# "THE EFFECT OF IRRIGATION OF SODIUM HYPOCHLORITE AT DIFFERENT TEMPERATURES AND CONCENTRATION ON THE ELIMINATION OF ENTEROCOCCUS FAECALIS FROM ROOTCANALS"

## AN IN VITRO STUDY

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# CERTIFICATE

*This is to certify that* **DR.RAKESH.R.RAJAN,** *post graduate student(2008-2011) in the Department Of Conservative Dentistry And Endodontics, J.K.K.Nataraja Dental College, Komarapalayam, Namakkal Dist – 638183, Tamilnadu. Has done the dissertation titled "***THE EFFECT OF IRRIGATION OF SODIUM HYPOCHLORITE AT DIFFERENT TEMPERATURES AND CONCENTRATION ON THE ELIMINATION OF ENTEROCOCCUS FAECALIS FROM ROOTCANALS" AN IN VITRO STUDY**. *Under my direct guidance and supervision in the partial fulfillment of the regulations laid down by* THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY, CHENNAI, FOR M.D.S BRANCH – IV CONSERVATIVE DENTISTRY AND ENDODONTICS DEGREE EXAMINATION.

It has not been submitted (partial or full) for the award of any other degree or diploma**.**

**Dr.J.V.Karunakaran M.D.S,**
Professor& Head,
Department of Conservative
Dentistry & Endodontics,
Komarapalayam,
Namakkal Dist – 638183,
Tamilnadu.

**Dr.Siva Kumar,M.D.S,**
Principal,
J.K.K.Nataraja Dental College
Komarapalayam,
Namakkal Dist – 638183,
Tamilnadu.

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# CONTENTS

Persistence of microorganisms or recontamination of the root canal system due to apical percolation has been long recognized as the primary etiological aspect for development of periapical lesions and failure of root canal therapy. Reinfection or chronic periapical infection may be due to viable bacteria residing within the ramifications of the root canal system and the dentinal tubules (Buck et al 2001) [4]. Eradication of bacteria from the root canal space is vital for successful outcome of root canal therapy.

Primary root canal infections are polymicrobial typically dominated by obligate anaerobic bacteria. As the host defense mechanisms lose the access to the radicular necrotic pulp space, opportunistic microorganisms and the low oxygen environment aggregate and these microbial communities may survive on organic remnants and exudates. Clusters of microorganisms in necrotic teeth and in teeth with failed root canal treatment are typically found in apical root canal area where they have access to apical tissue fluids.

In long standing infections these bacteria can invade adjacent dentin via open dentinal tubules. The obligate anaerobes are rather easily eradicated during root canal treatment. On the other hand facultative anaerobes are more likely to survive.

Studies investigating the role of microorganisms associated with treatment failures have reported the presence of microorganisms in 35% - 100% cases. The rate of recovery of microorganisms is also dependent on the type of technique used eg: molecular based or culture based.

Amongst this microbiota *Enterococcus faecalis* is of present interest as it is the most frequently detected species in endodontically treated teeth with persistent lesions (E. T. Pinheiro et al in 2004) [20]. Root canal treatment failures from persistent infection is a possibility. These organisms have demonstrated the capacity to survive the harshest of environments and the ability to evade and survive within the dentinal tubules. Invasion of dentinal tubules is brought about by virulence factors which promote adhesion to collagen and a collagen induced morphological growth response, tissue invasions, toxin mediated damage etc. They also gain nourishment from the tissue fluids (R.M.Love in 2001) [62], and also have the ability to survive in the root canal as a single organism without the support of other bacteria.

Once the microorganisms are established in the root canal system they cannot be reached by the host defense mechanisms. Among the various procedures involved in the control of endodontic infection, irrigation is a important step for elimination of the microorganisms from the root canal space. During the biomechanical preparation of the canal various irrigant solutions with different concentrations and antimicrobial activity has been tried.

0.5% Sodium hypochlorite as an aqueous solution was first used as an antiseptic solution by Henry Dakin during the first world war to irrigate open wounds. Sodium hypochlorite has been widely used as an endodontic irrigant since its introduction by Walker in 1936. This irrigant has got a strong bleaching, deodorizing, solvent and effective antimicrobial action (Gordon et al in 1981)[25].The choice of concentration of sodium hypochlorite is still a matter of debate. Sodium hypochlorite has been used in various concentrations ranging from 0.5% to 5.25%. Sodium hypochlorite solutions of higher concentration are advocated for root canal therapy. The concentration of sodium hypochlorite is directly proportional to its antimicrobial efficacy, tissue dissolution and caustic potential.

The effectiveness of sodium hypochlorite irrigants could be improved by increasing their temperature. Preheating sodium hypochlorite solutions appears to increase their antimicrobial efficacy and tissue dissolution capacity (George Sirtes et al in 2005) [22].

It has also been reported that sodium hypochlorite at lower concentration is (0.5%-1%) is biocompatible. Of all the currently used irrigants sodium hypochlorite appears to be the more ideal as it covers most of the requirements for endodontic irrigant than any other known compound.

The aim of this study was to evaluate the efficacy of 1%, 3% and 5% sodium hypochlorite as an intra canal irrigant at three different temperatures of $24^0$Celsius, $37^0$Celsius and $45^0$Celsius against *Enterococcus faecalis* within the root canal and dentinal tubules.

Timothy A. Svec et al in 1977 [71] evaluated the effectiveness of chemo- mechanical preparation with normal saline solution and with a combination of sodium hypochlorite and hydrogen peroxide microscopically. The results indicated that a combination of sodium hypochlorite and hydrogen peroxide was significantly more effective in cleansing the canal system at 1 and 3 mm from the apex. At the 5-mm level, normal saline solution was equally effective as an irrigant.

Byström A et al in 1981 [5] evaluated the procedure of biomechanical preparation in endodontic therapy. Mechanical instrumentation reduced the number of bacteria considerably. Bacteria were eliminated from some canals during the treatment; bacteria persisted in rest of the canals during treatment. However there was no evidence that specific microorganisms were implicated in the persistent infections.

Gordon TM et al in 1981 [25] studied the effect of various concentrations of sodium hypochlorite on vital and necrotic bovine tooth pulp which was exposed to 1%, 3%, and 5% sodium hypochlorite for two to ten minutes. However, 3% and 5% sodium hypochlorite were equally effective in dissolving about three fourths of the vital pulp after two minutes exposure. Sodium hypochlorite at 1%, 3% and 5% were equally effective in dissolving 90% of the necrotic pulp after five minutes exposure.

Byström A et al in 1985 [6] studied the antibacterial effect of irrigating infected root canals with 0.5% and 5 % sodium hypochlorite solutions and found that there was no difference between the antibacterial effect of these two solutions. The combined use of ethylenediamine tetraacetic acid and 5% sodium hypochlorite solution was more efficient than the use of sodium hypochlorite solution alone.

Orstavik D et al in 1990 [60] studied the effect of endodontic irrigants and dressings on bacteria in bovine dentin specimens experimentally infected with *Enterococcus faecalis, Streptococcus sanguis, Escherichia coli, or Pseudomonas aeruginosa*. *Enterococcus faecalis* persisted for at least 10 days after withdrawal of nutrient support, whereas the other 3 organisms died within 4 to 48 hours. Endodontic medicaments were applied to infected specimen for comparison of antibacterial potency. Camphor p-monochlorophenol was generally more efficient than Calasept, and of the irrigants tested, iodine potassium iodide appeared more potent than sodium hypochlorite or chlorhexidine. The presence of a smear layer delayed, but did not eliminate, the effect of the medicaments.

B D Jett et al in 1994 [7] described that enterococci are commensal organisms well suited to survival in intestines and the oral cavity. Enterococcal virulence relating to (i) adherence to host tissues, (ii) invasion and abscess formation, (iii) modulation of host inflammatory responses, and (iv) potentially toxic secreted products. Aggregation substance, surface

carbohydrates, or fibronectin-binding moieties may facilitate adherence to host tissues. Lipoteichoic acid, superoxide production, or pheromones and corresponding peptide inhibitors each may modulate local inflammatory reactions.

J. F. Siqueira J R et al in 1997 [36] evaluated the effectiveness of 4.0% sodium hypochlorite (NaOCl) used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal was tested *in vitro* and found that sodium hypochlorite applied by the three methods tested, was significantly more effective than the saline solution in disinfecting the root canal. He also observed that direct extrapolations to clinical conditions must be exercised with caution because of the obvious limitations of *in vitro* studies.

J. Huque et al in 1998 [39] evaluated intra-canal irrigation procedures in eradicating bacteria from surface, shallow and deep layers of root dentine using extracted human teeth and observed that ultrasonic irrigation with 5.5% and 12% sodium hypochlorite was efficient in eradicating bacteria from artificial smear layer whilst 12% sodium hypochlorite irrigation with a syringe was insufficient. Ultrasonic irrigation with 12% sodium hypochlorite appeared to eliminate bacteria efficiently from surface, shallow and deep layers of root dentine.

Molander A et al in 1998 [55] in their study examined the microbiological status of 100 root-filled teeth with radiographically verified

apical periodontitis. Enterococci were the most frequently isolated genera, showing 'heavy' or 'very heavy' growth in 25 out of 32 cases (78%) and concluded that the microflora of the obturated canal differs from that found normally in the untreated necrotic dental pulp, quantitatively as well as qualitatively. Nonsurgical retreatment strategies should be reconsidered.

Siqueira, José F. Jr et al in 2000 [67] in their in vitro study of intra-canal bacterial reduction produced by instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite (NaOCl) or saline solution. All test solutions significantly reduced the number of bacterial cells in the root canal. There was no significant difference between the three sodium hypochlorite solutions tested. The three sodium hypochlorite concentrations showed large zones of inhibition against *Enterococcus faecalis*.

Buck.R.A et al in 2001 [4] compared three endodontic irrigants for efficiency in killing bacteria established within human dentinal tubules, and found that sodium hypochlorite seemed to be superior to all other irrigants.

B. P. F. A. Gomes et al in 2001 [3] assessed, *in vitro*, the effectiveness of several concentrations of sodium hypochlorite (0.5%, 1%, 2.5%, 4% and 5.25%) and two forms of chlorhexidine gluconate (gel and liquid) in three concentrations (0.2%, 1% and 2%) in the elimination of *Enterococcus faecalis.* Chlorhexidine in the liquid form at all concentrations tested (0.2%, 1% and 2%) and sodium hypochlorite (5.25%) were the most

effective irrigants. The time required to eliminate *Enterococcus faecalis* depended on the concentration and type of irrigant used.

R.M.Love in 2001 [62] tried to identify a possible mechanism that would explain how *Enterococcus faecalis* could survive and grow within dentinal tubules and reinfect an obturated root canal. The results of the study demonstrate that *Enterococucus faecalis* cells remain viable, and maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum. This mechanism may explain why cells of *Enterococcus faecalis* within radicular dentinal tubules act as a pathogen in failed endodontically treated teeth.

Carlos Estrela et al in 2002 [10] in their review of sodium hypochlorite described the important properties of sodium hypochlorite: antimicrobial effect, tissue dissolution capacity and acceptable biologic compatibility in less concentrated solutions (0.5 -1%). In relation to antimicrobial effect, studies have shown that sodium hypochlorite decreases number of microorganism during the treatment of teeth with apical periodontitis. The concentration rise of sodium hypochlorite is directly proportional to the antimicrobial effect and tissue dissolution capacity.

Love.R.M et al in 2002 [42] reported that bacterial invasion of dentinal tubules commonly occurs when dentin is exposed following a breach in the integrity of the overlying enamel or cementum. Recent evidence suggests that

streptococci may recognize components present within dentinal tubules, such as collagen type I, which stimulate bacterial adhesion and intra-tubular growth. Specific interactions of other oral bacteria with invading streptococci may then facilitate the invasion of dentin by select bacterial groupings.

Michael Goldsmith et al in 2002 [54] studied the effect of root-canal irrigation with different concentrations of sodium hypochlorite (3%, 5.1%, 7.3% NaOCl) on the mechanical properties of teeth and found that there was no difference in the strain recorded after irrigation by the different irrigants within the experimental group.

Dr.M.Evans et al in 2002 [56] tried to identify the mechanisms that enable *Enterococcus faecalis* to survive the high pH of calcium hydroxide. Survival of *Enterococcus faecalis* in calcium hydroxide appears to be unrelated to stress induced protein synthesis, but a functioning proton pump is critical for survival of *Enterococcus faecalis*. A functioning proton pump with the capacity to drive protons into the cell and acidify the cytoplasm is critical for survival of *Enterococcus faecalis* at high pH value.

