THE DISINFECTION OF

DENTAL IMPRESSION MATERIALS

KEVIN JOHN JENNINGS

BDS, MSc, FDSRCPS, DRDRCS

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University of Glasgow, Dental Hospital and School.

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DECLARATION

This work was undertaken in the Department of Adult Dental Care and the Department of Oral Sciences in the University of Glasgow Dental Hospital and School in the period from June 1988 to December 1993. The material contained in this thesis is entirely original.

Some techniques used in the thesis are modifications of previously published work; some are original techniques developed in the Department of Adult Dental Care and the Oral Microbiology Unit, of Glasgow Dental Hospital and School. The application of the techniques described in the work was undertaken by the author, or undertaken under his direct supervision.

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SUMMARY

Heightened awareness the importance of cross-infection control in dentistry has resulted in the publication of guide-lines by bodies such as the General Dental Council, the British Dental Association, and the American Dental Association, and, as such advice is regularly upgraded in the light of new information, there is a continuing need for clinical research in this area.

Data, in the dental literature, on the contamination of impression materials by oral microorganisms, are limited, and the aim of the first part of this thesis was to assess microbial survival in impression moulds prepared from polysulphide, poly (vinyl siloxane) and alginate materials. The viability of five microbial species was tested over a five hour period. Rapid elimination of microorganisms was evident within moulds made from an alginate impregnated with sodium didecyldimethyl ammonium chloride (Blueprint Asept); a reduction in colonisation with time was less marked with conventional materials. In a further study of the disinfectant-containing alginate, high microbial concentrations were eliminated within 40 minutes, although varying susceptibility of different organisms, to this material, was evident.

In a further *in-vitro* investigation, the persistence of microorganisms following contact with impression surfaces for three minutes, was examined. With Blueprint Asept, there was almost total elimination on initial sampling, and no microbial growth was detectable after five minutes. With the conventional materials, microbial survival varied from 68.5% to 80.5%, five hours after the contaminating inocula were discarded. The retention of microorganisms was dependant upon the material type; initial contamination of the conventional alginate was substantially greater than rubber base materials, and bacteria, which were virtually eliminated from rubber base materials after five hours, survived to varying degrees on conventional alginate (Kromopan).

In an investigation of impressions from dentate patients, microbial growth on conventional alginate and rubber base specimens was evident, in some cases, five hours after impressions were recorded. Initial contamination levels of alginate considerably exceeded poly (vinyl siloxane), and there was a rapid reduction in the number of micro-organisms on the rubber base materials in comparison with alginate (when considered as a percentage of initial microbial loading). No growth was apparent from Blueprint Asept impressions. With edentulous patients, again no growth was recorded from Blueprint impressions, but five hours after Kromopan impressions were made, substantial

contamination was still evident. Comparison of Kromopan impressions from dentate and edentulous patients, showed slightly greater initial contamination from the dentate group.

The thesis was developed by examination of the removal of microorganisms from different impression materials, and by comparison of three commonly used disinfectant agents. In order to compare the impression materials, microbial persistence on alginate, polysulphide, and poly (vinyl siloxane) materials, following the application of weak disinfectants, was examined. After removal of the contaminating inocula, impression surfaces were left as non-disinfected control specimens, or were disinfected with dilute aqueous solutions of chlorhexidine gluconate. Initial contamination of poly (vinyl siloxane) was minimal, there was a rapid reduction in the numbers of microorganisms on non-disinfected control specimens of this material, and there was no growth after application of disinfectant. In comparison, there was substantially more loading of polysulphide rubber on initial sampling, and the microorganisms persisted to a greater extent on control samples. Disinfection resulted in substantial reduction, although not total elimination, of contaminants. A heavy initial colonisation of alginate increased with time on control samples, and disinfection was only partially successful. For all three materials,

initial colonisation with *C albicans* was less marked than with *Ps aeruginosa*.

The persistence of microorganisms on alginate (Kromogel), following the application of one of three commercially available disinfectants (2% glutaraldehyde, 0.2% chlorhexidine gluconate, or 0.0125% sodium hypochlorite) was assessed and, while all produced substantial reductions in colonisation, chlorhexidine gluconate was found to be the least effective agent.

An alternative method of using chlorhexidine, within impression materials, was then evaluated. Conventional and disinfectant-containing alginates were contaminated, and microbiological testing carried out after various time-intervals. Following inoculation with *C albicans*, the colonisation of conventional alginate (Kromogel) was greater than colonisation of a chlorhexidine-containing material (Hydrogum); conversely, with *Ps aeruginosa*, colonisation of Hydrogum was significantly greater than Kromogel. There was no clear distinction between these two materials for the carriage of *Staph aureus*. In all instances, the contamination of Blueprint (containing didecyldimethyl ammonium chloride) was significantly less than the other two materials. In a further laboratory assessment of chlorhexidine-containing, didecyldimethyl ammonium chloride-containing and standard alginates, a

cylinder assay plate method diffusion test was used. Discs of impression material were applied to culture plates inoculated with one of six microorganisms (*Strep sanguis*, *Strep mutans*, *Strep milleri*, *Strep salivarius*, *Actino viscous* or *Porph gingivalis*), and incubation was carried out under appropriate conditions. Susceptibility of each microorganism to each of the impression materials was evaluated. A similar degree of growth inhibition occurred with Hydrogum and Blueprint, but there was little evidence of inhibition around Kromogel samples.

In a final *in-vitro* assessment of the chlorhexidine-containing impression material (Hydrogum), the time course killing period was assessed (with Kromogel as the control material). Culture media, containing neutralisers for chlorhexidine, were used to allow assessment of the time after which the chlorhexidine-containing impressions could be considered decontaminated. *Staph aureus* and *Strep sanguis* were inoculated on the culture media used in the study, and growth on each media was found to be sustained. Blocks of impression material were immersed momentarily in brain heart infusion broth cultures of either *Staph aureus* or *Strep sanguis*, and microbial sampling carried out. After 60 minutes, the control alginate failed to eradicate the test microorganisms. With the chlorhexidine-containing alginate, all microorganisms were eliminated after 60

minutes, and in some cases there was no evidence of microbial growth after 30 minutes.

The use of chlorhexidine, to reduce the microbial contamination of impressions, was then examined in three *in-vivo* experiments, evaluating the effect of chlorhexidine incorporated within alginate powder, or its use as a mouth-rinse prior to recording impressions. From a preliminary study, undertaken to establish methods of investigation, it was apparent that comparison between subjects in the experimental group was not appropriate, and in the two main investigations, multiple impression samples were collected, under differing experimental conditions, for each of ten subjects in a controlled experimental population. There was no growth from Blueprint impressions, confirming the previously described antimicrobial effect of this material, and providing an effective control for assessment of the chlorhexidine-containing preparation. All Kromogel and Hydrogum impressions were contaminated, and examination of mean values for microbial growth for each subject, and assessment of the group as a whole, showed there was no significant difference between samples from these two materials.

In the second part of this study, the effect of a pre-impression mouth-rinse with 0.2% aqueous chlorhexidine

gluconate, was assessed. Rinsing with tap water, or with chlorhexidine, was carried out prior to recording the alginate (Kromogel) impressions and, for each subject, microbial colonisation was found to be significantly reduced after chlorhexidine rinsing. From results for the group as a whole, contamination of samples collected after chlorhexidine rinsing, was significantly less than occurred in standard Kromogel impressions, and in those recorded after rinsing with tap water. There were no differences between Kromogel impressions, and those recorded after tap water rinsing.

In dental practice, a variable time interval may elapse between recording impressions and pouring casts from them, and there are few published data on the dimensional stability properties of contemporary alginate materials on storage, particularly following immersion. The aims of the final study reported in this thesis, were to measure and compare linear accuracy, relative to time, of four contemporary alginate materials (including two which contained disinfectant agents), and to examine the effect of disinfection by immersion, followed by storage, on linear dimensional stability. Impressions of a steel standard, with abutments in the canine and first molar regions, were divided into two groups, and measurement over a 48 hour period was carried out on either, standard impression specimens (stored without prior immersion),

or, specimens immersed in hypochlorite disinfectant before storage and analysis.

There were significant variations between the different impression materials for each of the test measurements, and both storage and immersion produced statistically significant dimensional changes in all four materials. However it was apparent that the accuracy of impressions was, to some extent, more dependant upon the choice of material, than on the length of storage time before casts were made. In almost every case, the dimensional change following storage of non-immersed conventional alginate impressions (Palgat & Xantalgin), or Hydrogum (containing chlorhexidine), was less than the differences between the materials; impression dimensions were affected by storage to a surprisingly small degree. Immersion disinfection reduced the initial accuracy of all four materials.

Blueprint which was the least accurate immediately after the impressions were made, also showed most dimensional change over 48 hours, and the conventional material which was initially most accurate (Palgat), also showed the greatest dimensional stability over 48 hours.

CHAPTER 1

LITERATURE REVIEW

ASPECTS OF CROSS-INFECTION CONTROL IN PROSTHETIC DENTISTRY

1.1 INTRODUCTION

The importance of the regulation of standards in health-care is self-evident, and where the provision of care itself can be viewed as constituting a potential hazard to health, there exists a particular responsibility to identify and clarify risk factors. The General Dental Council has indicated its interest in these matters and in its guide-lines, "Professional Conduct and Fitness to Practise" [1], refers to three areas of dental practice in which there is a particular need to ensure that standards of patient protection are monitored closely. The areas highlighted are i) general anaesthesia and sedation, ii) dental radiography and radiation protection and, iii) cross-infection control.

In the course of their clinical activities, dentists are regularly exposed to a variety of pathogenic organisms from the blood and saliva of patients. These can be spread by direct contact with infectious lesions, blood or saliva, by airborne transfer of blood, saliva or nasopharyngeal secretion droplets (aerosols), or by indirect contact with contaminated intermediate objects (fomites). Such hazards constitute a risk not only for the dental surgeon, but also for ancillary dental staff and for patients subsequently attending the surgery.

Although the principles of disease transmission and the prevention of cross-infection by the use of disinfectants have been considered since the time of Lister [2], the perceived significance of infection control in dental practice has increased dramatically with the onset of the AIDS pandemic [3,4,5].

Public awareness of the dental surgery as an area of potential contamination has been heightened, particularly since the report of a case where human immunodeficiency virus was transmitted from dentist to patient, apparently during routine dental care [6,7,8]. However there have been a number of other studies involving patients of HIV-infected health workers, without any further report of human immunodeficiency virus transmission to a patient [9,10,11,12]. In response to reports from the Centres for Disease Control [13,14,15] and other publications, the American Dental Association has for some time advocated the use of specific cross-infection control procedures in the dental surgery and laboratory, and has issued guide-lines on the application of procedures to limit cross-infection in clinical dentistry [16,17,18, 19]. Matters considered in the ADA guide-lines include assessment of patients, preparation for dental treatment, procedures to be followed during treatment, handling of materials, instruments and equipment, and steps to be taken on completion of treatment.

The British Dental Association has issued, and regularly updates, advice on the control of cross-infection in dentistry [20,21,22]. Matters considered in the guidelines include surgery design, personal protection for members of dental staff, the sterilisation of instruments and equipment, and disinfection procedures. Advice, for all dental staff on practical steps for infection control, which deals with procedures routinely undertaken at the chair-side, has also been issued by the BDA [23, 24,25].

1.2 AREAS OF CONCERN

In the case of patients suffering from acute infections who seek dental treatment, steps to limit contamination can be taken before and during treatment, and appropriate medical advice can be sought. However, asymptomatic carriers of infectious disease, and patients in the prodromal phase of infection also present a potential cross-infection hazard in the dental surgery, and there may be instances when patients who constitute a potential infection risk, are unwilling to divulge or discuss full details of their medical history [26]. For these reasons, precautions to control cross-infection must be effective, and should be employed in the treatment of all patients [27,28,29].

Some diseases of bacterial origin, such as syphilis, are less common than once was the case and disorders of viral origin, in particular acquired immunodeficiency syndrome and hepatitis, are of most concern at the present time [30]. Secondary infection in immunocompromised patients is also an area of importance with respect to cross-infection control [31], and while the importance of tuberculosis has been highlighted in this context [32], more common conditions, such as *Staph aureus* infection, may be more significant when older patients, patients with underlying health problems, or patients on immunosuppresive medication, are concerned [33,34].

1.2.1 HEPATITIS

The term "hepatitis" is used to describe any diffuse inflammatory process affecting the liver, and its main causes are viral infections, certain drugs and other toxic substances (including alcohol).

Many different viruses can produce liver inflammation (eg coxsackie viruses and herpes viruses), and there are a number of different forms of specific hepatitis viruses, which are of varying significance to the dentist [35,36]. Transmission of hepatitis A virus, [37] and hepatitis E virus [38] in the dental surgery has not been recorded.

Hepatitis B virus (HBV) is a DNA virus found in blood, blood products and other body fluids (including saliva), and its importance in dentistry is well recognised [39, 40]. A relatively resilient, heat-resistant microorganism [41], HBV can survive for considerable periods of time on contaminated instruments [42]. Infection with HBV may occur when contaminated tissue fluids come in contact with the intact mucous membranes of the oral and nasal mucosae, or come in contact with skin lesions such as abrasions, or through punctures caused by needles or instruments [43,44]. Some 50% of hepatitis B infections are subclinical [45] and, in the remainder, jaundice is the main clinical sign. The liver is enlarged and tender, with malaise, nausea and anorexia being common features, and serum aminotransferase and bilirubin levels raised. In acute viral hepatitis, widespread liver cell necrosis occurs, for which no specific treatment exists, and possible sequelae include cirrhosis of the liver and primary hepatocellular carcinoma [46]. Some individuals continue to carry hepatitis B surface antigen (HBsAg) following recovery and are capable of transmitting the disease under appropriate circumstances. Some carriers have no history of viral hepatitis, because the initial infection was asymptomatic. The risk of hepatitis B infection is high for dental workers [47], and there is evidence of infection transmission from patients to dental staff [48,49], and from dental staff to patients

[50,51]. Dental technicians [52], clinicians in other medical disciplines [49,53,54,55], and medical laboratory personnel [55,56] are also at increased risk from the hepatitis B virus.

