosing occlusal caries in permanent molars and overdiagnosing in primary teeth. Even though this is inevitable, perhaps this may explain why the radiographic methods overestimated primary occlusal caries compared to histology in the silver standard analysis. If this is true, it is important to be aware of these effects when viewing radiographs of mixed dentitions in detecting occlusal caries.

Moreover, both modal vote and silver standard analyses found different performance results for scanned digital images. Compared to the histology gold standard, the silver standard analysis found scanned digital images to overestimate caries extent in both primary and permanent occlusal surfaces, while the modal vote analysis found them to underestimate primary and permanent occlusal caries extent. Also, when compared to conventional radiographs, the scanned digital images seemed to overestimate occlusal caries compared to conventional radiographs in both primary and permanent molars in the silver standard analysis. On the other hand, the scanned digital images were not different from conventional radiographs in detecting primary and permanent occlusal caries in the modal vote analysis. Again, observer variability justified such consistent differences between the two analyses that the radiology instructor overdiagnosed occlusal caries extent in scanned digital images compared to histology and conventional radiographs. Thus, similar to proximal caries detection (Moystad, Svanaes et al. 1995), magnification seems to affect the diagnostic accuracy of scanned digital images in detecting occlusal caries such that it subjectively enables some observers to detect more caries or less caries.

Lastly, both modal vote and silver standard analyses also found different performance results for direct digital images. Compared to the histology gold standard, the silver standard analysis found direct digital images to overestimate caries extent in primary surfaces but underestimate permanent occlusal surfaces, while the modal vote analysis found direct digital images were not significantly different from histology in primary surfaces but underestimated permanent occlusal caries extent. Also, when compared to conventional radiographs, the direct digital images seemed to equal to conventional radiographs in overestimating primary occlusal caries, but underestimated permanent occlusal caries in the silver standard analysis. On the other hand, the direct digital images underestimated primary occlusal caries compared to conventional radiographs, but equaled conventional radiographs in detecting permanent occlusal caries in the modal vote analysis. Like conventional radiographs (Kidd, Ricketts et al. 1993; Machiulskiene, Nyvad et al. 1999), this finding also reflects difficulty in diagnosing both primary and permanent occlusal caries together in direct digital images. Since there are only two studies focused on occlusal caries detection with direct digital radiography, the finding from the modal vote analysis confirmed findings of Hintze et al (1994) that there is no difference in detecting occlusal caries between conventional radiographs (D and E speed films) and direct digital images (Visualix) in permanent occlusal surfaces using histologic validation. However, the silver standard finding is also confirmed by the result from a recent study comparing F speed films with MPDx and 5 other direct digital radiography devices (Hintze and Wenzel 2002), which found that F-speed film was significantly more accurate than MPDx in detecting occlusal caries in permanent teeth. Nonetheless, there were no previous studies focused on diagnostic accuracy in detecting primary occlusal caries with direct digital radiography.

To summarize, occlusal caries was difficult to diagnose such that there was a wide range of diagnoses found due to observer dependence, caries lesion depth dependence, and radiographic/digital image density dependence. The silver standard evaluator seemed to be better at diagnosing permanent occlusal caries whereas the modal vote evaluators seemed to be better at diagnosing primary occlusal caries. Nonetheless, the modal vote analysis is probably better in representing the true diagnostic performance in the general dental practitioners. Therefore, compared to histology, all radiographic methods underestimated caries extent except conventional radiographs, which equaled histology in detecting primary occlusal caries. On the other hand, compared to conventional radiographs, there was no difference between conventional radiographs, scanned and direct digital images in detecting permanent occlusal caries, but both scanned and direct digital underestimated primary occlusal caries.