Goran Sundqvist et al in 2003 [24] reviewed the microbial flora in persistent infections. They identified the factors which help *Enterococcus faecalis* in persistent infection survival are: The serine protease and a collagen-binding protein (Ace) are involved in binding *Enterococcus faecalis* to dentine. The intrinsic capacity of *Enterococcus faecalis* to withstand a wide

pH range by Cell-wall-associated proton pump, which drives protons into the cell to acidify the cytoplasm, is important for survival of *Enterococcus faecalis* in a highly alkaline environment. Inherent characteristic of enterococci is an ability to adapt to fluctuating levels of nutrient supply and limitation, and this trait that may facilitate the persistence of *Enterococcus faecalis* in the canal long after root filling.

periodontitis. In mixed infections, *Enterococcus faecalis* typically is the dominant isolate.

J.F.Siqueira Jr et al in 2003 [37] found that endodontic flare ups don't have any significant influence in the outcome of endodontic treatment, its occurrence is extremely undesirable for both the patient and the clinician and can undermine the clinician - patient relationships. Microorganisms are arguably the major causative agents of flare ups, and adoption of preventive measures can significantly reduce the incidence of highly distressing and undesirable clinical phenomenon.

Leif Tronstad et al in 2003 [41] studied more than 700 different bacterial species, of which over 50% have not yet been cultivated, have been detected in the oral cavity. With regard to endodontic infections, to a great extent we are still in the era of bacterial cultivation, although a few groups have taken up molecular methods in their work. This has been verified with bacterial cultivation, checkerboard DNA–DNA hybridization, FISH and electron microscopic demonstration Many more bacteria are found with

hybridization studies than with cultivation Thus, a new understanding of endodontic infections is slowly evolving due to the results of molecular and electron microscopic studies.

Markus Haapasalo et al in 2003 [51] reviewed apical periodontitis and the microbiota responsible. *Enterococcus faecalis* is the dominant microbe in persistent apical periodontitis. *Enterococcus faecalis* penetrated the dentine even in the presence of the smear layer. *Enterococcus faecalis* was also shown to invade the dentinal tubules better than *Actinomyces israelii*. *Enterococcus faecalis* strains survived instrumentation and irrigation with sodium hypochlorite and ethylene diamine tetra acetic acid, none of the yeasts or coliform rods did. Intra-canal calcium hydroxide fails to eliminate *Enterococcus faecalis* from the infected dentine.

Christine Sedgley et al in 2004 [15] discussed the potential role of plasmids in oral and endodontic microbiology. Phenomenon responding plasmids in Enterococcus faecalis are capable of adaptability and stable maintenance in a wide spectrum of bacterial hosts. Clinical strains of *Enterococcus faecalis*, species commonly associated with root canal infections, can carry as many as six co-resident plasmids with different sizes and copy numbers. They also suggested that a combination of both molecular and cultural investigation will be required to provide a more comprehensive understanding of endodontic infection process.

C. E. Radcliffe et al in 2004 [14] determined the resistance of microorganisms associated with refractory endodontic infections to varying concentrations of sodium hypochlorite as a root canal irrigant and found that *Enterococcus faecalis* proved to be more resistant to sodium hypochlorite and this could at least partially be one of the reasons why *Enterococcus faecalis* is associated with refractory endodontic infections. Resistance of sodium hypochlorite to other species was not found in this study.

Dr David Figdor in 2004 [16] in his study on microbial etiology of endodontic failure found that *Actinomyces israeli* has an ability to establish itself in periapical tissues evading host response by collective cohesion. *Enterococcus faecalis* uses a proton pump to withstand a high pH and it can survive long-term starvation. These findings are relevant to the persistence of *Enterococcus faecalis* as a pathogen, since it is likely that *Enterococcus faecalis* may encounter periods of starvation in the root-filled canal. Even under such conditions, a small number of cells can gain the nutritional support required for survival and would therefore have the potential to maintain a periapical lesion.

E. T. Pinheiro et al in 2004 [20] studied the susceptibility of *Enterococcus faecalis* to antimicrobial agents. *Enterococcus faecalis* isolates were completely susceptible, in vitro, to amoxicillin, amoxicillin, clavulanic acid, vancomycin and moxifloxacin. Most isolates were susceptible to

chloramphenicol, tetracycline, doxycycline or ciprofloxacin. Erythromycin and azithromycin were least effective.

Gunnar Bergenholtz et al in 2004 [26] discussed the advances in the study of endodontic infections. Available knowledge in endodontic microbiology derives from classic sampling, laboratory processing and phenotypic identification of root canal bacteria. Advancement of improved methodologies for both sampling and laboratory processing has been crucial to the achievements in this field of endodontology. Molecular methods are the new advent in detection and identification of microorganisms.

In recent years genotypic identification has been applied and used as a tool for reclarification of root canal flora. An introduction of molecular biological technique to identify putative endodontic pathogens have opened up newer exciting prospective and is likely to further our understanding of the complex host, tissue, parasite interactions in apical periodontitis. It is known that adhesion of microorganisms to surfaces triggers altered expressions of a large number of genes which result from phenotypical changes.

These genes may be transferred and shared by different species in a biofilm community and may provide important survival properties to the recipient organism. In this context bacterial plasmids are a great significance as they participate in transfer of DNA.

Gunnel Svensater et al in 2004 [27] reviewed the role of biofilms in endodontic infection and states that surface-associated growth of microorganisms is the cause of most infections has put an emphasis on virulence properties and survival strategies of biofilm bacteria. The biofilm concept to endodontic microbiology will play a crucial role in helping us to understand, not only the pathogenic potential of the root canal microbiota, but also the basis for new approaches to infection control.

Güven Kayaoglu et al in 2004 [28] in their study of virulence factors noted that Enterococcus faecalis appears to possess the requisites to establish an endodontic infection and maintain an inflammatory response potentially detrimental to the host. Factors which help Enterococcus faecalis survive are - aggregating factors, surface adhesions, sex pheromones, lipotecholic acid, extracellular superoxide production, lytic enzymes, gelatinase and hyaluronidase and toxin cytolysin. They also note that primary periradicular lesions are due to mixed microbial flora rather than solely Enterococcus faecalis. Enterococcus faecalis is the frequently dominant and sometimes the only pathogen suggesting this species alone has the potential to maintain root canal infection and periradicular infection.

Ingar Olsen et al in 2004 [31] in their study on the salient virulent factors of anaerobic bacteria in endodontic infections found the microflora of infected root canals is complex. During treatment, the number of species

gradually decreases between appointments, and anaerobes are often eliminated or reduced. Facultative anaerobic and Gram-positive bacteria often predominate in canals of failed endodontic treatments, which may harbour no more than one to two species per canal with favourable environmental factors. Capsule, adhesion, invasion, toxins produced, protease/protease inhibitors, collagenase and immunosupression neutrophil monocyte are said to be some of the main virulance factors in selected endodontic organisms. Although the list of virulence factors and bacteria is not complete it goes some way in understanding the factors and mechanisms that can be involved in endodontic disease.

Luis Cha ´ Vez De Paz in 2004 [47] reviews the role of gram positive bacteria and their adaptive responses when exposed to stressful conditions such as endodontic treatment procedures. While gram negative anaerobes predominate in primary root canal infections, gram positive facultatives tend to become dominating in failing post treatment cases. Certain genera and some species seem to persist to a greater extent than others.

Robert M. Love in 2004 [63] in their study of invasion of radicular dentinal tubules by root canal bacteria state that it is a multi-factorial event that a limited number of oral bacterial species have the necessary properties to participate in. Enterococci possess a number of virulence factors that permit adherence to host cells and extracellular matrix and facilitate tissue invasion *Enterococcus faecalis*, which makes up a small percentage of the flora in

primary root canal infection, is the bacterial species most frequently recovered in root-filled teeth, and often as a pure culture. Recent advances also suggest that Streptococci and Enterococci may recognize components present within dentinal tubules like collagen Type I which stimulate adhesion and intra tubular growth. Specific interaction of other bacteria with invading Streptococci may then facilitate adhesion of dentin by selective bacteria.

Ashraf F. Fouad et al in 2005 [1] describes identification of Enterococcus species in non healing endodontic cases using polymerase chain reaction amplification and molecular sequencing. *Enterococcus faecalis* in primary cases been examined was very less when compared to its dominance in failed cases. *Enterococcus faecalis* was the only enterococcal species detected with an overall prevalence of 22%. Results of the study show that Enterococci may not be present in majority of cases with non healing periradicular lesions and more study are needed to characterize role of Enterococcus faecalis in persistent periradicular lesions.

A. Reynaud af Geijersstam et al in 2005 [2] measured the release of hydrolytic enzymes from human polymorphonuclear leukocytes (PMNs) during interaction with strains of Enterococcus faecalis isolated from endodontic infections. Majority of the *Enterococcus faecalis* strains induced little or no release of hydrolytic enzymes from the polymorphonuclear leukocytes cells. The finding may partly explain the clinical observation that

root canal infections dominated by *Enterococcus faecalis* are usually symptom free.

C. M. Sedgley et al in 2005 [15] studied the survival of *Enterococcus faecalis* in root canals and observed that *Enterococcus faecalis* inoculated into root canals maintained viability for 12-months ex vivo. The clinical implications are that viable *Enterococcus faecalis* entombed at the time of root filling could provide a long-term nidus for subsequent infection if the opportunity arises. Study reveals that *Enterococcus faecalis* has the capacity to recover from a prolonged starvation state in root filled teeth.

George Sirtes et al in 2005 [22] the study evaluated effects of preheating sodium hypochlorite solutions using a commercially available syringe heating device. Solutions remained stable during and after preheating. A 100-fold increase in killing efficacy was observed between corresponding sodium hypochlorite solutions at 20°C and 45°C. Preheating sodium hypochlorite solutions appears to improve their necrotic pulp tissue dissolution capacity and efficacy against stationary phase *Enterococcus faecalis* cells. Heating sodium hypochlorite chair side using a heating device bears the advantage that the desired irrigant temperatures can be reached within a relatively short period of time from stock solution stored in lower temperature.

J. F. Siqueira, Jr et al in 2005 [38] reviewed application of molecular methods in endodontic microbiology for comprehensive characterization of microbiota have revealed a higher complexity of the endodontic microbiota than previously reported by cultivation approaches. In addition to detecting some cultivable species in increased prevalence, molecular methods have expanded the list of putative endodontic pathogen by inclusion of some fastidious bacterial species or even uncultivable bacteria that have never been previously found for endodontic infections. In asymptomatic primary infections *Enterococcus faecalis* was less prevalent whereas asymptomatic persistent infection it had the highest incidence. *Propionibacterium Propionicum* was significantly associated with re treatment cases.

Mariam Abdullah et al in 2005 [49] studied the efficacy of selected root canal irrigants and a medicament on a clinical isolate of *Enterococcus faecalis* grown as biofilm or planktonic suspension phenotype. Irrigants used were: 3% sodium hypochlorite,10% povidoneiodine,0.2% chlorhexidine gluconate,17% ethylene-diamine tetra- acetic acid, calcium hydroxide at pH 12.3.

Sodium hypochlortite was the most effective agent and achieved 100% kills for all presentations of *Enterococcus faecalis* after a 2 min contact time. Based on single species model, the antimicrobial effectiveness of test agents was dependent on bacterial phenotype antimicrobial agent and duration

of contact with the agent. The known effectiveness of sodium hypochlorite was reiterated.

Markus Haapasalo et al in 2005 [52] states that cleaning and shaping of the root canal is the single most important factor in the prevention and treatment of endodontic diseases, and the effects of instrumentation and irrigation on intra-canal infection have been a focus of increased activity in endodontics. Although sterility of the root canal can occasionally be achieved by instrumentation and irrigation with antibacterial solutions, the protocols used today cannot predictably provide sterile canals. As none of the elements of endodontic therapy (host defense system, systemic antibiotic therapy, instrumentation and irrigation, intra-canal medicaments, permanent root filling, and coronal restoration) can alone guarantee complete disinfection.

N. Vivacqua-Gomes et al in 2005 [59] studied the recovery of *Enterococcus faecalis* after root canal treatment in single or multiple visits in an ex vivo model. Neither single- nor multiple-visit root canal treatment ex vivo, eliminated *Enterococcus faecalis* completely from dentinal tubules. Up to 60 days after root filling, *Enterococcus faecalis* remained viable inside dentinal tubules. When no sealer was used, *Enterococcus faecalis* presented a higher growth rate.