Hepatitis C virus, which has a parenteral mode of transmission [57], also poses a potential threat in the dental surgery. Sub-clinical infections are normal after infection with the hepatitis C virus, chronic liver disease is possible in the longer term [46], and there is an association with hepatocellular carcinoma [58]. In the UK, patients with hepatitis C usually belong to one of the high risk groups, which include intravenous drug abusers and recipients of blood or blood products [59, 60]. Although it has been suggested that dental treatment may be implicated in the transmission of hepatitis C, and that dentists constitute one of the higher risk groups for HCV infection [61], there is no recorded instance of this occurring in the UK [62], and recently it has been suggested that occupational HCV infections among dentists are not common [63]. There have been conflicting reports on the degree of prevalence of hepatitis C among health care workers with direct patient contact [61,64], and in a study of 100 UK hospital staff, hepatitis C virus antibodies were not detected in any [65]. Nonetheless, the hepatitis C virus is found in saliva [60,66,67,68], as well as blood, and there remains a possibility of

contamination from dental procedures. There are few data available concerning inactivation of hepatitis C agents, but routine cross-infection control measures have been recommended [69].

The hepatitis D virus causes disease in patients with a pre-existing or concurrent infection with acute or chronic hepatitis B. The virus, sometimes referred to as the delta agent [70], requires the hepatitis B surface antigen for replication. Superinfecion with HDV prolongs the course of HBV infection and leads to a more severe illness with a poorer prognosis and higher mortality. HDV is usually spread parenterally and is most common among drug addicts and haemophiliacs [71].

Rigorous application of sterilisation and disinfection measures are needed to prevent transmission of hepatitis viruses in the dental environment [72]. Immunisation, using HBsAg, is an effective and safe method of protecting against the hepatitis B virus, and as HDV infection is dependent upon pre-existing or concurrent infection with HBV, successful vaccination against HBV confers immunity against HDV (but not against HCV). As an increasing number of dental personnel has been immunised against hepatitis B [73,74,75,76], it may be that infection from hepatitis C virus, which is marginally more prevalent among the British population [77], will become the greater health risk in dentistry.

1.2.2 ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Since AIDS was originally reported [78,79,80] there have been dramatic advances in knowledge of epidemiological and clinical facets of the disease. Towards the end of 1991, almost half a million cases which met the clinical definition of AIDS had been reported to the World Health Organisation; there were approximately one and a half million adults with HIV infection in the western industrialised countries; six million in Africa; one million in Latin America, and over one million in Asia [81,82,83,84]. It is unlikely that these figures give a true representation of the scale of the AIDS problem, as it is accepted that there is a high degree of under-reporting of the condition, particularly in Third World countries.

In the UK, there has been a continued increase in the number of AIDS cases reported. While male homosexuals and intravenous drug abusers continue to be at greatest risk of infection, there has been a reported rise in the transmission incidence of the virus following hetero-sexual intercourse and in the newborn [85,86,87,88,89]. AIDS cases in the UK at the end of 1991 numbered 5,065 and, while the prevalence of HIV positive individuals is grossly underestimated, over 16,000 patients with HIV positive antibody status had been noted in the UK at that

time [90]. Over 1,200 of these were haemophiliacs, infected with HIV following treatment with contaminated blood factor VIII [91].

From the above it is clear that many dentists are seeing patients with HIV infection, often without realising that this is the case, although there are a number of oral manifestations which may help the practitioner recognise some subjects in this group. Hence candidal infection presenting as diffuse erythema of the palate or tongue, or in the form of white plaques (thrush), usually on the soft palate; hairy leukoplakia affecting lateral margins or ventral aspects of the tongue; Kaposi's sarcoma, often appearing as a (red, blue or purple) papule or nodule at the junction of soft and hard palate, or on the gingivae; severe acute necrotising ulcerative gingivitis (ANUG) in individuals with a reasonable standard of oral care, and severe aphthous-like ulceration or severe necrotising ulceration involving the fauces, have all been observed in HIV-infected patients [92].

HIV acts by depressing CD4 lymphocyte numbers, and the effects of the disease result from a lack of protective immunity in infected individuals. In a large proportion of cases the virus is latent within CD4 lymphocytes, and these carriers may be unaware they have been infected, or of their carrier status [93]. Most of the increasing

numbers of HIV carriers who attend for dental treatment are not readily identifiable, highlighting the need for routine application of cross-infection control measures. Semen and blood, containing the infected lymphocytes, are the normal modes of transmission [94], but the virus is found in most body fluids and has been identified in saliva [95].

In one case, transmission of the virus within the dental surgery has been reported [6,7,8], but in other studies involving patients of HIV-infected health workers, there has been no further report of human immunodeficiency virus transmission to a patient [9,10,11,12]. Nonetheless, while it is by no means certain that the AIDS virus can be transmitted during routine dental procedures, the AIDS patient constitutes an increased cross-infection risk within the dental surgery, because the oral cavity is a common site of secondary infection in such patients. Superficial fungal infections are common [96,97], and there is an increased risk of cross-infection from other pyogenic organisms and *mycobacterium tuberculosis* [98]. While it has been shown that HIV is inactivated by exposure to glutaraldehyde and sodium hypochlorite [99], the virus has been found to survive in blood for up to seven days at room temperature [100].

1.2.3 TUBERCULOSIS

A rising incidence of tuberculosis, attributed to the HIV epidemic, has been reported from the USA and several African countries [101,102,103], and it is predicted that in most advanced countries the HIV epidemic will lead to a number of additional cases of tuberculosis among HIV positive individuals, and a small additional risk to the HIV negative population [104]. A slight increase in the number of tuberculosis notifications in England and Wales has recently been observed, but there is no evidence that it is related to HIV [105]. However dentists are increasingly treating patients who may be predisposed to the disease, eg the elderly population with medically compromising conditions such as diabetes, chronic renal failure or lymphoma; patients on immuno-suppressive drugs, and individuals suffering from alcohol or drug abuse [32,106].

Most subjects infected with *Mycobacterium tuberculosis* are asymptomatic and develop a carrier state. If immunity diminishes, the microorganisms can produce active disease, (almost 90% of TB cases result from re-activation of an earlier infection [109]), and patients with active disease are infective. Tuberculosis is a highly infectious disease, typically involving the pulmonary system; but it can affect any organ or tissue

including the mouth [108]. Although airborne transmission is the most common route for TB infection, transmission can occur through direct contact [109,110]. Infection control measures in dentistry, including the disinfection of impressions, should aim to minimise the transmission of *Mycobacterium tuberculosis*; a previous outbreak of tuberculosis in the UK being related to dental treatment [111].

1.3 GENERAL APPROACH TO CROSS-INFECTION CONTROL

Because a high proportion of blood-borne viral carriers cannot readily be identified, a single tier standard to infection control should be adopted in dental practice [22]. Ideally surgery design should take account of disinfection requirements, and manufacturers now offer dental equipment constructed to ensure that work surfaces (in materials resistant to damage from disinfectant agents) are smooth and offer good access for cleaning. The use of easily cleaned, flat-surface control keys, rather than buttons to operate equipment, can contribute to effective disinfection around the dental chair, as can the use of detachable air and water syringes, and suction tips. Foot controls for the modification of chair position can help reduce contamination of the operator's hands, and those surfaces which have to be touched during

treatment, such as light handles and bracket tables, can have disposable coverings in the area of contact, to be changed between patients. If possible, surgeries should be designed so disinfection and sterilisation procedures can readily be incorporated within the normal routine [112]. It is good practice to have the working area beside the dental chair divided into zones, with a clear division between areas containing clean items and those which have been contaminated, to allow effective infection control during treatment of patients, and disinfection of the working area between patients [113].

Contamination from infectious patients is an ever present risk for all chairside dental staff, who should be aware of the need for personal protection. Vaccination against hepatitis B and *Mycobacterium tuberculosis* infection [1.2.1, 1.2.3], with regular monitoring of immune status, is essential, and vaccination against tetanus and poliomyelitis is advised [22]. Cuts and abrasions on the hands should be covered with waterproof dressings and gloves worn during treatment, and protective glasses and face masks donned for surgical procedures, or when aerosols are created [114].

Some contamination is inevitable during dental treatment, and a major part of cross-infection control is concerned with the removal of microorganisms left on surfaces,

instruments and equipment after dental treatment. Again a single tier standard is adopted, as patients constituting a high risk of infection will not normally be readily identifiable. Initial cleaning of contaminated items reduces the number of microorganisms to be killed and removes blood, saliva and other organic material which may insulate microorganisms from direct contact with a sterilisation or disinfection agent. The use of an ultrasonic cleaning bath is an effective method of debris removal which reduces direct handling of contaminated instruments and the likelihood of injury resulting from tissue puncture [115]. Not all items of dental equipment are suitable for ultrasonic cleaning (most notably dental handpieces and ultrasonic scalers) and, if doubt exists, manufacturers' advice on correct cleaning procedure is required.

The sterilisation process is intended to kill all micro-organisms, including high numbers of resistant bacterial spores [116]. In the dental surgery this is normally achieved by means of an autoclave which uses the latent heat of steam, usually under pressure; but dry heat, chemical vapour and gas sterilisers are also available. The effectiveness of these sterilisation procedures can be verified by spore testing and chemical monitoring. Immersion in a 2% solution of glutaraldehyde, with a contact time of about 10 hours, is capable of eliminating

bacterial spores and achieving sterilisation, but this method of sterilisation cannot be verified routinely by spore testing and is not recommended for items that can be heat sterilised. The American Dental Association has recommended that instruments which penetrate soft tissue or bone, or come in contact with the soft tissues, should be sterilised after each use, or discarded [19].

Although it has not been documented that dental handpieces have been responsible for the transmission of disease in the dental surgery, debris from operative dental procedures can enter the turbine chamber of high speed handpieces, potentially to be transmitted to the next patient [117], and it is now recommended by the ADA that handpieces are cleaned and heat sterilised between patients, to ensure internal and external sterility [118]. British Dental Association recommendations [21, 22] indicate that instruments must be disposable, or capable of being sterilised, and that handpieces must be cleaned, lubricated and sterilised after every patient.

Elimination of surface contamination in the surgery is achieved by the use of disinfectant chemical agents. The procedure is less effective than sterilisation, and while most important pathogenic organisms can be eliminated by surface disinfectants, there is no method of verifying the effectiveness of the techniques employed. Phenols,

iodophors, quaternary ammonium compounds, peroxygenated compounds, sodium hypochlorite and glutaraldehyde are disinfectants recommended for use in dentistry by the BDA [21,22], with sodium hypochlorite granules indicated for management of blood spills. It is pointed out that quaternary ammonium compounds are not effective against mycobacteria. The ADA no longer provides surface disinfectant agents with formal approval, but recommends that products for surface disinfection in the dental surgery are registered with the Environmental Protection Agency. There are legal implications for practising dentists who do not implement the appropriate guide-lines on infection control, both in the United States [119], and in the UK [120,121] where failure to employ adequate methods for cross-infection control may render a dentist liable to proceedings for misconduct from the General Dental Council [1]. Dentists are required to protect staff and patients attending the surgery, to review procedures which involve contact with any substances which may be hazardous to health (including micro-organisms), and to ensure that staff are trained to undertake procedures necessary for safe working.

1.4 THE PROBLEMS POSED BY DENTAL IMPRESSIONS

The use of dental impressions and the casts poured from them is a fundamental method of recording information in the dental surgery, and of transferring such information from the chairside to laboratory. Many different types of impression material, with differing physical and chemical properties, are in common use. Factors which affect the choice of such materials include dimensional accuracy and stability, compatibility with the oral tissues, cost, and ease of manipulation. No one class of impression material has ideal properties and the demand for differing types will continue for the foreseeable future.

Because dental impressions, and subsequently produced casts, have the potential to transmit microorganisms [122,123,124], methods of restricting the contamination risk have been considered. Recommendations from the American Dental Association [19] suggest that dental impressions should be rinsed to remove saliva, blood and debris, and should be disinfected in a suitable agent before being cast or transferred to the laboratory. It is recommended that work coming to the laboratory is received in a prepared area, that impressions are disinfected (unless this has been carried out prior to delivery to the laboratory), and staff handling

impressions wear protective clothing and disposable gloves. Advice issued by the British Dental Association [21,22] is less stringent with respect to handling of impression materials and laboratory items. It is recommended that impressions are rinsed under running water to remove any visible signs of contamination, that disposable impression trays are used, and that gloves be worn when impressions are poured.

Watkinson surveyed techniques for disinfection of impressions in UK Dental Schools and found a wide range of procedures in use [125]. There was an 83% response from Departments of Prosthodontics, Orthodontics and Conservative Dentistry and, in almost half (45%), no disinfection regime was followed. Impressions were rinsed under running water in 17.5% of departments, and in 37.5% chemical disinfection was undertaken routinely. Solutions of glutaraldehyde, chlorhexidine and hypochlorite were the disinfectant agents most commonly used. In a report issued by the American Association of Dental Schools [126] in which clinical guide-lines for infection control in dental education institutions are outlined, it is suggested that all patient-contaminated items to be transported to the laboratory should be disinfected using an Environmental Protection Agency-registered, hospital level, mycobacterial agent, capable of killing lipophilic and hydrophilic viruses.

1.5 AGENTS FOR DISINFECTION OF DENTAL IMPRESSIONS

As impression materials currently in use cannot be heat-sterilised, they are usually treated by immersion in an appropriate chemical disinfectant. Disinfectant agents must be effective in destroying microorganisms; should not affect adversely the dimensional stability or surface detail reproduction of impression materials; should not be toxic, and should not deteriorate with storage. Agents commonly used for immersion disinfection of dental impressions in the UK are glutaraldehyde, hypochlorite and chlorhexidine [22, 125], and chlorhexidine and sodium didecyldimethyl ammonium chloride have been incorporated within alginate impression materials (Hydrogum, Zhermack, Badia Polesine, Italy; Blueprint Asept, DeTrey Dentsply Co, Konstanz, Germany;)

Chlorine is an effective antimicrobial agent, which at appropriate concentrations is active against bacteria, fungi, spores and viruses (including hepatitis viruses and HIV). A range of hypochlorite solutions (including sodium and calcium hypochlorite) are used as a source of free chlorine for disinfection. Commercial products are available for specific purposes, such as the disinfection of infants' feeding bottles (where the application of solutions containing not less than $125/10^6$ parts of available chlorine has been found to have bactericidal

capacity in excess of 10^9 organisms/ml of hypochlorite solution [127]). For this application, solutions may be sodium hypochlorite (NaOCl) solutions, or hypochlorite solutions prepared from sodium dichloroisocyanurate tablets, which contain 2.5g of available chlorine, and are added to water to make up the desired quantity and strength of disinfectant solution. Both have a high disinfection capacity against a wide range of micro-organisms [128]. It has been reported that NaOCl and NaDCC solutions containing the same levels of available chlorine, exhibit similar bactericidal activity despite significant differences in pH [129]. However, NaDCC disinfectant solutions have a greater anti-microbial effect than calcium or sodium hypochlorite in the presence of organic matter. The pH of sodium dichloro-isocyanurate solutions is lower than sodium hypochlorite, which may be beneficial for anti-microbial activity under these conditions. Tablets of NaDCC are stable, but solutions produced by dissolving the tablets in water are unstable, and decompose more quickly than NaOCl solutions of the same strength.