## E.3. DIAGNODENT PERFORMANCE AND VARIABLES

### E.3.1. DIAGNOSTIC PRECISION OF DIAGNODENT

Lin's concordance correlation assessed intra-examiner reliability of the DIAGNOdent evaluator (D LIN). Lin's concordance correlations were calculated by assessing the duplicating measurements of DIAGNOdent readings in 5 different quadrant blocks (a total of 51 primary occlusal pits and 53 permanent occlusal pits) under controlled conditions of gamma irradiation, calibration with ceramic standardized disc only, and 100% moisture condition.

| | Tooth Type | | |
|---|---|---|---|
| | Primary Molar (51 occlusal pits) | Permanent Molar (53 occlusal pits) | Primary & Permanent (104 occlusal pits) |
| Lin's Concordance Correlation | 0.943 | 0.986 | 0.960 |

Table 22. Intra-rater reliability of DIAGNOdent.

This study showed that Lin's concordance correlations were almost perfect in measuring laser fluorescence for primary, permanent, and combined results. This high level of reliability is comparable to several published in vitro and in vivo studies using this device. For permanent molars, Lussi et al (Lussi, Imwinkelried et al. 1999) reported excellent (almost perfect) intra-examiner kappa scores of 0.88 (D2) and 0.90 (D3) with Spearman correlation of 0.97 in an in vitro study. Although Spearman correlation may overestimate repeatability, Lin's concordance correlation correctly estimates repeatability. Lussi et al (Lussi, Megert et al. 2001) also found an excellent (almost perfect) kappa value of 0.93 and Spearman's correlation of 0.98 in an in vivo study. For primary molars, Attrill el al (Attrill and Ashley 2001) showed a good (substantial) intra-examiner kappa value of 0.78 in an in vivo study. Another in vitro study focusing on primary molars also found good (substantial to almost perfect) intra-examiner kappa scores of 0.76-0.86 (D2) and 0.77-0.85 (D3) (Lussi and Francescut 2003). The clinical relevance for such high reproducibility of DIAGNOdent makes it suitable for the longitudinal monitoring of caries and thus also for assessing the caries activity and the outcome of preventive interventions (Lussi, Imwinkelried et al. 1999; Attrill and Ashley 2001; Lussi, Megert et al. 2001; Lussi and Francescut 2003).

### E.3.2. DIAGNODENT DIAGNOSTIC ACCURACY

#### <u>E.3.2.1. DIAGNOdent vs. Radiographic Methods</u>

| Teeth type and Surface<br>Enamel caries (%) | Modal Vote (Majority)<br>OR value | Silver Standard<br>OR value |
|---|---|---|
| Primary Occlusal Caries<br>56 | Conv=**Hist**≤ Digital< Scan< DD<br>0.94 1.44 2.12 3.02 | Scan < Conv=Digital< **Hist**< DD<br>0.02 0.20 0.45 2.35 |
| Permanent Occlusal Caries<br>63 | **Hist** <DD<Conv= Scan= Digital<br>2.66 9.88 11.7 22.6 | Scan < **Hist**=Conv≤ DD < Digital<br>0.35 1.45 2.94 4.32 |

Table 23. Occlusal caries detection findings in modal vote and silver standard analyses.

- **Hist**: histology gold standard; Conv: conventional radiographs; Scan: scanned digital images; Digital: direct digital images; DD: direct digital images
- Under histological validation, all radiographic and laser fluorescence methods were compared to conventional radiographs only.
- <: In the context of X < Conv < Y, means that Y underestimated caries extent compared to conventional radiographs but X overestimates compared to conventional radiographs.
- ≤: In the context of Hist=Conv≤Y, means that Y equals conventional radiographs, but underestimated caries extent compared to histology.
- =: In the context of X = Conv = Y, means that X and Y are not significantly different from conventional radiographs.

Using histology validation, DIAGNOdent significantly underestimated caries extent in primary and permanent occlusal surfaces in both modal vote and silver standard analyses. This finding was also reported in several in vitro studies (Lussi, Imwinkelried et al. 1999; Lussi, Megert et al. 2001; Sheehy, Brailsford et al. 2001) such that histologic validation revealed minute changes in dental tissues including demineralization in enamel and dentin. Ultimately, due to the validation technique, the cut-off limits as shown in the following section have been affected as well.

