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Coronal Microleakage of a Dentin Bonding Agent When Used in the Presence of a Eugenol Containing Endodontic Sealer

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Thesis Submitted to the
School of Dentistry
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Master of Science
In Endodontics

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Abstract

Coronal Microleakage of a Dentin Bonding Agent When Used in the Presence of a Eugenol Containing Endodontic Sealer

Jeffrey G. Minchau, DDS.

The propose of this dye and SEM study was to evaluate the cleansing capabilities of two solvents ethyl alcohol and chloroform, prior to dentin bonding in the coronal chambers of endodontically treated teeth obturated with either a eugenol or resin-based sealer. A total of 156 extracted human molar teeth were divided into 6 groups and obturated with either a eugenol-based (Roth's, Grossman), or a resin-based (AH Plus, Kerr) endodontic sealer. Samples were thermocycled and placed into India Ink or silver nitrate for (SEM samples). All teeth were cleared and evaluated for coronal microleakage. A significant difference was found between the six groups p= 0.002 (Legistic linear analysis). **Conclusion:** when using eugenol-based endodontic sealer it is imperative to clean the coronal chamber with either ethyl alcohol or chloroform prior to dentin bonding. When using a resin-based endodontic sealer it is not necessary to use a solvent to clean the coronal chamber.

Dedication

To Elizabeth, your unconditional love, support, and understanding have been invaluable. Our future together is my inspiration. To my parents, Thank-you for giving me every opportunity a son could ask for and instilling in me the desire to always do my best. I love you all and appreciate everything you have done for me.

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Chapter 1

Introduction

Successful endodontic therapy consists of three major components: removal of irritants from the root canal system, obturation of the cleaned and shaped system, and prevention of future contamination of the sealed root canal system(1). Complications most frequently arise when the sealing of the root canal is compromised. Temporary restorations are frequently placed as an inter-appointment medicament to prevent leakage of salivary contaminants into the obturated canal system until a permanent restoration is placed. If a portion of the obturated canal system is exposed to oral contaminants secondary to leakage of the temporary restoration, the prognosis of the endodontic therapy may be jeopardized.

To eliminate coronal microleakage through temporary restorations in obturated canal systems, the use of dentin bonding agents as a secondary coronal barrier has been advocated. Resin liners used for a secondary coronal barriers show superior sealing capability, are easily placed. However, previous research has shown that dentin bonding agents used in combination with a eugenol-based endodontic sealer may cause problems with incomplete curing.(2;3)

Eugenol containing sealers continue to be used as endodontic cements due to their superior sealing capabilities as well as their anti-bacterial properties.(4) Recent studies have promoted the use of various solvents to negate the effects of eugenol on resin bonding. A study conducted by Tjan et al.(2) stated that resin bonded post retention was restored when irrigating the canal with ethyl alcohol after the use of eugenol containing sealers. However, the results of this study were questionable due to aggressive post space preparations. A bacterial leakage study conducted by Wolanek et al.(5) promoted neutralizing the effect of eugenol by cleansing the coronal chamber with chloroform prior to placement of the dentin bonding agent. However their study did not compare a non-eugenol sealer in its treatment groups.

Resin-based sealers have shown reliability in providing adequate seal, increased setting time, for the root canal system(6). Clinicians have advocated the use of resin-based sealers to avoid the eugenol-resin interaction(7). However, Huang showed that resin-based sealers have shown unfavorable results leading to a dose-dependent increase in genotoxicity(8). Huang also noted that of the three sealer types evaluated, the highest level of DNA damage was induced by the resin-based sealers.

Many methods have been applied to evaluate the ability of root canal temporary materials and techniques of placement to prevent coronal microleakage(9-11). A popular model is through the linear measurement of the penetration of a dye along the canal wall and the temporary material(9;10). Another popular model for examination is thru the use of a scanning electron microscope (SEM). Recently Tay(11) has described an evaluation process using a silver nitrate stain to evaluate for leakage using a SEM. By using elemental analysis during SEM evaluation, traces of (Hg) silver particles along leakage paths have been detected proving the presence of leakage.

Currently there is no treatment protocol when dentin bonding in the presence of a eugenol-based sealer. The application of ethyl alcohol or chloroform

may enhance the sealing ability of resin bonding in the presence of eugenol. The potential effect that these solvents may have remains unproven, further investigation is warranted.

Significance of the Problem

Coronal seal of the restorative material and the gutta-percha seal against the canals are both important factors in root canal therapy. The success of endodontic therapy relies in the placement of the permanent restoration. Failure to restore the tooth within a timely manner may jeopardize the outcome of endodontic treatment due to coronal microleakage of the temporization materials. The advent of dentinal bonding has enhanced the protection of the temporized endodontically treated tooth from coronal microleakage. Literature has shown that dentinal bonding in the presence of a eugenol-based endodontic sealer results in leakage and poor sealing. The application of ethyl alcohol or chloroform may enhance the sealing ability of dentin bonding agents in the presence of eugenol. The potential effect that these solvents may have remains unproven, further investigation is warranted.

Statement of the Problem

1. Does the use of a eugenol-based endodontic sealer affect the sealing ability of a dentin-bonding agent and coronal microleakage following root canal therapy?
2. Does cleansing the coronal chamber with ethyl alcohol prior to resin bonding in the presence of a eugenol-based endodontic sealer enhance the sealing ability of a dentin-bonding agent to the dentin wall?

3. Does cleansing the coronal chamber with chloroform prior to resin bonding in the presence of a eugenol-based endodontic sealer enhance the sealing ability of a dentin-bonding agent to the dentin wall?
4. Does the use of a resin-based endodontic sealer affect the sealing ability of a dentin-bonding agent and coronal microleakage following root canal therapy?
5. Does cleansing the coronal chamber with ethyl alcohol prior to resin bonding in the presence of a resin-based endodontic sealer enhance the sealing ability of a dentin-bonding agent to the dentin wall?
6. Does cleansing the coronal chamber with chloroform prior to resin bonding in the presence of a resin-based endodontic sealer enhance the sealing ability of a dentin-bonding agent to the dentin wall?

Null Hypothesis

1. There is no statistical difference in the coronal linear dye leakage in teeth sealed with a eugenol-based sealer or a resin-based sealer.
2. There is no statistical difference between using either ethyl alcohol or chloroform to cleanse the coronal chamber with ethyl alcohol prior to resin bonding.
3. There are no morphological differences in dentin when teeth are exposed to different surface treatments before and after obturation and after dentin bonding.
4. There are no morphological differences in the sealer materials after exposure to different solvent cleansers.

Definition of Terms

Chloroform – A solvent used in this investigation. Chloroform, also known as trichloromethane, is primarily used in the manufacture of fluorocarbons for refrigerants, propellants, and plastics. The remainder is used for many purposes including extracting and purifying antibiotics, as well as an industrial solvent. Chloroform is one of the most popular solvents used in endodontics, however it is tissue toxic, and a possible carcinogen.

Coronal Microleakage – A term used to describe the unwanted passage of fluids, bacteria, and solution into the chamber of a crown thru a restorative material. Coronal microleakage has been attributed to causing root canal therapy failure when suspected.

Dentin Bonding Agent – A restorative material used in a dental procedure to produce an adhesive effect between the dentin and restoration. The dentin bonding agent used in this investigation is a one-step self-etch method, also called the "all in one method," contains etching, priming, and bonding functions in a single solution.

Endodontic Sealer – A material usually used with a core material (gutta-percha) in the obturation of a root canal system. Endodontic sealers are classified into three main categories based upon their composition; zinc oxide eugenol, calcium hydroxide, and resin-based.

Ethyl Alcohol – (Ethanol) is the alcohol found in alcoholic beverages such as beer and wine. It is a useful solvent in endodontics but may be harmful by inhalation, ingestion or if absorbed through skin when used in pure form.

Eugenol – A phenolic compound (oil of cloves) used as a component of many dental materials including many endodontic sealers.

Eugenol-based endodontic sealers – A group of endodontic sealers consisting of two parts a powder (zinc oxide) and a liquid (mostly eugenol) used to produce a seal during the obturation of a root canal. Roths 801 is the eugenol-based sealer used in this investigation.

Linear coronal leakage - A qualitative description of the presence of India ink dye or silver particles apical to the resin barrier, visualized microscopically. Will be recorded as the presence or absence of leakage.

Resin-based sealer – A group of endodontic sealers consisting of two-component paste: paste root canal sealer based on epoxy-amine resin chemistry. AH Plus is the resin-based sealer used in this investigation. Paste A consists of epoxy resins and paste B consists of amines.

Scanning Electron Microscope (SEM)- device which can magnify and show a very detailed 3-dimentional black and white image of a prepared specimen, created without the presence of light waves. SEM allows for the examination of the entire surface to an object both a low and very high magnifications. The depth of focus of the SEM is reported to be 300 times greater that that of conventional microscopes, and can offer resolution of 0.2 micrometers.

Solvent – A chemical agent used to dissolve gutta-percha and endodontic sealers. The solvents used in this investigation are Chloroform and Ethyl Alcohol.

Temporary restoration - A provisional restorative material placed to prevent coronal microleakage into a canal system, ideally replaced by a final restoration, within a time frame of 24 hours to six weeks.

Assumptions

1. The prevention of coronal microleakage is an important factor in the success of endodontic therapy.
2. Eugenol will inhibit the setting of dentin bonding agents used as sealers.
3. Poor sealing of coronal restoration will result in leakage into the canal system.
4. A coronal dye leakage study is an appropriate indicator of the quality of the coronal seal.

Limitations

1. *in vivo* conditions will not be able to be identically replicated when completing an *in vitro* investigation.
2. Internal morphology, chamber and canal size will differ among all teeth in the study.
3. Dyes or silver particles are used to expose to delineate extent of leakage, no bacteria or oral fluid is being included in the study.

Delimitations

1. Only freshly extracted, caries free human maxillary and mandibular molars will be used in this study.
2. All teeth will be carefully examined to exclude any teeth with enamel of dentinal damage or defects.
3. All teeth will be stored at 100% humidity in sterile saline solution before and after canal instrumentation and obturation.

4. The instrumentation and obturation of the extracted teeth will occur on the bench top to control variables.
5. One operator will perform all instrumentation and obturation of the teeth.
6. Thermocycling is used to simulate oral challenges *in vivo.*
7. Only one dye (India Ink) will be used in the coronal leakage observation. Only sliver nitrate (25N) will be used for the SEM investigation.

Chapter 2

Literature Review

The concept of coronal microleakage as a cause for root canal failure has been evaluated since 1961 when Marshall and Massler(12) questioned the effects that the alteration of the coronal seal may have. Allison et al.(13) in 1979 referred to the possibility that a poor coronal seal might contribute to an undesirable prognosis for root canal therapy. Numerous *in vivo* and *in vitro* investigations have recently been conducted to clarify the importance of coronal microleakage. Wu et al.(14) stated that in the 1990's one in four articles published in the *Journal of Endodontics* and the *International Journal of Endodontics* related to the study of coronal microleakage. The advent of new dental materials combined with the insight of research has allowed the dental profession to adopt updated treatment standards to minimize the effect of coronal microleakage.

Coronal microleakage has been evaluated by dye penetration(9;10;15), bacteria leakage(5;16;17), pressured water(18-20), animal histological(21), and human radiographic techniques(22). The penetration of dyes continues to be the commonly used technique. Gale(23) stated that dyes used to evaluate leakage have a much smaller particle size than bacteria in the order of nanometers rather than micrometers. Gale concluded that soluble dyes might be used to simulate leakage of bacterial substrates and products. The absence of dye penetration may warrant the absence of leakage and intact seal in a canal system.

Dye penetration has been used by several methods for visualization f leakage. Many early techniques advocated the longitudinal or cross sectioning of teeth for direct visualization of the penetrated dye. Robertson (24) developed a clearing technique where evaluation of leakage may be performed without altering any tooth structure. Roda and Gutmann(25;26) later developed a technique where an intra-oral microscope may be used for visualization of dye penetration thru the tooth structure.

Recently the use of a scanning electron microscope (SEM), has aided examiners in evaluating the structure of coronal chambers(27). The SEM allows for the examination of the entire surface to an object both a low and very high magnifications. The depth of focus of the SEM is reported to be 300 times greater that that of conventional microscopes, and can offer resolution of 0.2 micrometers(28).

Scanning electron microscopes were first commercially introduced around 1965(29). The SEM shows a very detailed 3-dimentional image, created without the presence of light waves(30). The image appears in black and white unlike those of a conventional microscope. Samples are prepared differently than those of a light microscope. All specimens are carefully dehydrated to prevent distortion. The samples are then coated with a very thin layer of gold and platinum using a sputter coater. This allows the samples surface to conduct electricity with the electrons of the SEM. Once prepared the sample is placed inside the microscope’s vacuum column through an air-tight door. After the air is pumped out of the column, the electron gun emits a beam of high energy electrons. The beam is directed through a series of magnetic lenses and focused on a very fine spot. At the bottom of the SEM, a set of scanning coils directs the focused beam across the entire sample surface. Deflected or secondary electrons are knocked loose from the sample surface. The secondary electrons are picked up by a detector, counted, and sent to an amplifier. The final image is built up from the number of electrons emitted from each spot on the sample(31).

Tay(11) has effectively evaluated microleakage observed in a total-etch wet-bonding technique under different handling conditions. In this evaluation the use of silver nitrate has been used to confirm microleakage. The unique property of SEM evaluation is the detection of leakage through the elemental scanning for silver staining particles. Tay showed that the detection of silver particles confirmed the presence of microleakage though a dentin-resin interface in his experiment. It can be concluded that the application of an SEM to evaluate the leakage as well as the surface structure be a valuable part in this investigation.

Various studies using saliva have demonstrated the devastating effects of coronal microleakage. In 1987, Swanson and Madison (9)showed that artificial saliva could penetrate up to 79-85% of an obturated canal system within three days. In animal studies with monkeys, Madison and Wilcox(15) found that all sixty four canals leaked regardless of the endodontic sealers type used. Magura et al.(32) evaluated obturated canal systems exposed to human saliva *in vitro*, and reported that a root canal should be retreated if exposed to the oral environment for 3 months. Khayat(33), using human saliva, noted that bacterial leakage occurred in less than 30 days regardless of the obturation technique used. Khayat also concluded that human saliva, which contains proteins enzymes and bacterial products, provides a better model for testing leakage.

A different means of evaluating coronal microleakage is thru the penetration of bacteria and endotoxins. Torabinejad et al.(17) evaluated, *in vitro*, the leakage of root filled teeth exposed to *Staphylococcus epidermidis*. Their results indicated that over 50% of the root canals were contaminated after 19 days of exposure. Trope et al.(16) was successful in presenting that endotoxin from *Actinobacillus actinomycetemcomitans* was able to pass thru obturated, un temporized root canals within 20 days. In a similar study by Carratu et al. stated that bacteria were able to penetrate thru canal systems within 13 to 37 days however, none of their samples subjected to endotoxin leaked after 31 days. This data shows that endotoxin was not be a reliable marker to study for microleakage.

The importance of establishing and maintaining a coronal seal throughout treatment has been shown by a number of authors. Saunders and Saunders(34) proposed that coronal microleakage may occur in any of three ways: Delay in placing a coronal restoration following root canal treatment; fracture of the coronal restoration or tooth, or inadequate root filling after post space preparation. Ray and Trope(22) stated that the quality of the coronal restoration may be more important than quality of endodontic therapy in relation to periapical health of the tooth. Likewise Klevant and Eggink(35) showed that initial healing took place in teeth that had a good coronal seal without containing an obturation material in the canals. In relation to surgically treated cases Rapp et al.(36) found significantly better healing was observed with teeth that were permanently restored following surgery.

After the importance of maintaining the coronal seal throughout treatment had been displayed, various temporary materials were tested to evaluate their sealing capability. Beckham et al.(37) evaluated unfilled resin and glass ionomer cement. Samples were immersed in artificial saliva for 7 days and then placed in methylene blue dye. Beckham found that glass ionomer cement demonstrated the greatest dye penetration and was statistically different from unfilled resin. Pisano et al.(38) evaluated Cavit (ESPE Dental, Norristown, PA), Zinc Oxide Eugenol (Intermediate Restorative Material (IRM) (Dentsply, Caulk, Milford DE), and Super-EBA (reinforced zinc oxide cement base with ethoxy benzoic acid (Harry J Bosworth Co, Skokie, IL) by bacterial leakage. At the end of 90 days, the results showed that 15% of the Cavit-filled orifices leaked, whereas 35% of the IRM and Super-EBA-filled orifices leaked. In a study conducted by Bobotis et al.(20) utilitizing a fluid filtration method, Cavit, glass ionomer cement, zinc phosphate cement, polycarboxylate cement, and IRM were evaluated. The results indicated that Cavit and glass ionomer cement provided leak proof seals during the 8-wk testing period, while leakage was observed in 4 of the 10 teeth restored with zinc phosphate cement. IRM and polycarboxylate cement were the least effective of the materials tested for preventing microleakage. In an in vivo study Beach et al.(39) evaluated bacterial leakage associated with three endodontic temporary restorative materials: Cavit, Intermediate Restorative Material and TERM. Three weeks after placement of each temporary restoration, bacterial leakage was evaluated by culturing the samples from the coronal portion of the temporary both aerobically and anaerobically. Cavit did not demonstrate leakage in any of the teeth in which it was used, and provided a significantly better seal. This may be due to hydroscopic expansion of Cavit during setting.

With the advent of current dentin bonding agents many publication have been reported for uses as a secondary seal against microleakage. Leonard(40) reported a significantly better seal in both the apical and coronal directions when using the dentine bonding agent and composite resin material. Beli et al.(41) concluded that adhesive resins should be considered as a secondary seal to prevent intraorifice microleakage, and later proved that high bond strengths can be achieved between adhesive resins and the various regions of the pulp chamber(42). Wells et al.(19) evaluated placement of two dental-resin cements (Principle or C&B Metabond) within the pulp canals or on the pulpal floor of the chamber. Wells found no statistically significant differences among the materials used or the location of the resin seal.

Recently the work of Wolanek(5) evaluated the effectiveness of a dentin bonding agent, Clearfil Liner Bond 2V (Kuraray) as a barrier to prevent coronal microleakage using a bacterial model. This system consists of a dual-curing bonding agent utilizing a self-etching primer and a bonding agent, both consisting of MDP (10-methacryloyloxydecyl dihydrogen phosphate) and HEMA (2-hydroxyethyl methacrylate). The biocompatibility of this bonding system has been proven by the work of Akimoto et al.(43) Wolanek also examined the effect of a eugenol-based sealer on the sealing ability of this resin adhesive. Wolanek concluded that the presence of eugenol in the sealer had no significant effect on the sealing ability of the resin adhesive. however, Wolanek removed the sealer with chloroform prior to placement of the resin adhesive.

Eugenol (2-methoxy-4-allyphenol),a phenolic compound, is the essential constituent of clove oil. Eugenol has been shown to diminish the adhesiveness of dentin bonding agents by inhibiting the polymerization of the resin.(3) Machhi et al. (44) found that eugenol-based sealers reduced the strength of the bond or even precluded dentin bonding. Hansen(45) stated in his conclusions that ZOE should not be used in cavities if dentin-bonding agent and resin restoration is anticipated at a later date. Yap(46) reported that pre-treatment with IRM mixed at a Powder: Liquid ratio of 10g:2g significantly increased microleakage and is not recommended clinically. Schwartz et al.(47) found that there was no difference in post retention when a eugenol-based sealer was used in the canal compared to a non-eugenol-based sealer. However, aggressive post space preparation resulted in variation in the results. To resolve the discrepancy Ngoh et al. (48) evaluated regional bond strengths of C&B Metabond resin to root canal dentin located in the coronal, middle, and apical third of the root. He found that the specimens treated with the eugenol liquid had significantly lower bond strengths than those without eugenol. Another significant finding was that the radicular region of the tooth tested had no effect on bond strength.

Two techniques have provided insight into possible treatments to negate the effect of eugenol on resin bonding. Tjan et al.(2) found that irrigation with ethyl alcohol (ethanol) restored the resistance to dislodgment of the posts. Wolanek(5) revealed that the use of a chloroform prior to resin bonding resulted in no leakage of bacteria into canal systems.

The use of root canal sealers is an important factor for maintaining the integrity of the seal of root canal fillings. Many articles demonstrate the composition, sealing properties, and biocompatibility of various sealers. Sealers may be broken down into four main groups: zinc oxide eugenol, epoxy resins, glass ionomer, and calcium hydroxide based. In a recent study conducted by Tagger et al.(49) the bond strength of various sealers to dentin were evaluated. Tagger believed that the more adhesion displayed by the sealers the more sealing ability of the sealer would be demonstrated, however, the zinc oxide eugenol-based sealer (Roth 801) had so little bond it could not be investigated by the study. Tagger contributed the long-term clinical success of Roth's cement due to its cohesive strength. Wu et al.(50) stated that some sealers leak less when used in a thin layer vs. a thick one.

Resin-based sealers have proven to be display adhesive properties to dentin and gutta-percha. Suprabha et. al(51) found that resin-based sealers showed superior sealing ability, compared to all other sealer types to gutta-percha. Taylor(52) reported the best adhesion of sealer to dentin occurred with a resin-based sealer.