Aqueous solutions of NaOCl, or NaDCC tablets or granules dissolved in water, are used in dentistry. Solutions should contain 10,000 parts per million of available chlorine when there is risk of contamination with blood or blood products [22], conditions which may apply to

contaminated dental impressions. Hypochlorite solutions are unstable and the chlorine level is indeterminable with time. A protracted time-interval may be required for effective use, due to ready inactivation by organic matter, which should be removed before disinfection.

Glutaraldehyde is a highly effective, broad-spectrum chemical disinfectant, active against viruses, bacteria, mycobacteria, fungi, and spores, over an extended period of time (10 hours). Immersion of instruments for 10 minutes in 2% glutaraldehyde is effective in eliminating fungi, viruses and bacteria [130], and the Council on Dental Therapeutics of the American Dental Association accepted glutaraldehyde for use in disinfection and sterilisation in 1973 [131]. Glutaraldehyde is not affected by organic matter, does not corrode metals, and it has been shown that its anti-microbial activity can be enhanced by use in conjunction with ultrasonic cleaning baths [132]. Glutaraldehyde is usually stored in an inactive form and activated with sodium bicarbonate before use. As it can be irritant and has sensitising properties, glutaraldehyde should be stored in closed containers and used in a well-ventilated environment. Prolonged exposure can lead to nasal and throat symptoms, nausea and headaches, and rashes which are thought to be due to direct irritation rather than to allergy [133]. Airway obstruction, eye irritation and dermititis [134]

and asthmatic reactions [135,136] have also been reported following exposure to glutaraldehyde.

Chlorhexidine is a broad spectrum biguianide antiseptic with bactericidal activity against Gram-negative and, particularly, Gram-positive microorganisms [137]. It has limited antiviral activity. It exerts its effect by absorbing on to the cell walls of microorganisms with resulting damage of permeability barriers, and in high concentration, precipitation of cytoplasmic contents is caused. It is accepted that the use of 0.2% chlorhexidine as a mouth rinse, twice daily, prevents the formation of dental plaque [138], and reduction of salivary bacteria by a pre-procedural rinsing with 0.12% chlorhexidine has been reported [139]. Clinical side-effects following the use of chlorhexidine are relatively localised and minor. They include staining of teeth, disturbances of taste, and, rarely, parotid gland swelling [140].

Evaluation of the activity of disinfectants against the viruses responsible for hepatitis and AIDS is incomplete, mainly because there is considerable difficulty in designing experimental models to yield data with valid clinical applications. However virus membrane solubility procedures, using a combination of a metabolisable detergent and ether, were reported to be successful in inactivation of hepatitis B virus, after 18 hours [141].

Bond and co-workers [142] examined the effect of 10 minutes exposure of hepatitis B virus, in dried human plasma, to 70% isopropyl alcohol; sodium hypochlorite (500mg free chlorine per litre); 2% aqueous glutaraldehyde; a combination of 2% glutaraldehyde and 7% phenol, or a 1:213 aqueous dilution of an iodophor detergent. Chimpanzees received the treated plasma intravenously, and none showed any sign of infection after five months. When re-challenged under similar conditions, but without inactivation of the hepatitis B virus, two of the animals were infected after four weeks. Kobayashi and co-workers, also using direct chimpanzee inoculation [143], found that exposure to 1% or 0.1% aqueous glutaraldehyde for five minutes, to 80% ethyl alcohol for two minutes, or to heat at 98^{o}C for two minutes inactivated HBV in human plasma. These studies indicate that resistance levels of HBV to disinfection are not extreme, and the likelihood is that chemicals with similar antimicrobial activity may be used safely against hepatitis B virus. Immersion in glutaraldehyde for 30 minutes was recommended in 1977 by the Centre for Disease Control in the USA [144].

1.6 THE DISINFECTION OF DENTAL IMPRESSION MATERIALS

1.6.1 Introduction

Dental impressions are made so that a replica of intra-oral structures can be produced, usually in dental stone or plaster of Paris, allowing analysis for diagnostic purposes or laboratory construction of an appliance. Despite these seemingly limited objectives, a large number of different types of material exist for recording impressions; these materials may be categorised by their mechanical properties or by chemical composition.

Impression materials may be considered as elastic or non-elastic. Non-elastic materials are confined to use in edentulous jaws as they will not reproduce accurately the undercuts found around or between the teeth. Elastic materials, which can regain their original form after removal from undercuts, may be used for cast production for both edentulous and dentate patients.

Impression materials may be classified according to viscosity. Viscous (mucocompressive, heavy bodied) materials displace or compress the soft tissues while recording an impression, but will flow under pressure without support from a well-fitting impression tray. Often they are not capable of recording fine detail.

Fluid (mucostatic, light bodied) materials will record impressions with minimal distortion of soft tissue, can record impressions in thin section and are more suited to recording fine detail. The main disadvantage in the use of fluid materials is that they require to be supported in function, either with a customised impression tray or by use in conjunction with a more viscous impression material.

Impression materials may be considered as either thermoplastic or chemically activated. To be effective in use, impression materials require to enter the mouth in a softened state, and harden or set before removal. Thermoplastic materials are softened by heating, usually in a water bath or the flame of a Bunsen burner. On cooling to mouth temperature (or below if water-cooled impression trays are used), the thermoplastic materials harden. The components of chemically-activated materials which react to produce a setting reaction, are combined by mixing immediately prior to insertion of the material into the mouth. Various methods of mixing are used depending on the impression material type. In some cases water is added to powder; drops of liquid catalyst may be added to a putty material; two putty materials may be kneaded together, or pastes may be mixed, either manually or by use of an automatic mixing gun.

Impression materials are commonly classified by chemical composition. Non-elastic materials include impression plaster and zinc-oxide & eugenol paste (which are chemically-activated, fluid materials), and impression compound (which is a thermo-plastic, viscous material). Elastic materials include irreversible and reversible hydrocolloids, and polysulphide, polyether, condensation silicone and poly (vinyl siloxane) rubber base materials. Hydrocolloid materials are usually of medium viscosity, and the rubber materials are manufactured in various viscosity types (eg light, medium and heavy bodied, and putty). With the exception of reversible hydrocolloid, which is thermoplastic, all elastic impression materials are chemically-activated.

The reaction of impression materials to disinfection is usually related to their chemical classification, and of particular importance is the effect of disinfection by immersion on dimensional stability and surface detail reproduction.

1.6.2 DISINFECTION OF NON-ELASTIC IMPRESSION MATERIALS

There is little information in the literature on the effect of disinfection regimes on non-elastic impression materials.

Storer & McCabe [145] evaluated the effect of immersion disinfection on impression compound and zinc oxide & eugenol impression paste. Impressions were soaked in hypochlorite (1% available chlorine), 2% glutaraldehyde, 4% formaldehyde, or water, for 16 hours. From subjective comparison with non-immersed impressions, they found that hypochlorite produced a detrimental effect on the surface detail reproduction of impression compound, but that zinc oxide & eugenol was clinically satisfactory. Dimensional stability assessment measurements were made of a test die and the casts produced from it, using a micrometer, and comparison was made between the immersed impressions and impressions stored under dry conditions for 16 hours. Impression compound was unsatisfactory following immersion in all agents tested. Zinc oxide & eugenol showed unsatisfactory dimensional change when soaked in hypochlorite, but was satisfactory following immersion in glutaraldehyde, formaldehyde or water.

Olsen, Bergman & Olsen [146] examined the effect of immersion disinfection on surface detail sharpness and dimensional stability of zinc oxide & eugenol, and found that immersion for one hour in any of seven different disinfectants (including 2% glutaraldehyde) produced no clinically significant changes. Fong & Walter [147] examined the effect of immersion for a 20 minute interval on dimensional stability and surface detail reproduction

of impression compound, impression plaster and zinc oxide & eugenol impression paste. The materials were immersed in a disinfectant containing quaternary ammonium compound and aldehydes. Variations in dimensional change between disinfected and control groups were considered to be of no clinical importance (although statistically significant at the 95% confidence level), and there was no adverse affect on surface detail reproduction.

1.6.3 DISINFECTION OF REVERSIBLE HYDROCOLLOID IMPRESSION MATERIALS

Hydrocolloid materials used in dentistry are colloidal suspensions of polysacharides in water, which exist in two forms (sol form or gel form). In the sol form, there is a random arrangement of the polysaccharide chains and the materials are fluid in consistency. In the gel form the polysaccharide chains become aligned, the materials become more viscous and may develop elastic properties [148].

The use of reversible agar hydrocolloid as a precision dental impression material was described in 1937 [149]. The development of elastic properties on alignment of the polysaccharide chains is induced by cooling of the sol. On reheating the gel, the bonds are destroyed and the material reverts to the sol form.

Olsen, Bergman & Bergman investigated the dimensional stability and surface detail sharpness of reversible hydrocolloid impression materials following immersion in disinfectant solutions [150], and found that immersion of agar materials for 1 hour was deleterious to dimensional stability.

Townsend, Nicholls, & Powell [151] examined the effect of iodophor, glutaraldehyde and phenol, and found the accuracy of reversible hydrocolloid materials was not affected by immersion for 10 minutes, or by a 10 minute contact with these agents applied as spray disinfectants. In a study by Merchant and co-workers [152], gold inlays were constructed from reversible hydrocolloid impressions of an inlay cavity preparation in an extracted maxillary first premolar. The experimental impressions were disinfected by immersion for 30 minutes in 2% acidic glutaraldehyde (diluted 1:4), 2% alkaline glutaraldehyde and a 1:10 dilution of commercial bleach, before being cast. Control specimens were made from non-immersed impressions. The fit of the inlays was assessed and subjectively graded. The fit of castings from those impressions immersed in alkaline glutaraldehyde was found to be significantly poorer than castings from the other disinfected impressions, but there was no significant difference between immersed and control specimens when alkaline glutaraldehyde was excluded from consideration.

1.6.4 DISINFECTION OF IRREVERSIBLE HYDROCOLLOID IMPRESSION MATERIALS

Irreversible hydrocolloid (alginate) impression materials have perhaps the most widespread use in dental practice. They are easy to manipulate and are readily accepted by patients. In addition, they are inexpensive and do not require elaborate equipment for their use. Alginate materials are usually supplied as a powder, to which room temperature tap water is added. The handling properties are dependant upon the molecular weight of the alginate compounds within the powder, which is controlled in the manufacturing process and which varies between different types and brands. The addition of water initiates an irreversible chemical setting reaction during which the material changes from a sol state to a gel state [153].

The alginate powder contains soluble sodium, potassium or triethanolamine salts of alginic acid. These react with calcium sulphate (contained within the alginate powder) when water is added, and insoluble calcium alginate is produced. This chemical reaction is responsible for the change in the physical state of the material from sol to gel. The speed at which this setting reaction occurs is controlled by the presence of a retarder (eg trisodium phosphate) in the material, which reacts preferentially with calcium sulphate. When the supply of retarder has

been exhausted the reaction to produce insoluble calcium alginate proceeds [153].

The dimensional accuracy of irreversible hydrocolloid materials has been subject to much study. In an early investigation of the effect of storage, Skinner & Pomes [154] found a degree of expansion on setting, shrinkage on dry storage, and expansion following immersion in water. It was found that the material was stable only if stored in an environment of 100% humidity, and that dimensional change could occur in impressions sent to commercial laboratories. Immediate pouring of casts from alginate impressions was recommended. Phillips and co-workers [155] also found dimensional change in alginate impression specimens on storage in different media, and they emphasised the necessity of immediate impression pouring. In this work, a number of different types of alginate material were investigated, and it is a complicating factor in the assessment of most alginate material studies, that different types and brands have been examined and a variety of techniques have been used in analysis.

Under ideal conditions, alginate materials have been found to be among the most accurate currently available [156], but it has also been found that they are adversely affected by immersion in disinfectants. As with studies

examining the dimensional stability of alginate, analysis of those examining the effects of disinfection on dimensional stability, is complicated by the variety of different materials (both impression and disinfection) investigated, and the differing methods of data gathering and analysis used.

Storer & McCabe [145] used an alginate material to examine the effect of 16 hour immersion in 2% glutaraldehyde, or a 10% solution of sodium hypochlorite (containing 1% available chlorine). Impressions of a steel mesh were assessed subjectively for surface detail reproduction. Both glutaraldehyde and sodium hypochlorite produced a colour change, but there was no apparent surface deterioration in the impression material. To assess dimensional stability following disinfection, impressions of a stainless steel cone were immersed for 16 hours in test disinfectants before stone casts were poured. Following immersion in glutaraldehyde, shrinkage of the alginate was evident, and the test dimensions were significantly different from those found in impressions stored under a damp gauze (which showed expansion). Following immersion in sodium hypochlorite, alginate specimens expanded, but showed no significant difference from impressions stored under damp gauze. The authors recommended the use of materials other than alginate when immersion disinfection is indicated.

Setcos, Peng & Palenik [157] reported on the effects of 30 minutes' immersion on alginate. Immersion was in distilled tap water or in disinfectant agents including chlorhexidine, glutaraldehyde and diluted bleach. Impressions were made of an engraved metal die, and casts poured in gypsum. Immersion in glutaraldehyde produced statistically significant changes in values for linear dimensions. Scanning electron microscopy indicated that immersion of the impressions dramatically changed the crystalline structure of casts.

Bergman, Bergman & Olson [158] examined the effect of disinfectant agents, including 2% glutaraldehyde and 0.5% chlorhexidine, on dimensional stability and reproduction of surface detail for four alginate materials. Impressions of a stainless steel block were mounted in a specimen holder to maintain 100% humidity. Immersion for one hour produced unacceptable dimensional change in all four impression materials, and it was concluded that alginate materials should not be immersed for this time-interval.

Herrera & Merchant [159] examined dimensional changes in different types of impression material after immersion for 30 minutes. The disinfectant agents included 0.5% and 1% sodium hypochlorite; 2% neutral glutaraldehyde; 0.13% neutral glutaraldehyde, and 0.5% povodine iodine.