Russell S. Eddy et al in 2005 [64] - Studied the ability of chlorine dioxide & sodium hypochlorite to eliminate *Enterococcus faecalis* from

dentinal tubules of bovine incisors. Chlorine dioxide and sodium hypochlorite were both effective in eliminating E. faecalis from the dentinal disks within 30 min. sodium hypochlorite had the lowest bacterial count and emerged a potent anti - microbial agent.

S. George et al in 2005 [69] studied effect of different growth conditions on the characteristics of *Enterococcus faecalis* biofilm on root canal, and the penetration of *Enterococcus faecalis* into dentinal tubules. Microscopic analysis highlighted a distinct variation in the ultrastructure of the biofilms formed under different experimental conditions. The study demonstrated distinct ultrastructural and physiochemical properties of biofilms formed and dentinal tubular penetration of *Enterococcus faecalis*. Study showed that development and modification of *Enterococcus faecalis* biofilm of root canal and its penetration into dentinal tubules was modulated by the prevailing environmental conditions.

Brenda P. F. A et al in 2006 [3] described the presence of *Enterococcus faecalis* in endodontic infections by culture and polymerase chain reaction analyses. Using a nested polymerase chain reaction technique, showed that *Enterococcus faecalis* is frequently detected not only in secondary endodontic infection but also in the primary infection. Therefore, *Enterococcus faecalis* involved in the etiology of endodontic failure may be those originally present in the necrotic pulps that have survived to the chemo-

mechanical procedures and intra-canal medicaments, which caused ecological changes in the root canals. Polymerase chain reaction technique only detected the target species, but did not enumerate the total number of bacteria present in the samples. *Enterococcus faecalis* was detected as frequently in teeth with necrotic pulp as in teeth with failing endodontic treatment when a polymerase chain reaction technique analysis was used.

Charles H. Stuart et al in 2006 [11] studied role of *Enterococcus faecalis* in root canal treatment failure and found prevalence of *Enterococcus faecalis* is low in primary endodontic infections and high in persistent infections (24 – 77%). *Enterococcus faecalis* is also more commonly associated with asymptomatic cases than with symptomatic ones. *Enterococcus faecalis* possesses several virulence factors, its ability to cause periradicular disease stems from its ability to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth. Currently, use of good aseptic technique, increased apical preparation sizes, and inclusion of full strength sodium hypochlorite and 2% chlorhexidine irrigants are the most effective methods to eliminate *Enterococcus faecalis*. Also noted that in the changing face of dental care continued research on *Enterococcus faecalis* and its elimination from the dental apparatus may well define the future of endodontic speciality.

G. O. Zoletti et al in 2006 [29] studied the prevalence of bacterial species in root-filled teeth with or without periradicular lesions by using culture dependent and independent approaches. Identification of *Enterococcus faecalis* was carried out by polymerase chain reaction (PCR) or conventional culture procedures. The molecular biology method revealed the occurrence of this species in 80% of the cases, while only 16% of the cases were positive after culture identification. One possible reason for differences is the ability of molecular methods to detect DNA from dead cells. The higher sensitivity of molecular biology methods, particularly polymerase chain reaction, when compared with culture. Regardless of the identification technique used, no significant difference was observed when comparing the occurrence of *Enterococcus faecalis* in root-filled teeth with and without periradicular lesions. Host resistance can vary from subject to subject and may have a different pattern of response to microbial infection. It's well known that not all clonal types within a given species are able to cause disease. The possibility exists that more virulent clones of Enterococcus faecalis were present in teeth evincing periradicular diseases. Till all these issues are properly addressed. A definitive conclusion that Enterococcus Faecalis is not the major pathogen associated with persistent periradicular diseases cannot be drawn.

Janir Alves Soares et al in 2006 [33] described the influence of sodium hypochlorite based irrigants on the susceptibility of intra canal

microbiota to biomechanical preparation.1%, 2.5% and 5% sodium hypochlorite (NaOCl) irrigants were used. After irrigation with 5% sodium hypochlorite, only structural arrangements consisting of Gram-positive cocci and bacilli persisted. Thus, BMP plus 5% sodium hypochlorite offered the best antiseptic potential because in the few positive cultures a significant reduction in the number of microbiological morphotypes was shown. Therefore biomechanical preparation with 5% sodium hypochlorite will decrease the number of microorganisms in the main root canal for the intra-canal dressing to eliminate.

John R. Bowden et al in 2006 [34] described a case report where sodium hypochlorite solution extruded beyond the apex of the root canal into the surrounding tissues. Where rapid, life-threatening swelling of the floor of mouth with airway secondary obstruction occurred following extrusion of sodium hypochlorite from the apex of a lower second molar. Management was done by intubation, decompression of tissue spaces and treatment with antibiotics and steroids.

Matthias Zehnder in 2006 [53] reviewed irrigants and recommends sodium hypochlorite solutions as the main irrigant solution. This is because of their broad antimicrobial spectrum as well as their unique capacity to dissolve necrotic tissue remnants. Efficacy of sodium hypochlorite can be increased by lowering their pH, pre heating, ultrasonic irrigation etc.

Seung-Eun Yang et al in 2006 [66] determined the effects of a smear layer and chlorhexidine (CHX) treatment on the adhesion of *Enterococcus faecalis* to bovine dentin. These results suggest that a smear layer enhances the adherence of *Enterococcus faecalis* to the dentin; chlorhexidine is effective in reducing the adherence of microorganisms. This study also showed that smear layer removal presented the initial colonization of *Enterococcus faecalis.*

Thomas R. Dunavant et al in 2006 [70] compared the efficacy of root canal irrigants against *Enterococcus faecalis* biofilms using a novel in vitro testing system. BioPure, MTAD, chlorhexidine, ethylene diamine tetra acetic acid, sodium hypochlorite were used as irrigants, 1% sodium hypochlorite and 6% sodium hypochlorite were more efficient in eliminating *Enterococcus faecalis* biofilm than the other solutions tested.

V. B. Berber et al in 2006 [72] evaluated the efficacy of 0.5%, 2.5% and 5.25% sodium hypochlorite (NaOCl) as intra-canal irrigants associated with hand and rotary instrumentation techniques against *Enterococcus faecalis* within root canals and dentinal tubules. At all depths and thirds of the root canals and for all techniques used, 5.25% sodium hypochlorite was shown to be the most effective irrigant solution tested when dentinal tubules were analyzed, followed by 2.5% sodium hypochlorite. No differences among concentrations in cleaning the canals were found. Especially at higher

concentration Sodium hypochlorite was able disinfect dentinal tubules, independent of canal preparation technique used.

William J. Kowalski et al in 2006 [73] evaluated the attachment by an enzyme linked immunosorbent developed to assess *Enterococcus faecalis* adhesion to particulate dentin. Definitive evidence states that collagen is an attachment site for *Enterococcus faecalis* and that Ace can mediate that attachment.

Chankhrit Sathorn et al in 2007 [13] compared the culturing method and treatment outcome and reported that intra canal sampling technique suffer from deficiencies that limit their predictive value. It emphases the need for more detailed clinical study of bacterial status and healing as well as refinement of technique for microbial sampling of canals. Microbial control fundamental to healing of apical periodontitis is central to endodontic practice. The effectiveness of antibacterial measures is generally monitored (in clinical research studies) by microbiological root canal sampling (MRS), which is often used as a predictor for healing. Chances of false negative, false positive, incidental false positive and incidental false negative results question the integrity of culturing.

Dilsah Cogulu et al in 2007 [17] studied the presence of *Enterococcus faecalis* in endodontic infections in both deciduous and permanent teeth by culture and polymerase chain reaction (PCR) methods. The results of the

present study confirm that both culture and polymerase chain reaction methods are sensitive to detect *Enterococcus faecalis* in root canals.

Radeva et al in 2007 [19] in this study on effectiveness of intra canal irrigants on *Candida Albicans* observed that among the solutions used for irrigating the root canals, 6% sodium hypochlorite was the most effective, followed by the 17% ethylene diamine tetra acetic acid. Frequently isolated pathogens from teeth with necrotic pulp include various enteric bacteria (*Klebsiella, Enterobacter*), fungi (especially *Candida spp.)* and enterococci (*Enterococcus faecalis*). When using different endodontic irrigants and intra-canal medicaments one has to keep in mind that in the infected root canal there are microorganisms that are quite resistant to the chemical and mechanical procedures (*Candida albicans, E. faecalis*). Decrease in the concentration significantly reduces the effectiveness of Sodium hypochlorite against *Candida Albicans*. The 2% Chlorehexidine is more effective than the 3% sodium hypochlorite in eliminating *Candida Albicans*.

J. Kampfer et al in 2007 [40] evaluated the hypothesis that food-borne viable *Enterococcus faecalis* cells could enter the root canal space via coronal leakage. Leakage of viable food-borne *Enterococcus faecalis* cells occurred in teeth with unsealed access cavities, as well as in some of the teeth sealed with temporary or even permanent filling materials. Results of study

substantiate the suspicion that food derived microbiota could enter the necrotic root canal system via microleakage.

Luciano Giardino et al in 2007 [44] compared the antimicrobial efficacy of 5.25% sodium hypochlorite, BioPure MTAD and Tetraclean against Enterococcus faecalis biofilm generated on cellulose nitrate membrane filters. 5.25% sodium hypochlorite can disgregate and remove the biofilm at every time; however, treatment with Tetraclean caused a high degree of biofilm disgregation in every considered time intervals as compared with MTAD. According to this work, further studies should be performed to understand the correct action and the correct sequence of different irrigants against bacteria both in the planktonic phase and organized in biofilm on the surface of the root canal wall or inside the dentinal tubules.

Luis Chávez de Paz in 2007 [47] reviewed possible role of biofilm communities observes that persisting infections subsequent to endodontic therapy are caused by one or two bacterial species that are “too robust” to be eliminated by conventional treatment measures. The ability of the organisms in such infections to form biofilms can be seen as the most important adaptive mechanism used by bacteria to survive the environmental changes resulting from the treatment protocol. Thus, from the realization that all oral microorganisms are capable of forming a biofilm and that such surface-associated communities exist in root canals, it is possible to apply the “biofilm

concept" to clinical treatment; that is, efforts should not be directed to specific individual organisms, but to a group of well-adapted organisms undoubtedly possessing increased resistance to a variety of antimicrobial agents.

L.W.M.Van Der Sluis et al in 2007 [48] described that passive ultrasonic irrigation can be performed with a small file or smooth wire (size 10–20) oscillating freely in the root canal to induce powerful acoustic microstreaming. Passive ultrasonic irrigation can be an important supplement for cleaning the root canal system and, compared with traditional syringe irrigation; it removes more organic tissue, planktonic bacteria and dentine debris from the root canal. Passive ultra sonic irrigation is more effective than syringe irrigation.

Makiko Iwanami et al in 2007 [50] evaluated the spreading of root canal irrigants on human root dentine. Irrigants used in the study where distilled water, sodium hypochlorite, ethylene diamine tetraacetic acid and cetrimide (Morhonine) individually the combination of Morhonine- sodium hypochlorite most significantly increased among all of the experimental groups.

M. Marending et al in 2007 [57] investigated the mechanical, chemical and structural alterations of human root dentine following exposure to ascending sodium hypochlorite concentrations. Observations made: Sodium hypochlorite caused a concentration dependent reduction of elastic modulus and flexural strength in human root dentine bars. Similar to the

effects on mechanical properties, the reduction of C and N analysis in the specimens was a function of hypochlorite concentration. Sodium hypochlorite made altered intertubular dentine permeable to basic fuchsin dye, although no effect of hypochlorite on inorganic dentine components could be detected in backscattered (SEM). As visualized on scanning electron microscope 3D reconstructions of the exposed dentine surface after demineralization, 5% sodium hypochlorite severely altered the peripheral dentine matrix. The current data link the concentration dependent sodium hypochlorite effect on mechanical dentin properties with dissolution of organic dentin components.

Saji George et al in 2007 [65] determined (1) the hydrophobicity of selected oral bacteria, (2) the influence of growth media (saliva and serum) and mode of growth (planktonic or biofilm) on the hydrophobicity of *Enterococcus faecalis*, and (3) the influence of growth media and conditioning fluids on the adherence of *Enterococcus faecalis* to dentin. The hydrophobicity of *Enterococcus faecalis* was significantly increased during starvation and biofilm mode of growth. The adherence of *Enterococcus faecalis* to dentin was appreciably increased after starvation and when dentin was conditioned with saliva. It was observed that surface conditioning of dentin with saliva and starvation can enhance the adherence of *Enterococcus faecalis* to dentin. The findings from this study indicated that the coronal leakage of saliva and the physiologic state of microbes might play

an important role in the adherence and biofilm formation of bacteria to root canal dentin.