In primary molars, both modal vote and silver standard analyses revealed that DIAGNOdent underestimated occlusal caries extent compared to conventional radiographs. This finding was not supported by previous studies focused on primary occlusal caries detection by DIAGNOdent and conventional radiograph. For example, Lussi et al (Lussi and Francescut 2003) reported that DIAGNOdent was not significantly different from conventional radiographs at the D3 level (outer dentin caries) in primary occlusal detection. Attrill et al (Attrill and Ashley 2001) found that DIAGNOdent was superior to conventional radiographs in detecting occlusal dentin caries in primary molars. Also, Anttonen et al (Anttonen, Seppa et al. 2003) suggested that the radiographic exam was the least accurate method compared to DIAGNOdent and visual exam in both diagnosing enamel and dentin occlusal caries in both primary and permanent teeth. Possible explanations for such different result found in this study are discussed in Section E.3.2.3.

Moreover, in detecting permanent occlusal caries, modal vote and silver standard analyses yielded different results. In the silver standard analysis, DIAGNOdent was not significantly different from conventional radiographs in permanent occlusal caries detection; whereas the modal vote analysis found DIAGNOdent overestimated permanent occlusal caries, detecting more caries extent compared to conventional radiographs. Previous studies supported the modal vote analysis findings in DIAGNOdent performance in permanent teeth as Lussi et al (2001) and Shi et al (2000) reported DIAGNOdent performed superior to radiography in detecting occlusal caries in permanent teeth.

Furthermore, since all statistical calculations only compared different methods to histology and conventional radiographs, there was no direct comparison of DIAGNOdent to scanned and direct digital images. Thus, diagnostic accuracy of DIAGNOdent cannot be compared to scanned and direct digital images.

E.3.2.2. ROC Analysis (Permanent & Primary Teeth Combined)

The ROC curve in Figure 14 demonstrated that DIAGNOdent readings had the highest area under the curve with dentinal (or deeper) caries, then inner enamel (or deeper) caries, and followed by outer enamel (any caries). DIAGNOdent performed better at detecting dentinal caries than inner enamel caries. This is in agreement with studies of Attrill et al (2001) and Lussi et al (2003) that DIAGNOdent was the most accurate system compared to radiographic and visual exam in detecting occlusal dentin caries in primary molars; however, at the noncavitated outer enamel and inner enamel level, it was not statistically significantly better that visual exam.

This finding also supports the fundamental mechanism of DIAGNOdent. It is important to keep in mind that DIAGNOdent measures the fluorescence of porphyrin (bacterial byproduct), and that it does not provide a direct measure of porosity or of demineralization. It simply measures the amount of specific bacterial by-products taken up by porous tissue. . Also, recall that the caries process involves demineralization in dental tissue before cavitation and bacterial infection occur. Early enamel demineralization in intact enamel surfaces may not contain bacteria since they are too large to fit through the much smaller diffusion channels (Young 2002). On the other hand, enamel cavitation is strongly associated with dentin involvement as shown by (van Amerongen, Penning et al. 1992) and that when definite cavitation is present, 75% of these teeth presented caries penetrated far into dentin. Significant enamel cavitation allows plaque accumulation and passage of bacteria and bacterial by-products into the more porous dentin (Young 2002). Therefore, it is logical to expect DIAGNOdent to detect dentin caries better than early enamel caries since bacteria and its byproducts porphyrins should be more easily found in infected dentin than in demineralized enamel; therefore, higher intensity of fluorescence should be found in infected dentin than demineralized enamel and in highly demineralized dentin below enamel caries that has extended into the dentin.