Comparison was made with non-immersed impressions and impressions immersed in distilled water. Of the materials tested, alginate alone showed statistically significant dimensional change between experimental and control groups, which occurred after immersion in 2% neutral glutaraldehyde and 0.5% povodine iodine. It was not indicated whether dimensional changes were likely to be of clinical importance.

Minagi and co-workers [160] examined the dimensional change occurring in poly (vinyl siloxane) and alginate materials, before and after immersion disinfection in agents containing 2% glutaraldehyde or 10,000 parts per million available chlorine. The distance between two markers, set in the materials, was assessed using a measuring microscope, and a range of immersion intervals was tested (5,10,15,20,30,60,120 minutes). The number of samples tested for each regime is not recorded, nor is the protocol followed for control samples. Alginate was less stable than silicone rubber, although the mean dimensional change of the alginate materials was less than 0.15%. It was concluded that disinfection procedures did not significantly affect the dimensional stability of the alginate under "simulated clinical conditions".

Durr & Novak [161] considered the effects of immersion disinfection on full arch impressions of a plaster cast

with embedded measurement posts. Impressions were made using an unspecified Type II alginate material, and disinfection was by immersion for 10 minutes in 1% sodium hypochlorite, or 2% glutaraldehyde. Measurements of distance between four reference points were made and casts from immersed alginate impressions showed some dimensional change when compared with casts from non-disinfected impressions, and with the master model. The dimensional change, although of statistical significance, was considered insignificant for clinical applications. The surface quality of the stone casts was assessed subjectively, and was not diminished following immersion in either disinfectant. Immersion in glutaraldehyde was found to produce an improvement in surface quality.

Wilson & Wilson [162] assessed the effect of immersion in chlorinated disinfectants, on alginate materials. After immersion for 10 minutes in either of two such solutions, dimensional change was found to be negligible, although there was considerable deterioration in the surface quality of the materials.

Jones and co-workers used computer-assisted microscopy to examine the effect of immersion in 2.2% glutaraldehyde on the dimensional stability of alginate [163]. Of the following regimes, one was applied to impressions of acrylic resin casts:

- impressions dipped momentarily in water, then cast,
- impressions dipped in glutaraldehyde, stored for 10 minutes under a damp gauze, then cast,
- impressions soaked in glutaraldehyde for 5, 10, 20, or 30 minutes, then cast.

The casts were poured in plaster, and measurement of 12 linear dimensions was recorded using a Reflex plotter linked to a computer. In a number of instances, rinsing or soaking of the impressions led to statistically significant changes in dimensional accuracy (eg soaking in disinfectant produced a significant reduction in over-jet measurements). However, the authors emphasised that the dimensional changes were quantitatively small.

Townsend and co-workers [151] examined the effect on alginate, of 10 minutes immersion in iodophor, phenol or glutaraldehyde/phenol. It was reported that dimensional accuracy was not affected, but in this abstract the detail of methodology was not described.

Peutzfeldt & Asmussen [164] examined the accuracy of alginate following immersion in a number of disinfectants including 2% glutaraldehyde (immersion for 60 minutes) and 0.13% phenate-buffered glutaraldehyde (immersion for 10 minutes). Control specimens were not immersed. The accuracy of stone dies poured from the impressions, was measured by assessing the fit of a steel ring, and a

significant improvement in accuracy was seen following immersion of Jeltrate (LD Caulk, USA) in 0.13% phenate-buffered glutaraldehyde ($p<0.05$). Immersion of Blueprint Regular (DeTrey Dentsply, England) in 2% glutaraldehyde, produced a statistically significant impairment of accuracy ($p<0.05$). The use of a mechanical method for measuring the clinical effect of the dimensional change enhanced the clinical relevance of this study. Under similar experimental conditions, and using the same hydrocolloid materials and disinfectant agents as above, but taking impressions of a stainless steel roughness standard block, Peutzfeldt & Asmussen [165] determined the effects of immersion on impression surface texture. Immersion of Alginoplast (Bayer Dental, Germany) in 2% glutaraldehyde for 60 minutes produced a significant improvement in surface detail ($p=0.05$). No other combination of impressions and disinfectants produced significant change in surface detail reproduction, when compared with the non-immersed control specimens. In both the above studies, disinfection took place immediately after the impressions were made and, in order "to imitate the clinical situation" specimens were stored (at 100% humidity) for 24 hours before casts were poured.

Jones and co-workers [166] examined the dimensional stability of a standard alginate impression material (Kromopan, Wright Dental, Scotland), which was subjected

to disinfection by dipping in 2.2% Glutaraldehyde or 2% Virkon (Antec International Ltd, England), or by immersion for 30 minutes in these agents. Comparison was made with a self-disinfecting alginate (Blueprint Asept, DeTrey Dentsply, England) and non-immersed control impressions of Kromopan. Impressions were made of an acrylic model and casts poured in plaster of Paris. Measurements were made with a Reflex plotter linked to a computer, and in only one of twelve different measurements recorded (upper inter-canine distance), was there any statistically significant dimensional variation between any of the six disinfection regimes recorded. It would appear that no clinically significant changes resulted from immersion of Kromopan for 30 minutes in either of the agents, and that the dimensional accuracy characteristics of the self-disinfecting alginate do not differ from the conventional material (disinfected or not).

In examining linear dimensional changes of an alginate material, following immersion in an iodophor for a period of either 10 or 30 minutes, Giblin and co-workers [167] compared the findings with those for immersed reversible hydrocolloid and poly (vinyl siloxane), and with non-disinfected control samples. Casts obtained from the immersed alginate samples showed greater percentage dimensional change than the other impression samples,

although with all three regimes, linear dimensional alterations were small (<0.5%).

Because a variety of materials and methods of testing has been used, and because of variations in the analytical methods, direct comparison of results from these studies is not possible. However it is apparent that a major factor to be considered in the disinfection of alginates, is the effect on dimensional stability. From studies in which prolonged immersion was carried out [145,158], dimensional stability was affected to a degree that was considered significant in clinical terms, and in one instance [162], immersion in chlorinating agents had a marked detrimental effect on surface detail reproduction (although this finding was not repeated elsewhere). With respect to dimensional stability, it appears that alginate behaves less favourably on immersion than some other elastic impression materials [160]; that spray disinfection is less detrimental than immersion [157], and that the combination of a particular disinfectant agent and a specific alginate material, may be important in producing inaccuracies [162].

In most instances, reproduction of surface detail was found to be satisfactory [145,161,162] and, although disinfection by immersion did induce some dimensional change, it was felt the degree was unlikely to affect

the clinical performance of the material [151,160,161, 162,163,166,167]. It would have been of value if in more cases, findings suggested by the experimental data were confirmed by clinical experiments, as in the case of the study by Peutzfelt & Asmussen [164] where impairment of the accuracy of Blueprint Regular following immersion in glutaraldehyde, was confirmed by assessment of the fit of a steel ring on a truncated steel cone abutment. Another important aspect of two of Peutzfelt & Asmussen's studies [164,165], was the design of the experimental procedure. Although disinfection took place immediately after impressions were made, all specimens were stored for 24 hours before casts were poured. It is an increasing trend for technical work to be undertaken by laboratories distant from the surgery, and a considerable period of time may elapse between recording alginate impressions and the pouring of casts. In addition there is little information on the carriage of microorganisms on these alginate materials, and although several studies have examined the effects of various disinfectants upon the dimensional stability of irreversible hydrocolloids, few have tested the efficacy of such procedures. Aspects of the effects of immersion, followed by storage, on the dimensional stability of alginate materials, the carriage of oral microorganisms on alginate materials, and the effect of disinfection on the colonisation of alginates, were investigated in the work described in this thesis.

1.6.5 DISINFECTION OF RUBBER BASE IMPRESSION MATERIALS

Brown [168] compared the ability of polysulphide rubber, condensation silicone and polyether rubber base materials to reproduce undercuts, and examined the dimensional stability of these materials during storage. He found that polysulphide and polyether reproduced undercuts on laboratory preparations accurately, and that polysulphide rubber was the most stable on dry storage. Eames and co-workers [169] examined linear dimensional change after storage (for 30 minutes, or 24 hours) in 34 different condensation silicone, polysulphide rubber and polyether elastomeric impression materials. Little difference was found between many of the materials, although expansion of polyether materials over a 24 hour period was highlighted.

Peutzfeldt & Asmussen [170] assessed the accuracy of poly (vinyl siloxane) materials, making impressions of a truncated steel cone. Accuracy was determined by measuring the closeness of fit of a steel ring (which matched the original steel cone) on the dies produced. Baysilex (Bayer Dental, Germany) and President (Coltene AG, Switzerland) were the most accurate of the materials tested. Polymerisation contraction of Mirror 3 Extrude (Kerr, USA) exceeded that of other formulations. With the exception of Mirror 3 Extrude, the poly (vinyl siloxane)

materials were more accurate than two polyether products tested by similar methods.

It has long been recognised that polyether materials absorb water [171], and Hembree & Nunez [172] found that moisture contamination adversely affected the dimensional accuracy of polyether impressions recorded under laboratory conditions.

Bergman, Olsen & Bergman [173] examined the effect of seven disinfectant agents (including 2% glutaraldehyde and 0.5% aqueous chlorhexidine) upon 13 different polysulphide, polyether, condensation silicone or poly (vinyl siloxane) elastomeric impression materials. Condensation silicones, polysulphides and polyethers exhibited shrinkage during storage for 24 hours after immersion. The least dimensional change occurred when poly (vinyl siloxane) was immersed in glutaraldehyde or chlorhexidine. Some specific combinations of impression material and disinfectant agent were more suitable for reproducing good surface detail than others.

Merchant and co-workers [174] examined the effects of immersion of poly (vinyl siloxane) and polysulphide impressions, of a metal mandibular dental arch, for 30 minutes (in a variety of disinfectants), on linear dimensional stability. The disinfectants included 2%

acidic glutaraldehyde, 1.3% neutral glutaraldehyde and sodium hypochlorite (0.5% and 1.0%). It was concluded that, with the possible exception of use as dies for cast restorations, the casts could be considered accurate.

Herrera & Merchant [175] evaluated the effect of 30 minutes immersion of alginate, polysulphide rubber, poly (vinyl siloxane) and polyether impression materials, and found minimal changes in the linear dimensions of the resultant casts.

Johansen & Stackhouse [176] measured linear dimensional change in different elastomeric materials following storage for 16 hours under dry conditions, or immersion for 16 hours in 2% glutaraldehyde. Poly (vinyl siloxane) products were stable during storage under wet and dry conditions, while polysulphide and condensation silicone specimens shrunk on storage under wet or dry conditions. Polyether shrunk under dry conditions and expanded on immersion, and the dimensional change between these was statistically significant.

Merchant and co-workers [177] determined the dimensional stability of polysulphide rubber materials following immersion in disinfectants (which included 2% alkaline glutaraldehyde and 0.5% sodium hypochlorite). Control specimens were immersed in distilled water, or stored at

room temperature without disinfection. Impressions of an inlay cavity were immersed for 30 minutes, and the fit of type II gold inlays assessed. There were no statistically significant differences between or amongst experimental groups, with respect to dimensional stability. Immersion in the glutaraldehyde and hypochlorite solutions tested was acceptable for polysulphide impression materials.

Johnson, Drennon & Powell [178] evaluated the effect of immersion disinfection on the accuracy and surface detail reproduction of polysulphide, poly (vinyl siloxane) and polyether impression materials. Glutaraldehyde (in which specimens were immersed for 10 minutes), and chlorine dioxide (in which specimens were immersed for 3 minutes) were among the immersion agents tested. Poly (vinyl siloxane) materials in combination with disinfectants other than neutral glutaraldehyde, produced casts with excellent dimensional accuracy; polyether materials were not accurate following disinfection by immersion, and polysulphide impressions were satisfactory with all the disinfectants. Minor changes in surface detail quality were observed following disinfection of poly (vinyl siloxane), polyether and polysulphide, but it was considered that these were unlikely to be of clinical significance.

Peutzfelt & Asmussen [164] examined three alginate and seven elastomeric materials, assessing accuracy with respect to the fit of a steel ring on dies produced from impressions of a truncated steel cone. They found that, whereas as alginate materials were affected to a considerable degree by immersion disinfection, the rubber base products were little affected, and they did not find the accuracy of polyether impaired by immersion disinfection. There were differences between impressions immersed in disinfectant agents and those immersed in de-ionised water, indicating that chemical composition of the disinfecting solution is a factor in producing dimensional change. In some cases, immersion compensated for polymerisation shrinkage, and gave rise to more accurate stone dies.

Langenwalter, Aquilino & Turner [179] evaluated the linear dimensional accuracy of poly (vinyl siloxane), polysulphide and polyether impression materials under dry conditions and after immersion for 10 minutes in agents which included 0.05% sodium hypochlorite and 2% glutar-aldehyde. Measurement was by examination of impressions of a test block (ADA standard No 19) using a travelling stage microscope at a magnification of x30. Polyether was the least accurate material, both on dry storage and with immersion. However, the linear accuracy of all three impression materials following disinfection, was within

0.5% of the test block, and was not affected by the choice of disinfectant agent. It was considered that dimensional stability was not affected to a degree which was clinically significant.

Drennon & Johnson [180] reported that surface detail reproduction from polyether and poly (vinyl siloxane) impressions was comparable, and that these materials produced significantly better surface detail than did polysulphide rubber. Immersion of all three rubber base formulations in acid glutaraldehyde resulted in enhanced line-detail reproduction, and poly (vinyl siloxane) and polyether impressions, immersed in acid glutaraldehyde, produced casts with surface roughness closest to the reference standard used in the investigation.

The consistent dimensional accuracy and stability of vinyl (poly siloxane) and polysulphide rubber base materials, are beyond doubt and, from this literature review, it is apparent they are not adversely affected by recommended immersion disinfection procedures. In no instance was such disinfection thought to have an significant detrimental effect on the clinical performance of these materials.