Carlos Estrela et al in 2008 [9] studied the efficacy of the sodium hypochlorite (NaOCl) and chlorhexidine (CHX) on *Enterococcus faecalis* was evaluated by systematic review and meta-analysis. The disinfection of the root canal system produced by emptying, enlargement and action of sodium hypochlorite reduces the remaining endodontic microbiota, which optimizes the efficacy of the intra-canal dressing and favors the achievement of a higher level of success of the endodontic treatment. Sodium hypochlorite and showed low ability to eliminate *Enterococcus faecalis* when evaluated by either polymerase chain reaction or culture techniques.

Ebtissam M. Al-Madi in 2008 [18] compared the intra-canal bacterial reduction using rotary instrumentation and intermittent passive ultrasonic irrigation (IPUI) with different concentrations and temperatures of sodium hypochlorite in different canal tapers. Use of passive ultrasonic irrigation of sodium hypochlorite for 30 second periods intermittently throughout canal preparation of infected root canals can be significantly enhanced by changing irrigant variables, as well as canal taper. Complete bacterial eradication can be achieved with intermittent passive ultra sonic irrigation and 5% sodium hypochlorite at room temperature or 2.5% sodium hypochlorite at 45$^{o}$Celsius. In addition, significant bacterial reduction in

contaminated root canals can be attained using intermittent passive ultra sonic irrigation with 2.5% sodium hypochlorite at 37$^{o}$Celsius or when canal taper is increased to 0.06.

Giampiero Rossi-Fedele et al in 2008 [23] evaluated the tissue dissolution ability of 4% sodium hypochlorite at increasing temperatures with the use of a baby bottle warmer. Heating sodium hypochlorite with baby bottle warmer increases bovine pulp dissolution speed, but reached a plateau when the temperature reached 60°Celcius – 75$^{0}$Celcius. The easy and safe use of such warmers could add to clinical efficiency of 4% sodium hypochlorite as regards to dissolution effect.

Hanan A. Balto in 2008 [30] evaluated in his study described the effect of different variables (delivery system, surface contact with the irrigant, the frequency of changing the irrigant and the total volume of the irrigant) of sodium hypochlorite on the elimination of *Enterococcus faecalis* using rotary instrumentation and intermittent passive ultrasonic irrigation. The flushing action, the larger volume of irrigant and increasing the contact time of the irrigant combined with intermittent passive ultra sonic irrigation and rotary instrumentation increased the elimination of *Enterococcus faecalis* from the root canals. Intermittent passive ultrasonic irrigation with larger volume of irrigant reduced bacterial colonization from the main root canal space in a short period of time.

Luciana M. Sassone et al in 2008 [43] evaluated the antimicrobial capacity of sodium hypochlorite (1% and 5%) and chlorhexidine (0.12%, 0.5% and 1%) with or without the addition of organic material (bovine serum albumin, BSA) against different bacterial samples. Using contact agar diffusion tests 0.12% CHX was ineffective against *Enterococcus faecalis* using the contact test while, in general, 0.5% chlorhexidine, 1% chlorhexidine, 1% sodium hypochlorite and 5% sodium hypochlorite was antibacterial against all tested bacterial strains.

Luciana M. Sassone et al in 2008 [45] studied the composition of the microbiota of primary endodontic infections associated with symptomatic teeth. The species found in higher counts in symptomatic cases were *Fusobacterium nucleatum ssp. vincentii, Veillonella parvula, Treponema socranskii, Enterococcus faecalis, and Campylobacter gracilis* and in asymptomatic cases were *Fusobacterium nucleatum ssp. Vincentii, Fusobacterium nucleatum ssp. Nucleatum, Enterococcus faecalis, Eubacterium saburreum, and Neisseria mucosa.* The data of the investigation suggested an association between higher total bacterial counts and levels of *Treponema forsythia* and the presence of pain.

P. Chivatxaranukul et al in 2008 [61] in their study of dentinal tubule invasion and adherence by Enterococcus faecalis observed that the Enterococcus faecalis readily invaded tubules, it did not adhere preferentially

to tubule walls. Initially bacteria were adhered to fractured odontoblasts then to dentinal tubule walls. Initial colonization of dentinal tubules by *Enterococcus faecalis* may depend primarily on other factors. Therefore, the initial colonization of dentinal tubules by *Enterococcus faecalis* may be primarily dependent on other factors such as the environmental conditions inducing surface receptor related gene expression.

In evidence based review in JOE 2009[21]of clinical studies an endodontic microflora observed that molecular analysis of the microflora associated with endodontic infections (from a limited patient population) has shown it to be far more diverse than shown by culture alone and to include numerous as-yet-uncultivable organisms. The application of molecular analysis and the identification by DNA sequence comparison allied with polyphasic taxonomic studies make the description of the oral microflora in its entirety a feasible objective in the short- to medium-term.

Jörg F. Schirrmeister et al in 2009 [35] attempted to isolate and detect new bacterial microorganisms of root-filled teeth associated with periradicular lesions. *Enterococcus faecalis* was the only detected species. *Enterococcus faecalis* was found to be the predominant species in root-filled teeth in which therapy had failed. *S. moorei, F. nucleatum, Atopobium rimae, Dialister invisus, and V. fluvialis. Olsenella uli, Slackia exigua, and Megasphaera spp.*where the other bacteria detected in this study.

M. Zehnder et al in 2009 [58] the pulpless root canal appears to be a habitat for *Enterococcus faecalis*. A more likely explanation for the high occurrence of enteroccci in filled root canals is that they enter after treatment. Enterococci do not appear to be colonizers of the oral cavity. They are merely transient oral bacteria, unless there is a predilection site such as the unsealed necrotic or filled root canal. The origin of this infection is most likely food. On a less sophisticated level, contamination of different enterococcal species in typical regional foods could be compared with the recovery of these species from root canals in a given country or area. Last but not least, it has been known for a long time that the healthy microbiota of the oral cavity can defend against potential pathogens. It would be interesting to identify the mechanisms preventing the colonization of the oral cavity by enterococci.

# ARMAMENTARIUM

**SAMPLE PREPARATION:**

1. Normal saline (Nirlife Health Care, Nirma Products, India)
2. 10% Hydrogen Peroxide (Nice chemicals Pvt Ltd, India)
3. 2.5% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
4. Ultra sonic Scaler (E.M.S)
5. Diamond water cooled disc
6. K file (Dentsply, Maillefer, Ballaigues, Switzerland)
7. Protaper system (Dentsply Maillefer, Ballaigues, Switzerland)
8. X –Smart (Dentsply Maillefer, Ballaigues, Switzerland)
9. Digital ultrasonic cleaner
10. Endosonic tips(E.M.S)
11. 5% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
12. 17% EDTA solution (Nice chemicals Pvt Ltd, India)
13. 5% Sodium Thiosulphate solution (Nice chemicals Pvt Ltd, India)
14. Distilled water

**GROUPING AND STERILIZATION:**

15. Composite resin (3M ESPE Filtek Z350)
16. Phosphate bonded investment material
17. Self sealing autoclavable pouches
18. Autoclave

**CULTURING & INOCULATION:**

19. Laminar air hood flow
20. Brain Heart Infusion Broth (HIMEDIA)
21. Incubator
22. 1ml tuberculine syringe (DISPOVAN ,India)

**IRRIGATION:**

23. 10ml syringe (DISPOVAN,India)
24. 5% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
25. 3% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
26. 1% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
27. 5% Sodium Thiosulphate solution (Nice chemicals Pvt Ltd, India)
28. Saline (Nirlife Health Care, Nirma Products, India)
29. Digital thermometer
30. Digital temperature controlled water bath

**SAMPLING & CULTURING:**

31. Paper point (Dentsply, Maillefer, Ballaigues, Switzerland)
32. Locking tweezers
33. Saline (Nirlife Health Care, Nirma Products, India)
34. Disposable petri dishes (HIMEDIA)
35. Glass “L” rod
36. Incubator

## COLLECTION OF SPECIEMEN

One hundred single rooted human upper anterior teeth were collected immediately after extraction and stored in normal saline (Nirlife Health Care, Nirma Products, India). The teeth were subsequently placed in solutions of 10% hydrogen peroxide (Nice chemicals Pvt Ltd, India) overnight, washed thoroughly and then placed in 2.5% sodium hypochlorite solution (Nice chemicals Pvt Ltd, India) for 24hrs.The specimens were then washed thoroughly and examined. All the external debris was cleaned using an ultra sonic device. Teeth with dilacerations, decreased root length, caries etc were discarded.

Sixty single-rooted human upper anterior teeth were selected for the study & were stored in normal saline. The root length was standardized to 15mm by horizontally sectioning the teeth at the cemento - enamel junction with water cooled diamond disc.

## SAMPLE PREPARATION

After ensuring apical patency with a 10 size K file (Dentsply, Maillefer, Ballaigues, Switzerland), the root canals and the apical foramen were enlarged with K-files up to size 20, under irrigation with distilled water at a working length 16mm to open out the apex. Then all samples were prepared by using Protaper system (Dentsply Maillefer, Ballaigues, Switzerland) and a X-Smart endomotor with 16:1 reduction handpiece (Dentsply, Maillefer, Ballaigues, Switzerland) was used at 250rpm with

distilled water irrigation. Canals were enlarged up to size F3 as per the manufacturer's recommendation.

After completion of the preparation, the enlarged apical foramen was sealed with composite resin (3M ESPE Filtek Z350) to prevent bacterial leakage. Ultrasonic irrigation was done with 5.25% sodium hypochlorite (Nice chemicals Pvt Ltd, India) samples were irrigated with distilled water. Subsequently, the samples were then placed in an ultrasonic bath with 17% ehlyene diamine tetraacetic acid solution (Nice chemicals Pvt Ltd, India) for 10mins. They were subsequently placed in 5.25% sodium hypochlorite solution for 10mins in the ultrasonic bath. Sodium hypochlorite was deactivated with 5% Sodium thiosulphate (Nice chemicals Pvt Ltd, India) solution. The samples were then washed and stored in distilled water for 24hrs.

**GROUPING AND STERILIZATION**

Teeth were separated into groups of 5 teeth each which were mounted in blocks made of phosphate bonded investment material. They were allowed to set and were stored in sterile autoclavable pouches.

All the blocks were autoclaved at $134^0$Celsius, 1 atm, for 20 min. Then, they were kept in an incubator at $37\ ^0$Celsius for 48 hrs.

## INOCULATION OF SAMPLES WITH ENTEROCOCCUS FAECALIS

*Enterococcus faecalis* in lyophilized form was acquired from Microbial Type Culture Collection and Bank, Institute Of Microbial Technology, Chandigarh, India. (S.T NO: AAATC 2716 R ST005)

100ml of Brain Heart Infusion broth (BHI) (HIMEDIA) was prepared and sterilized in a conical flask. Lyophilized culture of *Enterococcus faecalis* was inoculated into the broth and kept for incubation for 48hrs.

After 48hrs of incubation it was streaked on Brain Heart Infusion agar plate for a single isolate. Then the isolate was transferred to Brain Heart Infusion broth and incubated at 37 $^{0}$Celsius for 48 hrs and used for the study.

Uniform suspension of *Enterococcus faecalis* was made by placing the culture tubes in vortex mixture. Each root canal was completely filled with *Enterococcus faecalis* suspension using sterile 1 ml tuberculin syringe. The blocks were then placed in an incubator at 37° Celsius for 48 hours.

## GROUPING AND PROTOCOLS

Samples were divided into three groups, each group comprising of three blocks of five teeth each and fifteen teeth were taken as control comprising of three blocks of five teeth each.

The groups are as follows:

- Group I where 5% sodium hypochlorite was used to irrigate infected tooth samples at varying temperatures of $37^0$ Celsius, $24^0$ Celsius and $45^0$ Celsius.
- Group II where 3% sodium hypochlorite was used to irrigate infected tooth samples at varying temperatures of $37^0$ Celsius, $24^0$ Celsius and $45^0$ Celsius.
- Group III where 1% sodium hypochlorite was used to irrigate infected tooth samples at varying temperatures of $37^0$ Celsius, $24^0$ Celsius and $45^0$ Celsius.
- Group IV – Control - where saline was used to irrigate infected tooth samples at varying temperatures of $37^0$ Celsius, $24^0$ Celsius and $45^0$ Celsius these samples acted as the control samples.