Resin-based sealers have been shown to have excellent anti microbial effects. Lai et.al(53) reported the excellent antimicrobial properties displayed by resin-based sealers when challenged against four facultative anaerobic species (Streptococcus mutans, Streptococcus sanguis, Escherichia coli, and Staphylococcus aureus) and four obligate anaerobic species (Porphyromonas gingivalis, Porphyromonas endodontalis, Fusobacterium nucleatum, and Prevotella intermedia).

The biocompatibility of sealers remains an important factor in endodontic sealer selection. The use of epoxy based sealers remains in controversy. Oztan(54) reported that epoxy resin-based sealer AH Plus and the silicone-based sealer RSA have similar levels of cytotoxicity. In recent articles Huang et al.(6;8) showed that epoxy resin-based sealers demonstrated both cytotoxicity and genotoxicity in vitro, however long term in vivo studies have yet to be conducted.

Various solvents have been tested in the past, however most of these solvents are toxic or otherwise hazardous. Chloroform has proven to be the most effective solvent for removal of gutta-percha and sealers(55-57), however it remains to be tissue-toxic(58) and a possible carcinogen(59). In a recent study by Schafer(60) the solubility of 8 different root canal sealers in chloroform and in eucalyptus oil was compared. Schafer concluded that chloroform was a far more effective solvent of root canal sealers than eucalyptus oil.

The removal of the smear layer appears to be an important factor in the reduction of coronal leakage when using a resin-based sealer. Taylor(52), and Saunders(61) both found the removal of the smear layer with 17% EDTA (ethylene diamine tetra acetic acid) resulted in significant less leakage. However, Gettleman (62)stated that the only significant difference with regard to the presence or absence of the smear layer was found with a resin-based sealer, which had a stronger bond when the smear layer was removed with 17% EDTA.

This study will examine the effects of two commonly used solvents (in an endodontic office) on coronal microleakage of canal systems sealed with eugenol-based and resin-based endodontic sealers. This study will establish a treatment protocol for endodontists when using resin restorations immediately following obturation of a root canal system.

Chapter 3

Material and Methods

Sample Description

Recently extracted teeth were collected and stored in sterile saline. A total of 156 maxillary or mandibular molars with minimal restorations or carious lesions present were selected. When viewed with an optical microscope, any teeth with visible fractures present or damaged during the extraction procedure were eliminated. Any teeth that contain abnormal morphology in canal structures such as c-shaped or fins present in canal anatomy were eliminated. An exemption from the Institutional Review Board for the Protection of Human Research Subjects was requested since the teeth were not identified to a patient.

Research Design

This study compared the sealing effectiveness of a dentin-bonding agent immediately placed in the coronal chamber of six experimental groups and three control groups (Table 1). A eugenol-based endodontic sealer was compared to a resin-based endodontic sealer and two solvents, chloroform and ethyl alcohol, were evaluated to compare effectiveness in chamber cleansing prior to placement of the dentin bonding agent for each sealer type. 156 maxillary and mandibular teeth were thermocycled for 580 cycles with one-minute dwell time between water baths 5^o and 55^oC. Leakage was evaluated visually after the tooth was rendered transparent using a clearing process first published by Robertson(24).

Microscopic leakage was studied with a scanning electron microscope using silver nitrate penetration as an indicator for leakage following the protocol reported by Tay et al.(27)

Table 1. India Ink Sample Description

Group	Sealer	Sealer Type	Surface Treatment	Dentin Bonding Agent	Coronal Barrier
1	Roth’s	Eugenol-based	Cotton	Brush & Bond	Flowable resin
2	Roth’s	Eugenol-based	100% Ethyl Alcohol	Brush & Bond	Flowable resin
3	Roth’s	Eugenol-based	Chloroform	Brush & Bond	Flowable resin
4	AH Plus	Resin-based	Cotton	Brush & Bond	Flowable resin
5	AH Plus	Resin-based	100% Ethyl Alcohol	Brush & Bond	Flowable resin
6	AH Plus	Resin-based	Chloroform	Brush & Bond	Flowable resin

Control Groups

Group	Sealer	Sealer Type	Surface Treatment	Dentin Bonding agent	Coronal Barrier
Positive Control	Roth’s	Eugenol-based	None	None	None
Positive Control	AH plus	Resin-based	None	None	None
Negative Control	None	None	None	Brush & Bond	Flowable resin

Instrumentation Methodology

Non-carious human maxillary and mandibular molar teeth were examined for structural integrity utilizing a surgical microscope. A total of 156 teeth were divided in to six groups each containing 25 teeth and three control groups containing 2 teeth each. Once selected by meeting all criteria above, the teeth were placed in 5.25% sodium hypochlorite (The Clorox Company, Oakland, CA) for 24 hours prior to instrumentation to dissolve any tissue from the root surfaces. Any remaining tissue was removed with a periodontal scaler. Teeth were stored at 100% humidity in sterile saline until further instrumentation.

Coronal access was completed to expose the pulpal chamber using 1958 beaver burs (Dentsply, Midwest York, PA) and Endo Z burs (Dentsply, Maillefer, Tulsa, Oklahoma) cooled with water. Occlusal surfaces were removed leaving 5mm of the crown above the CEJ. The pulp tissue was removed using Sure flex ISO endodontic hand files (Dentsply Milford, DE). The remaining apical portion of the canals were enlarged with a step-back series of #2, 3, and 4 Gates Glidden burs (Dentsply, Milford, DE) to the level of the chamber floor. The pulp chamber was treated with 5.25% sodium hypochlorite for 30 minutes, followed by 17% EDTA (Pulpdent Corporation, Watertown, MA) for 2 minutes. Each canal was dried with paper points before obturation.

Experimental Group Preparation

Experimental groups 1-3 were obturated using a eugenol-based endodontic sealer Roth Root Canal Cement (Type 801 Elite grads, Roth International, Chicago, IL) and gutta-percha from the Obtura II (Obtura Spartan, Fenton, MO). Experimental groups 4-6 were obturated using a resin-based endodontic sealer AH plus Root Canal Sealing

Material (Dentsply, Miolford DE) and gutta-percha from the Obtura II. All sealers were added to the canals with a paper point prior to placement of the gutta-percha. The heated gutta-percha was vertically compacted with Schilder pluggers (Obtura Spartan, Fenton, MO) incrementally until the canals were filled to the level of the coronal chamber. Excess gutta-percha was removed by using heated pluggers to the level of the coronal chamber of each canal.

Cleansing Methodology

Experimental Group 1 & 4

Immediately after obturation for each tooth, removal of the excess endodontic sealer from the coronal chamber was completed with a series of cotton pellets until no visible evidence of any opaque sealer remained. When a cotton pellet was inserted and removed without any visible trace of sealer, the chamber was deemed cleansed.

Experimental Group 2 & 5

Immediately after obturation for each tooth, removal of the excess eugenol-based endodontic sealer from the coronal chamber was completed using 98% ethyl alcohol. The coronal chamber was filled with 98% ethyl alcohol to the brim. A series of cotton pellets were used to clean the chamber until no visible evidence of any ethyl alcohol or opaque sealer remained. When a cotton pellet was inserted and removed without containing any visible trace of sealer or ethyl alcohol, the chamber was deemed cleansed.

Experimental Group 3 & 6

Immediately after obturation for each tooth, removal of the excess endodontic sealer from the coronal chamber was completed using chloroform. The coronal chamber was filled with chloroform to the brim. A series of cotton pellets were used to clean the chamber until no visible evidence of any chloroform or opaque sealer remained. At this point, once a cotton pellet was inserted and removed without containing any visible evidence of sealer or chloroform, the chamber was deemed cleansed.

Bonding Methodology

Once cleansing was completed, the chamber was ready to receive the Brush & Bond dentin bonding agent (Parkell, Farmingdale, NY) One drop of bonding liquid was mixed with the primed mixing tip of the brush and bond system for ten seconds. The dentin bonding agent was applied throughout the coronal chamber and then allowed to set for ten seconds. The treated surface of the chamber was lightly air dried for five seconds to not allow any of the dentin bonding agent to pool. The dentin bonding agent was then light-cured for 20 seconds. The application a flowable composite resin Natural Elegance (Henry Schein, Melville, NY) was placed on the chamber floor to an average depth of 1.5mm then light cured for 30 seconds.

Following placement of the resin barrier, the apical portion of each root was removed leaving 6 mm of the root structure apical to the chamber floor. A 2mm deep retro-preparation was placed into each root tip and filled with a dual cured glass ionomer Fuji II Lc (GC America, Alsip, IL).   The teeth were stored for 72 hours at 100% humidity, 37degrees C, to allow the sealers to set up.

Controls

Positive controls for the experiment consisted of 4 teeth. Two teeth were obturated with gutta-percha sealed with eugenol-based sealer, and two with resin-based sealer. The teeth were not covered with a coronal barrier.

Negative controls for the experiment consisted of 2 teeth. Both teeth were obturated without the presence of sealer. The coronal barrier consisted of a dentin bonding agent and flowable composite placed over the coronal chamber for each tooth.

Thermocycling Methodology

The six experimental groups plus the three control groups were thermocycled for 24 hours (580 cycles) in deionized water using the thermocycling unit. Temperature ranged between 5^o and 55^o Celsius between the two baths. Each cycle consisted of submerging the samples for 30 seconds in the 55^o Celsius thermal bath, then submerging them into the 5^o bath with ice for 30 seconds, with a traveling time of 20 seconds.

Visual Leakage Detection Methodology

The teeth were air dried and coated with two applications of nail polish including the apical portion of the teeth so that only the coronal chamber was left. The teeth were removed, air dried and placed into Higgins India Ink (Sanford, Bellwood, IL) for 48 hours. The teeth were then rinsed with tap water, and the nail polish was removed by placing each tooth in acetone for 20 minutes and scrubbed with a tooth brush, to allow for the clearing chemicals to contact the tooth surface. The teeth were demineralized by placing them in 5% nitric acid for 48 hours. The teeth were then rinsed with tap water and dehydrated with 80% ethyl alcohol for 24 hours, followed by 24 hours in 100% ethyl alcohol. Clearing of the roots was accomplished by placing them into methyl salicylate, oil of wintergreen (VWR Scientific West Chester, PA) for a period of 48 hours. Each tooth was assigned an identification number for data collection.

Two endodontic residents independently evaluated leakage using a 10X magnification intraoral microscope. Each tooth was evaluated for dye leakage passing through the coronal barrier in to the level of the gutta-percha. The presence or absence of leakage was recorded for each tooth as compared to the control groups and used for statistical analysis.

SEM Methodology

Part I

A total of five teeth from each group were selected for Scanning Electron Microscope evaluation (SEM). The technique for tooth preparation and sectioning for the SEM evaluation was described by Tay et al(27). The five teeth from each group were air dried and covered with two coats of nail varnish to ensure no leakage from any portion of the radicular system. The teeth were then placed into a 50 wt% silver nitrate aqueous solution for 2 hours in total darkness(63). Following retrieval they were rinsed in distilled water and placed into a photo-developing solution and exposed under a fluorescent light for 6 hours. This was to ensure that the silver ion reduction would be complete. Following removal the teeth were immersed in acetone for 20 minutes to dissolve the layer of nail varnish. The teeth were demineralized by placing them in 5% nitric acid for 48 hours. The teeth were then rinsed with tap water and dehydrated with 80% ethyl alcohol for 24 hours, followed by 24 hours in 100% ethyl alcohol. Clearing of the roots was accomplished by placing them into methyl salicylate, oil of wintergreen, for a period of 48 hours. All teeth were air dried and embedded in a epoxy resin to allow for sectioning. Three plano-paralled buccal-lingual sections were obtained from each tooth with copious irrigation by a diamond blade microsectioner (Buehler, Lake Bluff, IL). Samples were air dried for 48 hours prior to evaluation under the SEM. The Samples were then coated with a gold platinum layer using a SPI=Module Carbon Coater (SPI, West Chester, PA). The prepared samples were then evaluated using a Joel JSM-6400 Scanning Electron Microscope (Joel, Tokyo, Japan).

Detection of silver nitrate was completed by performing a backscatter elemental analysis for silver particles in the evaluation of each specimen. Once the silver particles were detected visually within the samples, confirmation by the elemental analysis was completed. A print out of the elemental components of the scanned surface was recorded, and a photograph of the scanned area was made.

Part II

A total of twelve teeth from the experimental group were selected for coronal chamber surface examination using a SEM. The twelve teeth were divided into the same treatment groups as in the India ink section of the evaluation. All teeth were accessed with 1958 beaver burs (Dentsply, Midwest York, PA) and Endo Z burs (Dentsply, Maillefer, Tulsa, Oklahoma). The teeth were sectioned in half in a plano-parallel buccal-lingual cut, to reveal the coronal chamber, using a diamond blade microsectioner (Buehler, Lake Bluff, IL). Teeth were irrigated with sodium hypochlorite for a total of thirty minutes and exposed to 17% EDTA for two minutes. The coronal chambers and coronal portion of the canals were obturated with gutta-percha and sealer and cleansed immediately with wither cotton, ethyl alcohol, or chloroform (Table 2). Samples were air dried for 48 hours prior to evaluation under the SEM.

The Samples were then coated with a gold platinum layer using a SPI=Module Carbon Coater (West Chester, PA). The prepared samples were then evaluated using a Joel JSM-6400 Scanning Electron Microscope (Tokyo, Japan).

Table 2. SEM Sample Description

Group	Sealer	Sealer Type	Surface Treatment
1	Roth's	Eugenol-Based	Cotton
2	Roth's	Eugenol-Based	100% Ethyl Alcohol
3	Roth's	Eugenol-Based	Chloroform
4	AH Plus	Resin-Based	Cotton
5	AH Plus	Resin-Based	100% Ethyl Alcohol
6	AH Plus	Resin-Based	Chloroform
Control	Roth's	Eugenol-based	None
Control	AH Plus	Resin-based	None
Control	None	None	Gutta-percha

Statistical Analysis

Logistic linear analysis detected a significant difference between the sealer type and the cleanser at the level of p= 0.296 (Appendix B) warranted further examination using a Pearson’s chi square. The six experimental groups were analyzed using Pearson’s Chi square.

Equipment and Materials

- 90 extracted human maxillary of mandibular molar teeth.
- Standard School of Dentistry Endodontic Set Up
- Gates Glidden Burs (Dentsply, Milford, DE)
- Sure flex ISO endodontic hand files (Dentsply Milford, DE)
- RC prep (Premier, Norristown, NJ)
- Endodontic Irrigation Syringe (Monoject, Sherwood Medical , St. Louis, MO)
- Obtura II warm vertical obturation system (Obtura Spartan, Fenton, MO)
- Obtura II gutta-percha (Obtura Spartan, Fenton, MO)
- AH plus Root Canal Sealing Material (Dentsply, Milford DE)
- Roth Root Canal Cement (Type 801 Elite grads, Roth International, Chicago, IL)
- Cavit (ESPE, Germany)
- Fixation Solution, a 10 % neutral buffered Formalin Solution (Hydrol Chemical Company, Yeadon, PA)
- Nitric Acid (VWR Scientific Products, West Chester, PA)
- Ethyl Alcohol (Fisher Scientific, Fair Lawn, NJ)
- Higgins India Ink (Sanford, Bellwood, IL)
- Methyl Salicylate (JT Baker, Philipsburg, NJ)
- Zeiss Intraoral Microscope (Carl Zeiss international, Oberkochen Germany)
- Boley Gauge (William Dixon, Carlstadt, NJ)
- Paper points (Dentsply, Milford, DE)
- 17% EDTA (Pulpdent Corporation, Watertown, MA)
- 5.25% NaOCl (The Clorox Company, Oakland, CA)
- Salivart Synthetic Saliva, Aqueous Solution (Gebauer Company**,** Cleveland, OH)
- Thermocycling Unit (West Virginia University, School of Dentistry)
- SPI=Module Carbon Coater (West Chester, PA)
- Joel JSM-6400 Scanning Electron Microscope (Tokyo, Japan).
- Flowable Composite Natural Elegance, (Henry Schein, Melville NY)
- Brush & Bond dentin bonding agent (Parkell, Farmingdale, NY)
- Fuji II Lc (GC America, Alsip, IL).
- Diamond blade microsectioner (Buehler, Lake Bluff, IL).
- 1958 beaver burs (Dentsply, Midwest York, PA)
- Endo Z burs (Dentsply, Maillefer, Tulsa, Oklahoma).

Chapter 4

Results and Discussion

Results

The results of this study supported that there is statistical difference in the coronal linear dye leakage among the treatment groups. Therefore, we will reject the null hypothesis. The results of the control teeth supported the research design. The positive controls showed complete dye penetration and the negative controls showed no dye penetration. Table 3 and Figure 1 display the results for all of the groups combined. As seen the teeth in group 1 leaked more than any other group. Group 2 was the best group with only four of its samples that displayed leakage.

Table 3. India Ink Coronal Microleakage

Experimental Group	Treatment	Teeth Showing Microleakage	Total
1	Roth	17	20
2	Roth & EtOH	4	20
3	Roth & Chloroform	7	20
4	AH Plus	9	20
5	AH Plus & EtOH	6	20
6	AH Plus & Chloroform	8	20

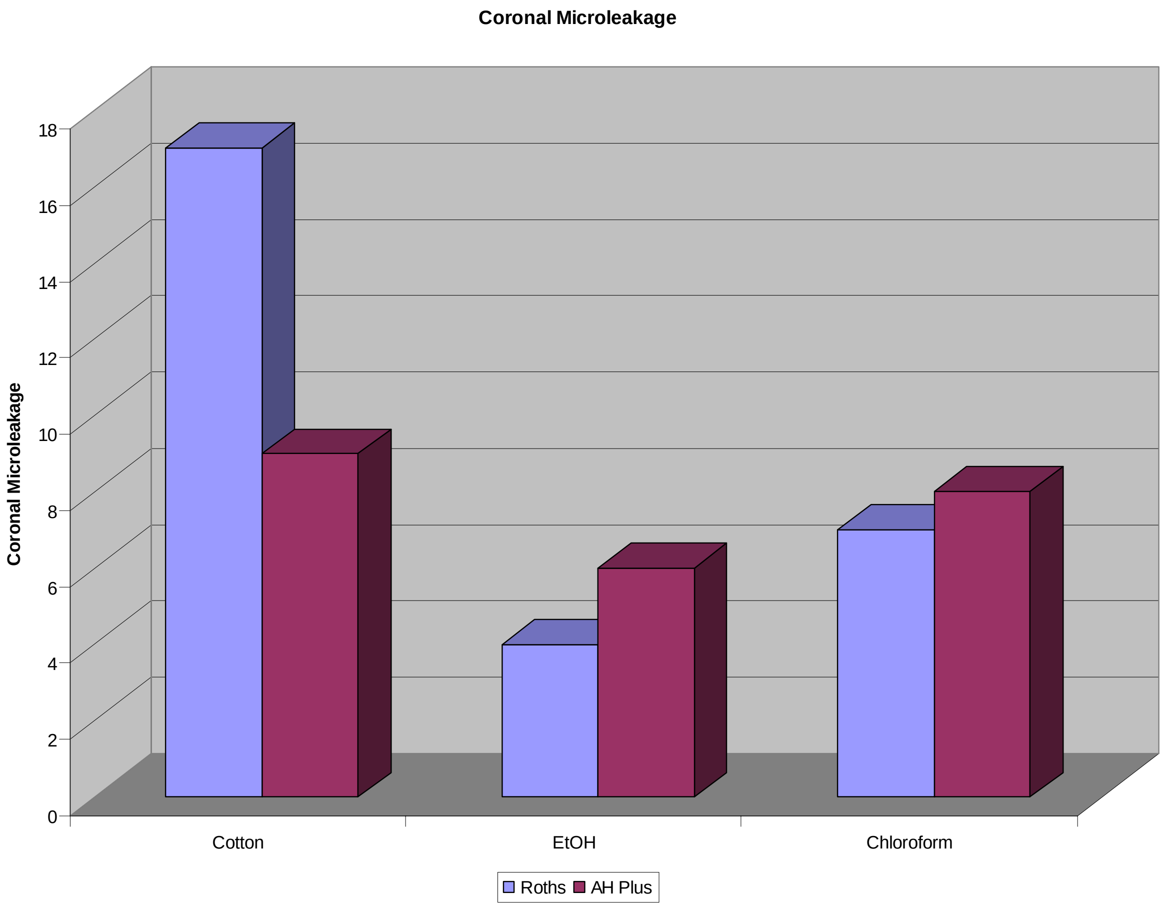


Figure 1. India Ink Coronal Microleakage

Eugenol-based Sealer (Groups 1-3)

- Table 4 Provides the number of samples which showed coronal microleakage that occurred comparing the groups treated with a eugenol-based endodontic sealer. Group 1 displayed the most leakage with nearly all of the samples, n=17 out of 20 showed the presence of leakage. In contrast Group 2 had only 4 out of 20 and Group 3 had 7 out of 20 leaked. A statistical difference exists in comparing these three groups at a level of $p < 0.0001$. (Pearson chi square)

- A significant difference was found when comparing group 1 (cotton) and group 2 (ethyl alcohol) $p< .0001$ (Pearson chi square)
- A significant difference was found in comparing group 1 (cotton) and group 3 (chloroform). Pearson chi square of $p < 0.0001$
- Table 4 and Figure 2 compare the cleansing agent that provided the best result between Group 2 and Group 3. In Group 2 (ethyl alcohol) only 4 of 20 samples leaked, showed better cleansing capabilities than Group 3 (chloroform) where 7 of 20 samples leaked. Pearson chi square found no significant difference with a value of $p = 0.2881$.