1.6.6 THE ANTIMICROBIAL EFFECT OF IMMERSION DISINFECTION

Studies examining the carriage of microorganisms on dental impression materials, or the efficacy of various disinfection procedures, are few. Drennon and co-workers [181] found immersion, for 10 minutes in 2.5% glutaraldehyde, effective in the elimination of *Ps aeruginosa*, *Staph aureus* and *Salmonella choleraesius* from polysulphide rubber impressions, and McNeill and co-workers demonstrated that, although a large number of micro-organisms were retained on the surface of impression materials after rinsing in tap water, immersion in 2% glutaraldehyde for 20 minutes, or in a sodium chloride solution containing 1,000 ppm available chlorine, for 7.5 minutes, was effective in eliminating artificially inoculated micro-organisms [182]. Westerholm and co-workers [183] evaluated the effect of eight disinfectant sprays on irreversible hydrocolloid impressions which had been artificially contaminated with 3 bacterial species (*Staph aureus*, *Mycobacterium phlei*, *Bacillus subtilis*), and with mixed oral flora. There was a wide variation between the different agents, although, full strength sodium hypochlorite (5.25%) was found to be an effective antimicrobial agent.

The incorporation of an antibacterial agent within Blueprint Asept was tested by Ghani and co-workers [184],

who reported a high degree of disinfection against mixed flora of human saliva, compared with standard alginate, while Tyler and co-workers [185] found no useful clinical effect from the use of the same material against herpes simplex virus type 1 and poliovirus type 1.

While no specific directions have been issued on the disinfection of dental impressions in the UK, guide-lines on chemical disinfection in hospitals, published by the Public Health Laboratory Service [186], indicate that exposure, to 2% alkaline glutaraldehyde or chlorine-based agents containing 10,000 ppm available chlorine, will inactivate viruses, including HIV, in about 1-2 minutes. Mycobacterium tuberculosis is more resistant, and 20 minutes exposure to 2% alkaline glutaraldehyde may be required to eliminate this microorganism.

Investigations of the carriage of microorganisms on alginate and rubber base impression materials, and the effect of various disinfectant regimes on microbial colonisation and dimensional stability, were further developed in the work of this thesis.

CHAPTER 2

CARRIAGE OF ORAL FLORA ON IRREVERSIBLE HYDROCOLLOID AND ELASTOMERIC IMPRESSION MATERIALS

INTRODUCTION

While guide-lines on the control of cross-infection in dentistry, including the disinfection of impression materials, have been issued by the American and British Dental Associations [19,21], there is little information in the literature on the carriage of microorganisms on the surface of dental impressions.

AIMS OF STUDY

The main objective of this study was to investigate the persistence of microorganisms on a number of impression materials in order to provide some indication of their relative potential for transmission of infection. There were *in-vitro* and *in-vivo* components to the study.

In the *in-vitro* investigation, microbicidal properties of poly (vinyl siloxane), polysulphide and two alginate materials were investigated and compared by *in-vitro* assay. The alginate material "Blueprint Asept" (Table 2.1), claimed by the manufacturer to be totally microbicidal, was of particular interest, and a variety of microbial strains were examined. With respect to *in-vivo* aspects, the transfer and persistence of microorganisms on dental impressions, from dentate and edentulous subjects, was assessed.

From these experiments it was hoped to establish whether a need for disinfection of impression materials exists, and whether the use of the "disinfectant" impression material in question was an effective antimicrobial measure.

2.1 EXPERIMENT 1 - MATERIALS AND METHODS

2.1.1 IMPRESSION MATERIALS AND MOULD PREPARATION

In this laboratory investigation, the influence of impression material type on the survival time of a number of microorganisms was assessed. Microorganisms were held in suspension in moulds of the impression materials. Three conventional impression materials (representative of the main types in common use in dental practice), and a material reported to have disinfectant properties, were investigated (Table 2.1). Moulds (Fig 2.1) were prepared for each impression material, using a cylindrical perspex vial (diam 2.8cm, height 2.0cm), a graduated test-tube (height 10cm), and a vertical measuring stand, calibrated in centimetres.

Prior to use, instruments and apparatus were disinfected using wipes impregnated with 70% ethyl alcohol (Azowipes, Vernon-Carus Ltd, Preston, England) to minimise the introduction of any contaminants. Impression materials were proportioned, mixed and manipulated according to manufacturers' instructions, and spatulated into perspex vials in such a way as to minimise the introduction of air bubbles.

The regular viscosity polysulphide rubber material was loaded into a syringe and injected into the vial. Poly (vinyl siloxane) was used in a two stage (putty and wash) system; putty was loaded into the vial and manipulated to create a central reservoir into which low viscosity paste could be injected. The base of the test-tube was placed centrally over the perspex vial (loaded with impression material) and submerged to create a well corresponding to a depth of 2.0ml on the test-tube. Due to displacement of the impression material from the vial during this process, the test-tube markings were obscured. To help overcome this problem, a vertical measuring device was adjusted to a pre-determined height of 13cm, indicating the level to which the test-tube should be submerged to create a well of standardised depth (Fig 2.2). The test-tube was maintained at the established height until the impression material set. It was then withdrawn and excess material trimmed from the periphery with a sterile blade.

The moulds were sealed in sterile humid containers at room temperature for the short period until they were inoculated with the appropriate experimental or control suspensions (2.1.3).

2.1.2 MICROORGANISMS AND CULTURE CONDITIONS

The *in-vitro* persistence of microorganisms within the impression moulds was tested with five microbial species, representative of the oral flora. These comprised four species of bacteria and one yeast (Table 2.2). Pure cultures were grown; the bacterial species (*E coli, Ps aeruginosa, S aureus, S mutans*) on blood agar plates incubated aerobically for 24 hours at 37^{o}C; the yeast (*C albicans*) on Sabouraud's medium (Oxoid Ltd, Basingstoke, England), each being incubated for 48 hours at 37^{o}C.

The microorganisms for inoculation were prepared as follows:

a) Using a sterile loop, several colonies (one loopful) were taken from a culture plate and inoculated into a sterile universal container filled with 9ml sterile phosphate buffered saline (pH 7.2, 0.1M).

b) The inoculum was dispersed by vortex mixing using a "whirlmixer" for 60 seconds, and the suspension was serially diluted (1:10) with more sterile saline to reduce the microbial count to approximately 10^3/ml - 10^4/ml.

2.1.3 INOCULATION AND SAMPLING OF IMPRESSION MOULDS

For each material, 1.8ml of prepared inoculum was delivered into an impression mould using a sterile pipette. The container in which the moulds were stored was re-sealed to prevent further contamination, and a timer was started. Control suspensions were prepared by dispensing 1.8ml samples of inocula into sterile vials. The survival rate of organisms in the suspensions was examined over a 5 hour period. Immediately following inoculation of the moulds (0 mins) a 0.2ml sample was pipetted from the control suspension and transferred to a labelled sterile bijou container. Subsequent 0.2ml samples were taken from both control and experimental suspensions at 30 mins, 1, 3 and 5 hours. The samples were stirred gently with a sterile pipette for 20 secs, to homogenise the suspensions prior to sampling.

2.1.4 CULTURE

A "spiral plater" (Spiral System Marketing, Baltimore, USA) was used to quantitatively assess the colony forming units of microorganisms per millilitre of the samples (CFU/ml). The spiral plater (Fig 2.3) is a specialised dispenser which mechanically distributes an adjustable volume of liquid sample on to the surface of a rotating 10cm culture plate in an Archimedean spiral.

Every sample is distributed such that the volume of microbial suspension in a unit area is constant and quantifiable, allowing accurate and rapid assessment of microorganisms in the sample. The plater was adjusted to deliver 50ul in each sample. Suspensions of *C albicans* were delivered on to Sabouraud's dextrose agar, and suspensions of *E coli, Ps aeruginosa, S aureus* and *S mutans* delivered on to blood agar. Between samples the plater was sanitised by drawing disinfectant solution, followed by sterile distilled water, through the stylus tube, thus preventing organisms from being retained and false readings obtained. Blood agar plates were incubated aerobically for 24 hours at $37^{o}C$, and Sabouraud's plates, similarly for 48 hours at $37^{o}C$.

2.1.5 COLONY COUNTS

Following incubation, the microorganisms produced colony forming units on the culture plates along the lines created by the stylus of the spiral plater (Fig 2.4). Whenever possible, the entire number of CFU on the culture plate in question was counted. When the number of CFU was high, the culture plates were sectioned using a specialised cutting device, resulting in division of the culture plates (Fig 2.5). Each area marked on the grid represented a known and constant volume of the sample deposited on the culture plate.

Thus the microbial density of the original sample could be determined by counting colonies on a unit area of the plate and dividing by the volume of the sample contained in the area analysed (Fig 2.6). When culture plates were sectioned several areas were assessed to increase the accuracy of the results. Procedures for EXPERIMENT 1 (2.1.1 - 2.1.7) were carried out twice for each microbial strain, using fresh inocula.

2.2 EXPERIMENT 1 - RESULTS

After counting of the colony forming units, the results were standardised by converting the data into percentage values. The initial concentration of microorganisms in the suspensions (CFU/ml at 0 mins) was taken to represent the 100% value, and subsequent readings obtained over the five hour period in control and experimental suspensions were expressed as a percentage of the initial value.

In control suspensions of *C albicans* (Fig 2.7, Table 2.3) the number of colonies cultured after 5 hours was 52% of the initial value (at 0 minutes). For the suspensions held in the Blueprint impression material moulds, the CFU value after 10 minutes was 13% of that found initially at 0 minutes and no growth was evident after 30 minutes. With the conventional materials there was a consistent

pattern of microbial retention, in that there was gradual diminution in CFU with time; the highest CFU values were evident in alginate, and the smallest in the poly (vinyl siloxane) material.

For *E coli* (Fig 2.8, Table 2.4), the number of CFU in the control specimens after 5 hours was 77.5% of the original value. CFU values for the suspensions held in moulds of Blueprint impression material were reduced by over 98% within 10 minutes, but a small number of CFU was evident after 3 hours. Conventional materials again showed a consistent pattern of microbial retention; there was a gradual diminution with time.

Ps aeruginosa (Fig 2.9, Table 2.5) showed high survival rates in the control suspensions, with a CFU value after 5 hours that was only 15% less than the initial value at 0 minutes. There was almost no growth from suspensions held in wells of Blueprint material after 3 hours, but high CFU values were found in those suspensions sampled after shorter time intervals; over 50% of the original CFU value seen at 10 minutes. The conventional impression materials showed a consistent pattern of microbial retention, with a relatively small diminution in CFU values with time. The lowest CFU values were found with alginate, the highest with poly (vinyl siloxane).

For control suspensions of *Staph aureus* (Fig 2.10, Table 2.6), CFU values after 5 hours were 52% of the value seen at 0 minutes. All growth within the Blueprint moulds was eliminated after 10 minutes. There was a gradual diminution in CFU values with time in the impression moulds of the conventional materials, with CFU values for alginate being less than the rubber base materials.
For *Strep mutans* (Fig 2.11, Table 2.7) the number of CFU found for control specimens after 5 hours was 52% of the initial value seen at 0 minutes, and Blueprint eliminated all microbial growth within 10 minutes. Growth on the conventional materials diminished with time and was more persistent on polysulphide rubber than on the two other materials.

Examination of the control suspensions (Fig 2.12) showed that *E coli* and *Ps aeruginosa* were more persistent over the five hour test period than the other microorganisms, although there was a reduction in CFU values for *E coli* after 30 minutes. There was a gradual uniform reduction in CFU values for *C albicans*, *Staph aureus* and *Strep mutans* over the five hour period, CFU values being halved in that time. *Ps aeruginosa* (and to a lesser extent *C albicans*) resisted the antimicrobial action of Blueprint more than the other test microorganisms (Fig 2.13), with an appreciable number of colonies cultured after 30 minutes.

With respect to the other three impression materials, (other than a rapid reduction in the number of colonies from suspensions of *Staph aureus* in wells of Kromogel impression material) there was a uniform pattern of reduction in CFU values over the five hour test period, with none of the microorganisms showing any consistent variation in response (Figs 2.14, 2.15, 2.16).

2.3 EXPERIMENT 2 - MATERIALS AND METHODS

As results from EXPERIMENT 1 showed that the disinfectant alginate (Blueprint Asept) destroyed virtually all the organisms tested within a period of 1 hour, a further investigation was conducted using Blueprint Asept alone. In EXPERIMENT 2, the inocula within the impression moulds were observed in greater detail over a shorter period of time. Experimental procedures as above (2.1.1 - 2.1.7) were carried out with the five microbial species, with impression moulds made only from Blueprint Asept. Sampling was undertaken at five minute intervals, over a 35 or 40 minute period depending upon the survival rate of the microorganisms. Control samples of inocula within sterile vials were also sampled at five minute intervals for the experimental period. Procedures for EXPERIMENT 2 were carried out three times for each microbial strain, using fresh inocula.

2.4 EXPERIMENT 2 - RESULTS

The results were standardised by conversion of the data for persistence of microorganisms, into percentages of the initial CFU values observed at 0 minutes (Tables 2.8 - 2.12), allowing comparison of the effect of Blueprint on the five microbial strains examined. In the control suspensions more than 50% of all microorganisms remained after 35 minutes, and *Staph aureus* and *Strep mutans* diminished more rapidly than the other micro-organisms (Fig 2.17).

For *Staph aureus* and *Strep mutans* the value for the number of CFU occurring after five minutes within moulds of the disinfectant alginate Blueprint were less than 1% of the initial value in the control samples. There was no growth evident from *Strep mutans* suspensions held in Blueprint impression moulds, after 15 minutes, and no growth from *Staph aureus* suspensions after 30 minutes. There was a marked reduction of CFU values from *E coli* suspensions in Blueprint moulds; 98% elimination after 15 minutes and no growth from *E coli* inoculated suspensions after thirty minutes. *C albicans* and *Ps aeruginosa* were found to be most resistant to the microbicidal effects of Blueprint. *C albicans* was least affected in the short term; after 10 minutes in the Blueprint Asept impression moulds, the CFU value from suspensions inoculated with

C albicans was 38% of the value found in initial control suspensions. This value (38%) was considerably higher than found for other microorganisms after 10 minutes in the impression moulds. *Ps aeruginosa* persisted as long as *C albicans*, although there was no growth evident from suspensions inoculated with either microorganism after 40 minutes (Fig 2.18).

2.5 EXPERIMENT 3 - MATERIALS AND METHODS

The objective of this laboratory investigation was to examine the persistence of the selected microorganisms on the surface of each of the impression materials following contact between impression and microbial suspension for a limited period of time (3 minutes). This was intended to simulate clinical conditions during impression taking.