However, dentin demineralization also occurs before bacterial infection. Ricketts el al expressed that surgical intervention is not necessary when dentin is merely demineralized and uninfected (Ricketts, Kidd et al. 1995). About 60% of the radiolucencies seen in the outer half of the dentin are likely to be noncavitated (Anusavice 1997; Wenzel and Hintze 1999), and in this demineralized dentin should be remineralized and restored only when cavitation has occurred. The question is whether or not bacterial byproducts (porphyrins) can easily pass through these enamel diffusion channels in demineralized enamel so DIAGNOdent can detect the fluorescence. What about demineralized dentin? If porphyrin cannot pass through these diffusion channels, then DIAGNOdent is selective in detecting infected dentin. This would greatly help make the restorative decisions easier. On the other hand, if porphyrin can pass through these diffusion channels in demineralized enamel and dentin, then it is important to establish clinically relevant cut-off limits of laser fluorescence to differentiate demineralized enamel, demineralized dentin and infected dentin. This would greatly benefit early caries detection in longitudinal caries activity monitoring and in assessing the outcome of preventive interventions.

Porphyrin may actually pass through demineralized enamel and dentin since fluorescence can be detected in inner enamel and outer dentin when validating caries extent in histological sections. Therefore, ideally it is best to establish a cut-off limits for demineralization in outer and inner enamel, as well as outer and inner dentin, if these fluorescence signals can be quantified to depth or severity of carious lesions. However, some early preliminary studies have reported that DIAGNOdent seems to be unsuitable for the detection of initial carious changes in enamel (Lussi, Imwinkelried et al. 1999) and difficult to distinguish clearly between truly deep dentinal caries and superficial dentinal caries (Lussi, Megert et al. 2001).

E.3.2.3. DIAGNOdent reading vs. Histology

Table 18. OR and 95% confidence interval for DIAGNOdent readings vs. histology.

| DIAGNOdent Readings | Odds Ratio (OR) | 95% Confidence Interval | p-Value |
|---|---|---|---|
| Primary Occlusal Surfaces | 8.69 | 5.37-14.08** | <0.0001** |
| Permanent Occlusal Surfaces | 2.78 | 1.64-4.71** | 0.0002** |

** Statistical significance vs. histology gold standard with $p \leq 0.05$

Compared to histology, DIAGNOdent significantly underestimated occlusal caries extent in both primary and permanent occlusal surfaces as shown in Table 18. Furthermore, since the 95% confidence interval of primary teeth did not overlap that of permanent teeth, DIAGNOdent underestimated caries extent in primary occlusal surfaces significantly more than permanent occlusal surfaces. In the laboratory setting, the mean value for sound surfaces was significantly lower in primary teeth than in permanent teeth as described by Anttonen et al (Anttonen, Seppa et al. 2003). Although many studies have reported that DIAGNOdent has similar diagnostic performance in detecting primary occlusal caries as in permanent occlusal caries by comparing two different studies (Anttonen, Seppa et al. 2003; Lussi and Francescut 2003), the above direct comparison in this study illustrated that DIAGNOdent significantly underestimated caries extent in primary molars compared to permanent molars.

Considering the basic mechanism of the DIAGNOdent (to detect fluorescence of bacterial porphyrin), different macro and micromorphological characteristics in primary teeth can affect its ability to detect bacterial porphyrin. Macro-morphologically, the enamel and dentin layers of the primary dentition are known to be much thinner than those of its permanent successor (Hunter, Westb et al. 2000). Compositionally, primary teeth demonstrate less calcium and phosphate ions in dentin (Nor, Feigal et al. 1996), overall a lower degree of minerals (Wilson and Beynon 1989), and micro-morphologically exhibit a higher degree of enamel porosity (Shellis 1984) than permanent teeth. Lussi et al (Lussi and Francescut 2003) suggested that primary teeth have greater enamel porosity (more light scattering leading to less fluorescence) and thinner enamel (less masking dentin fluorescence leading to more fluorescence). They suggested that due to the canceling effects of these two optical properties of primary teeth, there was a small overall change of the fluorescence signal of primary teeth compared to the permanent teeth. However, even though the optical property of macro- and micromorophology may have balanced out, there are other factors that may affect bacterial porphyrin fluorescence of primary teeth in this study.