Table 4. Coronal Microleakage of Eugenol-Based Sealers

Eugenol-based sealer

Experimental Group	Treatment	Teeth Showing Microleakage	Total
1	Roth	17	20
2	Roth & EtOH	4	20
3	Roth & Chloroform	7	20

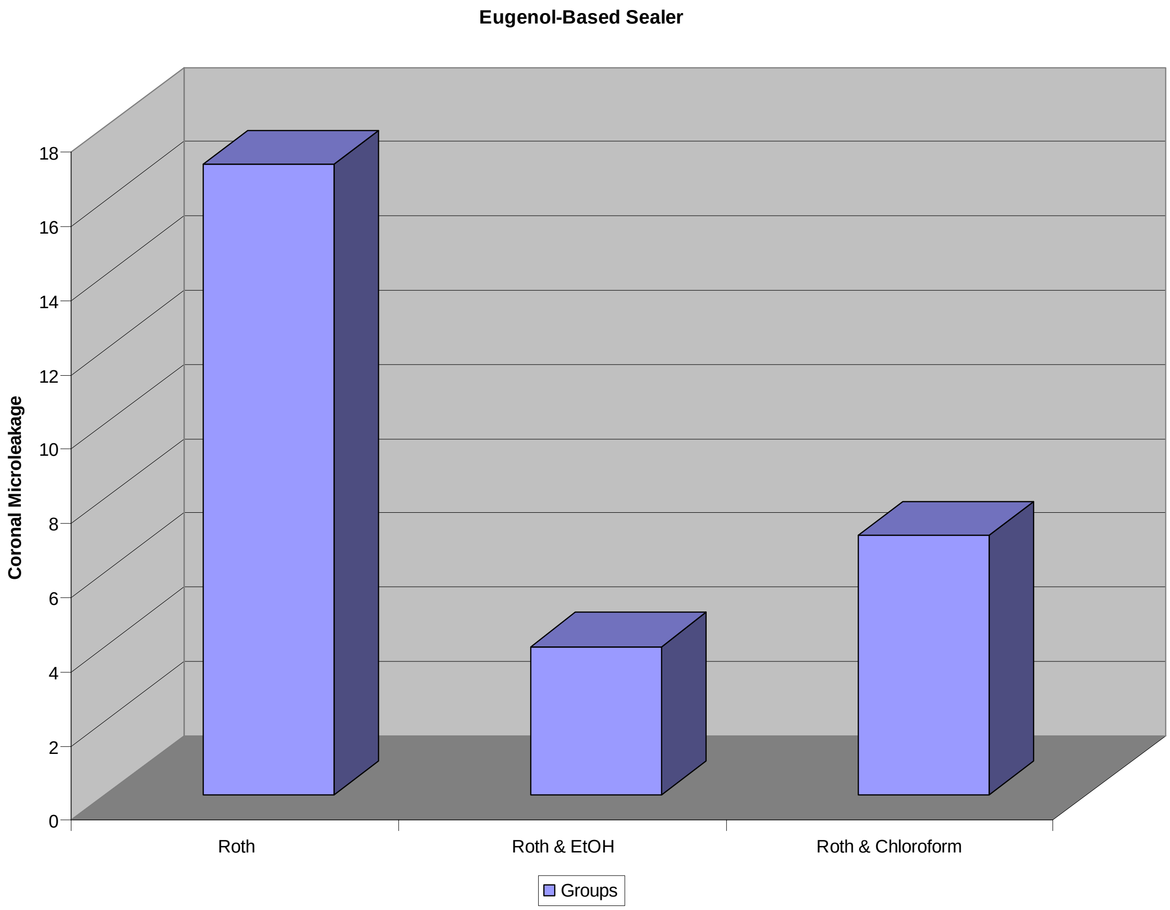


Figure 2. Coronal Microleakage Eugenol-Based Sealers

Resin-based Sealer (Groups 4-6)

- Table 5 provides the number of samples that showed coronal microleakage that occurred comparing the groups treated with a resin-based endodontic sealer. Group 4, 5, and 6 each had approximately equal numbers of samples that leaked. There was no statistical difference found between any of the resin-based treated Groups, (Pearson chi square p = .4526). Figure 3 shows that the cleansing agents did not contribute to the number of teeth that leaked in groups 4, 5, and 6.

- There was no significant difference between groups 4 and Groups 5. (Pearson chi square p = 0.3272).
- There was no significant difference between groups 4 and groups 6. (Pearson chi square p = 0.7491).
- There was no significant difference found between group 5 and group 6. (Pearson chi square p = 0.5073).

Table 5. Coronal Microleakage Resin-Based Sealers

Resin-Based Sealer

Experimental Group	Treatment	Teeth Showing Microleakage	Total
4	AH Plus	9	20
5	AH Plus & EtOH	6	20
6	AH Plus & Chloroform	8	20

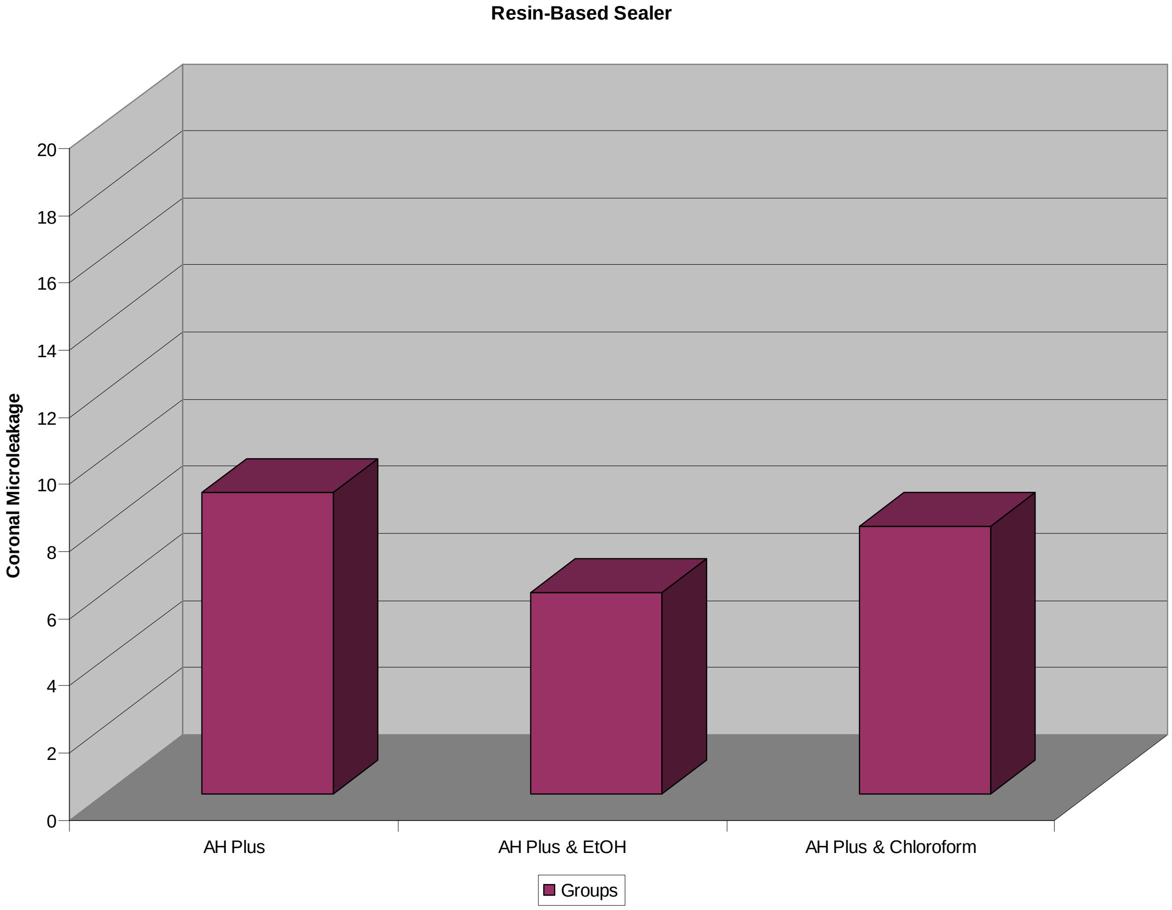


Figure 3. Coronal Microleakage Resin-Based Sealers

Eugenol-based vs. Resin-based

Group 1 vs. Group 4 (Cotton)

- Table 6 and figure 4 provide the number of tooth in each group that displayed leakage when cleaned with cotton. In Group1, the eugenol-based endodontic sealer, 17 of 20 displayed leakage. In Group 4, the resin-based endodontic sealer, only 9 of 20 displayed leakage. This difference was found to be significant at p= 0.0067 (Pearson chi square)

Table 6. Coronal Microleakage Eugenol vs. Resin-Based

Eugenol vs Resin

Experimental Group	Treatment	Teeth Showing Microleakage	Total
1	Roth	17	20
4	AH Plus	9	20

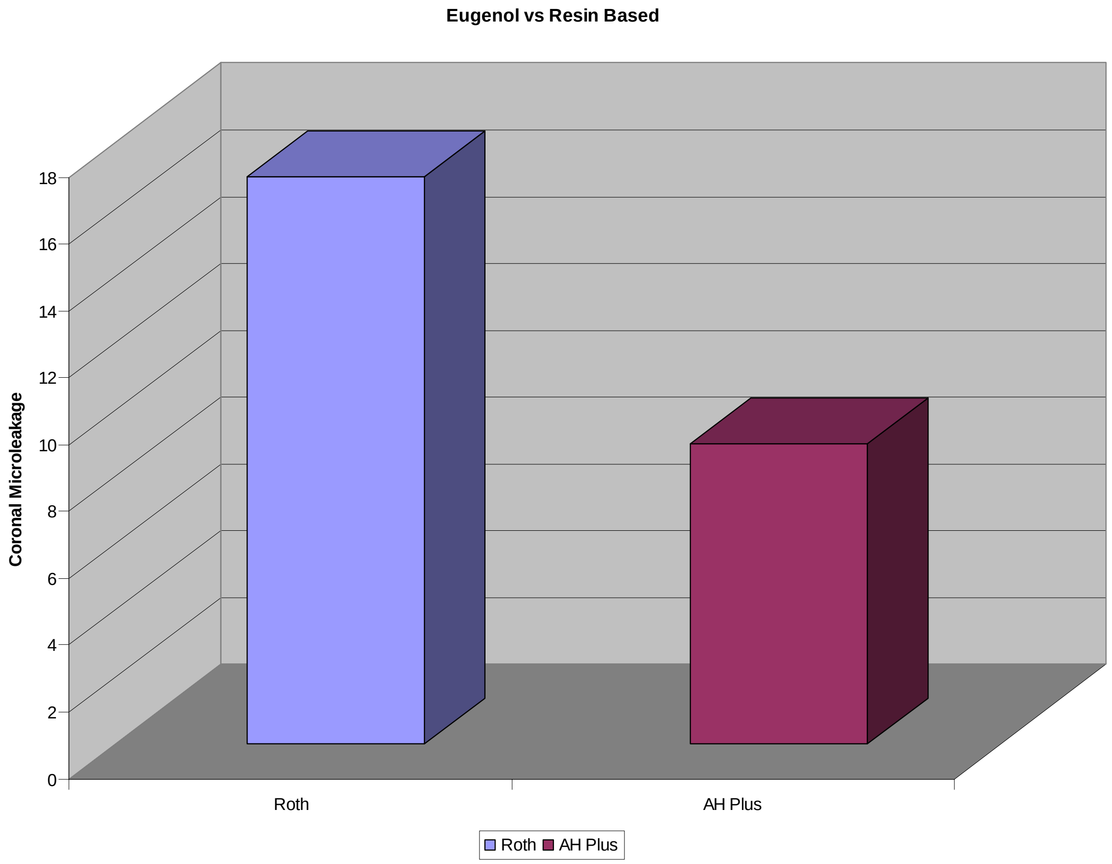


Figure 4. Coronal Microleakage Eugenol vs. Resin-Based

Ethyl Alcohol Eugenol-based vs. Resin-based

Group 2 vs. Group 5 (Ethyl Alcohol)

- Table 7 provides the number of tooth in each group that displayed leakage when cleaned with ethyl alcohol. As seen in figure 5, no significant difference, p = 0.4652 (Pearson chi square) exists between the two groups.

Table 7. Ethyl Alcohol Eugenol-based vs. Resin-based

Ethyl Alcohol

Experimental Group	Treatment	Teeth Showing Microleakage	Total
2	Roth & EtOH	4	20
5	AH Plus & EtOH	6	20

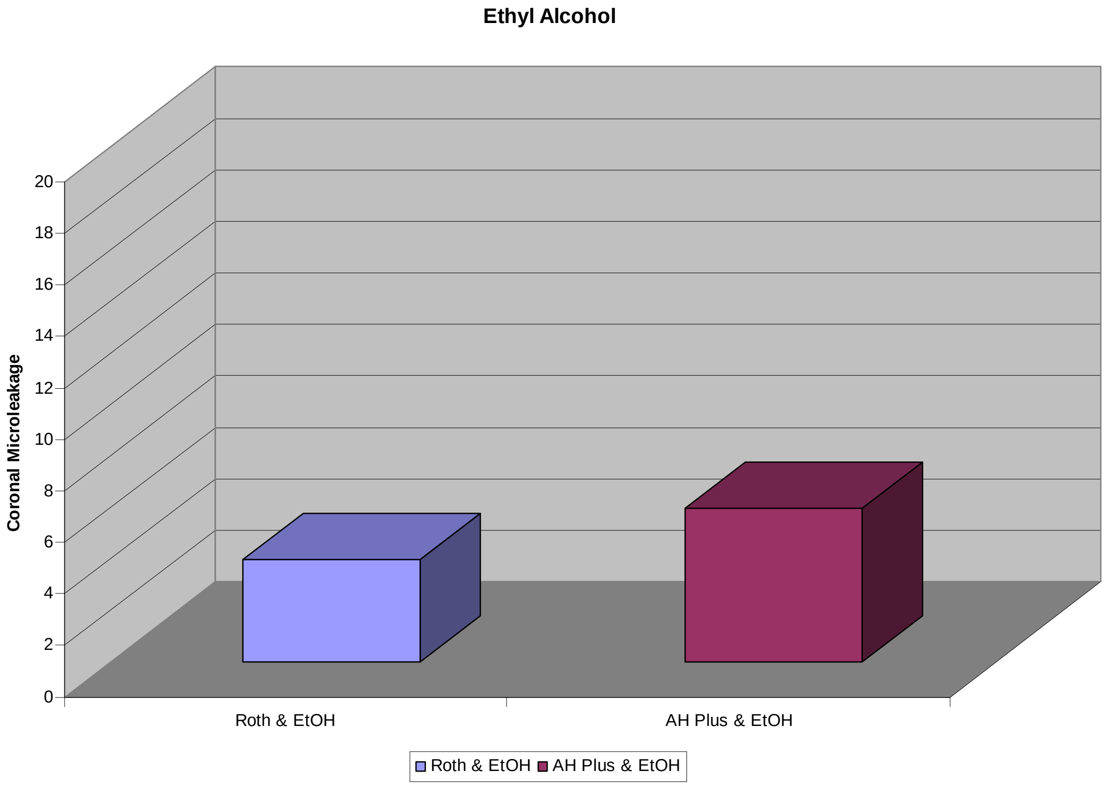


Figure 5. Ethyl Alcohol Eugenol-based vs. Resin-based

Chloroform Eugenol-based vs. Resin-based

Group 3 vs. Group 6 (Chloroform)

- Table 8 provides the number of tooth in each group that displayed leakage when cleaned with chloroform. As seen in figure 6, no significant difference p = .7440 (Pearson chi square), exists between the two groups.

Table 8. Chloroform Eugenol-based vs. Resin-based

Chloroform

Experimental Group	Treatment	Teeth Showing Microleakage	Total
3	Roth & Chloroform	7	20
6	AH Plus & Chloroform	8	20

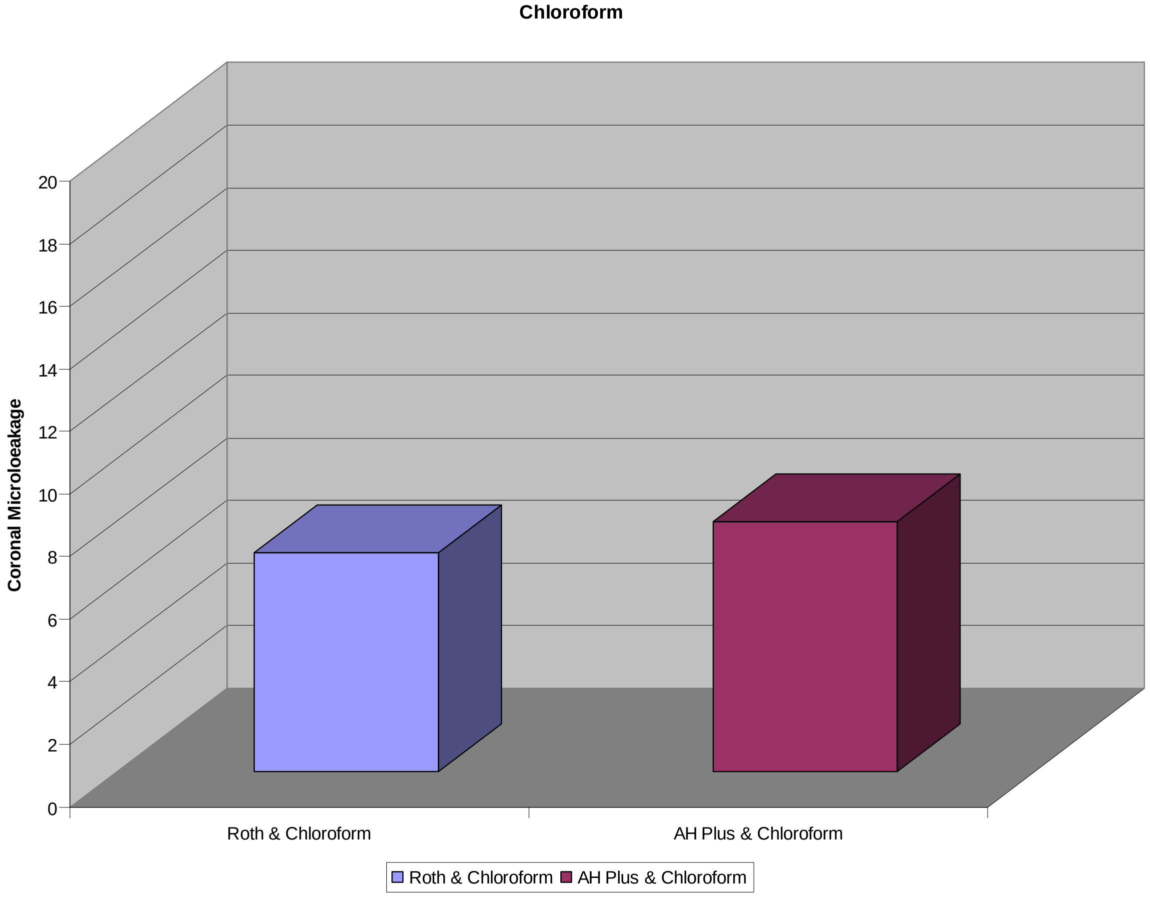


Figure 6. Chloroform Eugenol-based vs. Resin-based

SEM Part 1

Figure 7 shows results taken from Group 1. This sample showed the most leakage present through the coronal chamber. Detection of silver with elemental analysis was confirmed thru leakage paths along the resin-dentin interface (figure 8) and along the gutta-percha-dentin interface (figure 9). The results indicate a positive leakage path for the sample taken for group 1.