2.5.1 IMPRESSION MATERIALS AND MOULD PREPARATION

The same impression materials as used in EXPERIMENT 1 were selected (Table 2.1), but in EXPERIMENT 3 the impression moulds in poly (vinyl siloxane) were made using only low viscosity material. Impression moulds were made from each of the four materials in sterile 10cm diameter petri-dishes (Fig 2.19). The impression materials were proportioned, mixed and manipulated in

accordance with manufacturers' recommendations and loaded in to the shallow (6mm deep) lid of the petri-dish. The base, with a smaller diameter than the lid, was pressed on to the impression material, to make a flat mould with a smooth surface and uniform depth of 4mm. After setting, excess material was trimmed from around the periphery, and to help prevent surface contamination, the base of the petri-dish was left in position until the microbial suspension was introduced.

2.5.2 MICROORGANISMS AND CULTURE CONDITIONS

The *in-vitro* persistence of the species of yeast and four species of bacteria used in EXPERIMENT 1 (Table 2.2), was tested, and suspensions for inoculation were prepared as in EXPERIMENT 1 (2.2.1).

2.5.3 INOCULATION OF IMPRESSION MOULDS

Impression moulds were uncovered by removing the base of the petri-dish, and 7ml of inoculum was introduced on to the surface of the test impression material. The entire surface was left submerged for three minutes, after which time the inoculum was discarded carefully and a timer started. Control suspensions of 7ml of inoculum were introduced into sterile vials for each of the test micro-organisms.

2.5.4 SAMPLING, CULTURE, INCUBATION AND COLONY COUNTS

The persistence of microorganisms on the surface of the impression materials was examined by removing samples from the impression moulds at regular time intervals. Sampling was carried out using a 13mm diameter cork borer, which was sterilised between the collection of samples by heating in it the flame of a Bunsen burner. It was then cooled in sterile phosphate buffered saline. Impression samples were removed immediately after the inoculum was discarded (0 mins) and deposited in a labelled universal container containing 20ml sterile phosphate buffered saline. Further samples were removed after 30 mins, and 1, 3 and 5 hours. The universal containers were vortex-mixed for 60 seconds to displace microorganisms from the impression surface into the saline, and the resultant suspension immediately decanted into a sterile universal container to prevent re-settling of microorganisms on to the surface of the impression samples. The control suspension was sampled at time intervals corresponding with those for the experimental impression materials. A 50ul sample of each suspension was plated (using the spiral plater) on the appropriate culture medium, incubated aerobically and the resulting surface growth recorded (ie, as for EXPERIMENT 1: 2.1.5; 2.1.6; 2.1.7). EXPERIMENT 2 was carried out twice with each microbial strain, using fresh inocula.

2.6 EXPERIMENT 3 - RESULTS

To allow comparison of survival rates for the different microbial species, percentage values for the number of microorganisms persisting on the impression surfaces, after appropriate experimental periods, were calculated.

Data from EXPERIMENT 3 are shown in Tables 2.13 - 2.17. In control samples, microbial suspensions were inoculated into sterile vials, and there was a reduction in the numbers of microorganisms (CFU) with time. After five hours, percentage CFU value for microorganisms remaining in the control samples varied from 68.5% with *E coli*, to 88.5% with *S aureus* (Fig 2.20).

Blueprint Asept caused almost total elimination of all micro-organisms tested, with only traces of *Ps aeruginosa* and *E coli* evident on initial sampling. No microorganisms of any strain were detectable after five minutes.

There were differences in survival rates for the various microorganisms when each of the conventional impression materials was examined. Following inoculation on petri-dishes containing alginate (Kromopan), *E coli* (with a 27% survival rate after 5 hours) was the most persistent species; *Staph aureus* (8.5% survival after 5 hours) and *Strep mutans* (5% survival after 5 hours) were the least persistent species (Fig 2.21).

C albicans was the only microorganism to survive in substantial numbers on rubber base impression materials. After five hours the survival rate of *C albicans* was 28% on the surface of poly (vinyl siloxane) and 22% on the surface of polysulphide rubber (Figs 2.22, 2.23). The other microorganisms were almost totally eliminated on rubber base materials after five hours, the only growth detected being with *S mutans*, which showed some residual activity (1% of the initial value for colony forming units) on polysulphide rubber.

The initial retention of each microorganism appeared to be affected by the type of impression material on which it was inoculated. As numbers of microorganisms delivered on to the impression surfaces was not standardised, the numbers retained on different impression surfaces were not directly comparable. However it was noted in all instances that the initial microbial loading of alginate (Kromopan) was substantially greater than that found on either of the rubber base materials.

Comparisons of the viability of the microbial species on impression surfaces was made by reference to percentage reduction of microorganisms with time. The survival pattern of *C albicans* was distinctive, in that an appreciable quantity of the yeast was recovered from the rubber base materials after five hours (22-28%),

ie substantially greater than the 15% recovered from alginate (Kromopan) (Table 2.13).

All four species of bacteria were virtually eliminated from the surface of both rubber base materials after five hours but survived to varying degrees on Kromopan (Tables 2.14 - 2.17). *E coli* and *Ps aeruginosa* persisted in considerable numbers on Kromopan; the persistence of *E coli* (27%) was greater than *Ps aeruginosa* (12%) after five hours, but *Ps aeruginosa* was initially more profuse (Fig 2.21). Both *S aureus* and *S mutans* were cultured from Kromopan after five hours (8.5% and 5% respectively), and a high percentage value of the initial CFU count was recorded for both materials after one hour (Table 2.16, 2.17).

2.7 EXPERIMENT 4 - MATERIALS AND METHODS

2.7.1 SUBJECTS

In this study retention of microorganisms on alginate and elastomeric impression materials was examined in dentate and edentulous subjects. The dentate group consisted of twenty-one senior students at Glasgow Dental Hospital and School, selected on a voluntary basis.

The subjects were between 20 and 22 years of age; seven were male and 14 were female, all of whom were healthy. The edentulous group consisted of four male and four female patients attending Glasgow Dental Hospital and School for the provision of complete dentures. The age range of the edentulous group was from 68 to 79 years, and all were in good health.

2.7.2 IMPRESSIONS

In the first part of the study, involving 21 dentate subjects, three impression materials were examined viz: Blueprint Asept, New Kromopan and Provil (Table 2.1). For each subject a maxillary impression was recorded in one impression material only. Thus, seven impressions were obtained for each of the test materials, and no subject was sampled on more than one occasion. In the second part of the study, with eight edentulous patients, only the alginate impression materials were investigated. Four maxillary impressions were recorded with Kromopan, and four with Blueprint Asept. Again no subject was sampled on more than one occasion.

The impressions were taken in adhesive-coated perforated plastic stock trays. In the dentate group, Provil impressions were made using a single stage putty and wash technique; the low viscosity material was supported by

the high viscosity putty. In all cases, the impression samples for the production of microbial suspensions, were removed from the palatal area of maxillary impressions (Fig 2.24) using a 13mm diameter sterile cork borer. Samples were taken from Provil and Kromopan impressions immediately on removal from the mouth; after 10 mins; 30 mins; 1 hour; 3 hours, and after 5 hours. With Blueprint Asept, as the material had been observed to be effective in rapidly removing microorganisms in the *in-vitro* study, sampling was performed at 0, 10, 20 and 30 minute intervals. Samples of impression material were placed in 10ml phosphate buffered saline and whirlmixed, and the resultant suspension was immediately decanted into bijoux containers, to prevent any settling of microorganisms.

In the intervals between sampling, to prevent drying of the materials, impressions were stored in sealed plastic bags containing damp gauze, in such a fashion as to ensure no contact with the palatal area.

2.7.3 CULTURE, INCUBATION AND COLONY COUNTS

Duplicate samples were distributed on blood agar plates using the spiral plater, incubated for 24 hours, and the number of colony forming units on each plate counted. Microbial growth was such that the use of the sectioning device was not required.

2.8 EXPERIMENT 4 - RESULTS

The number of organisms recovered from the impressions was examined and, in the dentate group of patients, no growth was recorded from impressions made with Blueprint Asept (Table 2.18a). Growth was apparent in samples from both Kromopan (Table 2.18b) and Provil (Table 2.19c), and in some cases organisms were cultured five hours after the impressions were recorded. As the experimental groups were comprised of different individuals, comparison between groups gave only an indication of the trends for microbial retention for each material. Initial microbial loading of Kromopan considerably exceeded Provil (Table 2.18d) and there was a more rapid reduction in the number of microorganisms (CFU) cultured with Provil than with Kromopan, when considered as a percentage of initial microbial loading (Table 2.18e).

In the edentulous patients, no growth was recorded from impressions made with Blueprint (Table 2.19a). In the four edentulous subjects for whom Kromogel impressions were recorded, considerable growth of microorganisms was evident (Table 2.19b). Hence five hours after recording the Kromopan impressions, the mean value of CFU cultured from samples was 36% of the value recorded at 0 minutes (Table 2.19c).

Comparison of the Kromopan impressions from dentate and edentulous patients showed there to be slightly greater mean initial microbial loading of impressions from the dentate group, and the percentage reduction in CFU values over a five hour period to be comparable between the two groups. However, the small size of experimental groups, and single sampling, makes this observation of limited value.

2.9 DISCUSSION

2.9.1 EXPERIMENT 1

Impression wells from the three conventional impression materials (Kromopan, Provil and Permlastic) produced similar reactions when inoculated with the various micro-organisms. Over the five hour test period there was a reduction in numbers of organisms within the impression wells which, with one exception, fell below 50% of the original values for colony forming units. Poly (vinyl siloxane) provided the exception following inoculation with *Ps aeruginosa*, when the bacterial count after five hours was 52% of the original value. The reduction in microbial numbers with these materials was considerably greater than was found with the control suspensions. There was a rapid reduction of the microorganisms in

moulds made with Blueprint Asept impression material. Within ten minutes *S aureus* and *S mutans* were eliminated; *E coli* was reduced to 1.8% of its original value, and *C albicans* to 13% of its original value. *Ps aeruginosa* demonstrated greater resistance to Blueprint Asept; the bacterial count was halved within ten minutes and after one hour 8.3% of the original number of colony forming units was evident following culture.

2.9.2 EXPERIMENT 2

Findings in EXPERIMENT 1 suggested that more detailed study of the effects of the disinfectant alginate (Blueprint Asept) would be of value in determining its clinical value, and the findings of this more detailed investigation confirmed trends apparent in EXPERIMENT 1. Even with a high concentration of microorganisms, as used in this study, all were eliminated within 40 minutes. However variable susceptibility to the disinfectant alginate was evident, the more virulent *E coli*, *C albicans* and *Ps aeruginosa* persisted for longer periods than *S aureus* and *S mutans*.

2.9.3 EXPERIMENT 3

From EXPERIMENT 3, where microbial suspensions were in contact with flat impression surfaces for a time interval

similar to that expected in recording impressions, there was confirmation of the effective antimicrobial activity of Blueprint. The high initial microbial loading of the conventional alginate (Kromogel) and the persistence of microorganisms on this material over an extended period of time are also noteworthy, and are in contrast with the rubber base impression materials.

2.9.4 EXPERIMENT 4

It is accepted that, because of limitations in study design, results from this preliminary *in-vivo* study indicate trends worthy of further investigation, rather than permit clear conclusions to be drawn. However the results correspond to some extent with the findings of the *in-vitro* studies, and the effect of Blueprint in eliminating all microorganisms in the *in-vivo* study is clear, particularly when the level of microbial retention on the conventional alginate material (Kromopan) is considered. The high initial level of microbial loading of Kromopan (in comparison with Provil) in the dentate subjects, is also noteworthy, as is the high retention level of microorganisms on Kromopan over the five hour period. Because each experimental group was composed of different individuals, the value of comparing the effect of the impression materials between groups is limited; perhaps examination of the action of different materials

in a single experimental group of subjects would be more informative.

Although of interest, the finding of higher mean values for the number of microorganisms (CFU) colonising impressions from the dentate group, in comparison with the edentulous group, requires clarification in a larger and more detailed study, utilising multiple samples for each subject, and larger experimental populations.

2.10 CONCLUSIONS

The experiments detailed in this chapter confirmed the considerable antimicrobial effect of the alginate impression material containing didecyldimethyl ammonium chloride (Blueprint Asept), on the microorganisms tested. Even when the impression moulds were in the form of wells of material with limited direct contact between the impression material and inoculated microbial suspensions, the antimicrobial effect of Blueprint was striking.

It was also evident that the microorganisms could survive for some considerable time on the conventional impression materials examined (ie Kromopan). There was little difference between the conventional materials when the inoculum was stored in impression material wells.

Although further clarification is required, it appeared that the carriage of microorganisms on the conventional alginate material (Kromogel) was more pronounced than on the rubber base materials after the inoculum was discarded from the flat impression surfaces, perhaps suggesting that the surface properties of the respective materials may have had an influence on microbial retention, and also suggesting that conventional alginate materials may have greater potential for cross-infection than other commonly used materials.

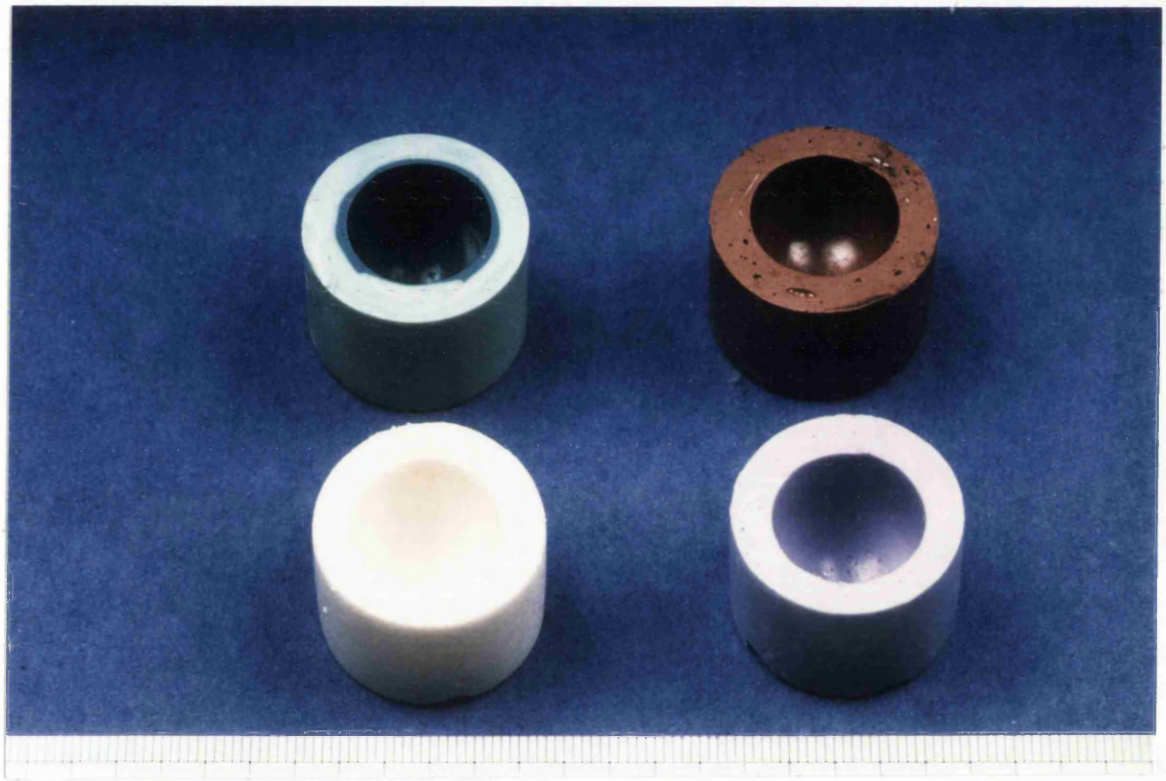


FIGURE 2.1

Impression moulds,

into which microbial suspensions were inoculated.