There are two studies focused on fluorescence detection of porphyrin-producing bacteria in human caries (Koenig, Hibst et al. 1993; Koenig, Schneckenburger et al. 1994). They reported that the bacteria *Streptococcus mutans* and various *lactobacillus* species showed no typical porphyrin fluorescence, but *Actinomyces odontolyticus*, *Bacteroides intermedius (Prevotella intermedia)*, and *Pseudomonas aeruginosa* showed strong fluorescence at 635 nm. *Actinomyces odontolyticus* is more abundant in plaque from sound surfaces (Boue, Armau et al. 1987; Marchant, Brailsford et al. 2001). *Prevotella intermedia* is an obligate anaerobic gram negative rod that is frequently associated with periodontal disease such as adult periodontitis, acute necrotizing ulcerative gingivitis, and pregnancy gingivitis (Fukui, Kato et al. 1999; Maida, Campus et al. 2003). Moreover, *Pseudomonas aeruginosa* is a known pulmonary pathogen, which has been isolated from patients with "refractory periodontitis" (Colombo, Haffajee et al. 1998; Barbosa, Mayer et al. 2001) or necrotizing gingivostomatitis among immunocompromised patients (Myoken, Sugata et al. 1999). So, even though *Streptococcus mutans* and the *lactobacilli* are the acidogenic organisms most commonly associated with human dental caries (van Houte 1994; van Palenstein Helderman, Mattee et al. 1996), they are not responsible for producing porphyrin, which fluoresce from laser light at 655 nm.

Additionally, another study focusing on the predominant microflora of nursing caries (early childhood caries) lesions (early childhood caries) (Marchant, Brailsford et al. 2001) reported that *Streptococcus mutans, Actinomyces israelii, Actinomyces gerencseriae, Candida albicans, lactobacilli* and *veillonellae* were isolated more frequently ($p<0.05$) from infected dentin taken from the actual nursing caries lesion while

*Streptococcus oralis*, *Streptococcus sanguis, Streptococcus gordonii, Actinomyces naeslundii* and *Actinomyces odontolyticus* were isolated more frequently from the plaque from the caries-free children. While many cross-sectional studies focused on the role of *mutans streptococci* and *lactobacilli* and considered other components of the infecting flora to be insignificant, Marchant et al (Marchant, Brailsford et al. 2001) showed a diverse microflora and supported a non-specific etiology for nursing caries in which the physiological characteristics of the infecting flora, not its composition, was the major determinant underlying the disease process.

In summary, (1) DIAGNOdent does not measure the major players of caries causing bacteria such as *Strepococcus mutans, Actinomyces israelii, Actinomyces gerencseriae, Candida albicans, lactobacilli* and *veillonellae* which are commonly found in infected dentin of nursing caries; (2) DIAGNOdent indirectly measures caries activity such that it is measuring the diffused porphyrin (produced by noncarious causing bacteria of *Actinomyces odontolyticus, Bacteroides intermedius (Prevotella intermedius)*, and *Pseudomonas aeruginosa*) into established carious lesions; and (3) *Acitnomyces odontolyticus* are frequently found in plaque samples in caries-free children and not in infected dentin.

Based on the above information, one may make a few inferences: (1) carious primary teeth have less *Actinomyce odontolyticus* than carious permanent teeth since *Actinomyce odontolyticus* are more frequently found in plaque samples of caries free children as reported by Marchant et al (Marchant, Brailsford et al. 2001); (2) DIAGNOdent's ability to detect porphyrin is dependent on whether or not *Actinomyces odontolyticus, Prevotella intermedius*, and *Pseudomonas aeruginosa* are part of host's normal flora; and (3) *Prevotella intermedius* is typically found in adult periodontitis and *Pseudomonas aeruginosa* is typically found in refractory periodontitis which are not usually found in children. So, DIAGNOdent could detect more porphyrin fluorescence produced by *Actinomyce odontolyticus, Prevotella intermedius*, and possibly *Pseudomonas aeruginosa* in permanent teeth compared to primary teeth. The observed low fluorescence signals obtained in the primary teeth are most likely a combination of the optical properties described above and the lesser likelihood of porphyrins in children's' mouths.