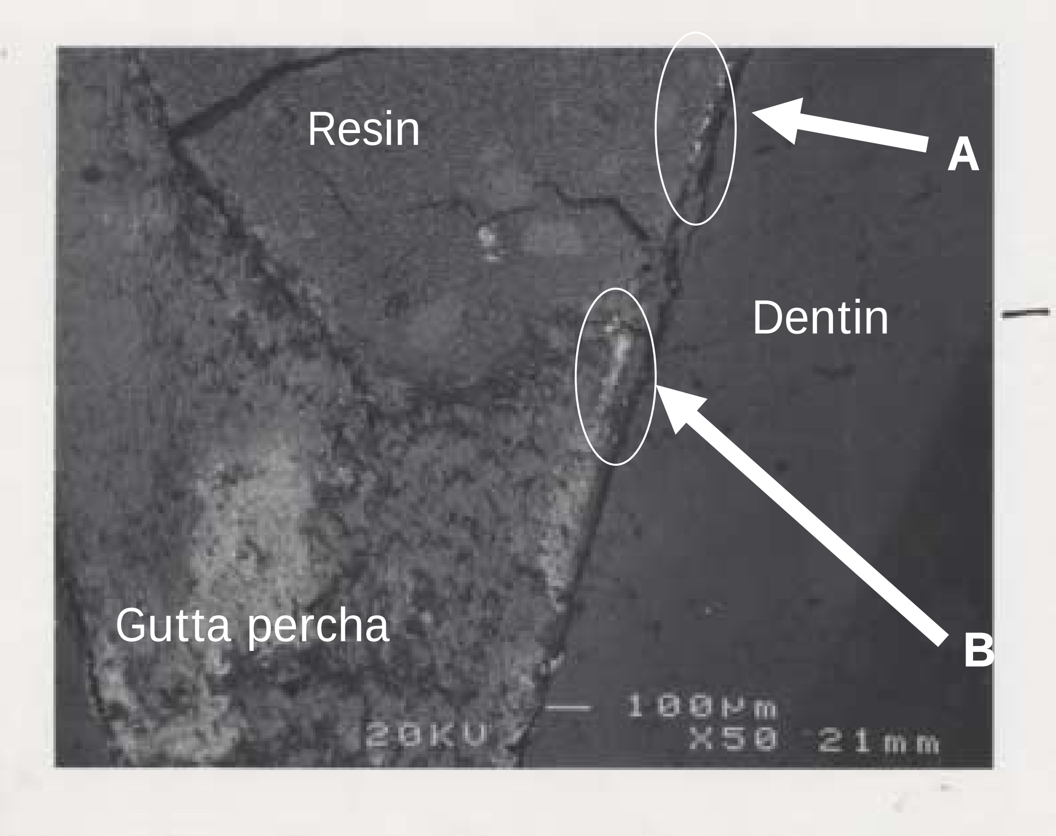
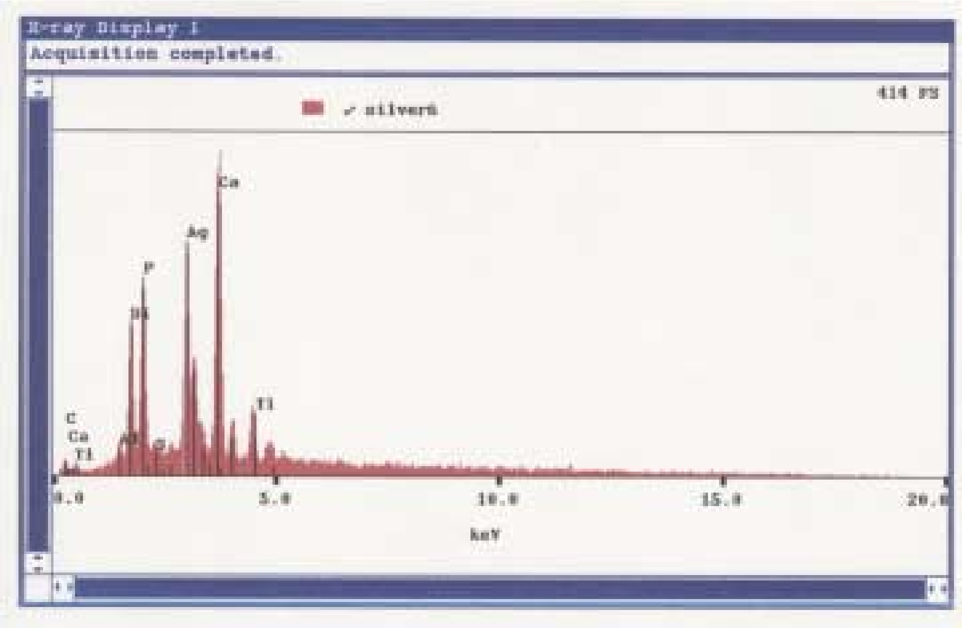


Figure 7. Group 1 Leakage



Acquisition completed.

Figure 8. Figure A

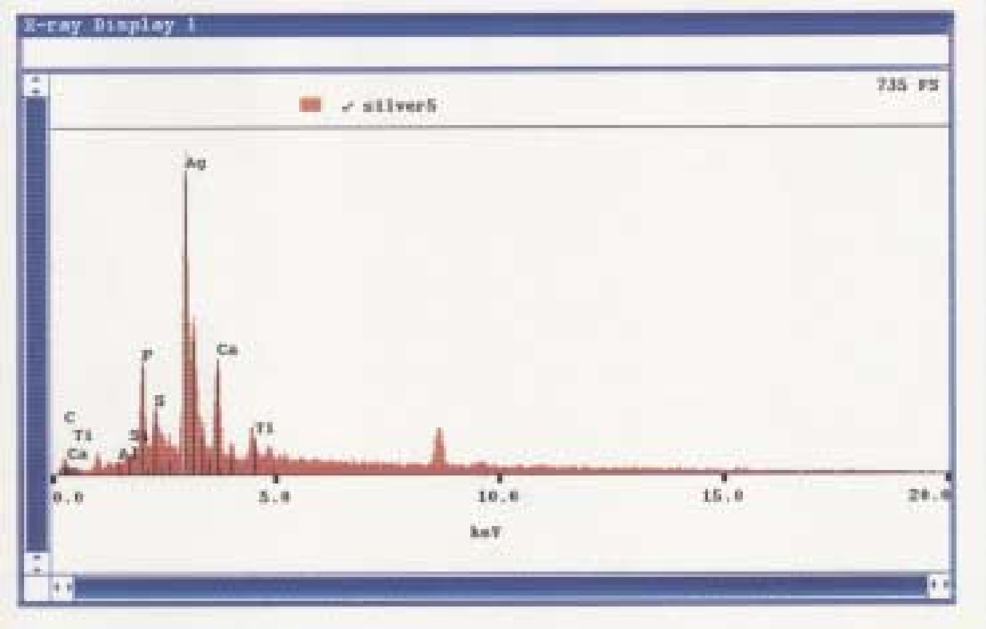


Figure 9. Figure B

Figure 10 shows the results taken from group 6. This sample showed the least amount of leakage present through the coronal chamber. Elemental analysis resulted in the detection of silica particle fillers uniformly scattered along the entire bonding surface.

Figure 10. Group 6 No Leakage

SEM Part 2

Figure 11 depicts the uniform dentinal surface of the control group. This group was treated with sodium hypochlorite for 30 minutes. As seen the smear layer around dentinal tubules remains partially cleansed with sodium hypochlorite.

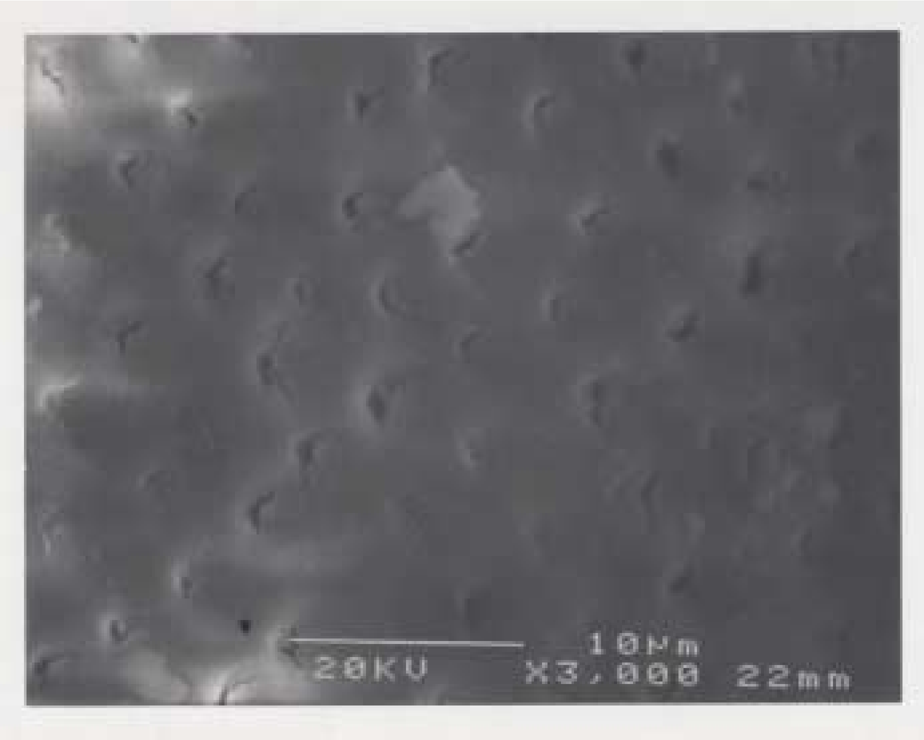


Figure 11. 5.25% Sodium Hypochlorite

Figure 12 depicts the uniform dentinal surface of the second control group. This group was treated with 5.25% sodium hypochlorite for 30 minutes followed by 17% EDTA for 2 minutes. As seen the smear layer has partially been removed. This sample is viewed at looking slightly parallel to the chamber floor.

Figure 12. 5.25% Sodium Hypochlorite + 17% EDTA

Figure 13 depicts the dentinal surface completely covered by gutta-percha. This sample was taken from the chamber floor.

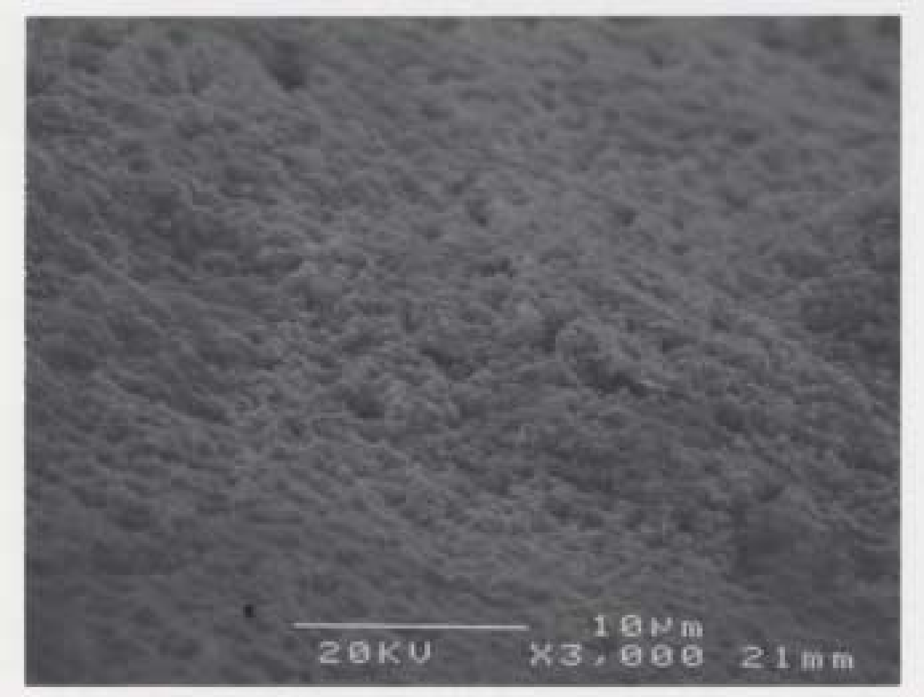


Figure 13. Gutta - Percha

Figure 14 depicts the dentinal surface completely covered by the eugenol-based sealer (Roths). The eugenol-based sealer contains many filler particles primarily composed of zinc oxide.

Figure 14. Eugenol-Based Sealer (Roths)

Figure 15 depicts the dentinal surface completely covered by the resin-based sealer (AH Plus sealer). As seen many filler particles of the sealer are present. The sealer consists of epoxy resins, zirconium oxide and silica.

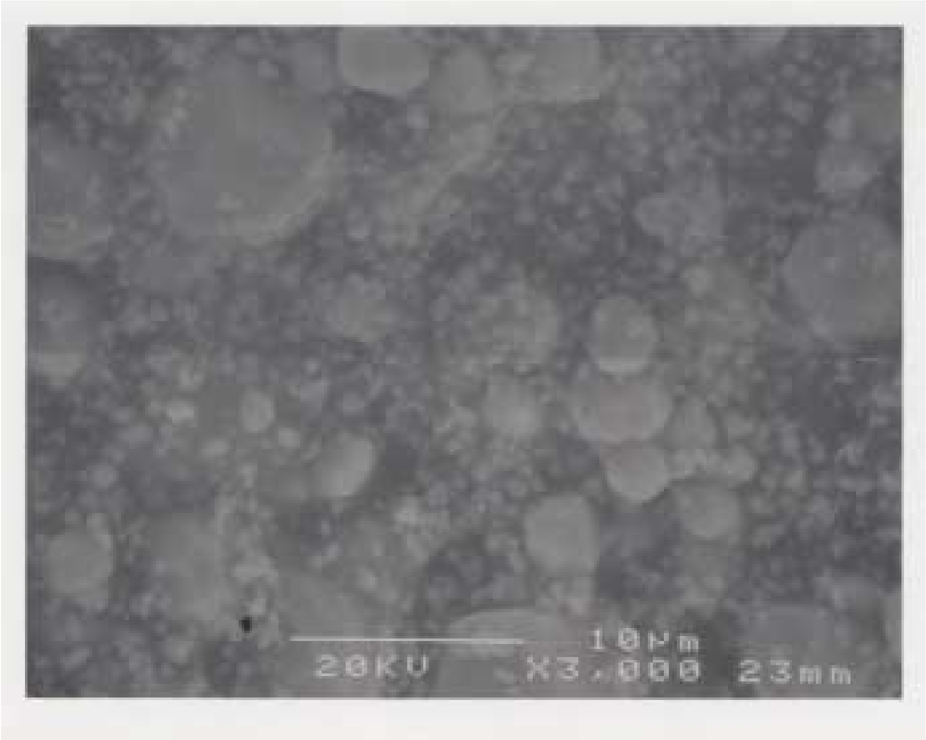


Figure 15. Resin-Rased Sealer (AH Plus sealer)

Figure 16 depicts the dentinal surface of group 1 treated with the eugenol-based sealer cleansed with cotton. A smear layer has covered the dentinal tubules and sealer particles remain in the viewed surface.

Figure 16. Group 1 Treated With the Eugenol-Based Sealer Cleansed With Cotton

Figure 17 depicts the dentinal surface of group 2 treated with the eugenol-based sealer cleansed with ethyl alcohol. The dentinal surface appears more cleansed, however residual sealer particles remain imbedded inside the dentinal tubules.

Figure 17. Group 2 Treated With the Eugenol-Based Sealer Cleansed With Ethyl Alcohol

Figure 18 depicts the dentinal surface of group 3 treated with the eugenol-based sealer cleansed with chloroform. A consistent smear layer remains covering the entire dentinal surface. This layer may consist of gutta-percha particles left over form the cleansing process. The dentinal tubules look dramatically different from the other groups.

Figure 18. Group 3 Treated With the Eugenol-Based Sealer Cleansed With Chloroform

Figure 19 depicts the dentinal surface of group 4 treated with the resin-based sealer cleansed with cotton. As seen the sealer particles remain along the dentinal surface. No dentinal tubules appear cleansed in the sample surface.

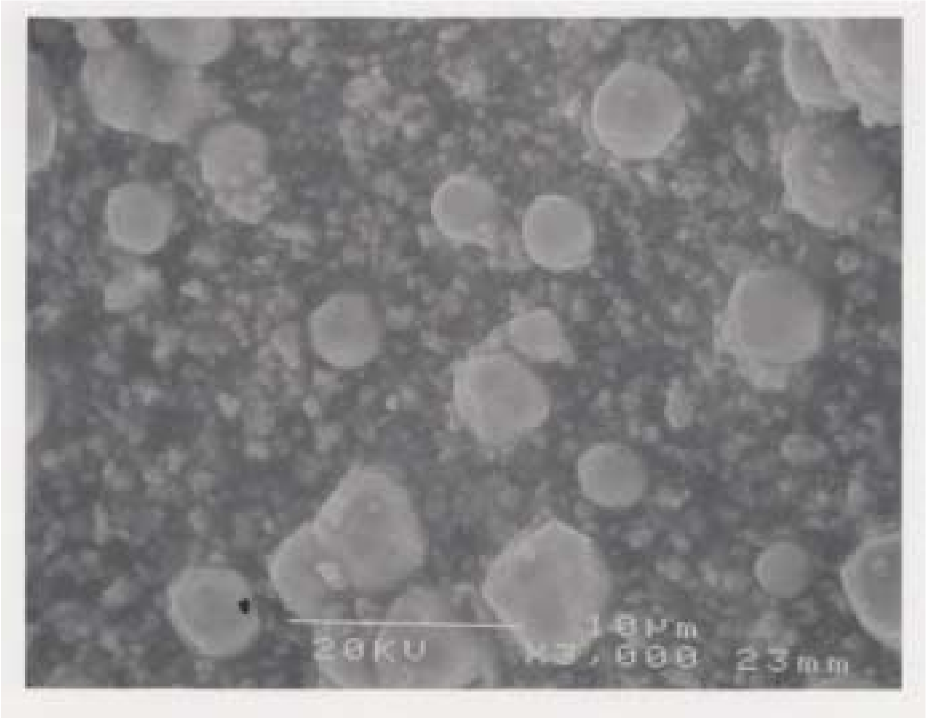


Figure 19. Group 4 Treated With the Resin-Based Sealer Cleansed With Cotton

Figure 20 depicts the dentinal surface of group 5 treated with the resin-based sealer cleansed with ethyl alcohol. Although particles in the sealer do not dissolve in ethyl alcohol, the surface appears cleansed. The dentinal tubules do appear to have some resin particles that remain.

Figure 20. Group 5 Treated With the Resin-Based Sealer Cleansed With Ethyl Alcohol

Figure 21 depicts the dentinal surface of group 6 treated with the resin-based sealer cleansed with chloroform. The presence of a smear layer possible consisting of a mixture of resin-based sealer and gutta-percha particles remains. A similar smear layer appeared in group 3 which also used chloroform to cleanse the chamber.

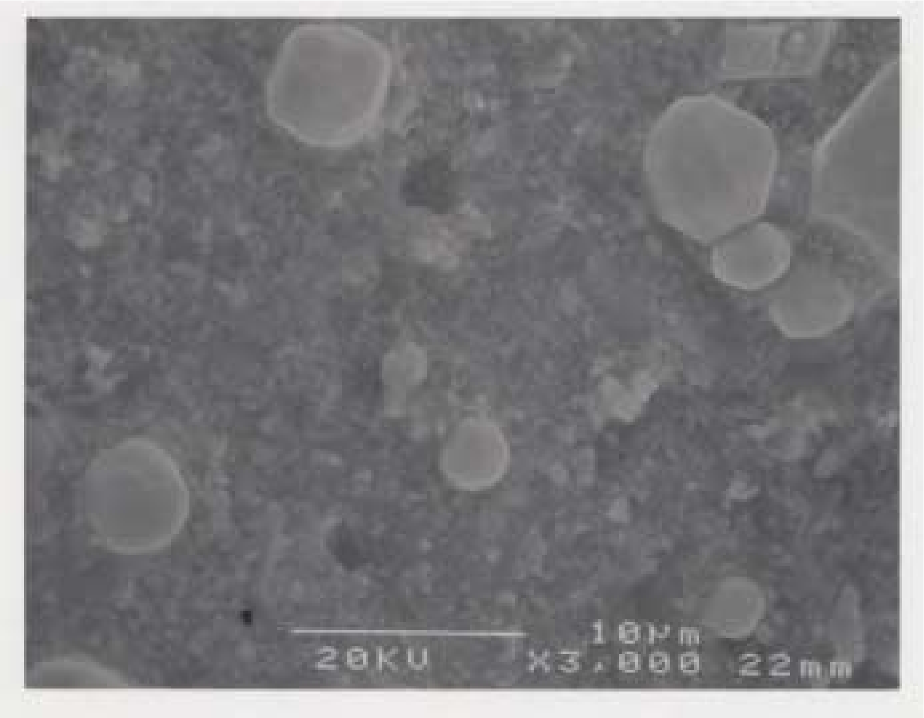


Figure 21. Group 6 Treated With the Resin-Based Sealer Cleansed With Chloroform

Discussion

Successful endodontic therapy consists of three major components: removal of irritants from the root canal system, obturation of the cleaned and shaped system, and prevention of future contamination of the sealed root canal system(1). Removal of irritants from the root canal system is completed through effective mechanical instrumentation using files, combined with chemical irrigation of sodium hypochlorite. Once the removal of irritants is completed, obturation of the canal system can be completed.

A variety of techniques and materials may be used to successfully obturate a canal system. The combination of gutta-percha with an endodontic sealer is traditionally used to complete the obturation process. The use of endodontic sealers ensures the complete seal of the canal with the gutta-percha. The composition of the endodontic sealers differ in the chemical make up from either a eugenol, resin, or calcium hydroxide. The selection of the sealer type will vary in different situations, and is ultimately left to the discretion of the clinician.

Once obturation of the tooth is completed, an interim restoration is placed to prevent future contamination of the sealed root canal system. The interim restoration will remain until placement of a permanent restoration. If a delay in the placement of the permanent restoration occurs, leakage of oral contaminants into the finished root canal system will require the re-treatment of the root canal system. This outcome is unfortunate, and will frustrate both the clinician and the patient.

To avoid this unfavorable complication, the immediate placement of a resin barrier under the interim restoration in the coronal chambers has been advocated. It is widely recognized that the chemical composition of eugenol-based endodontic sealers inhibit dentin bonding. As seen in the results of this study, the type of sealer used will affect the adhesive ability of the dentin-bonding agent to bond to dentin. To solve this dilemma various researchers have recommended alternative treatment techniques to enhance resin bond after obturation. In order to investigate this problem, two solvents, ethyl alcohol and chloroform, were used to clean the coronal chamber dentin surface prior to dentin bonding. No research exists today compares the effect of two different cleansers on the two sealers. The results of this study suggest that not only the type of sealer used, but also the surface treatment of the coronal chamber prior to resin bonding play a role in the outcome of dentin seal.