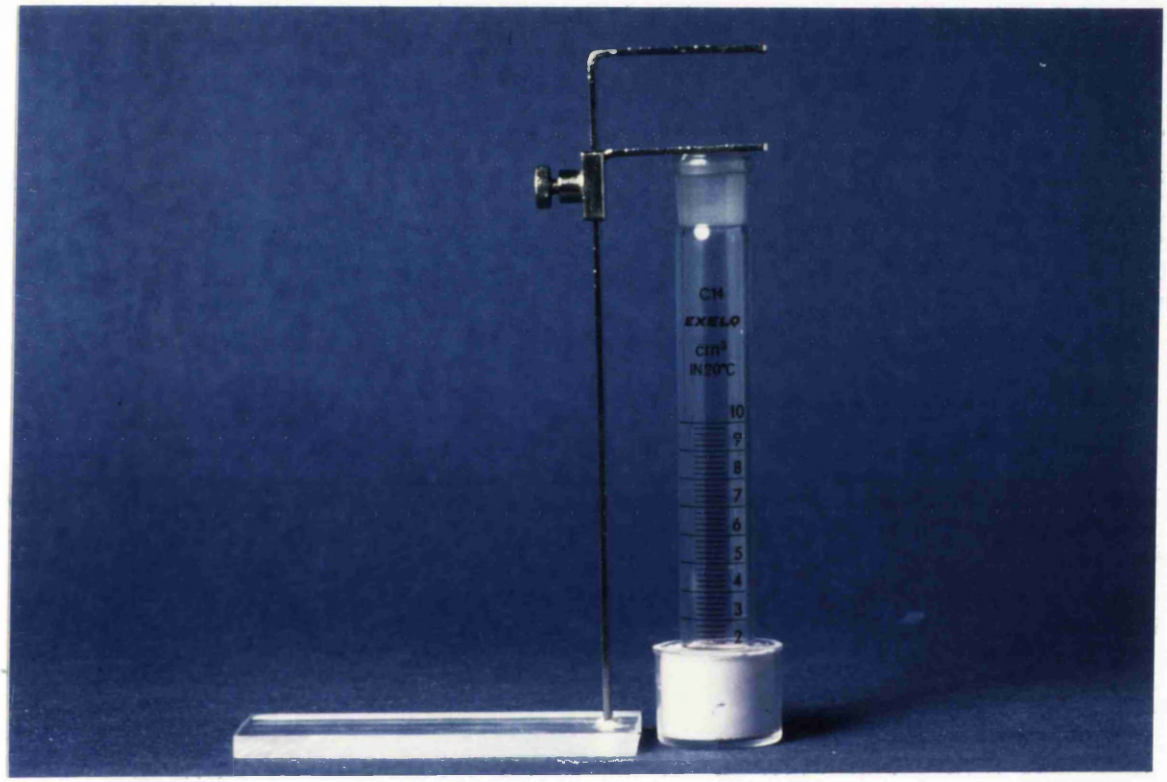


FIGURE 2.2

Vertical measuring device,

adjusted to height of 13cm.

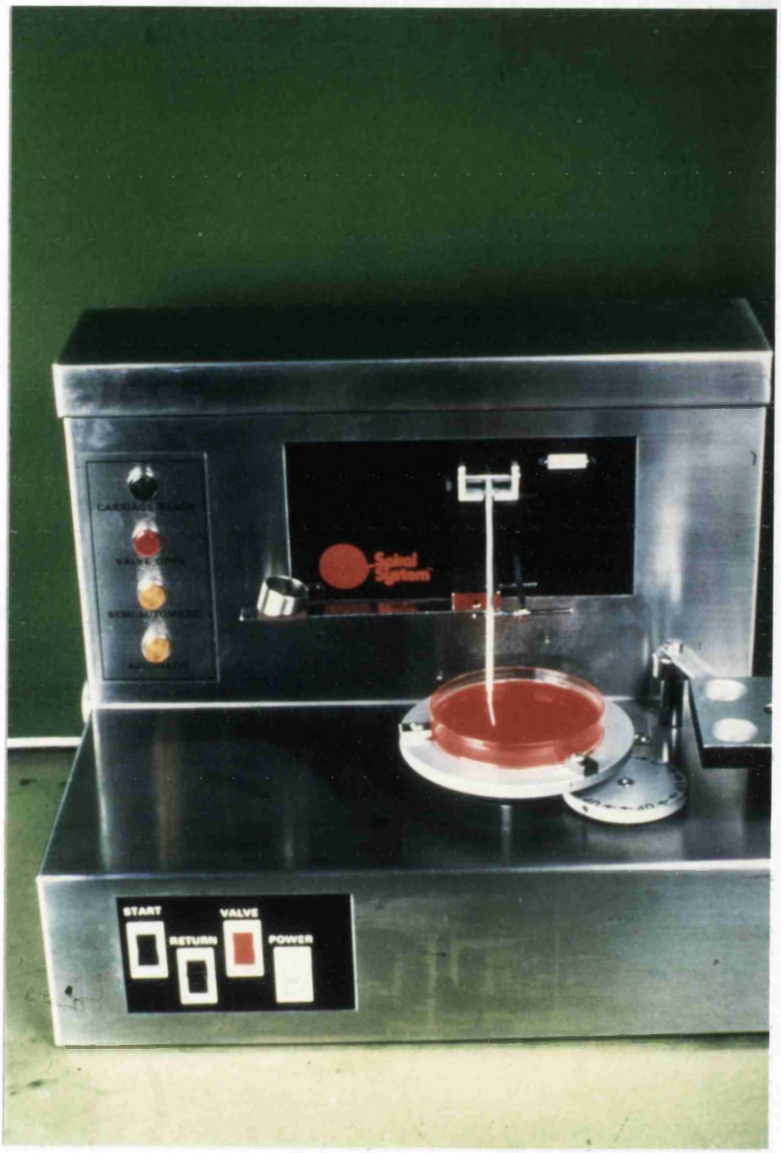


FIGURE 2.3

The spiral plater.

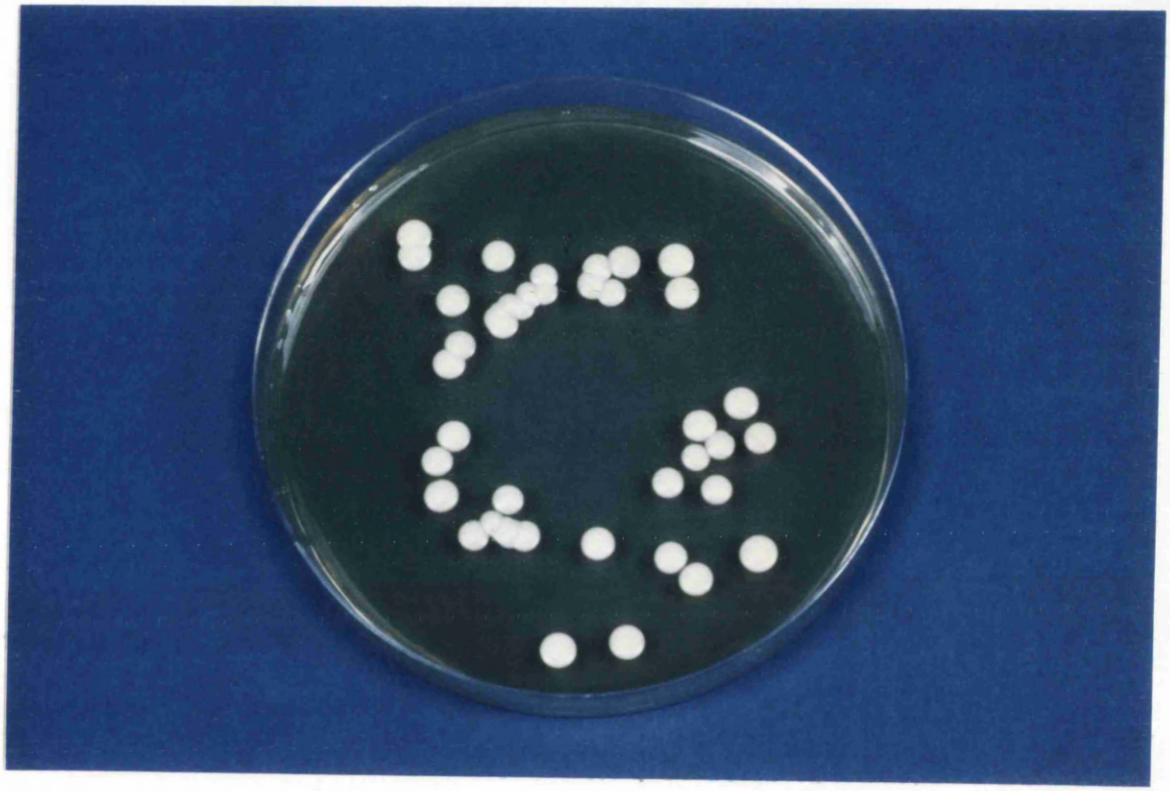


FIGURE 2.4

Colony Forming Units on culture plate,

(Candida albicans - Sabouraud's dextrose agar).

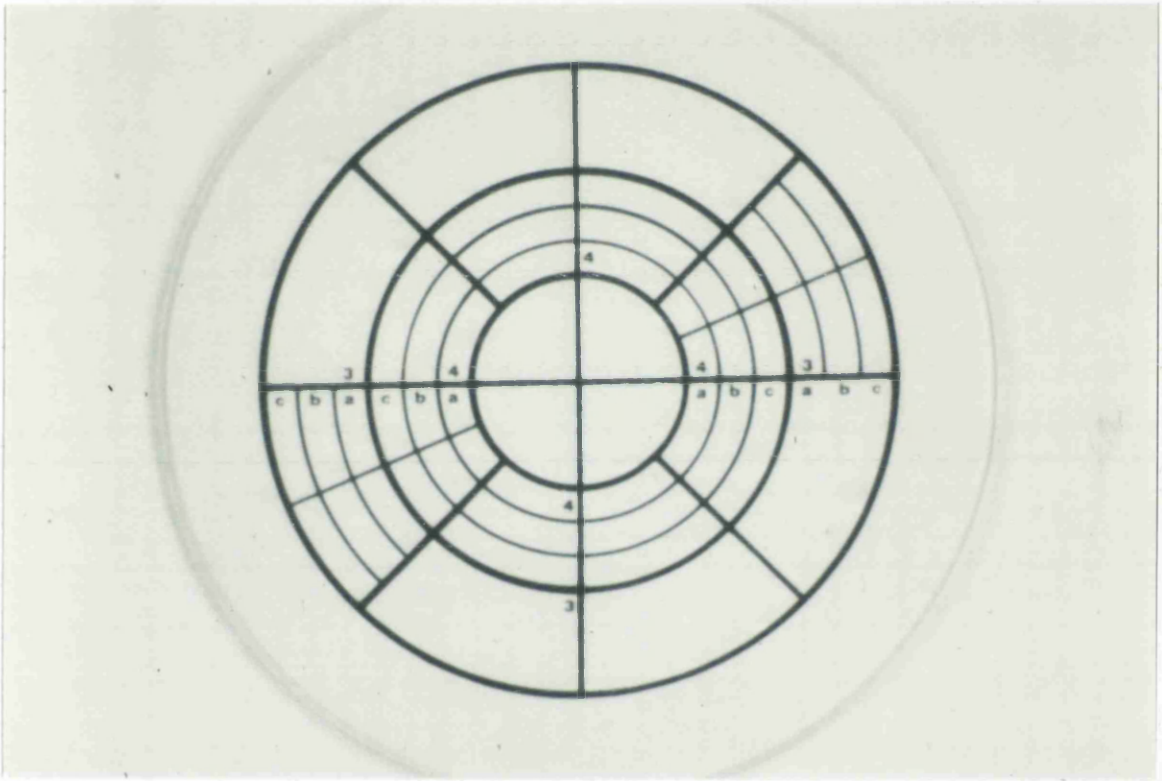


FIGURE 2.5

Template for assessment of growth

in sections of culture plate.