### E.3.3. CUT-OFF LIMITS

Table 24. Cut-off limits of different studies.

| Studies | Type/ Teeth | No Caries | Outer Enamel | Inner Enamel | Outer Dentin | Inner Dentin |
|---|---|---|---|---|---|---|
| This Study | In Vitro Permanent | 0-5 | 6-8 | 8-11 | >11 | |
| Kavo's Instruction | ------------ | 0-14 | | 15-20 | 21-99 | |
| Lussi et al 1999 | In Vitro Permanent | 0-4 | 4.01-10 | | 10.01-18 | >18.01 |
| Lussi et al 2001 | In Vivo Permanent | 0-13 | 14-20 | | >20 | |
| Attrill et al 2001 | In Vitro Primary | 0-9 | 10-17 | | 18-99 | |
| Lussi et al 2003 | In Vitro Primary | 0-4 | | 5-12 | >12 | |

This study found similar DIAGNOdent cut-off limits to two in vitro studies by Lussi (Lussi, Imwinkelried et al. 1999; Lussi and Francescut 2003). However, these cut-off limits were different from those of Kavo (the manufacturer) and other in vivo studies. Thus, as suggested by Lussi et al (Lussi, Imwinkelried et al. 1999), Sheehy et al (Sheehy, Brailsford et al. 2001), and Lussi et al (Lussi, Megert et al. 2001) the differences found in cut-off limits between in vitro and in vivo/clinical study may be due to (1) extracted teeth were stored in thymol solution or formalin for a period of time; (2) in vitro teeth were professionally cleaned; and (3) histology validation can detect minute demineralization under the microscope.

Lussi et al also suggested that histologic dentin caries should not indicate immediate operative intervention in all circumstances. As far as a dentist making a treatment decision is concerned, the decision should depend on a range of other variables such as a patient's case history, fluoride and dietary status, as well as perceived caries activity. Many researchers have concluded that the DIAGNOdent should always be used in conjunction with a visual exam because a high laser fluorescence reading may indicate caries, hypominerlization, and/or staining that is not caries related(Lussi, Imwinkelried et al. 1999; Shi, Welander et al. 2000; Lussi, Megert et al. 2001). Also, Sheehy el al (Sheehy, Brailsford et al. 2001) suggested that when using DIAGNOdent in the clinical setting, the cut-off points recommended by the manufacturers should be used to interpret results.

E.3.4. GAMMA IRRADIATION EFFECT

Gamma irradiation is one of the most effective sterilization techniques to avoid cross-contamination in the laboratory settings. It is important to make sure that gamma irradiation sterilization does not affect laser fluorescence readings by changing the physical properties of teeth (enamel and dentin) or bacterial byproducts (porphyrin).

In a study performed by White el al (White, Goodis et al. 1994), Fourier transform infrared spectroscopy (FTIR), and optical properties of dentin blocks were studied on 4 different sterilization techniques: (1) gamma irradiation; (2) ethylene oxide; (3) dry heat; and (4) autoclaving. It was found that no detectable changes were found with gamma irradiation, but all other methods introduced some detectable change in the spectra. So, White et al have found that gamma irradiation at 173 krad with use of a Cesium (Cs137) radiation source introduced no detectable changes in dentin blocks as measured by FTIR, UV/VIS/NIR, or permeability. Another study tested sterilization effectiveness of gamma irradiation (congruent with 25kGy), steam autoclaving (121 degrees C for 15 min), sodium hypochlorite (NaOCl) (12% w/v for 24h) and povidone-iodine (7.5% w/v for 24h) on dental enamel (Amaechi, Higham et al. 1998; Amaechi, Higham et al. 1999). They found that these four sterilization techniques affected the enamel surface as follows: gamma irradiation (cream discoloration), NaOCl (bleaching), and povidone-iodine (white spot-like lesion). The numerical values of mineral loss and lesion depth in groups were ranked as following: gamma irradiation <povidone-iodine <control <autoclave <NaOCl. They concluded that the four sterilization methods were all effective to sterilize enamel, but gamma irradiation was the most acceptable method for enamel to be used in cariogenicity tests having the least adverse effect.