India Ink

The use of India Ink as a dye in this investigation to detect for coronal microleakage proved to be a satisfactory way of examining the differences between the six treatment groups and the controls. This finding agrees with that of Swanson, Madison and Wilcox.(9;10). However, according to the research of Wu(14), The reliability of these results is questionable. Wu also concluded that evaluation of through the use of dye penetration techniques might give little relevant information. Despite the findings of Wu, the results of this study seemed to present a challenge that adequately tested the concept of coronal microleakage. Until other methods are presented and proven to effectively evaluate coronal microleakage, dye penetration studies will continue to be used.

Comparing the six treatment groups, the eugenol-based sealer in combination with the cotton cleansed chamber did the worse with nearly all of the samples in the group displaying leakage. This finding states that the immediate dentinal bonding to a eugenol-contaminated field will result in a breakdown of the bond. This finding disproves the misconception that pretreatment acid etching and rinsing will remove the contaminated layer, allowing dentin bonding to occur. It is imperative that the use of a cleanser be used prior to dentin bonding with the use of an eugenol-based sealer.

The bonding agent used during the investigation is a sixth generation self-etching primer. The old process of a three-step process has been re-invented to include the combination of the etchant mixed with the primer/bonding agent. This new development offers a simple one step application of the chemically activated solution followed by a 10 second light cure. The self etch-primer bonding system was chosen for this investigation due to the ease of placement and being less technique sensitive. The control groups provided the verification of the validity of the product prior to the start of the treatment groups. The use of positive and negative control groups will also be able to isolate the effect of leakage from the coronal sealing resin and canal sealers.

One drawback that was noted in the use of this bonding technique is the absence of the additional rinsing steps found in traditional multi-step techniques. The application of a separate etchant with rinsing may cause more of the eugenol material to be removed, enhancing the strength of the bond. An investigation into this possibility is currently underway and will provide a unique insight to the effect of different generations of dentin bonding agents have on successful bonding in a eugenol contaminated field.

The addition of a chemical cleanser has proven to be beneficial to obtaining a successful dentin bond. Both ethyl alcohol and chloroform prove to be effective in the removal of the eugenol contamination as seen in the dye portion of the investigation. This result agrees with the findings of Tjan(2) and Wolanek(5). However, the results of this investigation noted that ethyl alcohol slightly outperformed chloroform for removal of the eugenol-based sealer. This result proved to be weak and further investigation may be needed with an increased sample size to confirm this finding.

During the chamber cleansing process it was noted that the chloroform produced a pink smear layer of partially dissolved gutta-percha and sealer. The brief placement of chloroform into the chamber seemed to dissolve the gutta-percha. This finding agrees with the results of Schafer(60), who proved the aggressive nature of using chloroform to remove eugenol-based sealers in the retreatment of endodontically treated teeth. The cotton pellets used through the chamber changed in color form darker to lighter pink with each subsequent swipe. Although the chambers appeared visually clear at the end of the cleansing process, remains of the gutta-percha smear may have occluded dentin tubules resulting in slightly more leakage. This is seen in the SEM figures for Group 3 and 6, where visibly more particles remain blocking dentin tubules.

The differences in the resin-based sealer group proved to be less significant the than that of the eugenol-based group in respect to their leakage potential. As the results indicate, a similar number of teeth displayed leakage in each of the three groups. The dentinal bonding was not effected by significantly by the presence of contaminant particles from the resin-based sealer. The ethyl alcohol cleansed chamber had less number of teeth displaying leakage compared to that of the chloroform, however, the difference was not statistically significant.

Ethyl alcohol and chloroform seemed to not be as effective in clearing the resin-based sealers from the coronal chambers. Slightly more numbers of resin-based sealer groups leaked more than the eugenol-based sealer groups. However the numbers were not found to be statistically different.

The time required for both sealers to complete set may have influenced the results noted between the two groups. The setting time through polymerization of the resin-based sealer is much faster, in the order of minutes, compared to that of several hours for the eugenol-based sealer. Surface cleansing was performed immediately, more of the resin sealer was setting compared to that of the eugenol sealer.

A significant difference was found when comparing groups 1 and 4, the eugenol and the resin-based sealer types without the use of a solvent. The eugenol- based sealer did significantly worse in leaking that that of the resin-based sealers. It can be concluded that the use of a solvent when using a eugenol-based sealer is essential prior to bonding. When using a resin-based sealer it may not be as important to consider the use of a solvent, since small percentages of unreactive monomers will be left on the surface after polymerization.

The behavior of groups 1 and 4 reaffirmed the findings that Tjan and Nemetz(2) found, which outlined the interaction of resin bonding in the presences of a eugenol-containing sealer. Tjan and Nemetz concluded that the phenolic compound of eugenol does inhibit polymerization of the resinous restorative materials. Their conclusions also stated that residual eugenol should be removed whenever an endodontic sealer is used and a resin restoration is anticipated.

Tjan and Nemetz recommended the use of ethyl alcohol for the removal of the eugenol-based endodontic sealer. Eugenol is fully soluble in ethyl alcohol, and only sparingly soluble in water. In addition alcohol spreads readily over the entire dentinal surface because of excellent wetting properties according to Van de Ryke(64).

When comparing the performance between the two sealer types when cleaned with ethyl alcohol, although not statistically significant, the best results were displayed when clearing the eugenol-based sealer. No significant difference was observed when no surface treatment was used compared to solvent cleansed surface. The results of this portion are also similar to the work of Tjan and Nemetz, who found that ethyl alcohol consistently removed the residual eugenol sealer from post space preparations that were contaminated with a eugenol-based sealer.

The selection for the use of chloroform was guided by the work of Wolanek et al.(5) Although potentially carcinogenic, and extremely tissue-toxic, chloroform is the most common solvent used in endodontics. Chloroform proved to be efficient in the removal of eugenol-containing sealers immediately prior to bonding with a similar sixth generation dentin bonding system.

When comparing the performance between the two sealer types when cleaned with chloroform, no significant difference was found in regards to leakage. The results in this investigation would agree with that of Wilcox(65), who found that chloroform did not have marked solvent action on a resin-based sealer.

The lack of interaction between chloroform and a resin-based sealer may be explained through the work Schafer and Zandbiglari(60). They concluded that resin-based endodontic sealers were completely soluble in chloroform after a period of 10 minutes. The contact time of the chloroform and the two sealers in our investigation was 1 minute. No visible damage should be detected, perhaps a statistically significant result would have occurred if the chloroform had an extended contact time with the resin-based sealer. Future investigation with longer contact time may be warranted.

SEM Part 1

Confirmation of leakage through SEM was proven to be an effective tool. The detection of silver particles using elemental analysis was shown in figure 7. Coronal microleakage passed from the dentin-resin barrier and extended into the gutta-percha-dentin barrier. In backscattering technique, silver particles appear to be bright dots clustered along the dentin-restorative and dentin gutta-percha interface. Both clusters of silver were analyzed and detection was verified.

The second sample for elemental analysis was selected to show the absence of leakage. The selection came from group 6 (resin-based sealer and chloroform). As seen in figure 10, similar dots do exist passing through the dentin-resin barrier, into the gutta-percha. The dots are more dispersed. Elemental analysis indicates the presence for silica. The dots seemed to have a granular appearance and it may be from the fillers form the sealer or flowable. A cluster of charging is noted in the sample in the resin layer. This sample confirms the absence of leakage through elemental analysis.

SEM Part 2

Figure 11 depicts the uniform dentinal surface of the control group. This group was treated with sodium hypochlorite for 30 minutes. As seen in the figure the smear layer has occluded all of the dentinal tubules to some degree. Figure 12 depicts the uniform dentinal surface of the second control group. This group was treated with 5.25% sodium hypochlorite for 30 minutes followed by 17% EDTA for 2 minutes. The use of

EDTA to remove the smear layer has been proven effective. Compared to figure 10, the opening of tubules is evident.

Figure 13 depicts the dentinal surface completely covered by gutta-percha. Mainly uniform in appearance, this sample was taken from the chamber floor. Cleansers affect the gutta-percha in different ways. Ethyl alcohol appeared to be more passive and not interact with the gutta-percha during the cleansing process. Chloroform had the opposite effect during the cleansing effect and caused the gutta-percha to begin to dissolve.

Figure 14 depicts the dentinal surface completely covered by the eugenol-based sealer (Roth's). This sealer is composed of mixing a powder and liquid. Many small particles are present within the sealer. The liquid component of the sealer is eugenol. The powder is primarily composed of zinc oxide. The zinc oxide particles seem to make up the granular component of the sealer. However, the granular structure of the eugenol-based sealer is significantly smaller in size. The eugenol-based sealer appeared to react better to the cleansing solutions as compared to the resin-based, however when comparing the results, no statistical difference was noted.

Figure 15 depicts the dentinal surface completely covered by the resin-based sealer (AH Plus sealer). The sealer is composed of a paste-paste combination. Larger particles are noted with the resin-based sealer. The primary content of the sealer is epoxy resins, zirconium oxide, and silica.

Figure 16 depicts the dentinal surface of group 1 treated with the eugenol-based sealer cleansed with cotton. As seen in the sample a large amount of sealer debris remains on the surface of the tooth. Many of the dentin tubules appear occluded with the remains of the sealer.

Figure 17 depicts the dentinal surface of group 2 treated with the eugenol-based sealer cleansed with ethyl alcohol. Many of the dentin tubules are visible. Although many of the tubules still have some sealer components present, more dentinal surface is exposed to allow enhanced dentinal bonding.

Figure 18 depicts the dentinal surface of group 3 treated with the eugenol-based sealer cleansed with chloroform. Although much of the sealer layer has been removed, the dentinal surface now appears to be covered by gutta-percha remnants. This could be due the dissolution property that the chloroform had on gutta-percha. Although bonding was not statistically affected by the use of chloroform over ethyl alcohol, slightly more leakage occurred with chloroform group.

Figure 19 depicts the dentinal surface of group 4 treated with the resin-based sealer cleansed with cotton. Although no dentinal tubules visibility appear in the sample, dentin bonding was not effected by the presence of the resin-based sealer. The cotton cleansed surface does appear to reveal some open dentin tubules for mechanical and chemical retention in bonding.

Figure 20 depicts the dentinal surface of group 5 treated with the resin-based sealer cleansed with ethyl alcohol. As seen in the sample, dentin tubules have reappeared and are readily able to participate in dentin bonding. Some residual particles remain, however the lack of eugenol in the resin-based sealer may enhance the bonding capabilities. Many of the larger particles are gone when comparing the treated surface.

This may be due to the large particle size being more susceptible to being mechanically removed.

Figure 21 depicts the dentinal surface of group 6 treated with the resin-based sealer cleansed with chloroform. As seen in the sample, very few dentin tubules are visibly present and a residual layer of gutta-percha blanket over the dentinal surface. Some residual resin-based sealer particles remain present. Despite the visibly contaminated surface, dentinal bonding was not significantly affected.

The sample teeth in this study were chosen to replicate the variables often seen in clinical situations. The samples included both human maxillary and mandibular molars. This was done to provide a group of teeth, which reflected different chamber and canal sizes to that which we treat in our daily practices. However this variable may also affect the consistency and reliability of the results obtained. A much larger sample size may be needed.

Clinically, it seems logical to conclude from this investigation that

- The use of a cleansing agent, either ethyl alcohol or chloroform, in the presence of a eugenol containing endodontic sealer prior to immediate dentin bonding is paramount.
- The selection of ethyl alcohol might outperform that of chloroform, in removing the unwanted eugenol compound.
- When using a resin-based endodontic sealer cleansing of the coronal chamber may not be necessary.

Further investigations may be necessary to evaluate the *in vivo* success rates of the results obtained.

Chapter 5

Summary and Conclusion

Summary

The prevention of coronal microleakage through temporary after completion of a root canal procedure is an important factor contributing to the success of endodontic therapy. The advent of current bonding techniques allow for a secondary resin bonded seal to be placed under a temporary to protect against coronal microleakage. Eugenol-based endodontic sealers have been shown to inhibit polymerization of immediately placed resin bonding. The propose of this study was to evaluate the cleansing capabilities of two solvents ethyl alcohol and chloroform, prior to dentin bonding in the coronal chambers of endodontically treated teeth obturated with either a eugenol or resin-based sealer.

It was hypothesized that there is no difference in the leakage between the two sealer types used. In addition it was hypothesized that there is no difference in the solvent used to clean the coronal chamber prior to resin bonding.

A total of 156 extracted human teeth were prepared for root canal therapy. The teeth were divided into six groups and three control groups. The six groups were obturated as follow: 1) eugenol-based sealer with cotton, 2) eugenol-based sealer with ethyl alcohol, 3) eugenol-based sealer with chloroform, 4) resin-based sealer with cotton, 5) resin-based sealer with ethyl alcohol, 6) resin-based sealer with chloroform. Application of a sixth generation dentin-bonding agent followed by placement of a 2mm layer of flowable composite was placed immediately after obturation. The teeth were thermocycled for for 24 hours (580 cycles) in deionized water. All teeth were then exposed to India ink for a period of 48 hours. The teeth were then cleared and leakage was measured. The presence of leakage was recorded and statistically compared for each group. SEM analysis was utilized to confirm leakage through elemental analysis. Samples from each group were visually evaluated for surface configuration. The results of the study supported to reject the null hypothesis for each case.

Conclusions

This *in vitro* study supports: 1) the use of a cleansing agent, either ethyl alcohol or chloroform, in the presence of a eugenol containing endodontic sealer prior to immediate dentin bonding is paramount. 2) The selection of ethyl alcohol might outperform that of chloroform, in removing the unwanted eugenol compound. 3) When using a resin-based endodontic sealer cleansing of the coronal chamber may not be necessary. Further investigation may be necessary to evaluate the *in vivo* success rates of the results obtained.

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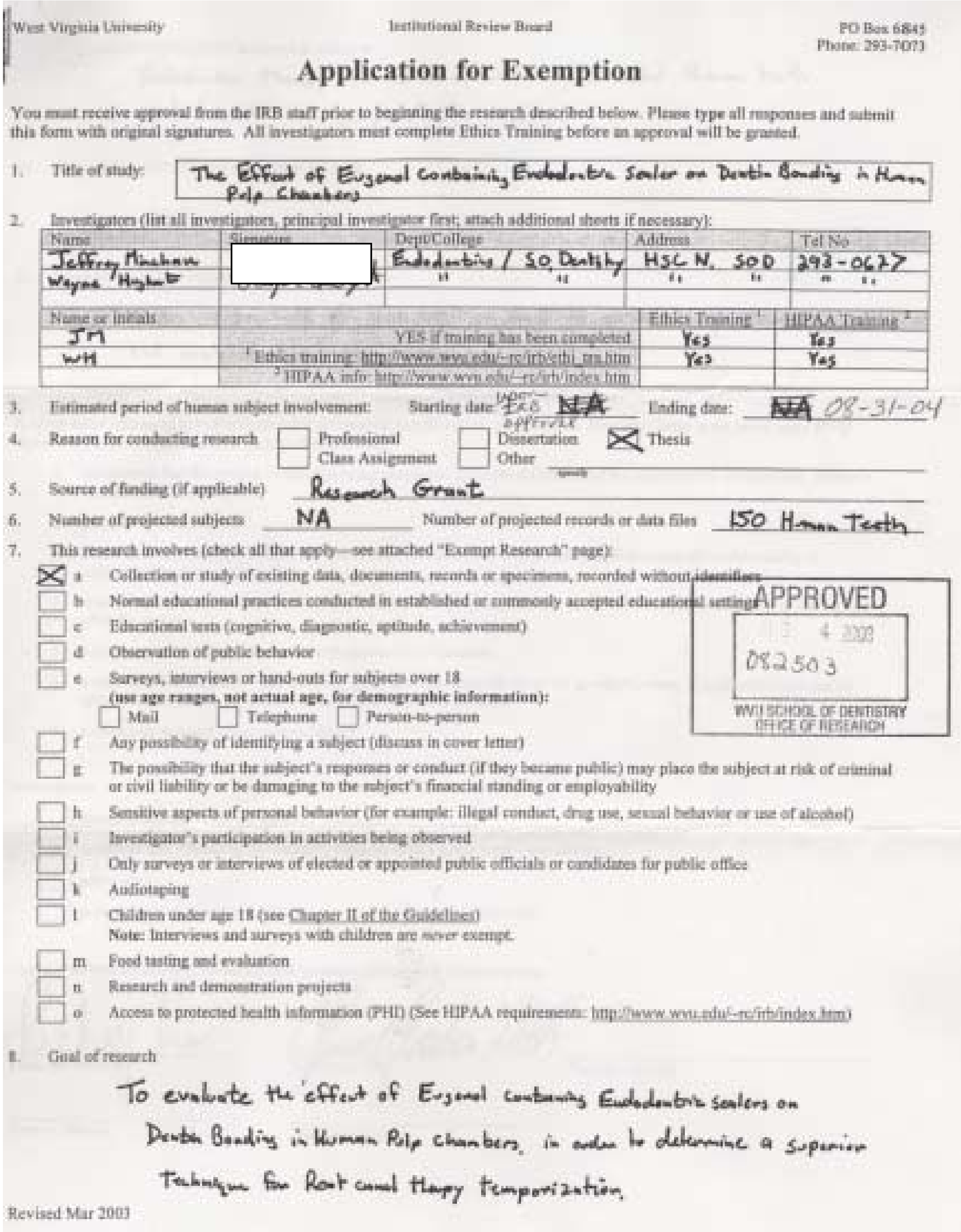
Appendix A



West Virginia University Institutional Review Board PO Box 6845
Phone: 293-7073

Application for Exemption

You must receive approval from the IRB staff prior to beginning the research described below. Please type all responses and submit this form with original signatures. All investigators must complete Ethics Training before an approval will be granted.

1. Title of study: The Effect of Eugenol Containing Endodontic Sealer on Dentin Bonding in Human Pulp Chambers

2. Investigators (list all investigators, principal investigator first; attach additional sheets if necessary):

Name	Signature	Dept/College	Address	Tel No
Jeffrey Mischan	[redacted]	Endodontics / SO Dentistry	HSC N. 500	293-0627
Wayne Hughes		" "	" "	" "

Name or initials		Ethics Training [1]	HIPAA Training [2]
JM	YES if training has been completed	Yes	Yes
WH	[1] Ethics training: http://www.wvu.edu/~rc/irb/ethi_tra.htm	Yes	Yes
	[2] HIPAA info: http://www.wvu.edu/~rc/irb/index.htm		

3. Estimated period of human subject involvement: Starting date: upon IRB approval ~~NA~~ Ending date: ~~NA~~ 08-31-04

4. Reason for conducting research ☐ Professional ☐ Class Assignment ☐ Dissertation ☐ Other ______ (specify) ☒ Thesis

5. Source of funding (if applicable) Research Grant

6. Number of projected subjects NA Number of projected records or data files 150 Human Teeth

7. This research involves (check all that apply—see attached "Exempt Research" page):

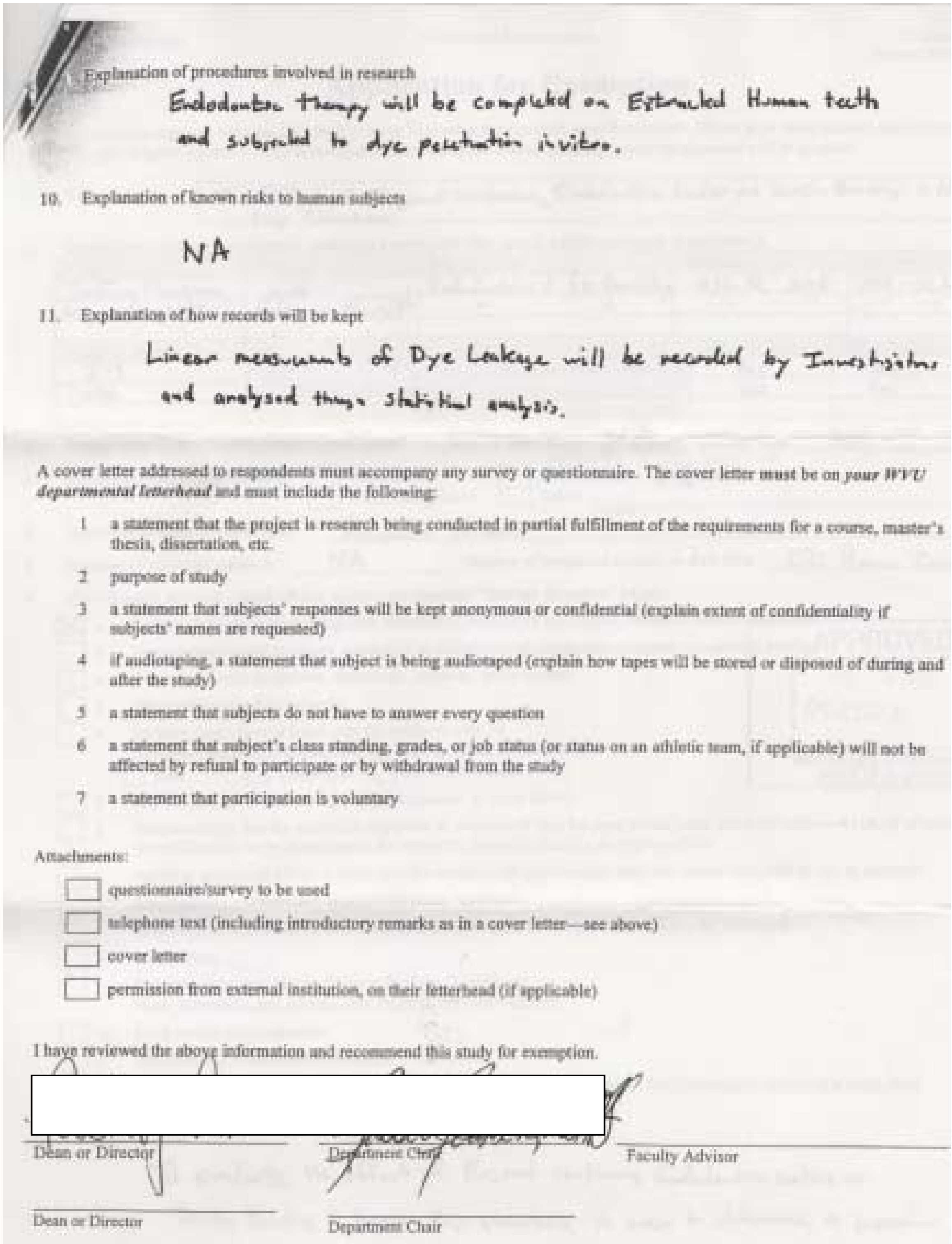
- ☒ a Collection or study of existing data, documents, records or specimens, recorded without identifiers
- ☐ b Normal educational practices conducted in established or commonly accepted educational settings
- ☐ c Educational tests (cognitive, diagnostic, aptitude, achievement)
- ☐ d Observation of public behavior
- ☐ e Surveys, interviews or hand-outs for subjects over 18 **(use age ranges, not actual age, for demographic information):**
  - ☐ Mail ☐ Telephone ☐ Person-to-person
- ☐ f Any possibility of identifying a subject (discuss in cover letter)
- ☐ g The possibility that the subject's responses or conduct (if they became public) may place the subject at risk of criminal or civil liability or be damaging to the subject's financial standing or employability
- ☐ h Sensitive aspects of personal behavior (for example: illegal conduct, drug use, sexual behavior or use of alcohol)
- ☐ i Investigator's participation in activities being observed
- ☐ j Only surveys or interviews of elected or appointed public officials or candidates for public office
- ☐ k Audiotaping
- ☐ l Children under age 18 (see Chapter II of the Guidelines)
  Note: Interviews and surveys with children are *never* exempt.
- ☐ m Food tasting and evaluation
- ☐ n Research and demonstration projects
- ☐ o Access to protected health information (PHI) (See HIPAA requirements: http://www.wvu.edu/~rc/irb/index.htm)

APPROVED
[illegible] 4 2003
082503
WVU SCHOOL OF DENTISTRY
OFFICE OF RESEARCH

8. Goal of research

To evaluate the effect of Eugenol containing Endodontic sealers on Dentin Bonding in Human Pulp chambers, in order to determine a superior Technique for Root canal therapy temporization.