The irrigants in the respective groups were heated to the desired temperatures using a water bath. The actual temperatures were continuously monitored by using a digital thermometer with a probe. 10ml of each irrigant was used for irrigation and the solution left insitu for 15minutes.After 15minutes the samples were rinsed with equal amounts of 5% sodium thiosulphate solution to deactivate the sodium hypochlorite.

**SAMPLING AND CULTURE**

1200ml of Brain Heart Infusion agar was prepared and poured into 60 disposable petri dishes and kept for solidification under laminar air flow hood. Canals were filled with Brain Heart Infusion broth. Samplings of the canals were done by inserting sterile paper point (Dentsply, Maillefer, Ballaigues, Switzerland). Tweezers used for manipulating the paper points were sterilized over open flame before using. Sampling was done by inserting paper points to the full working length and rotating them. Each paper point was transferred to 10ml of sterile saline solution and vortexed . 1ml of sample was taken and serially diluted in order to get a dilution of $10^{-1}$,$10^{-2}$...$10^{-5}$.

From the dilutions 0.1ml of sample was taken using micro pipette and inoculated into Brain Heart Infusion agar plates. Using glass "L" rods the sample was uniformly spread (Spread plate method). Plates were incubated at 37 $^{0}$C for 48 hrs.

After 48hrs of incubation typical *Enterococcus faecalis* colonies were counted in all plates and results were tabulated. ANOVA was used with logarithmic transformation (P = 0.05).

All the procedures above were performed under a laminar airflow hood under aseptic conditions.

Table : I

| 45 Degree Celsius | | | | | |
|---|---|---|---|---|---|
| IRRIGANTS | **S1** | **S2** | **S3** | **S4** | **S5** |
| 5% SODIUM HYPOCHLORITE | 1 | 0 | 0 | 0 | 0 |
| 3% SODIUM HYPOCHLORITE | 4 | 1 | 2 | 1 | 2 |
| 1% SODIUM HYPOCHLORITE | 5 | 3 | 3 | 1 | 3 |
| SALINE | 5 | 6 | 8 | 8 | 10 |

## STATISTICAL ANALYSIS

## ANOVA

| | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Saline | Between Groups | 7.200 | 1 | 7.200 | 2.700 | .019 |
| | Within Groups | 8.000 | 3 | 2.667 | | |
| | Total | 15.200 | 4 | | | |
| 3% | Between Groups | 5.000 | 1 | 5.000 | 15.000 | .030 |
| | Within Groups | 1.000 | 3 | .333 | | |
| | Total | 6.000 | 4 | | | |
| 1% | Between Groups | 5.000 | 1 | 5.000 | 5.000 | .011 |
| | Within Groups | 3.000 | 3 | 1.000 | | |
| | Total | 8.000 | 4 | | | |

Dependent Variable – 5% Sodium Hypochlorite

Independent Variable – 1% Sodium Hypochlorite, 3% Sodium Hypochlorite and Saline

At 5% level of significance, it is found that there is a significant difference between the dependent variable and the independent variables.

It can be concluded that 5% Sodium Hypochlorite is comparatively better than the other.

**Interpretation:**

1. The significant value between Saline and 5% Sodium hypochlorite is **0.019**, which means that there is a significant difference between them.

2. The significant value between 3% and 5% Sodium hypochlorite is **0.030**, which means that there is a significant difference between them.

3. The significant value between 1% and 5% Sodium hypochlorite is **0.011**, which means that there is a significant difference between them.

Table : II

| 37 Degree Celsius | | | | | |
|---|---|---|---|---|---|
| IRRIGANTS | **S1** | **S2** | **S3** | **S4** | **S5** |
| 5% SODIUM HYPOCHLORITE | 2 | 1 | 1 | 2 | 2 |
| 3% SODIUM HYPOCHLORITE | 4 | 3 | 4 | 5 | 8 |
| 1% SODIUM HYPOCHLORITE | 8 | 7 | 8 | 7 | 12 |
| SALINE | 21 | 15 | 11 | 9 | 8 |

## STATISTICAL ANALYSIS
## ANOVA

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 3% | Between Groups | 5.633 | 1 | 5.633 | 11.266 | .031 |
| | Within Groups | 1.500 | 3 | .500 | | |
| | Total | 7.133 | 4 | | | |
| 1% | Between Groups | 14.500 | 1 | 14.500 | 16.111 | .022 |
| | Within Groups | 2.700 | 3 | 0.900 | | |
| | Total | 17.200 | 4 | | | |
| Saline | Between Groups | 34.800 | 1 | 34.800 | 5.800 | .039 |
| | Within Groups | 18.000 | 3 | 6.000 | | |
| | Total | 112.800 | 4 | | | |

Dependent Variable – 5% Sodium Hypochlorite

Independent Variable – 1% Sodium Hypochlorite, 3% Sodium Hypochlorite and Saline

At 5% level of significance, it is found that there is a significant difference between the dependent variable and the independent variables.

It can be concluded that 5% Sodium Hypochlorite is comparatively better than the other.

**Interpretation:**

1. The significant value between Saline and 5% Sodium hypochlorite is **0.039**, which means that there is a significant difference between them.

2. The significant value between 3% and 5% Sodium hypochlorite is **0.031**, which means that there is a significant difference between them.

3. The significant value between 1% and 5% Sodium hypochlorite is **0.022**, which means that there is a significant difference between them.

Table : III

| 24 Degree Celsius | | | | | |
|---|---|---|---|---|---|
| IRRIGANTS | **S1** | **S2** | **S3** | **S4** | **S5** |
| 5% SODIUM HYPOCHLORITE | 2 | 2 | 4 | 1 | 5 |
| 3% SODIUM HYPOCHLORITE | 2 | 2 | 2 | 3 | 3 |
| 1% SODIUM HYPOCHLORITE | 6 | 6 | 6 | 7 | 8 |
| SALINE | 21 | 10 | 8 | 17 | 15 |

## STATISTICAL ANALYSIS
## ANOVA

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 3% | Between Groups | 15.603 | 3 | 5.201 | 3.467 | .041 |
| | Within Groups | 1.500 | 1 | 1.500 | | |
| | Total | 17.103 | 4 | | | |
| 1% | Between Groups | 24.480 | 3 | 8.160 | 4.800 | .040 |
| | Within Groups | 1.700 | 1 | 1.700 | | |
| | Total | 26.180 | 4 | | | |
| Saline | Between Groups | 56.349 | 3 | 18.783 | 4.174 | .046 |
| | Within Groups | 4.500 | 1 | 4.500 | | |
| | Total | 60.849 | 4 | | | |

Dependent Variable – 5% Sodium Hypochlorite

Independent Variable – 1% Sodium Hypochlorite, 3% Sodium Hypochlorite and Saline

At 5% level of significance, it is found that there is a significant difference between the dependent variable and the independent variables.

It can be concluded that 5% Sodium Hypochlorite is comparatively better than the other.

**Interpretation:**

1. The significant value between Saline and 5% Sodium hypochlorite is **0.046**, which means that there is a significant difference between them.

2. The significant value between 3% and 5% Sodium hypochlorite is **0.041**, which means that there is a significant difference between them.

3. The significant value between 1% and 5% Sodium hypochlorite is **0.040**, which means that there is a significant difference between them.

Table : IV

| 5% SODIUM HYPOCHLORITE AT VARIOUS TEMPERATURES | | | | | |
|---|---|---|---|---|---|
| IRRIGANTS | **S1** | **S2** | **S3** | **S4** | **S5** |
| 5% SODIUM HYPOCHLORITE | 1 | 0 | 0 | 0 | 0 |
| 5% SODIUM HYPOCHLORITE | 2 | 1 | 1 | 2 | 2 |
| 5% SODIUM HYPOCHLORITE | 2 | 2 | 4 | 1 | 5 |

## STATISTICAL ANALYSIS
## ANOVA

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 37 Degrees | Between Groups | 6.000 | 3 | 2.000 | 1.000 | .046 |
| | Within Groups | 2.000 | 1 | 2.000 | | |
| | Total | 8.000 | 4 | | | |
| 24 Degrees | Between Groups | 5.500 | 3 | 1.833 | 3.667 | .032 |
| | Within Groups | .500 | 1 | .500 | | |
| | Total | 6.000 | 4 | | | |

Dependent Variable – 5% Sodium Hypochlorite at 45 degrees.

Independent Variable – 5% Sodium Hypochlorite at 24 degrees and 5% Sodium Hypochlorite at 37 degrees.

At 5% level of significance, it is found that there is a significant difference between the dependent variable and the independent variables.

It can be concluded that 5% Sodium Hypochlorite at 45 degrees is comparatively better than the other.

**Interpretation:**

1. The significant value between 5% Sodium Hypochlorite at 45 degrees and 5% Sodium hypochlorite at 37 degrees is **0.046**, which means that there is a significant difference between them.

2. The significant value between 5% Sodium Hypochlorite at 45 degrees and 5% Sodium hypochlorite at 24 degrees is **0.032**, which means that there is a significant difference between them.

In both the cases, the 'p' value is less than 0.05, so there is a significant difference between 5% at 45 degrees and the other two, which means that 5% Sodium Hypochlorite at 45 degrees is better.

**Note:**

**If the 'p' value is less than 0.05. we conclude that there is a significant difference between the two variables.**

**If the 'p' value is more than 0.05. we conclude that there is no significant difference between the two variables.**

The primary objective of the root canal therapy is to three dimensionally obturate the debrided canals with a biocompatible filling material, in order to eliminate a source of infection from the residual organic material or from apical percolation. The most common cause of apparent failure in endodontically treated teeth is apical percolation resulting from incomplete canal obturation.

There are various causes by which the pulp gets infected, it could be a sequelae to a profound carious lesion or cracks of the crown extending to the pulp chamber etc. Regardless of the pathway of entry of microorganisms a clear distinction should be made between a vital and a non vital tooth. Pulpitis is the host reaction to opportunistic pathogen entering the endodontium. Normally vital pulp tissue can defend against microorganisms and is largely non infected until it gradually becomes necrotic. Once teeth become nonvital the pulp space of teeth with radiographic signs of periapical rarefaction always harbours cultivable microorganisms.

In treatment of vital teeth focus is always on the prevention of entry of microorganisms entering a primarily sterile environment which is the apical portion of root canal (Asepsis). In nonvital teeth focus shifts to removal of all microorganisms from within the root canal space (Anti sepsis). As the host defense loses its access to the pulp space opportunistic microorganisms which can survive harsh ecological conditions and low oxygen environment aggregate in the root canal space. They primarily survive on organic pulp tissue remnants and exudates from periodontium.

Clusters of microorganisms in necrotic teeth with failed root canal treatment are typically found in the apical root canal area where they have access to tissue fluids.

In long standing infections these microorganisms in root canal space invade the adjacent dentin via the open dentinal tubules (Love R.M 2001)[62]. Primary root canal infections are polymicrobial, typically dominated by obligately anaerobic bacteria. The most frequently isolated microorganisms before root canal treatment include *Gram negative anaerobic rods, Gram positive anaerobic cocci, Gram positive and anaerobic facultative rods, Actinomyces, Propionicum bacterium, Bifidobacterium, Rothia, Eubacterium, Lactobacillus species and Gram positive facultative Streptococcus species*.(Love.R.M 2001) [62] (Leif Tronstad et al 2003) [41].

The obligate anaerobes are rather easily eradicated during root canal treatment. The facultative bacteria such as *Nonmutans Streptococci, Enterococci, Lactobacilli and Actinomyces* once established are more likely to survive chemo-mechanical instrumentation and root canal medication.

Life is not easy for an endodontic pathogen. Microbes seeking to establish in the root canal must leave the nutritionally rich and diverse environment of oral cavity, breach enamel, invade dentin, overwhelm the immune response of the pulp and settle in the remaining necrotic tissue in root canal. They have to compete in a limited space with other microbes for the available nutrition. Through genetic exchange and mutation microbes have

developed specialized system that facilitate their ability to find, compete and survive in these very specific environments. Infection of the root canal is no random event. The type and mix of the microbial flora develop in response to the surrounding environment. Factors that influence whether the species die or survive are the particular ecological niche, nutrition, anaerobiosis, pH and competition or cooperation with other microorganism. Species that establish a persistent root canal infection are selected by phenotypic traits that they share in common and that are suited to the modified environment. Some of these shared characteristics include the capacity to penetrate and invade dentin, a growth pattern of chains and cohesive filaments, the resistance to antimicrobials used in endodontic treatment as well as an ability to grow in monoinfection, to survive periods of starvation and to evade the host response. Microorganisms that establish themselves in the untreated root canal would experience an environment of nutritional diversity that changes with time. In contrast the well filled root canal offers the microbial flora a little more than shelter from the host and microbial competitors but in a small dry nutritionally limited space. Invariably in all the cases it is the environment that selects for microorganisms those posses traits suited to establishing and sustaining the disease process. (Goran Sundqvist et al 2003) [24].