Some teeth sterilized in this study were found to be slightly cream discolored after gamma sterilization, but not all teeth. The results from this study showed that the paired t-test revealed that gamma irradiation does not significantly alter the laser fluorescence measurements for either calibration techniques, but the Wilcoxon signed ranked test also found no statistical significance when using the calibration disc only and was significantly different when the ZeroIn technique used. Thus, gamma irradiation may have some effect on the fluorescence of the teeth and may affect the laser fluorescence readings through either the optical property of the teeth or bacterial byproduct (porphyrin). Since sterilization is a must for laboratories handling of biological specimens, gamma irradiation sterilization was utilized for this study instead of other sterilization methods. The statistical analysis performed for the main study used measurements with calibration via calibration disc only since there was no statistical significance in both analyses for these experimental conditions.

E.3.5. MOISTURE EFFECT

In this study, all 3 moisture conditions (moist 100, moist water/air, and moist air) were statistically different from each other. As a result, moist air had the highest mean values, then moist water/air and moist 100. So, the DIAGNOdent values increased as teeth were exposed to dryer conditions. This finding was also reported in other studies (Shi, Welander et al. 2000). Possible reasons may be that moisture from water/saliva absorbed or deflected fluorescence in other directions that the DIAGNOdent handpiece could not measure. Thus, this study is in agreement with Shi et al (2000) and suggested that moist 100 (moist) and moist water/air (dry) were not different to a clinically relevant extent, but moist air was different from moist 100 and moist water/air. Nonetheless, the statistical analysis performed for the main study used measurements with moist water/air condition (1 drop of water and 3 second of air blast dry) instead. It is important for future in vitro DIAGNOdent studies to avoid letting teeth sit out the bench more than 10 min prior to measuring laser fluorescence. It is also important for clinicians to be aware that over drying teeth intraorally may also affect the DIAGNOdent readings, which require supports by future investigations.

### E.4. LIMITATION OF THIS STUDY

Even though a pilot study set out to ensure proper design in simulating clinical situations, there were inevitable intrinsic limitations associated with this in vitro study.

(1) Study sample: From previous studies, the status of proximal or occlusal surfaces (demineralized vs. cavitated) has a direct impact on the diagnostic accuracy (Lussi 1996). When comparing diagnostic accuracy among different methods or different studies, it is important to consider the condition of proximal or occlusal surfaces being compared (demineralized vs. cavitated). The sample obtained in this study included a range of noncavitated to 1/3 cavitated proximal and occlusal lesions since reasons for extraction were either near exfoliation or gross caries. In these cavitated teeth, the bacterial porphyrin may have leaked out to storage media (0.1% Thymol) resulting in overall lower DIAGNOdent readings.

(2) Storage media (0.1% thymol): Extracted teeth were stored in 0.1% thymol solutions for a period of 6 months prior DIAGNOdent measurements. Storage for a long period of time may cause porphyrin to leak out from cavitated lesions. Also a previous preliminary study has shown that storage media such as thymol and formalin may alter the fluorophers thus affecting DIAGNOdent performance (Lussi, Megert et al. 2001).

(3) Gamma irradiation sterilization: Compared to other sterilization techniques (ethylene oxide, dry heat, autoclaving, sodium hypochlorite, and povidone-iodine), gamma irradiation sterilization seemed to be an acceptable method for enamel to have the least adverse effect. Some teeth sterilized by gamma irradiation turned slightly cream color and slightly increased in DIAGNOdent values. In the gamma irradiation effect study, results showed that gamma irradiation does affect laser fluorescence when

calibrated with the ZeroIn technique, but it does not affect laser fluorescence reading when calibrated only with the ceramic standardized disc. Since sterilization of extracted teeth is a must for laboratory study, DIAGNOdent was calibrated with the ceramic standardized disc only after gamma irradiation sterilization was performed.