Revised Mar 2003



Explanation of procedures involved in research

Endodontic therapy will be completed on Extracted Human teeth and subjected to dye penetration invitro.

10. Explanation of known risks to human subjects

NA

11. Explanation of how records will be kept

Linear measurements of Dye Leakage will be recorded by Investigators and analysed through statistical analysis.

A cover letter addressed to respondents must accompany any survey or questionnaire. The cover letter **must** be on ***your WVU departmental letterhead*** and must include the following:

1. a statement that the project is research being conducted in partial fulfillment of the requirements for a course, master's thesis, dissertation, etc.
2. purpose of study
3. a statement that subjects' responses will be kept anonymous or confidential (explain extent of confidentiality if subjects' names are requested)
4. if audiotaping, a statement that subject is being audiotaped (explain how tapes will be stored or disposed of during and after the study)
5. a statement that subjects do not have to answer every question
6. a statement that subject's class standing, grades, or job status (or status on an athletic team, if applicable) will not be affected by refusal to participate or by withdrawal from the study
7. a statement that participation is voluntary

Attachments:

☐ questionnaire/survey to be used

☐ telephone text (including introductory remarks as in a cover letter—see above)

☐ cover letter

☐ permission from external institution, on their letterhead (if applicable)

I have reviewed the above information and recommend this study for exemption.

[redacted]

Dean or Director

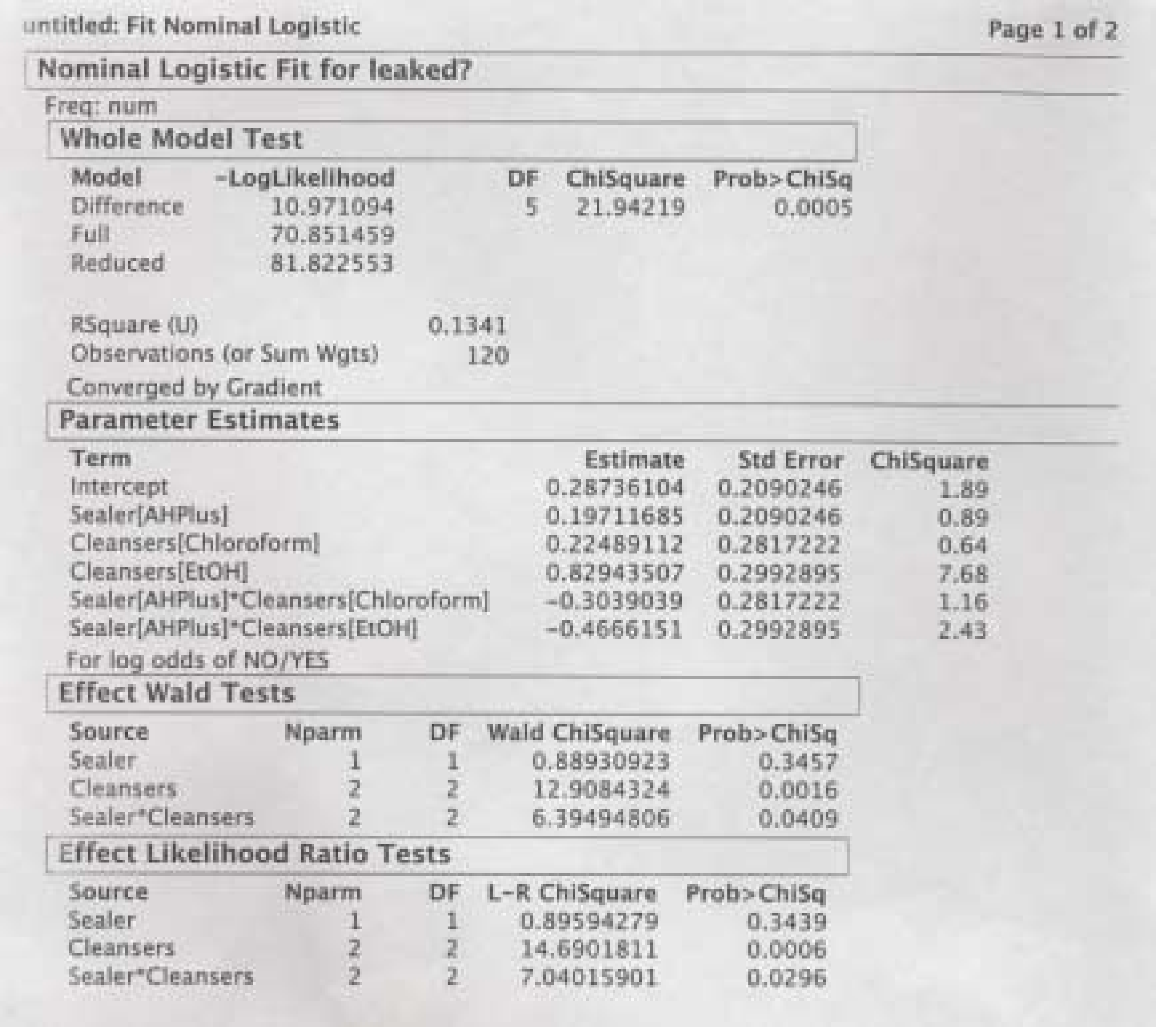
Department Chair

Faculty Advisor

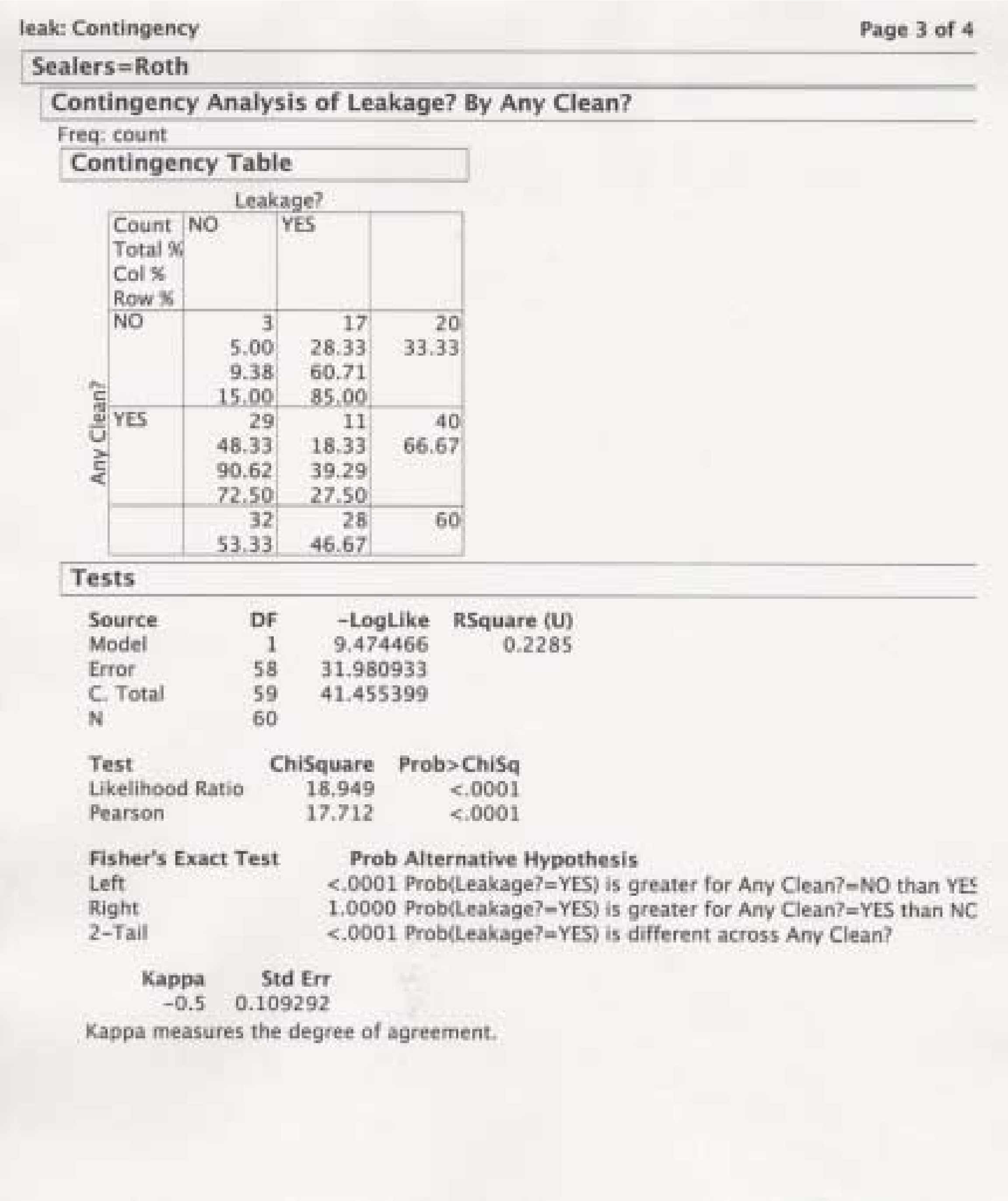
Dean or Director

Department Chair

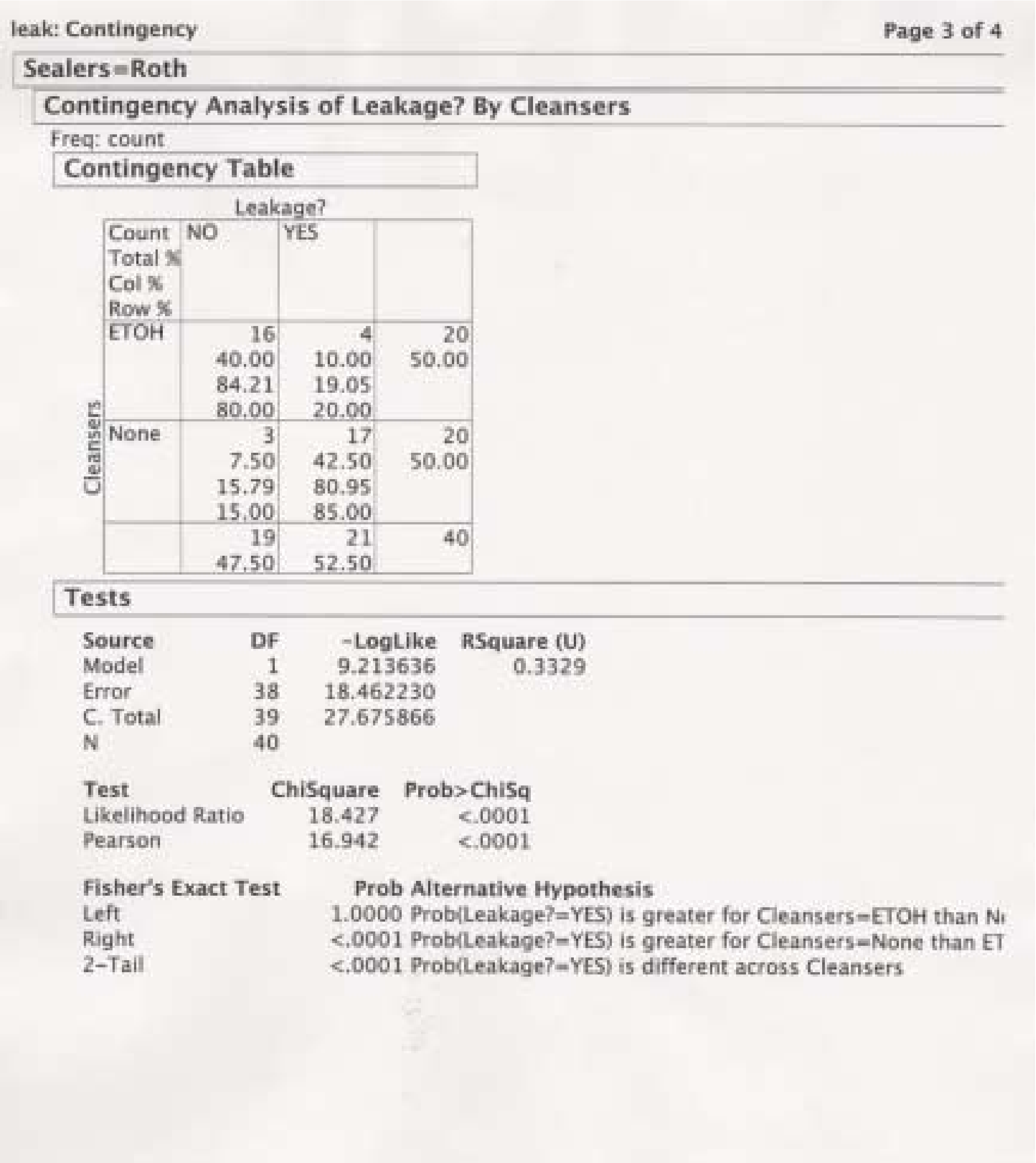
Appendix B



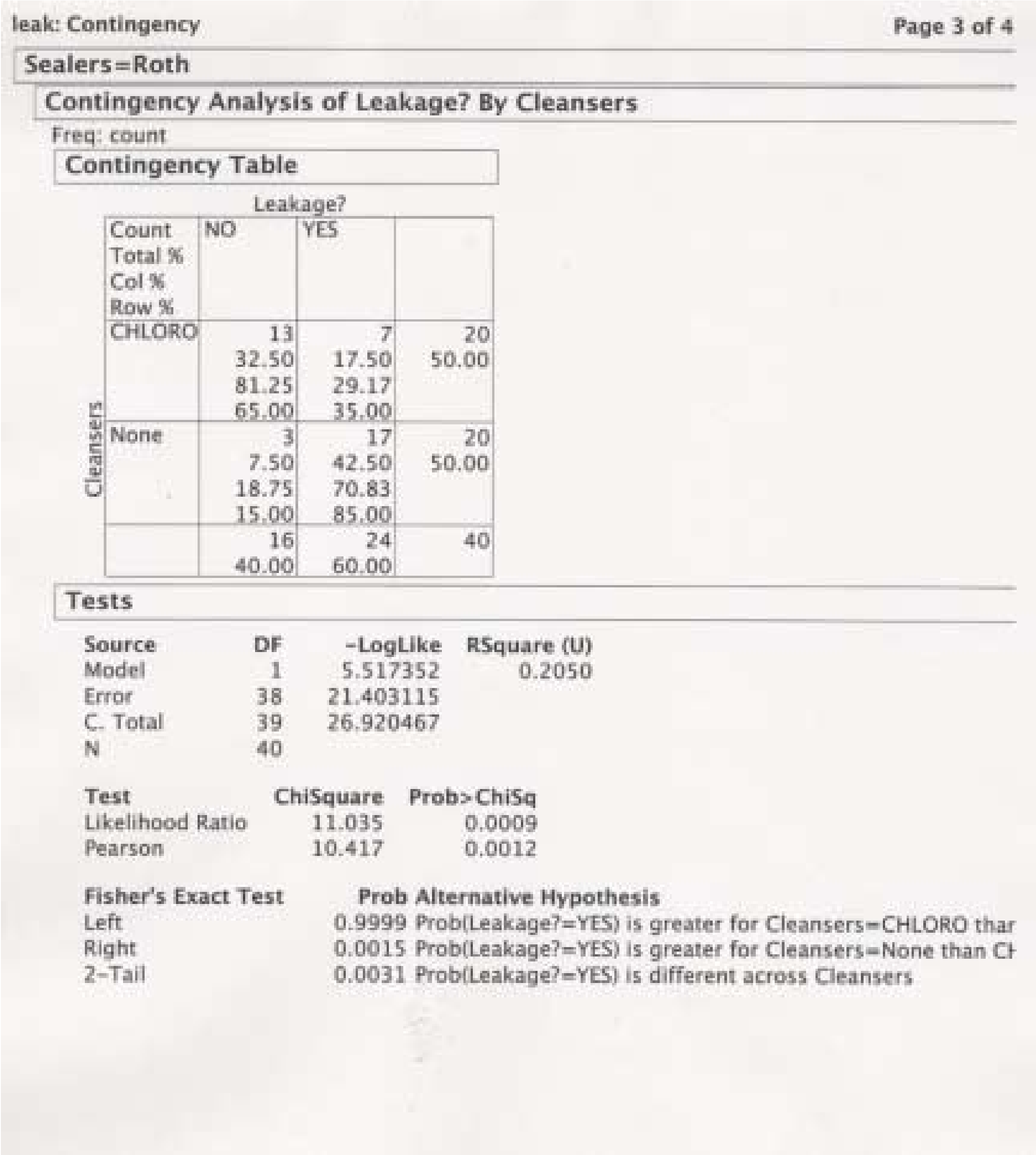
```
untitled: Fit Nominal Logistic                                              Page 1 of 2
Nominal Logistic Fit for leaked?
 Freq: num
 Whole Model Test
   Model          -LogLikelihood          DF    ChiSquare   Prob>ChiSq
   Difference         10.971094            5     21.94219       0.0005
   Full               70.851459
   Reduced            81.822553

   RSquare (U)                      0.1341
   Observations (or Sum Wgts)          120
  Converged by Gradient
 Parameter Estimates
   Term                                        Estimate    Std Error   ChiSquare
   Intercept                                 0.28736104    0.2090246        1.89
   Sealer[AHPlus]                            0.19711685    0.2090246        0.89
   Cleansers[Chloroform]                     0.22489112    0.2817222        0.64
   Cleansers[EtOH]                           0.82943507    0.2992895        7.68
   Sealer[AHPlus]*Cleansers[Chloroform]     -0.3039039     0.2817222        1.16
   Sealer[AHPlus]*Cleansers[EtOH]           -0.4666151     0.2992895        2.43
  For log odds of NO/YES
 Effect Wald Tests
   Source             Nparm     DF   Wald ChiSquare   Prob>ChiSq
   Sealer                 1      1       0.88930923       0.3457
   Cleansers              2      2       12.9084324       0.0016
   Sealer*Cleansers       2      2       6.39494806       0.0409
 Effect Likelihood Ratio Tests
   Source             Nparm     DF   L-R ChiSquare   Prob>ChiSq
   Sealer                 1      1      0.89594279       0.3439
   Cleansers              2      2      14.6901811       0.0006
   Sealer*Cleansers       2      2      7.04015901       0.0296
```