As long as the carious lesion has not entered the pulp, the pulpal, inflammation can be reversible. The inflammation being brought about by bacterial antigens interacting with local immune system. As the bacterial cells enter the superficial layers of pulp which even though heavily inflamed is

considered to be relatively bacteria free as long as it remains vital. In pulpitis the inflammation is surprisingly localized even after the bacteria has invaded the pulp space. Dental caries remains the major pathway through which the bacteria enter the pulp and root canal space. The gram positive rods invariably are the frontline invaders of pulp.

At a distance of 2- 3mm from the necrosis and bacteria, the pulp tissue as judged from the histological sections appears to be healthy. Eventually the diseased area becomes larger and bacteria evade deeper into the root canal space. Dynamics of this phase may take few days, weeks to several years. From the clinical point of view it is important to know that as long as there is vital pulp tissue there seems to be a limited number of bacteria in root canal space and that the infection has not spread into root dentin. Therefore outcome of endodontic treatment in tooth with pulpitis is excellent.

The fate of the bacteria entering root canal space is dependent on: redox potential, access to an availability of nutrients, gram positive and negative bacterial interactions and hosts defense system. The redox potential in necrotic root canal is very low favouring the growth of anaerobic microorganisms.

Apical periodontitis is an inflammatory process in periradicular tissue caused by microorganisms in the necrotic root canal. In primary apical periodontitis an aerobic dominance is a typical feature. The majority of organisms in primary apical periodontitis reside in main root canal system.

The location of where the bacteria are found, be that in lateral canals near the apex or the furcation area or in other parts of the root that has not been studied. However, lateral periodontitis lesions often detected in radiographs indicate the presence of bacteria and bacterial products in the lateral canals.

The etiology of apical periodontitis in filled teeth (post treatment disease; PTD) is generally the same as primary apical periodontitis that is microbial infection of root canal. The root filled teeth have already undergone a variety of procedures and consequently secondary factors are often high lightened and presence of disease is analyzed.

Without the presence of a microbial infection, mechanical complications due to technical procedures and use of materials do not cause more than temporary problems such as transient inflammatory reactions and occasional pain.

The host defense system is a key factor in preventing the spread of infection from root canal to periapical tissues and surrounding bone. A lack of circulation in the necrotic root canal makes it impossible for the phagocytes and rest of the immune system to penetrate into the root canal space for more than a few 100μm. The defense system is limited to achieving a balance between the microbial intruders and the body, but unfortunately cannot eliminate infection in root canal (Markus Haapasalo et al 2003) [51].

In chronic apical periodontitis the main mechanism responsible for destruction of the normal bone structure is activation of the bone osteoclasts

and inhibition of the osteoblastic activity. The sequence of the events resulting in osteoclastic stimulation is a network of immunological chain reaction, where inflammatory cytokines play a role. Although alternate theories of the osteoclast activation have been proposed, the fact remains that the host's own osteoclastic cells that remove the bone around the root tip.

Removal of bone is understood to be an important defense strategy, as bone has poor capacity to defend itself against bacterial intrusion and osteomyelitis might ensue if infection is allowed to spread. Bone is removed by the body's own defense mechanism before infection reaches the periapical tissues. In apical periodontitis the lesion is filled with phagocytes and other defense cells which effectively prevent further spread of microbial infection.

When the root canal space is filled with a root filling material, bacterial presence in the canal is not always accompanied by the presence of disease. The absence of infection and disease can be explained by lack of communication between the bacteria in the root canal and the host tissue. Research in endodontic microbiology has clearly been characterized by primary apical periodontitis than in post treatment apical periodontitis. The organisms most commonly found in root filled teeth apical periodontitis are *α and non hemolytic Streptococci, Actinomyces, Lactobacillus, Propioni bacterium, all facultative gram negative enteric rods and facultative gram negative rods*. eg: *Proteus mirabilis, Klebsiella, Escherichia colli* etc.

Biofilm was term introduced to designate the thin layered condensation of microbes that may occur on various surface structures in nature. Free floating bacteria existing in an aqueous environment, are called Planktonic microorganisms which are a prerequisite for formation of biofilms. These films may thus become established on any organic and inorganic surface substrate where planktonic microorganisms prevail in a water based solution eg: attachment of bacteria to teeth to form dental plaque.

The excretion of adhesive substances is crucial for initial attachment of organisms as well as holding the biofilm bacteria together. The structure per se will then provide better protection and may allow a better resistance to adverse external influences for the organisms incorporated as compared to planktonic state. The growing knowledge suggests organisms in biofilm assume a stronger pathogenic potential than in planktonic state. Thus these biofilms have clinical significance in that not only the host defense mechanism but also therapeutic efforts have a most difficult task to deal with microorganisms that are gathered in biofilm. (Gunnel Svensa¨ter et al 2004) [27] (S. George et al 2005) [69].

Biofilms in root canal have been confirmed by examination of extracted teeth with periapical lesions. The ability of the organisms in recurrent infections to form biofilms can be seen as the most important adaptive mechanism used by bacteria to survive the environmental changes resulting from treatment protocol (Luis Chávez de Paz 2007)[47].

*Enterococcus faecalis* is the dominant species present in post treatment infections. It is most frequently isolated species and is also predominant isolate in the canal (J. F. Siqueira et al 2005) [38],(Jörg F. Schirrmeister et al 2009) [35],(E. T. Pinheiro et al 2004) [20].A recognized pathogen in post treatment endodontic infections, *Enterococcus faecalis* is frequently isolated In both mixed and mono cultures. *Enterococcus faecalis* is probably species which can best adapt to and tolerate the ecologically demanding conditions in the filled root canal. Eradication of *Enterococcus faecalis* from the root canal with chemo-mechanical preparation and using disinfecting irrigants and antibacterial dressing is difficult.

Enterococcal cells are spherical or ovoid, occurring in pairs / short chains in liquid media. They form creamy whitish colonies, are gram positive, catalase negative and able to grow in 6.5% sodium chloride , at temperatures ranging from $10^0$Celsius to $45^0$ Celsius, and they can survive 30 minutes at $60^0$ Celsius and at a pH over 9.6. Most *enterococci* are facultative anaerobes, but some species are strict aerobes.

*Enterococcus faecalis* possess several virulence factors that permit adherence to host cells and extra cellular matrix, facilitate tissue invasion, effect immune modulation and cause toxin mediated damage. The factors are:

1. Aggregation substance(AS)
2. Enterococcal surface protein (Esp)

3. Gelatinase
4. A cytosine toxin
5. Extracellular superoxide production
6. Capsular polysaccharides
7. Antibiotic resistance determinant (Isabelle Portenier et al 2003) [32]

Although *Enterococcus faecalis* is dominant species in root treated teeth with infections there is no evidence that it is responsible for severe acute infections. (E. T. Pinheiro et al 2004) [20].

These organisms under starvation conditions change to stationary phase (non–growth phase), with increase in time of starvation the cells decrease in size. After a long period of starvation the number of culturable cells decreases. Once the nutrition is available the starved cells are able to recover by using serum as a nutritional source. Total number of bacteria is similar to initial levels. The cells have entered a state in which they are viable but not culturable with standard microbial techniques. Starvation may be one of the major factors resulting in high resistance of *Enterococcus faecalis* to endodontic medicaments.These organisms have been known to form biofilm (Luis Chávez de Paz 2007) [47].

The formation of biofilm helps it to resist destruction by enabling these bacteria to become 1000 times more resistant to phagocytosis,

antibodies and antimicrobials than non biofilm producing organisms. Calcium hydroxide a commonly used intra canal medicament has been shown to be ineffective in killing *Enterococcus faecalis* on its own especially when a high pH is not maintained, due to following reasons:

a) *Enterococcus faecalis* maintains pH homeostasis.

b) *Enterococcus faecalis* has a proton pump – additional way of maintaining pH homeostasis.

c) At a high pH 11.5 or greater *Enterococcus faecalis* cannot survive.

However due to buffering capacity of dentin it is very unlikely that pH of 11.5 can be maintained in dentinal tubules with current calcium hydroxide utilization technique.

There are additional survival and virulence factors for *Enterococcus faecalis* namely:

a) Ability to bind to dentin and invasion of dentinal tubules.

b) Alteration of the host response.

c) Suppression of the action of lymphocytes.

d) Presence of lytic enzymes, cytolysin, pheromone and lipoteicholic acid. (Charles H. Stuart et al 2006) [11].

Adherence is considered to be the first step for bacterial colonization of host tissue, including tubule invasion and is mediated by bacterial specific cell

– surface component (adhesins). It has been shown that Ace (collagen binding protein of enterococci) promoted *Enterococcus faecalis* adhesion to some extracellular matrix proteins including collagen Type I. As collagen type I is the main organic component of dentin. It is widely considered to be a major substrate for *Enterococcus faecalis* (P. Chivatxaranukul et al 2008) [61].

It has been shown that the initial colonization of *Enterococcus faecalis* can be prevented and controlled by removal of smear layer or treatment with chlorehexidine.

Factors such as coronal leakage of saliva and physiological state of microbes might play an important role in adherence of bacteria and biofilm formation on root canal dentin (Saji George et al 2007) [65].

The majority of *Enterococcus faecalis* strains induced little or no release of hydrolytic enzymes from the PMN cells (polymorpho nuclear cells). This partly explains the clinical observation that root canal infection dominated by *Enterococcus faecalis* are usually symptom free (A. Reynaud af Geijersstam et al 2005) [2].

Enterococci may not be present in majority of cases of non healing periradicular lesions. *Enterococcus Faecalis* was the only enterococcal species detected with an overall prevalence of 22%. (Ashraf F. Fouad et al 2005) [1].

Culture methods have provided a great contribution to, and have much still to offer for elucidation of endodontic disease. However molecular

approaches to detect and identify microbial species have several advantages when compared to culture.

Molecular methods particularly polymerase chain reaction are more specific, accurate, sensitive and rapid than culture, and can detect uncultivable and fastidious microorganisms. Polymerase chain reaction(PCR) is a technique which uses DNA polymerase enzyme to make a huge number of copies of virtually any given piece of DNA or gene. Polymerase chain reaction technique is generally more sensitive than the culture technique. Polymerase chain reaction can detect non viable / viable as well as non culturable cells also. It should be emphasized that polymerase chain reaction technique only detects the target species, but does not enumerate the total number of bacteria present in the sample. This points out the importance of considering when using molecular assays not only the presence of a specific microorganisms but also the number of cells in a sample in order to associate it with different types of endodontic infections (Güven Kayaoglu et al 2006) [28].

Various methods have been suggested to eradicate or prevent *Enterococcus faecalis* from gaining access to the root canal space. Preparation of the apical portion of the root canal to a larger instrument size will help prevent growth of intra-canal microorganisms by making the apical portion more accessible. It also facilitates removal of innermost radicular dentin which in turn provides the potential to remove intra tubular

bacteria and open the dentinal tubules for antimicrobial action. Sodium hypochlorite if used in 3% to full strength, in adequate amounts and exchanged regularly has the ability to destroy *Enterococcus faecalis* in the root canal. It is an effective irrigant for all presentations of Enterococcus *faecalis* including its existence as a biofilm. MTAD has been found to be effective against *Enterococcus faecalis* in preliminary studies. It has anti collagenase activity, low pH and ability to release gradually over time.

Its effects are enhancing when used in conjugation with sodium hypochlorite. 2% chlorehexidine in a gel or liquid formulation is effective at reducing / completely eliminating *Enterococcus faecalis* from the superficial layer of dentinal tubules upto 100μm. the gel is effective for up to 15days this is impart attributed to its substantive anti microbial activity (Charles H. Stuart et al 2006) [11.]

Historically countless compounds in aqueous solution have been tried as root canal irrigants. Ideally root canal irrigants should:

a) Have broad anti-microbial spectrum.

b) Have tissue dissolving capacity.

c) Inactivate endodotoxins.

d) Prevent smear layer formation during instrumentation / dissolve the latter once it has formed.