(4) Teeth are more clean in an in vitro study: Similar to other in vitro studies, extracted teeth were scrubbed with a toothbrush to remove blood and debris prior to gamma irradiation; they were cleaned after mounting in dental stone, and professionally cleaned with prophy pumice. Thus, these extracted teeth may have been cleaner than teeth in an actual clinical conditions resulting in lower DIAGNOdent readings as suggested by Lussi eve al (Lussi, Imwinkelried et al. 1999; Lussi, Megert et al. 2001).

(5) Histologic gold standard: Histologic validation allows observation of minute changes in demineralization (Lussi, Imwinkelried et al. 1999; Lussi, Megert et al. 2001). In this study, the histological validation was provided by polarized light microscopy and stereomicroscopy. Polarized light microscopy is known as the histological validation technique for early demineralization (Gustafson and Gustafson 1961; Ricketts, Watson et al. 1998); stereomicroscopy is the most trustworthy histological validation method compared to film radiography, microradiography, and naked-eye inspection for the detection of caries in occlusal tooth surfaces. Investigators have suggested demineralization in enamel and dentin without cavitations does not require surgical intervention if dentin is not infected (Ricketts, Kidd et al. 1995). It is unclear whether the histologic caries could differentiate demineralized and infected dental tissue. Thus, when using histologic validation, caries is revealed more and larger than radiography resulting in higher OR values.

## E.5. FUTURE STUDY SUGGESTIONS

(1) Establishment of a more refined histologic validation with microbiological sampling

(2) Comparison of primary occlusal caries detection in radiographic, visual and laser fluorescence in noncavitated teeth with histologic validation.

(3) Comparison of primary occlusal caries detection in radiographic, visual, and laser fluorescence in cavitated teeth with histologic validation.

(4) Longitudinal study of clinical effectiveness in monitoring progression of incipient occlusal and proximal caries.

## E.6. CONCLUSIONS

As the disease burden of caries has changed in recent decades, the treatment philosophy has changed to a minimal intervention approach. However, such an approach is only effective if caries can be diagnosed and monitored at an early state. Early caries detection in primary teeth is especially crucial due to the rapid rate of caries progression as a result of the reduced enamel thickness of primary teeth. Thus, when new diagnostic systems (flatbed scanner, CCD direct digital radiography, and laser fluorescence device) are introduced, it is essential to first validate the diagnostic performance of these new diagnostic systems in the laboratory setting where accurate validation can be confirmed. This study supported the following null hypotheses:

For proximal caries detection:

- There is no difference in detecting proximal caries in the mixed dentition between indirect (Epson Expression 1600), direct digital (MPDx), and conventional radiography (F-speed film).

For occlusal caries detection:

- There is no difference in detecting <u>overall</u> occlusal caries between indirect (Epson Expression 1600), direct digital (MPX), conventional radiography (F-speed film) in permanent teeth.
- When using laser fluorescence (DIAGNOdent), there is no difference in detecting <u>occlusal caries</u> in different moisture conditions of 100% moisture condition and 1 drop of water with Air blast 3 seconds.

This study rejected the following null hypotheses:

For occlusal caries detection:

- Indirect and direct digital both underestimated caries compared to conventional radiographs in primary teeth.
- Laser fluorescence (DIAGNOdent.) overestimated and detected more permanent occlusal caries extent than all radiographic methods, but it underestimated primary occlusal caries extent more than all radiographic methods.
- Ten minutes air dry was significantly different from 100% moisture and 1 drop of water with air blast 3 seconds conditions.
- Gamma irradiation does not significantly alter the laser fluorescence readings when DIAGNOdent is calibrated with a standardizing ceramic disc only. However, overall gamma irradiation does affect laser fluorescence in teeth.

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