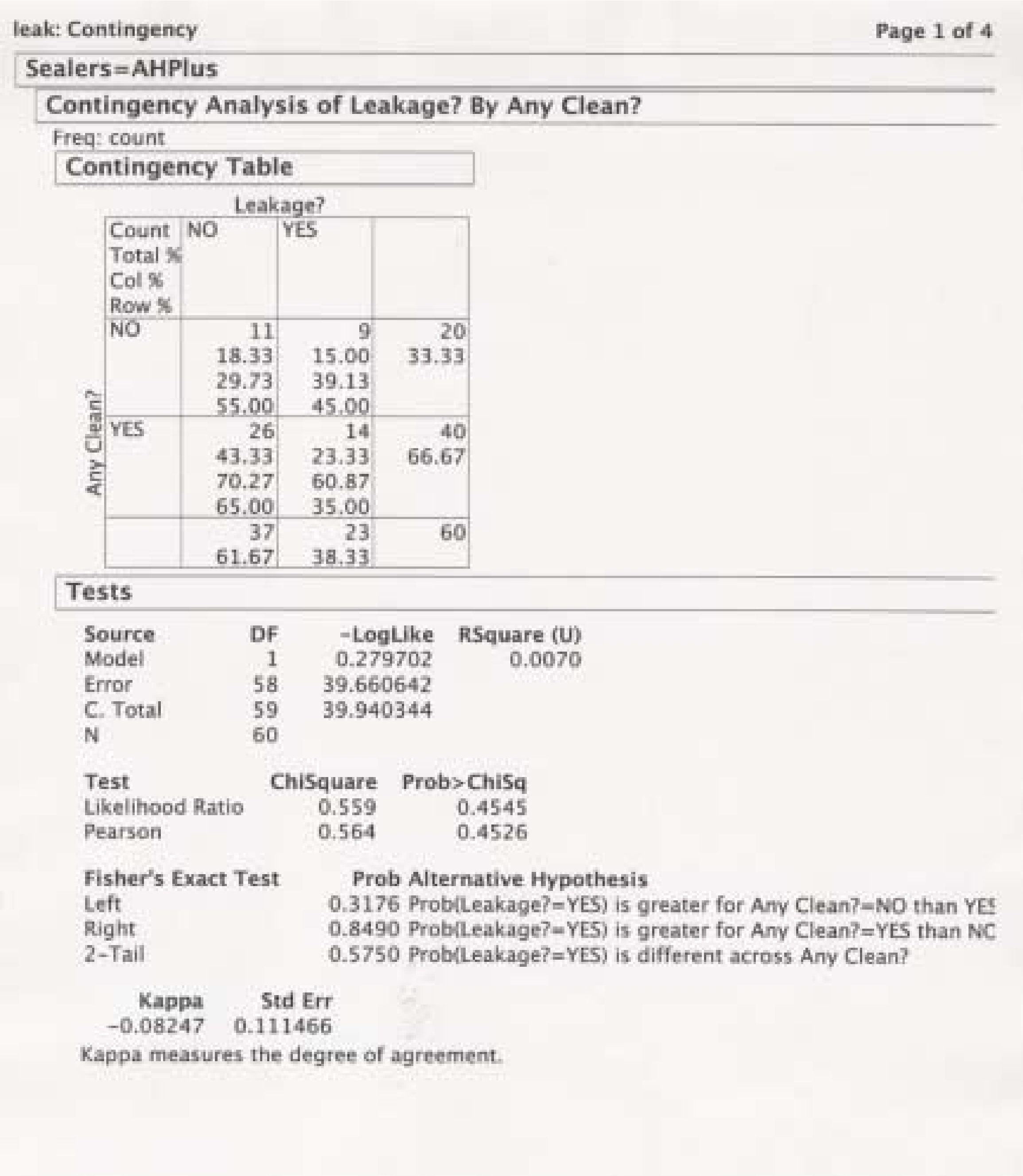
```
leak: Contingency                                                    Page 3 of 4
Sealers=Roth
  Contingency Analysis of Leakage? By Any Clean?
   Freq: count
   Contingency Table
                    Leakage?
            Count   NO        YES
            Total %
            Col %
            Row %
            NO            3        17        20
                       5.00     28.33     33.33
                       9.38     60.71
                      15.00     85.00
  Any Clean? YES         29        11        40
                      48.33     18.33     66.67
                      90.62     39.29
                      72.50     27.50
                         32        28        60
                      53.33     46.67
   Tests
     Source          DF      -LogLike    RSquare (U)
     Model            1      9.474466        0.2285
     Error           58     31.980933
     C. Total        59     41.455399
     N               60

     Test              ChiSquare   Prob>ChiSq
     Likelihood Ratio     18.949       <.0001
     Pearson              17.712       <.0001

     Fisher's Exact Test        Prob Alternative Hypothesis
     Left                      <.0001 Prob(Leakage?=YES) is greater for Any Clean?=NO than YE
     Right                     1.0000 Prob(Leakage?=YES) is greater for Any Clean?=YES than N
     2-Tail                    <.0001 Prob(Leakage?=YES) is different across Any Clean?

          Kappa     Std Err
           -0.5    0.109292
     Kappa measures the degree of agreement.
```



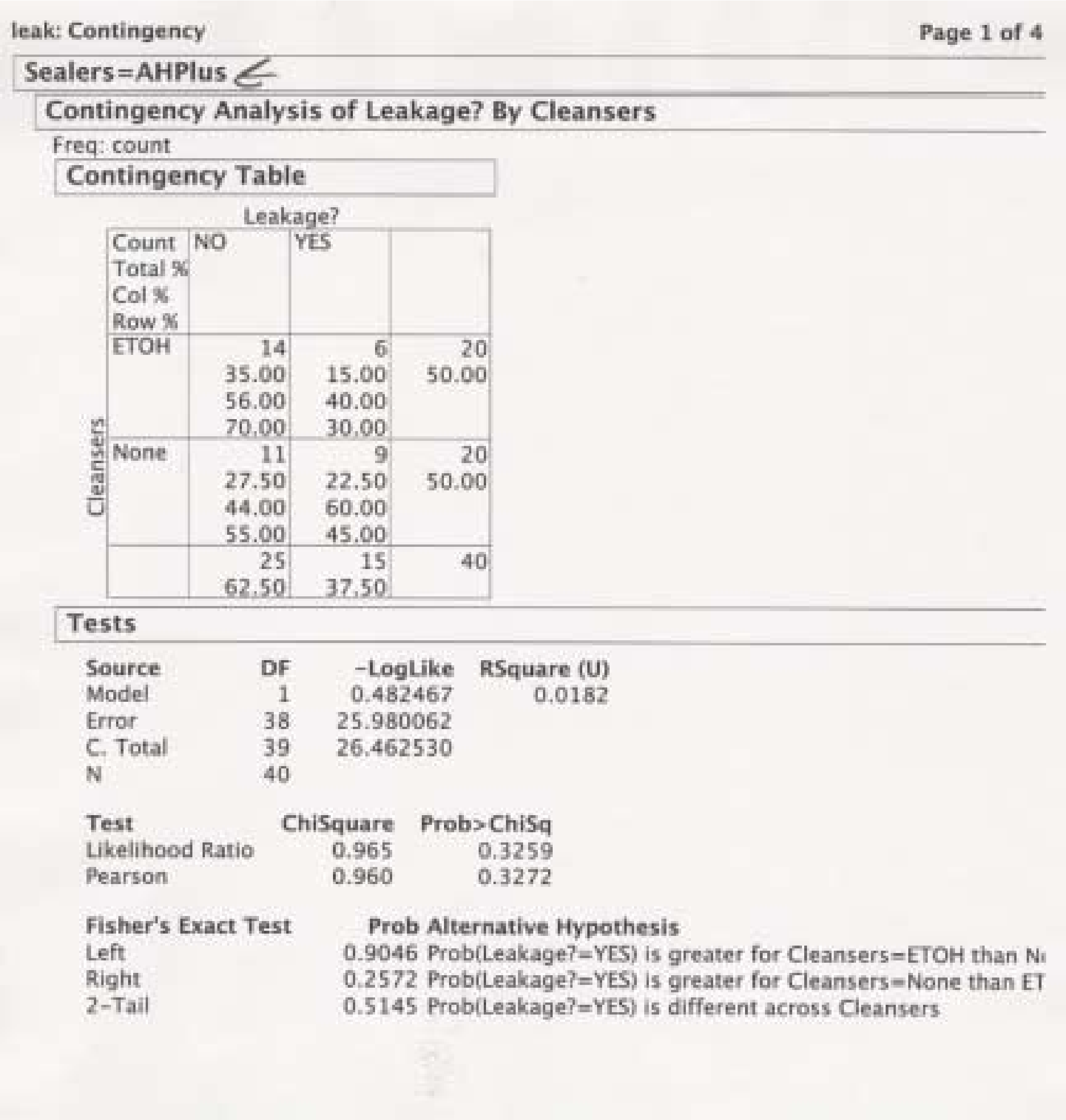
```
leak: Contingency                                                    Page 3 of 4
Sealers=Roth
  Contingency Analysis of Leakage? By Cleansers
   Freq: count
   Contingency Table
                   Leakage?
          Count   NO        YES
          Total %
          Col %
          Row %
          ETOH        16          4        20
                   40.00      10.00     50.00
                   84.21      19.05
                   80.00      20.00
 Cleansers None        3         17        20
                    7.50      42.50     50.00
                   15.79      80.95
                   15.00      85.00
                      19         21        40
                   47.50      52.50
   Tests
     Source          DF      -LogLike   RSquare (U)
     Model            1      9.213636       0.3329
     Error           38     18.462230
     C. Total        39     27.675866
     N               40

     Test               ChiSquare   Prob>ChiSq
     Likelihood Ratio      18.427       <.0001
     Pearson               16.942       <.0001

     Fisher's Exact Test        Prob Alternative Hypothesis
     Left                     1.0000 Prob(Leakage?=YES) is greater for Cleansers=ETOH than N
     Right                    <.0001 Prob(Leakage?=YES) is greater for Cleansers=None than ET
     2-Tail                   <.0001 Prob(Leakage?=YES) is different across Cleansers
```



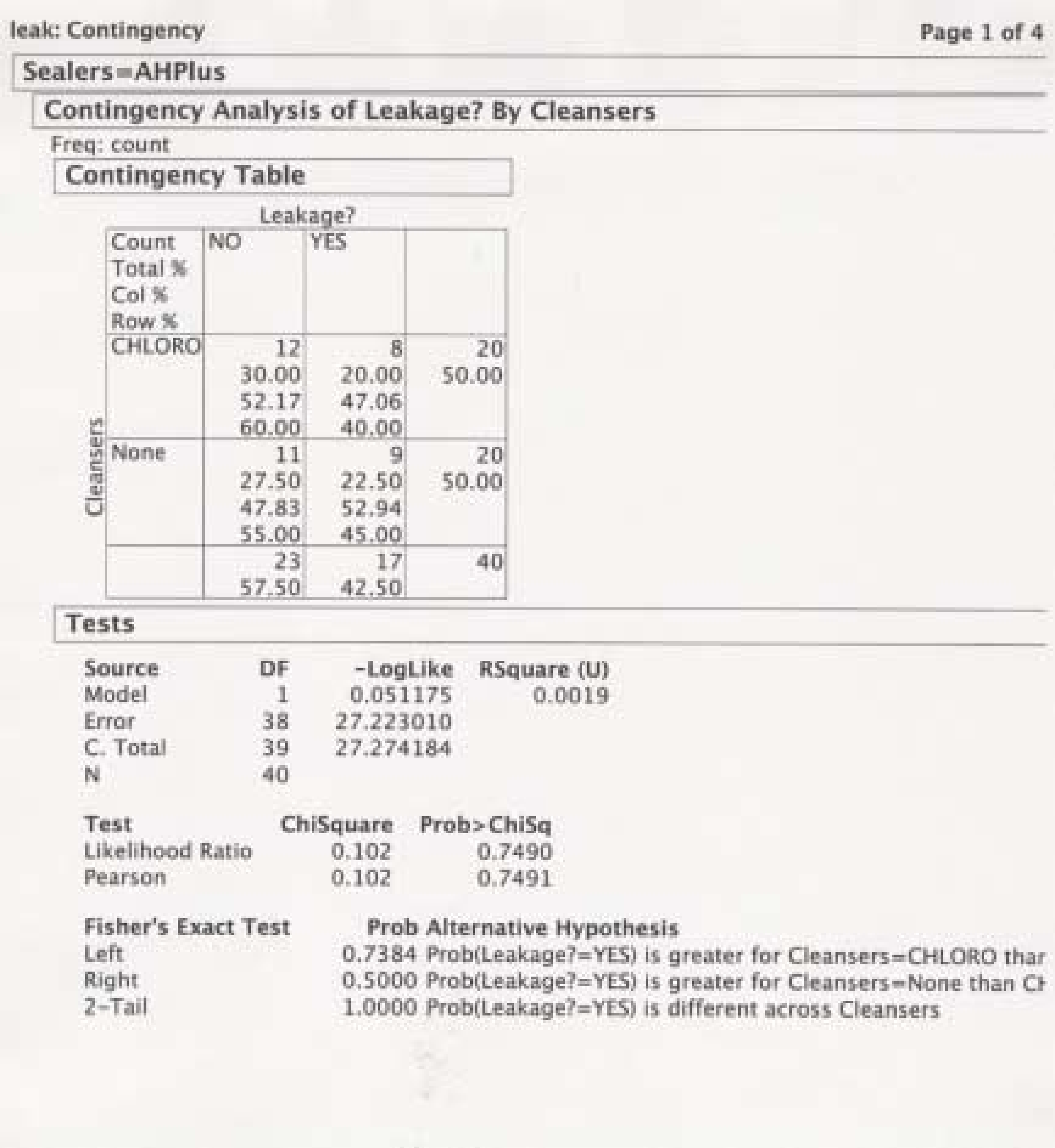
```
leak: Contingency                                                    Page 3 of 4
Sealers=Roth
  Contingency Analysis of Leakage? By Cleansers
   Freq: count
   Contingency Table
                  Leakage?
          Count    NO       YES
          Total %
          Col %
          Row %
          CHLORO       13        7       20
                    32.50    17.50    50.00
                    81.25    29.17
                    65.00    35.00
Cleansers None          3       17       20
                     7.50    42.50    50.00
                    18.75    70.83
                    15.00    85.00
                       16       24       40
                    40.00    60.00
   Tests
     Source        DF     -LogLike   RSquare (U)
     Model          1     5.517352       0.2050
     Error         38    21.403115
     C. Total      39    26.920467
     N             40

     Test               ChiSquare   Prob>ChiSq
     Likelihood Ratio      11.035       0.0009
     Pearson               10.417       0.0012

     Fisher's Exact Test        Prob Alternative Hypothesis
     Left                     0.9999 Prob(Leakage?=YES) is greater for Cleansers=CHLORO tha
     Right                    0.0015 Prob(Leakage?=YES) is greater for Cleansers=None than C
     2-Tail                   0.0031 Prob(Leakage?=YES) is different across Cleansers
```



```
leak: Contingency                                              Page 1 of 4

Sealers=AHPlus
  Contingency Analysis of Leakage? By Any Clean?
   Freq: count
    Contingency Table
                  Leakage?
            Count   NO       YES
            Total %
            Col %
            Row %
            NO          11        9       20
                     18.33    15.00    33.33
                     29.73    39.13
                     55.00    45.00
 Any Clean? YES         26       14       40
                     43.33    23.33    66.67
                     70.27    60.87
                     65.00    35.00
                        37       23       60
                     61.67    38.33

    Tests
      Source          DF      -LogLike   RSquare (U)
      Model            1      0.279702       0.0070
      Error           58     39.660642
      C. Total        59     39.940344
      N               60

      Test              ChiSquare   Prob>ChiSq
      Likelihood Ratio      0.559       0.4545
      Pearson               0.564       0.4526

      Fisher's Exact Test       Prob Alternative Hypothesis
      Left                    0.3176 Prob(Leakage?=YES) is greater for Any Clean?=NO than YE
      Right                   0.8490 Prob(Leakage?=YES) is greater for Any Clean?=YES than N
      2-Tail                  0.5750 Prob(Leakage?=YES) is different across Any Clean?

           Kappa      Std Err
        -0.08247     0.111466
      Kappa measures the degree of agreement.
```



```
leak: Contingency                                                     Page 1 of 4
 Sealers=AHPlus
  Contingency Analysis of Leakage? By Cleansers
   Freq: count
   Contingency Table
                 Leakage?
           Count   NO       YES
           Total %
           Col %
           Row %
           ETOH        14        6       20
                    35.00    15.00    50.00
                    56.00    40.00
                    70.00    30.00
 Cleansers None        11        9       20
                    27.50    22.50    50.00
                    44.00    60.00
                    55.00    45.00
                       25       15       40
                    62.50    37.50
   Tests

    Source          DF     -LogLike   RSquare (U)
    Model            1     0.482467       0.0182
    Error           38    25.980062
    C. Total        39    26.462530
    N               40

    Test              ChiSquare   Prob>ChiSq
    Likelihood Ratio      0.965       0.3259
    Pearson               0.960       0.3272

    Fisher's Exact Test     Prob Alternative Hypothesis
    Left                  0.9046 Prob(Leakage?=YES) is greater for Cleansers=ETOH than N
    Right                 0.2572 Prob(Leakage?=YES) is greater for Cleansers=None than ET
    2-Tail                0.5145 Prob(Leakage?=YES) is different across Cleansers
```



leak: Contingency Page 1 of 4

Sealers=AHPlus

Contingency Analysis of Leakage? By Cleansers

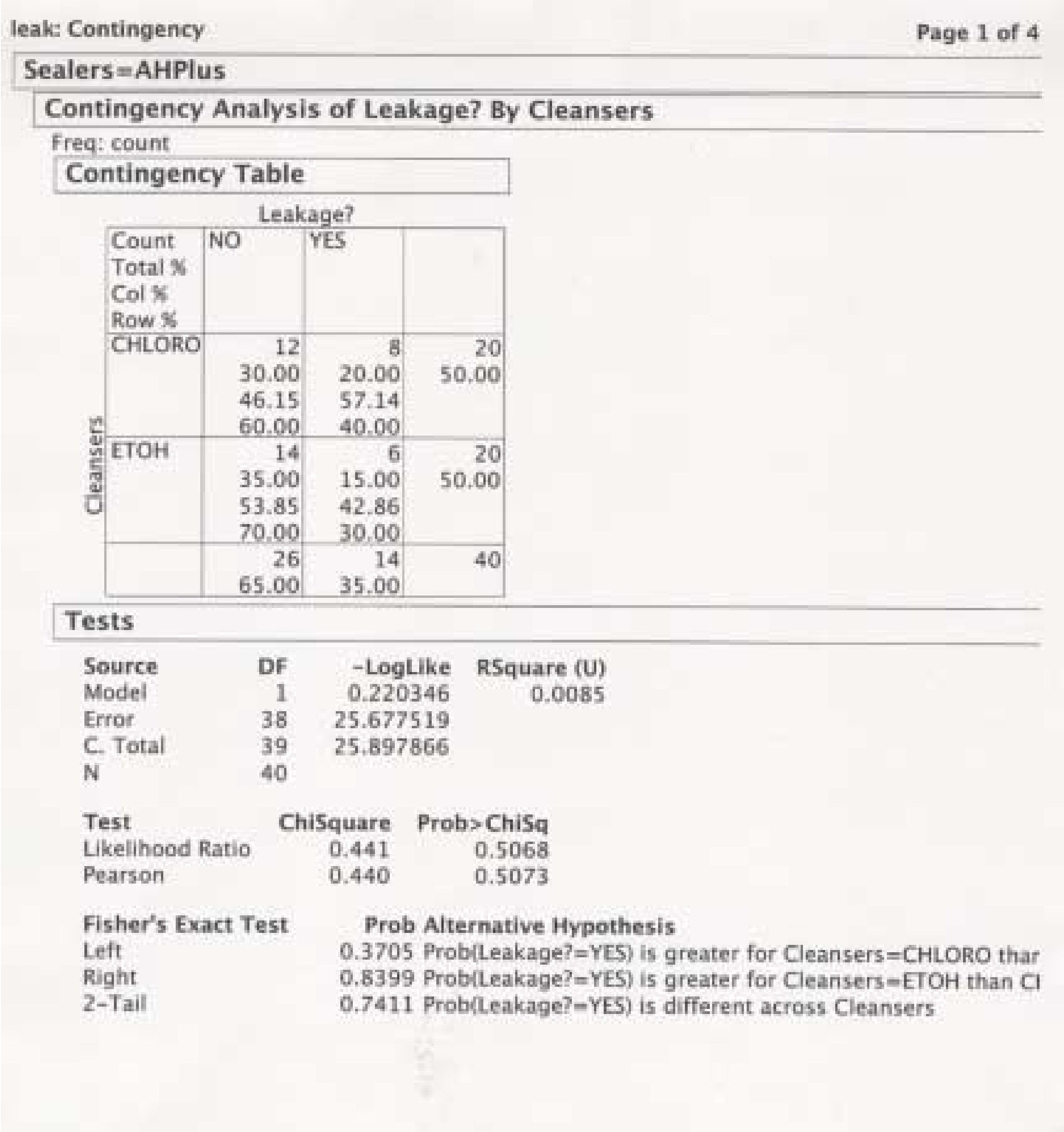
Freq: count

Contingency Table

		Leakage?		
	Count Total % Col % Row %	NO	YES	
Cleansers	CHLORO	12 30.00 52.17 60.00	8 20.00 47.06 40.00	20 50.00
	None	11 27.50 47.83 55.00	9 22.50 52.94 45.00	20 50.00
		23 57.50	17 42.50	40