Of all the currently used substances sodium hypochlorite appears to be the most ideal as it covers more of the requirements for endodontic irrigant than any other known compound.

Sodium hypochlorite and chlorehexidine are agents frequently used in the treatment of endodontic and periodontal infections.

The use of sodium hypochlorite as an antimicrobial solution began at the end of the $18^{th}$ century, with the "Water of Javele in 1972" a solution containing sodium hypochlorite and potassium hypochlorite. Labaraque's solution, a solution containing 2.5% sodium hypochlorite appeared in 1820. A solution containing 0.5% of available chlorite with boric acid to reduce its pH was proposed by Dakin in order to disinfect wound during world war I. Walker introduced the use of 5% sodium hypochlorite in dentistry, and this was reinforced by Grossman & Maiman.

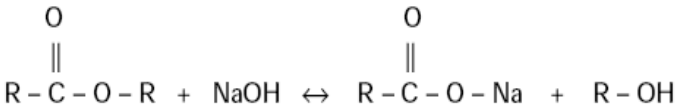
Sodium hypochlorite is the present irrigant of choice due to its antimicrobial effectiveness, tissue dissolving effects and its tissue tolerance at adequate clinical concentrations. One of the problems with the use of sodium hypochlorite concerns its appropriate concentration due to cellular damage caused by its extrusion into the periapical tissue (Carlos Estrela et al 2002) [10].

In this present study the effectiveness of varying concentrations of sodium hypochlorite (1%, 3%, and 5%) was evaluated as a root canal irrigant at the three different irrigant temperatures mainly $24^0$Celsius, $37^0$Celsius and $45^0$Celsius in an in vitro setting.

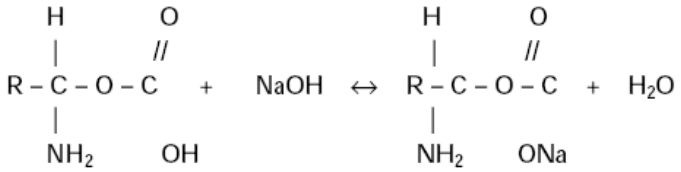
Sodium hypochlorite exhibits a dynamic balance as shown:

$$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$$

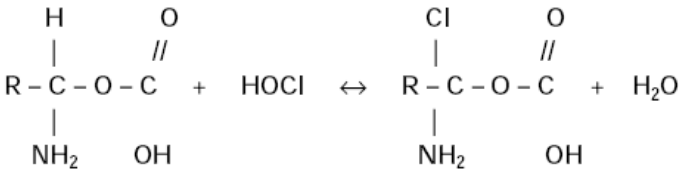
Scheme 1. Saponification reaction.




Scheme 2. Amino acid neutralization reaction.




Scheme 3. Chloramination reaction.



The efficacy of sodium hypochlorite preparation could be enhanced in a number of ways:

a) By lowering the pH value – by buffering. However this method has its own draw backs of a decrease in shelf life.

b) Increasing the temperature of low concentration sodium hypochlorite solutions, improves their immediate tissue dissolution capacity, also removes organic debris from the dentin shavings more efficiently than unheated counterparts. The bactericidal rates for sodium hypochlorite solutions are more than doubled for each $5^0$Celsius rise in temperature range of $5^0$Celsius – $60^0$Celsisus. The systemic toxicity of preheated sodium hypochlorite should be lower than one or more concentrated non heated counterparts. As a temperature equilibrium is reached quickly.( George Sirates et al 2005) [22]. The tissue dissolution ability of sodium hypochlorite reached a plateau between $60^0$Celsius – $75^0$Celsius. This could be explained in two ways : either the temperature rise has a limit to exert its dissolving properties, or the sodium hypochlorite became exhausted with time, with no more extra chlorine available, could further be investigated in other studies (Giampiero Rossi-Fedele et al 2008) [23].

c) Ultrasonic activation of sodium hypochlorite has also been advocated as this would accelerate chemical reaction. This creates acoustic streaming cavitation effect which enhance and achieves a superior cleansing action. Ultrasonic energy also produces heat thus rendering the sodium hypochlorite slightly more active. If ultrasonic activation of sodium hypochlorite is to be used, it is important to apply the ultrasonic instrumentation after the canal preparation has been completed.

d) It should also be noted that time is the factor which has gained little attention. Even fast acting irrigant like sodium hypochlorite need adequate working time to reach their potential. Optimal time a sodium hypochlorite irrigant at a given concentration needs to remain in the canal is an issue yet to be resolved. (Matthias Zehnder,2006) [53].

e) PUI – Passive ultrasonic irrigation – relies on transmission of acoustic energy to an irrigant in root canal without cutting action. This induces acoustic streaming and cavitation of the irrigant. Intermittent Passive ultra sonic irrigation with a large volume of irrigant reduced bacterial colonization from main root canal space in short period of time.( Hanan A. Balto,2008) [30].

f) The increase of concentration of sodium hypochlorite is directly proportional to anti – microbial, tissue dissolution capacity, bleaching and deodorizing effect.

Some authors have reported resistance of certain strains of *Enterococcus faecalis* particularly in refractory post – treatment infections to be highly resistant to sodium hypochlorite.

In the present study 5% sodium hypochlorite at $45^0$Celsius was found to be the most efficient in eliminating *Enterococcus faecalis* from the root canals. The solutions of sodium hypochlorite at other temperatures of $24^0$Celsius and $37^0$Celsius at the same concentration of 5% were found to be most effective in their respective group in controlling growth of *Enterococcus faecalis.*

However at $45^0$Celsius the 5% sodium hypochlorite solution eradicated the *Enterococcus faecalis* from the root canals in all the samples except one where only one colony was found.

The positive control groups (Saline) in all temperatures produced the highest growth of colonies.

Warming of sodium hypochlorite in a clinical setting could be done easily by using a temperature controlled bath or heating in a syringe warming device or a baby bottle warmer. The baby bottle warmer however does not maintain constant temperature though different settings are offered. Warming is best performed for each case with a fresh solution as questions have been raised as regards to availability of active chlorine with passage of time. Stock solutions could be prepared and stored at low temperature for chair side use, depending on clinical requirements ( Ebtissam M. Al-Madi 2008)[18].

Though in this study the 5% sodium hypochlorite was found to be most effective against *Enterococcus faecalis* this was an invitro setting. At 5% concentrations utmost care needs to be exercised not to accidentally push the hypochlorite solution beyond the apex which could have undesirable side effects as the solution is highly active.

A higher temperature and a lower concentration could be more than sufficient for a comparable antimicrobial effect, as it would render the solution more biocompatible.

The time of exposure of the solution to the canal contents is also of importance. When deemed safe 5% sodium hypochlorite solution warmed to $45^0$Celsius could be used for optimal antimicrobial effect.

Though various techniques have been advocated for bacterial culturing molecular assay based identification technique like polymerase chain reaction has their own limitations. In that it cannot distinguish between DNA from viable / dead cells and thus it's unclear whether the results from this method truly represent the authentic living endodontic flora.

As this is an in vitro study, further studies evaluating the use of heated sodium hypochlorite in a clinical setting are recommended.

Sixty single rooted anterior teeth were collected, cleaned and stored in normal saline. They were standardized to a length of 15mm and the root canals preparation was done using Protaper instruments as per the manufacturer's instructions.

The teeth were then divided into groups and inoculated with *Enterococcus faecalis*. Followed by irrigation with varying concentrations of Sodium hypochlorite (1%, 3% and 5%) at temperatures of ($24^0$Celsius, $37^0$Celsius,$45^0$Celsius).

Sampling was done by inserting paper points followed by culturing. After 48hrs of incubation typical *Enterococcus faecalis* colonies were counted in all plates, results tabulated and statistically analyzed.

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On completion of the study on the effect of varying concentration of sodium hypochlorite at three different temperatures on *Enterococcus faecalis* within the canals.

The following conclusions were made:

- 5% sodium hypochlorite at $45^0$Celsius was found to be the most effective irrigant used.
- Statistical analysis with ANOVA at 5% level of significance showed there is a significant difference between 5% sodium hypochlorite at $45^0$Celsius and other two groups of 5% sodium hypochlorite at $37^0$Celsius and $24^0$Celsius which means that 5%sodium hypochlorite at $45^0$ is better.
- Based on the results of the study chair side pre-warming sodium hypochlorite before use with a temperature controlled device is recommended.
- 5% Sodium hypochlorite at $24^0$Celsius and $37^0$Celsius were also able to inhibit the growth of *Enterococcus faecalis* efficiently in comparison to the control group. Effectiveness of sodium hypochlorite as an irrigant at lower concentration could be enhanced by increasing the temperature.

- The effect of varying concentrations of sodium hypochlorite as an irrigant at varying temperature in a clinical setting should be evaluated.

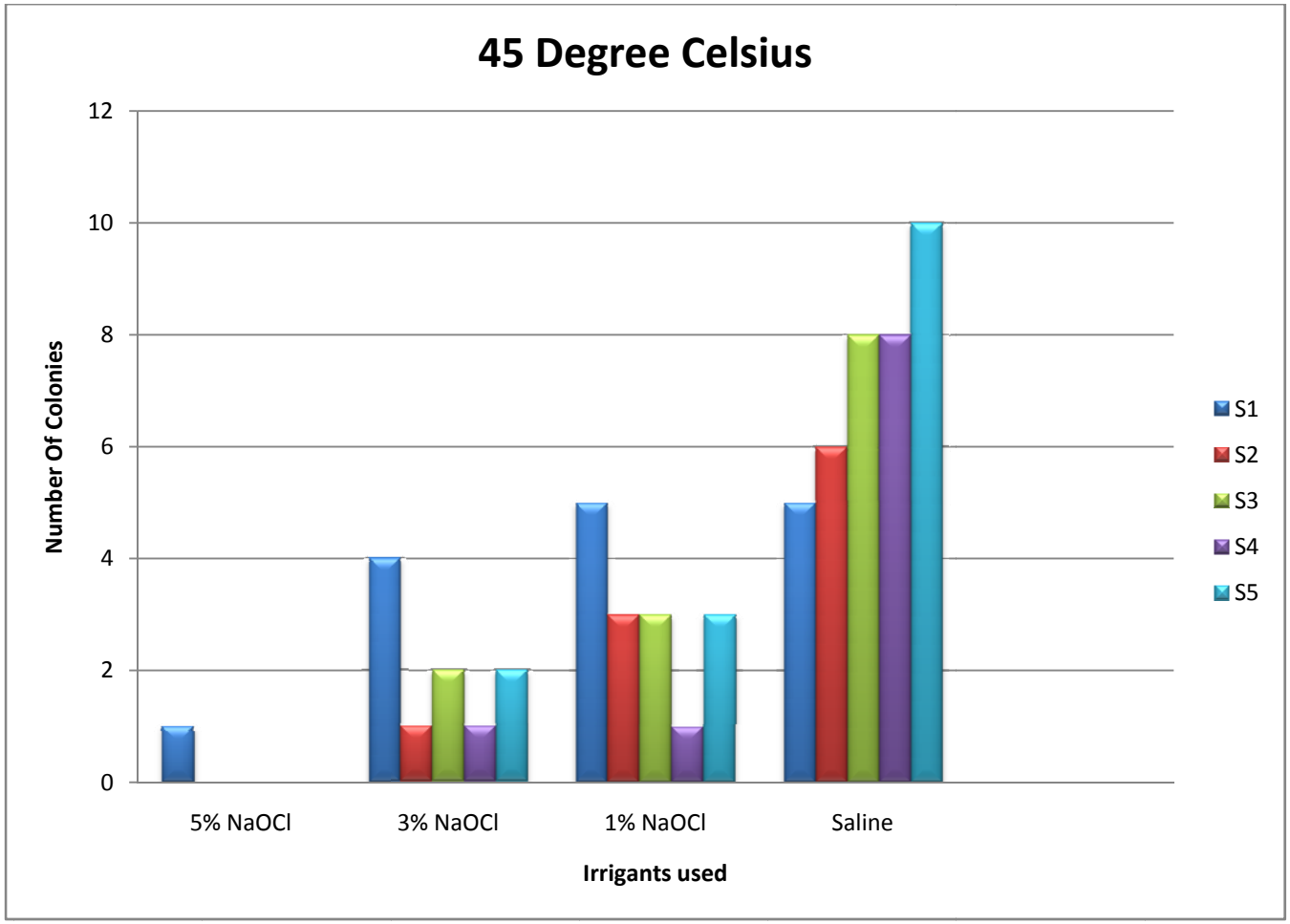
45 Degree Celsius
12
10
8
6
4
2
0
Number Of Colonies
5% NaOCl
3% NaOCl
1% NaOCl
Saline
Irrigants used
S1
S2
S3
S4
S5

Chart : 1

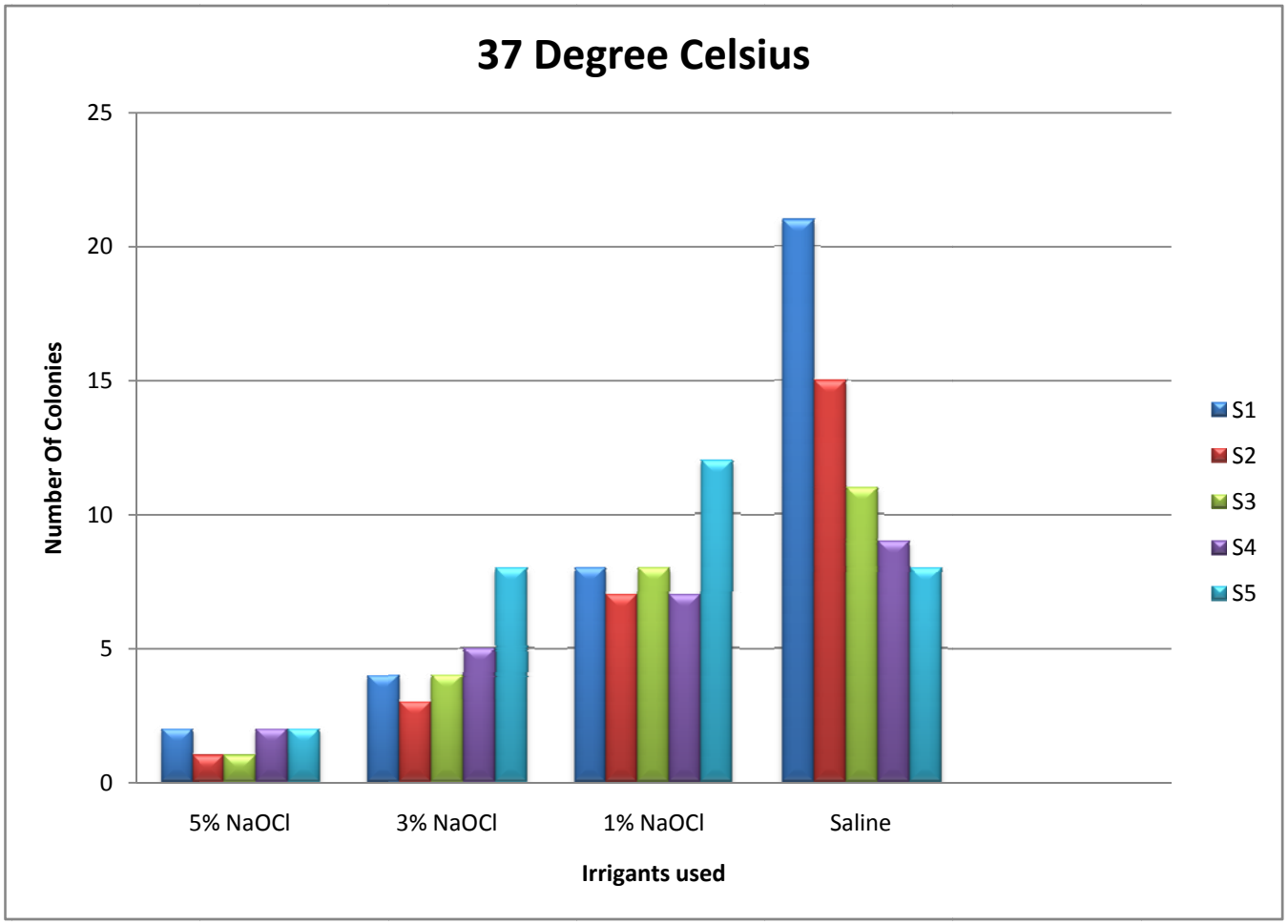
37 Degree Celsius
25
20
15
10
5
0
Number Of Colonies
5% NaOCl
3% NaOCl
1% NaOCl
Saline
Irrigants used
S1
S2
S3
S4
S5

Chart-2

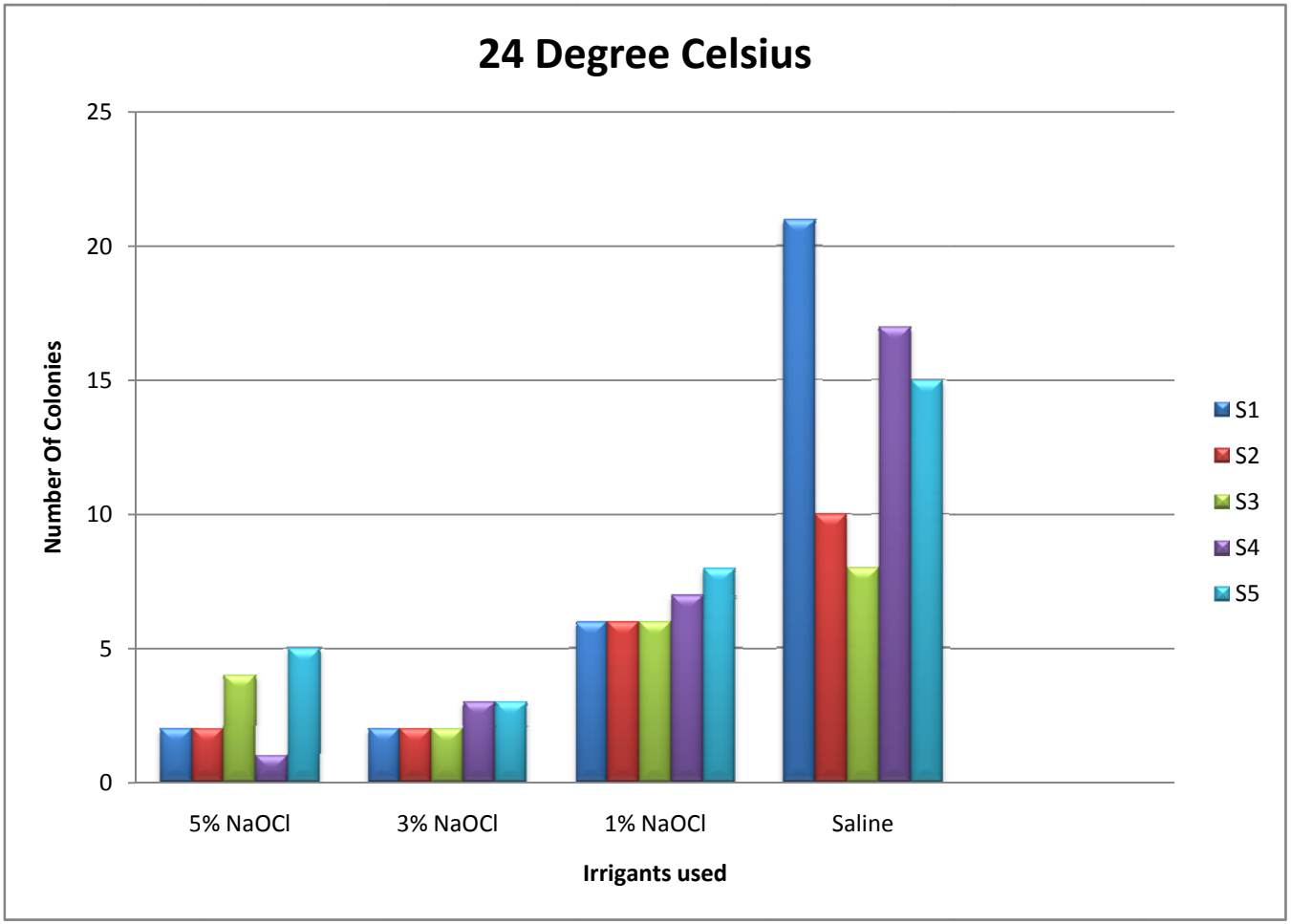
24 Degree Celsius
25
20
15
10
5
0
Number Of Colonies
5% NaOCl
3% NaOCl
1% NaOCl
Saline
Irrigants used
S1
S2
S3
S4
S5

Chart - 3

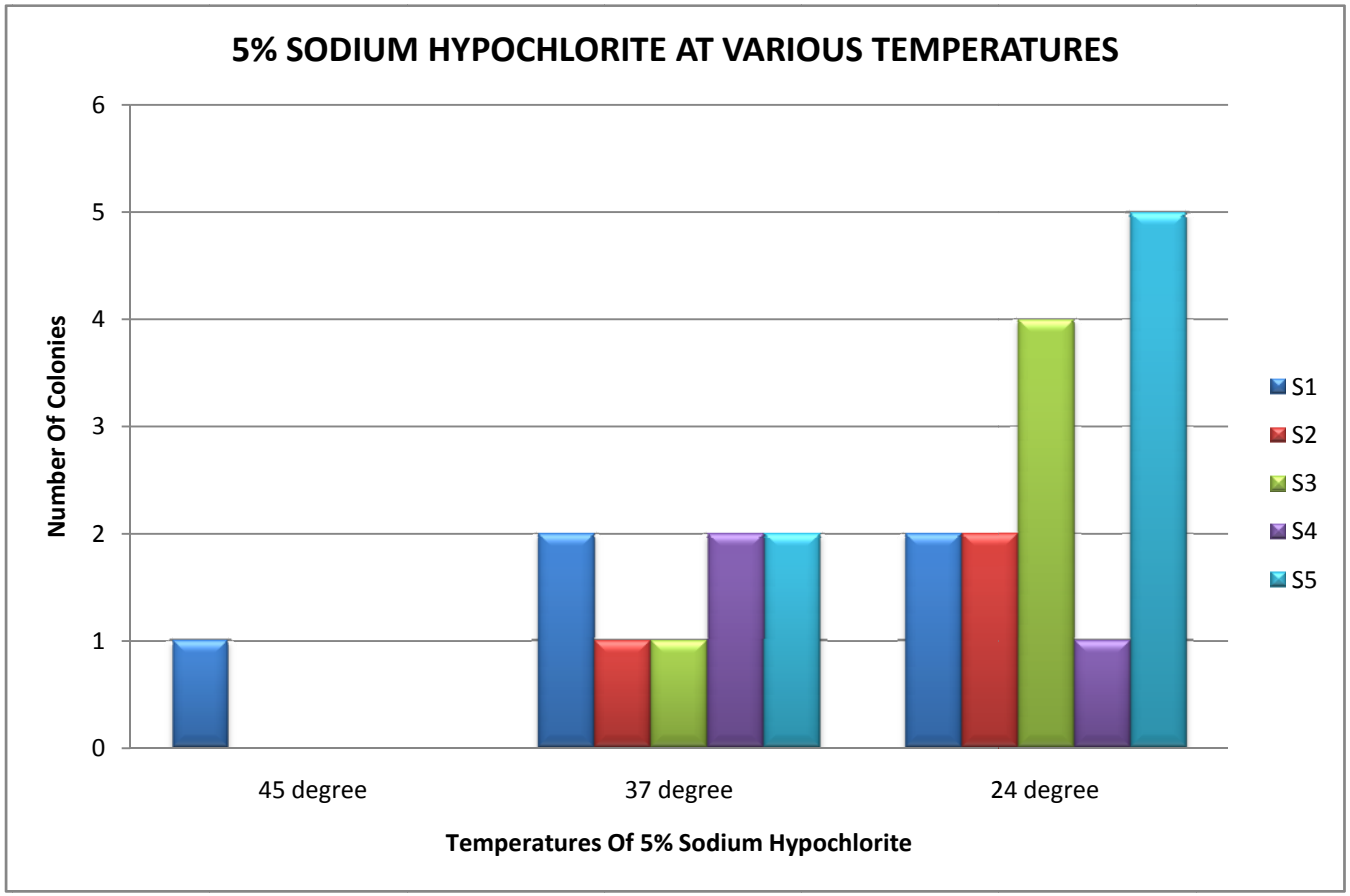
5% SODIUM HYPOCHLORITE AT VARIOUS TEMPERATURES
Number Of Colonies
0
1
2
3
4
5
6
45 degree
37 degree
24 degree
Temperatures Of 5% Sodium Hypochlorite
S1
S2
S3
S4
S5

Chart - 4