Tests

```
Source          DF      -LogLike   RSquare (U)
Model            1      0.051175       0.0019
Error           38     27.223010
C. Total        39     27.274184
N               40

Test               ChiSquare   Prob>ChiSq
Likelihood Ratio       0.102       0.7490
Pearson                0.102       0.7491

Fisher's Exact Test    Prob Alternative Hypothesis
Left                 0.7384 Prob(Leakage?=YES) is greater for Cleansers=CHLORO thar
Right                0.5000 Prob(Leakage?=YES) is greater for Cleansers=None than C
2-Tail               1.0000 Prob(Leakage?=YES) is different across Cleansers
```



leak: Contingency Page 1 of 4

Sealers=AHPlus

Contingency Analysis of Leakage? By Cleansers

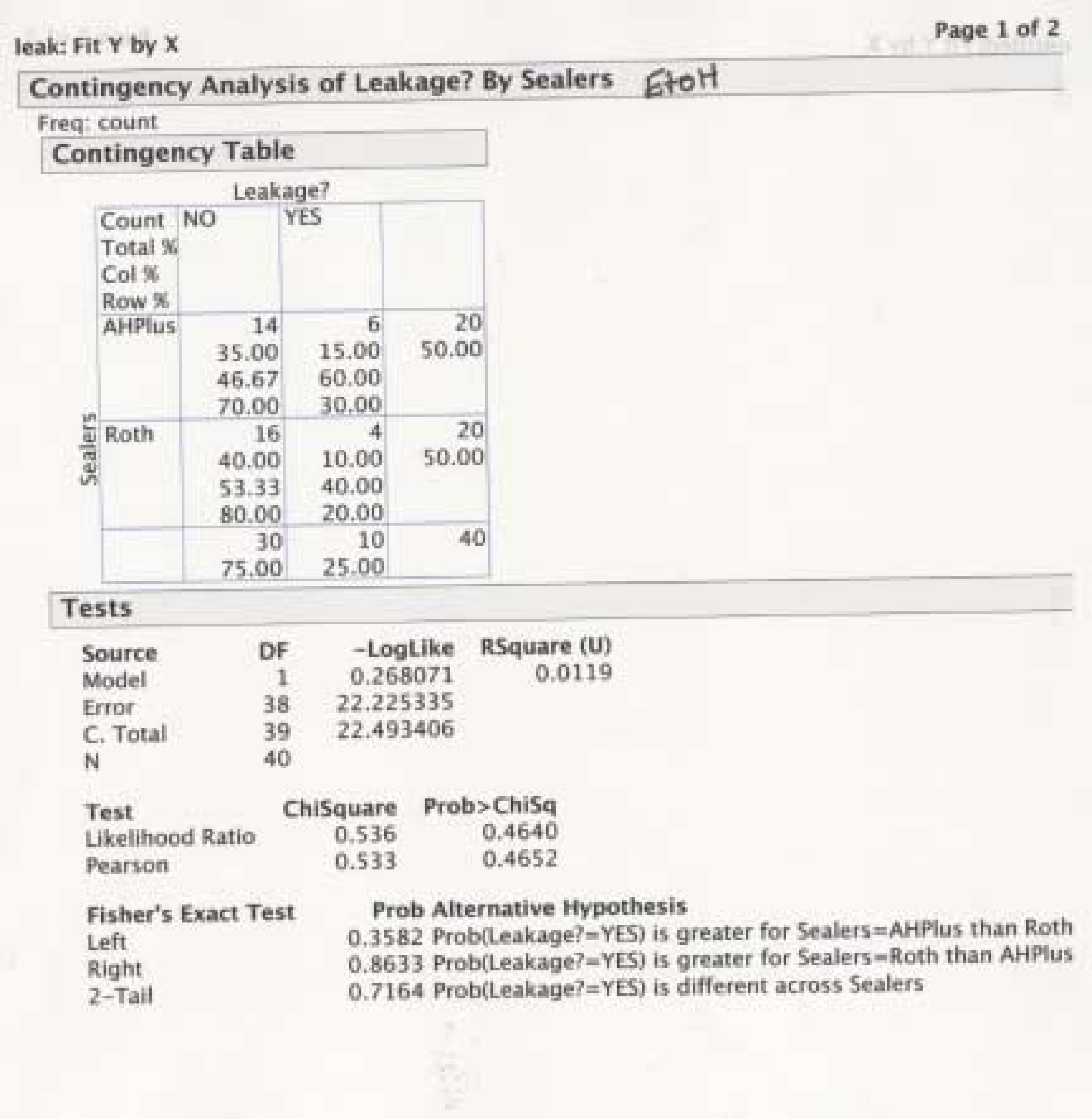
Freq: count

Contingency Table

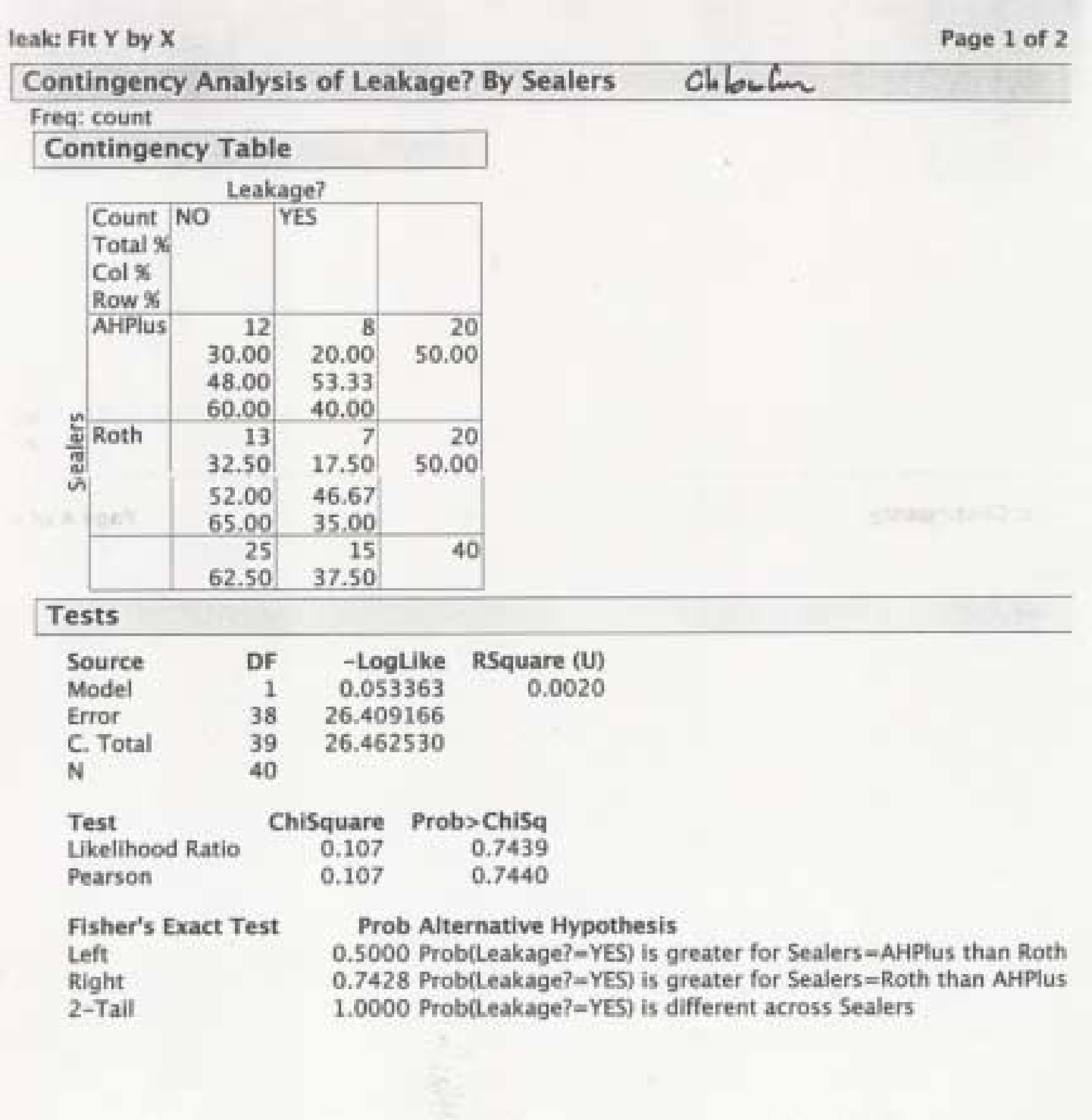
		Leakage?		
	Count Total % Col % Row %	NO	YES	
Cleansers	CHLORO	12 30.00 46.15 60.00	8 20.00 57.14 40.00	20 50.00
	ETOH	14 35.00 53.85 70.00	6 15.00 42.86 30.00	20 50.00
		26 65.00	14 35.00	40

Tests

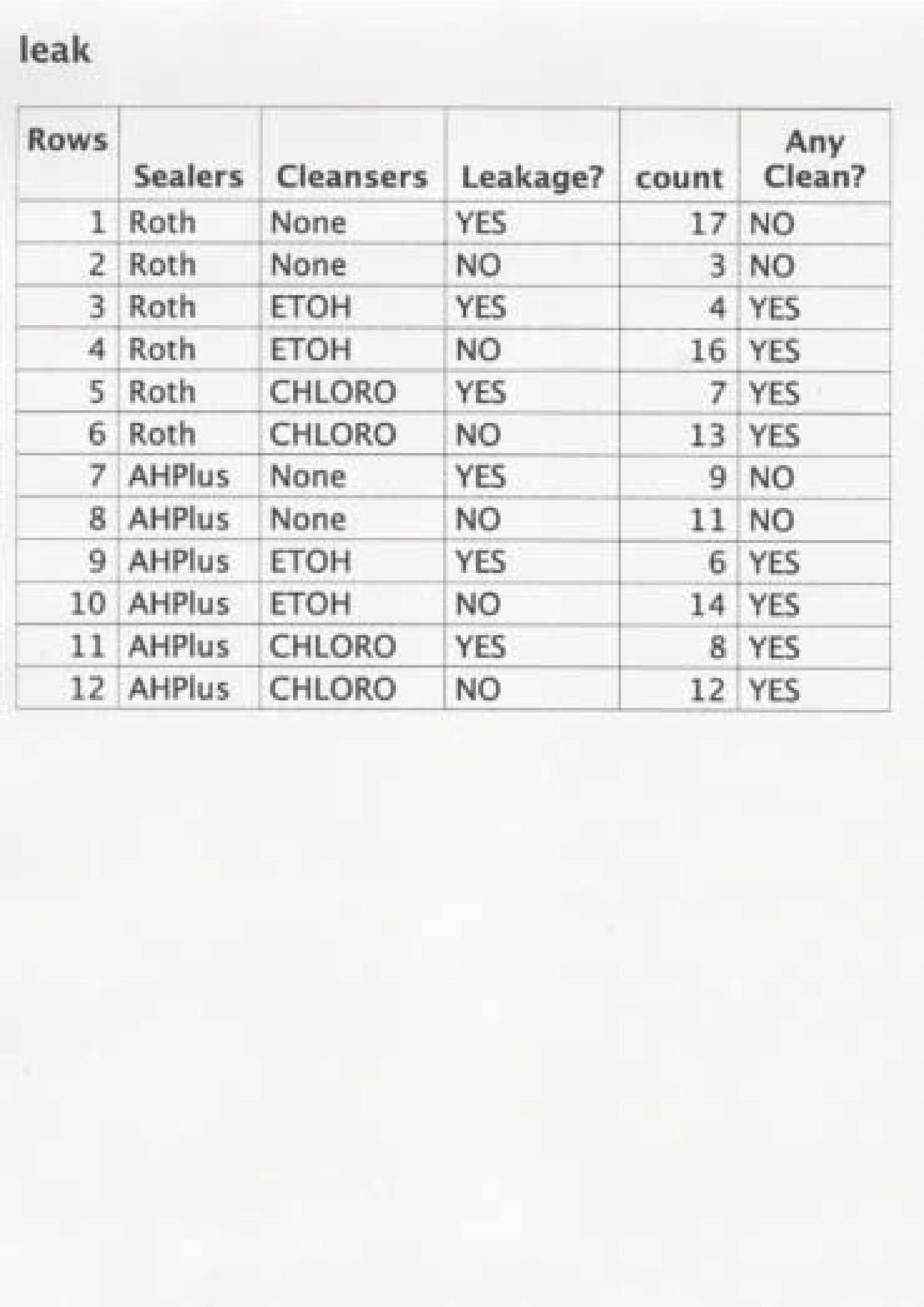
```
Source            DF      -LogLike   RSquare (U)
Model              1      0.220346        0.0085
Error             38     25.677519
C. Total          39     25.897866
N                 40

Test              ChiSquare   Prob>ChiSq
Likelihood Ratio      0.441       0.5068
Pearson               0.440       0.5073

Fisher's Exact Test       Prob Alternative Hypothesis
Left                    0.3705 Prob(Leakage?=YES) is greater for Cleansers=CHLORO thar
Right                   0.8399 Prob(Leakage?=YES) is greater for Cleansers=ETOH than Cl
2-Tail                  0.7411 Prob(Leakage?=YES) is different across Cleansers
```



```
leak: Fit Y by X                                                    Page 1 of 2

Contingency Analysis of Leakage? By Sealers   EtoH

 Freq: count
 Contingency Table
                     Leakage?
          Count    NO        YES
          Total %
          Col %
          Row %
          AHPlus        14        6       20
                     35.00    15.00    50.00
                     46.67    60.00
                     70.00    30.00
 Sealers  Roth          16        4       20
                     40.00    10.00    50.00
                     53.33    40.00
                     80.00    20.00
                        30       10       40
                     75.00    25.00

 Tests

   Source          DF      -LogLike   RSquare (U)
   Model            1      0.268071       0.0119
   Error           38     22.225335
   C. Total        39     22.493406
   N               40

   Test                ChiSquare   Prob>ChiSq
   Likelihood Ratio        0.536       0.4640
   Pearson                 0.533       0.4652

   Fisher's Exact Test       Prob Alternative Hypothesis
   Left                      0.3582 Prob(Leakage?=YES) is greater for Sealers=AHPlus than Roth
   Right                     0.8633 Prob(Leakage?=YES) is greater for Sealers=Roth than AHPlus
   2-Tail                    0.7164 Prob(Leakage?=YES) is different across Sealers
```



```
leak: Fit Y by X                                                    Page 1 of 2
Contingency Analysis of Leakage? By Sealers        Chloroform
 Freq: count
 Contingency Table
                  Leakage?
          Count    NO       YES
          Total %
          Col %
          Row %
          AHPlus      12        8       20
                   30.00    20.00    50.00
                   48.00    53.33
                   60.00    40.00
  Sealers Roth        13        7       20
                   32.50    17.50    50.00
                   52.00    46.67
                   65.00    35.00
                      25       15       40
                   62.50    37.50
 Tests
   Source          DF     -LogLike   RSquare (U)
   Model            1     0.053363      0.0020
   Error           38    26.409166
   C. Total        39    26.462530
   N               40

   Test              ChiSquare   Prob>ChiSq
   Likelihood Ratio      0.107       0.7439
   Pearson               0.107       0.7440

   Fisher's Exact Test       Prob Alternative Hypothesis
   Left                    0.5000 Prob(Leakage?=YES) is greater for Sealers=AHPlus than Roth
   Right                   0.7428 Prob(Leakage?=YES) is greater for Sealers=Roth than AHPlus
   2-Tail                  1.0000 Prob(Leakage?=YES) is different across Sealers
```



leak

Rows	Sealers	Cleansers	Leakage?	count	Any Clean?
1	Roth	None	YES	17	NO
2	Roth	None	NO	3	NO
3	Roth	ETOH	YES	4	YES
4	Roth	ETOH	NO	16	YES
5	Roth	CHLORO	YES	7	YES
6	Roth	CHLORO	NO	13	YES
7	AHPlus	None	YES	9	NO
8	AHPlus	None	NO	11	NO
9	AHPlus	ETOH	YES	6	YES
10	AHPlus	ETOH	NO	14	YES
11	AHPlus	CHLORO	YES	8	YES
12	AHPlus	CHLORO	NO	12	YES

Appendix C

Coronal Microleakage

	Group 1			**Group 2**			**Group 3**	
	Roth			**Roth & EtOH**			**Roth & Chloroform**	
1	Yes	1	1	No	0	1	No	0
2	No	0	2	No	0	2	No	0
3	Yes	1	3	No	0	3	No	0
4	Yes	1	4	Yes	1	4	Yes	1
5	Yes	1	5	No	0	5	Yes	1
6	Yes	1	6	No	0	6	No	0
7	No	0	7	No	0	7	No	0
8	Yes	1	8	No	0	8	No	0
9	Yes	1	9	Yes	1	9	No	0
10	Yes	1	10	Yes	1	10	Yes	1
11	Yes	1	11	No	0	11	No	0
12	No	0	12	No	0	12	Yes	1
13	Yes	1	13	No	0	13	No	0
14	Yes	1	14	Yes	1	14	Yes	1
15	Yes	1	15	No	0	15	Yes	1
16	Yes	1	16	No	0	16	No	0
17	Yes	1	17	No	0	17	No	0
18	Yes	1	18	No	0	18	Yes	1
19	Yes	1	19	No	0	19	No	0
20	Yes	1	20	No	0	20	No	0
	# Leaked 17/20			**# Leaked 4/20**			**# Leaked 7/20**	

	Group 4			**Group 5**			**Group 6**	
	AHPlus			**AHPlus & EtOH**			**AHPlus & Chloroform**	
1	Yes	1	1	No	0	1	No	0
2	No	0	2	No	0	2	Yes	1
3	No	0	3	No	0	3	Yes	1
4	Yes	1	4	No	0	4	No	0
5	No	0	5	No	0	5	No	0
6	Yes	1	6	No	0	6	No	0
7	Yes	1	7	Yes	1	7	No	0
8	No	0	8	No	0	8	Yes	1
9	No	0	9	No	0	9	Yes	1
10	No	0	10	Yes	1	10	Yes	1
11	No	0	11	Yes	1	11	No	0
12	Yes	1	12	No	0	12	Yes	1
13	No	0	13	Yes	1	13	No	0
14	No	0	14	No	0	14	No	0
15	Yes	1	15	No	0	15	No	0
16	No	0	16	No	0	16	No	0
17	Yes	1	17	Yes	1	17	Yes	1
18	Yes	1	18	Yes	1	18	No	0
19	No	0	19	No	0	19	No	0
20	Yes	1	20	No	0	20	Yes	1
	# Leaked 9/20			**# Leaked 6/20**			**# Leaked 8/20**	

Curriculum Vitae

Jeffrey G. Minchau, D.D.S.

Education/Degrees:

Doctor of Dental Surgery
West Virginia University School of Dentistry
Morgantown, WV
May 2000

Bachelor's of Science in Biology
University of Pittsburgh
Johnstown, PA
May 1996

Professional Experience:

Associate Dentist, Hilltop Dental Associates
Johnstown, PA
June 2000-2002

Dental Lab Assistant, Hilltop Dental Associates
Johnstown, PA
Part-time and summers 1992-1996

Awards/ Honors:

American Association of Endodontists Achievement Award, May 2000
Pierre Fauchard Academy Induction, May 2000
American Academy of Gold Foil Operators Award, May 2000
American Academy of Oral & Maxillofacial Pathology Award, May 2000
American Association of Dental Research Award, May 2000
American Association Oral & Maxillofacial Surgeons Award, May 2000
Quintessence Book Student Award for Research, May 2000
Clinical Competency Award, May 2000
Monongalia County Dental Society Student Award, May 1999

Research/Publications:

Accuracy of Poly(vinyl) Siloxane Dies Fabricated from Alginate and Polyether Impressions
Co-investigator, 2000
Published in: Journal of Dental Research, 2000, #2433

Presented:
The American Academy of Dental Research, International Convention
Washington, DC
April, 2000

West Virginia University School of Dentistry, Research Convocation
Morgantown, WV
March, 2000

Accuracy of Fit of Resin Inlays Fabricated From Poly(vinyl) Siloxane Dies Using Alginate and Polyether Impression Material
Co-investigator, 1998
Published in: Journal of Dental Research, 1999, #1022

Presented:
The American Academy of Dental Research International Convention
Minneapolis, MN
March 1999

American Association of Cosmetic Dentistry
Vancouver, British Colombia
June 1999

Leadership:

American Dental Association New Dentists Committee
7th District Delegate 2001-Present

Cambria County Dental Society
Vice President 2001-Present
Treasurer/Secretary 2000-2001

Delta Sigma Delta Dental Fraternity
West Virginia University School of Dentistry
Morgantown, WV
President 1999-2000
Vice President 1998-1999
Treasurer 1997-1998

American Student Dental Association
West Virginia University School of Dentistry
Morgantown, WV
Vice-President 1999-2000
Treasurer 1998-1999
Class Representative 1996-1998

Student Research Group

West Virginia University School of Dentistry
Morgantown, WV
President 1998-1999
Vice-President 1997-1998

Conferences:

American Association of Endodontics
Annual Convention
Tampa, Fla.
May 2003

American Academy of Operative Dentistry
Annual Convention
Chicago, IL
February 2001, 2000

Peter E. Dawson
Concept of Complete Dentistry, Seminar II
St. Petersburg, FL
November 2000

Peter E. Dawson
Concept of Complete Dentistry, Seminar I
St. Petersburg, FL
February 1999

American Student Dental Association
Quad-Regional Convention
Atlanta, GA
January 1998

American Student Dental Association
Quad-Regional Convention
Charleston, SC
March 1997

Professional Membership:

American Association of Endodontists, 2000-present
American Dental Association, 2000-present
Pennsylvania Dental Association, 2000-present
Cambria County Dental Society, 2000-present
American Association of General Dentists, 2000-present
American Academy of Operative Dentistry, 1998-present