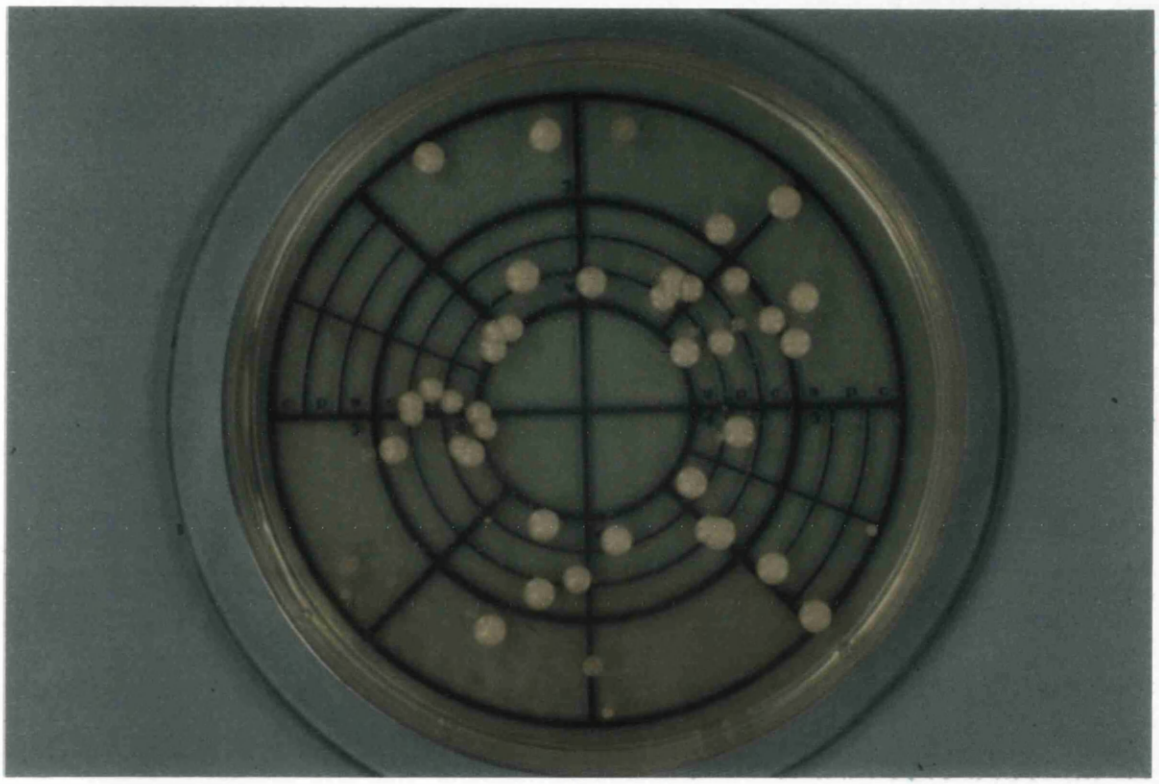


FIGURE 2.6

Culture plate superimposed on template,
showing divisions for counting
when CFU values were high.

Figure 2.7 (Experiment 1)

C Albicans : Percentage survival with time
(4 Impression Materials and Control)

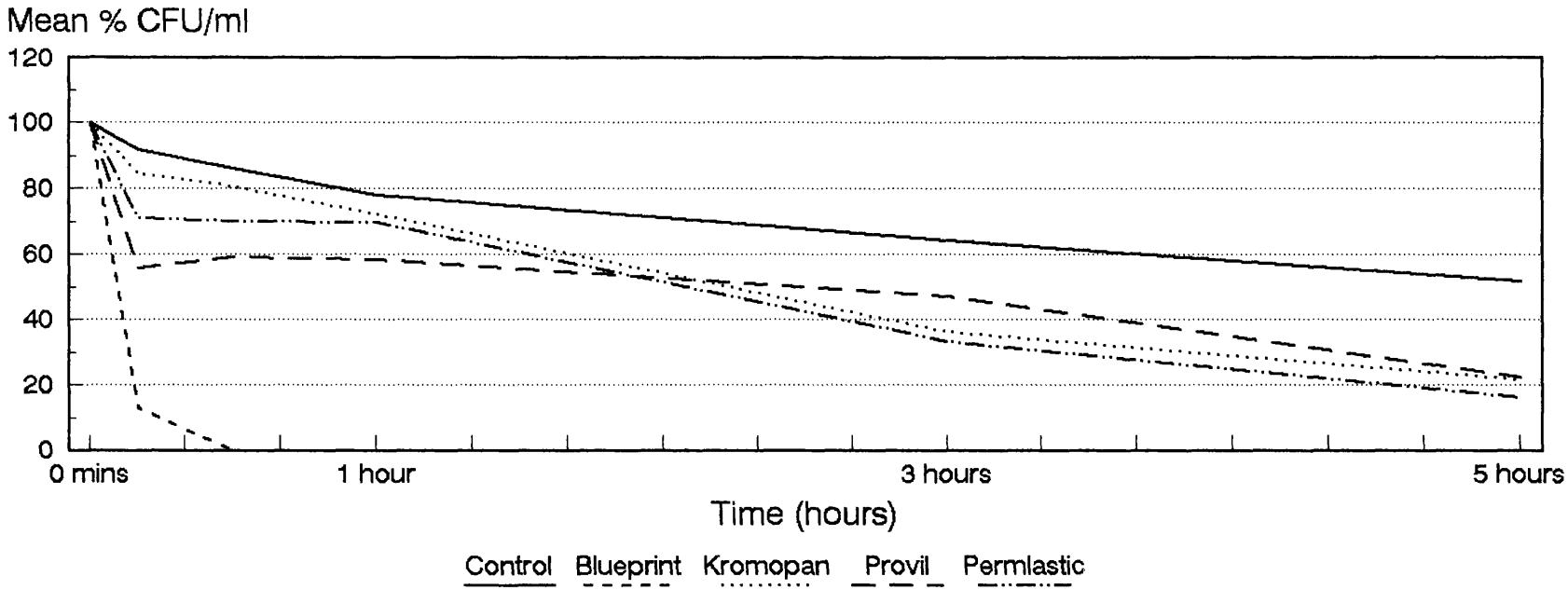


Figure 2.8 (Experiment 1)

E Coli : percentage survival with time
(4 Impression Materials and Control)

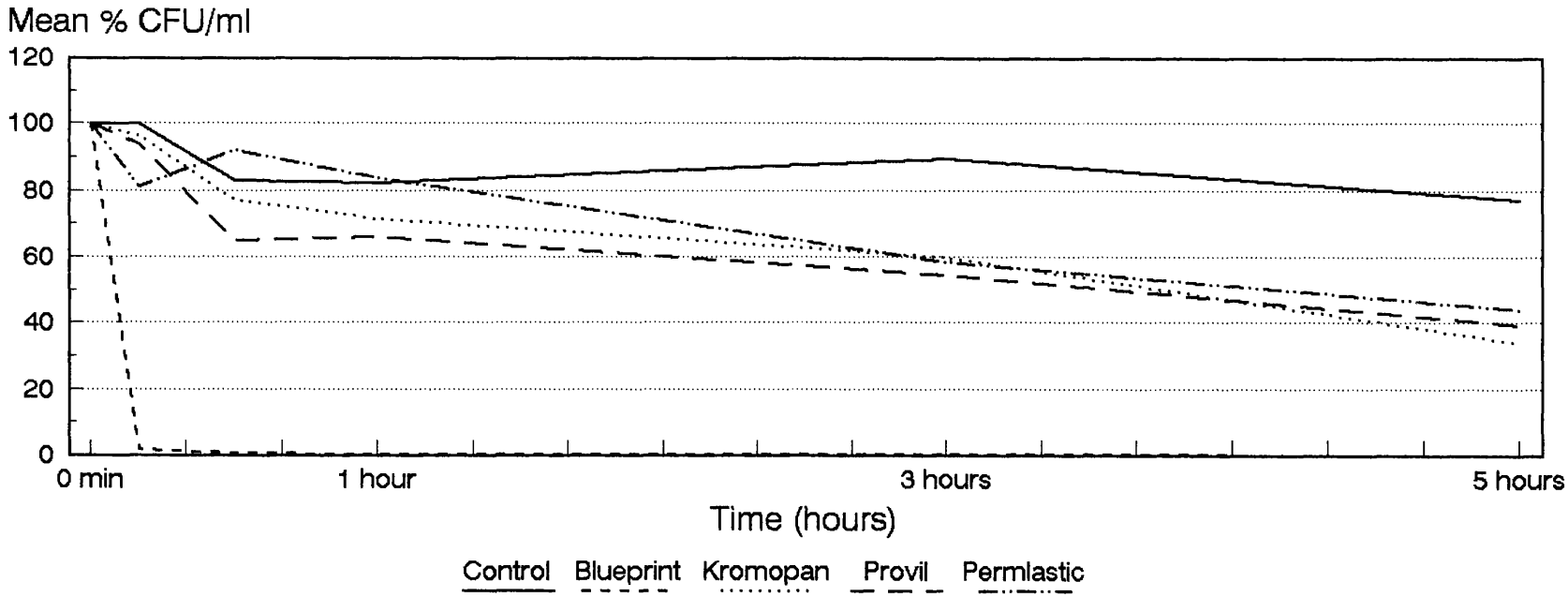


Figure 2.9 (Experiment 1)

Ps Aeruginosa : Percentage survival with time
(4 Impression Materials and Control)

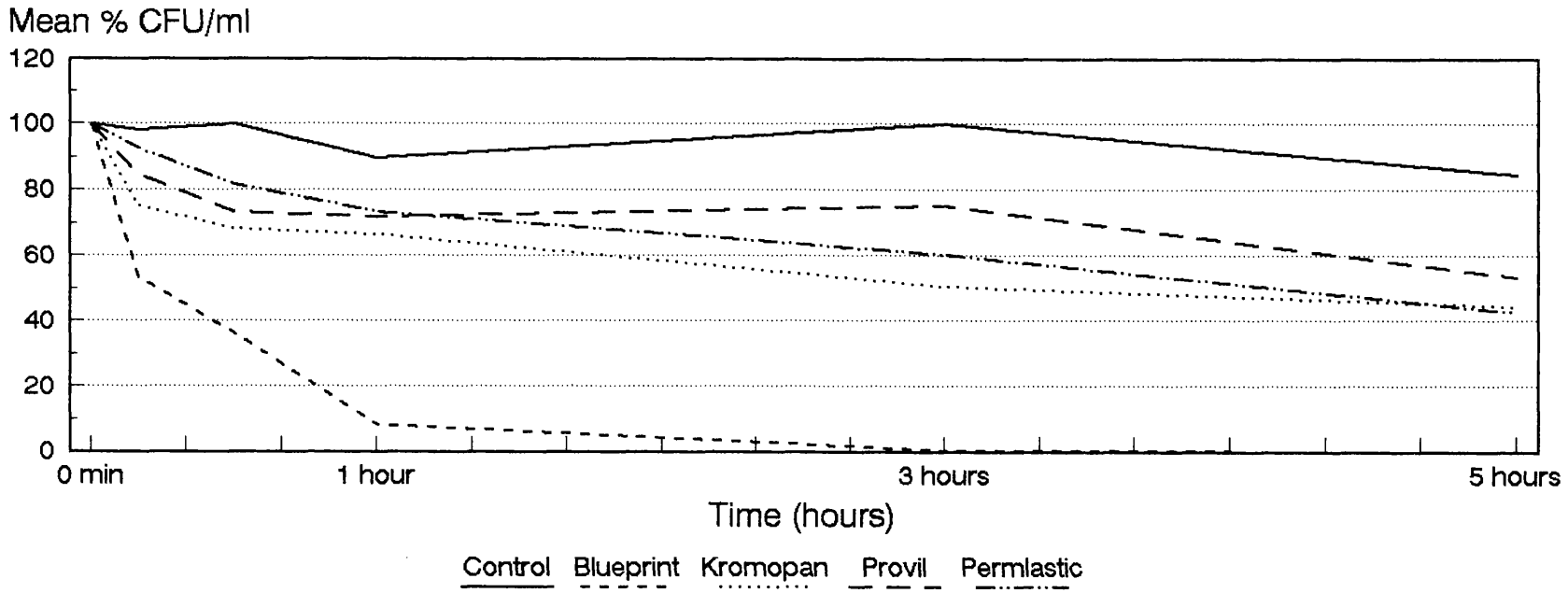


Figure 2.10 (Experiment 1)

Staph Aureus : Percentage survival with time
(4 Impression Materials and Control)

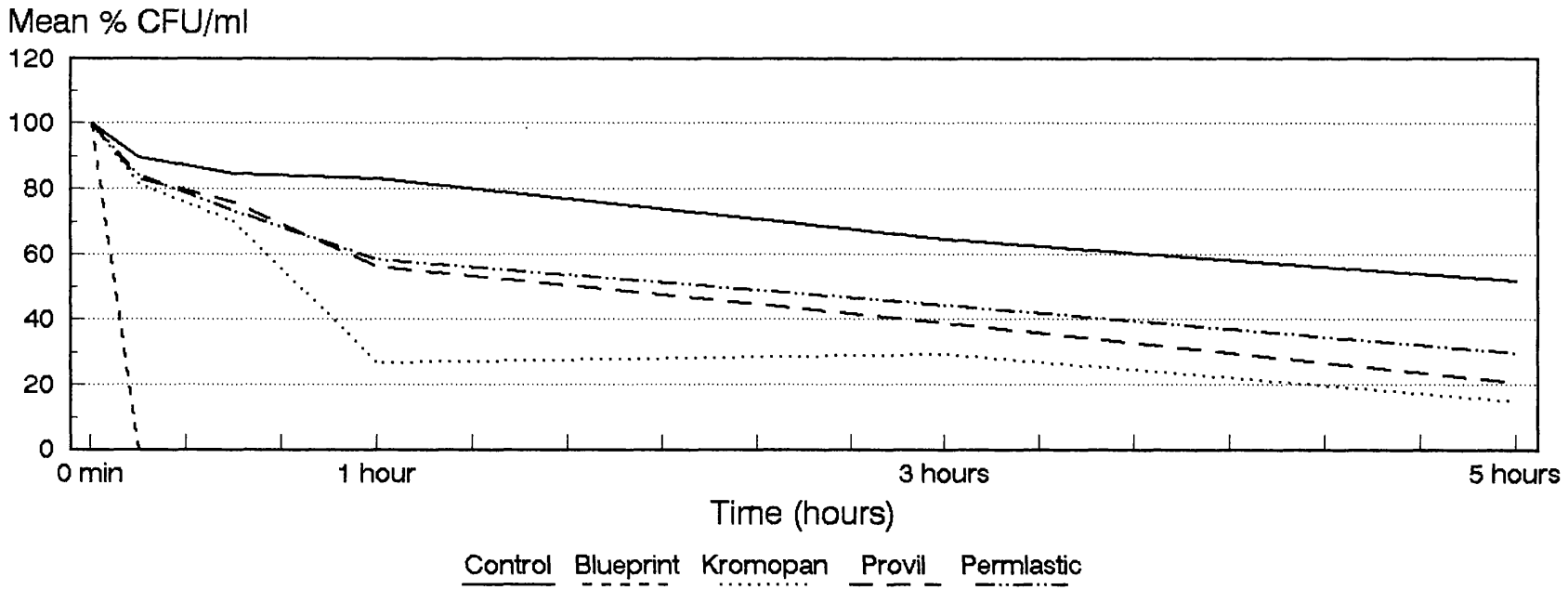


Figure 2.11 (Experiment 1)

Strep Mutans : Percentage survival with time
(4 Impression Materials and Control)

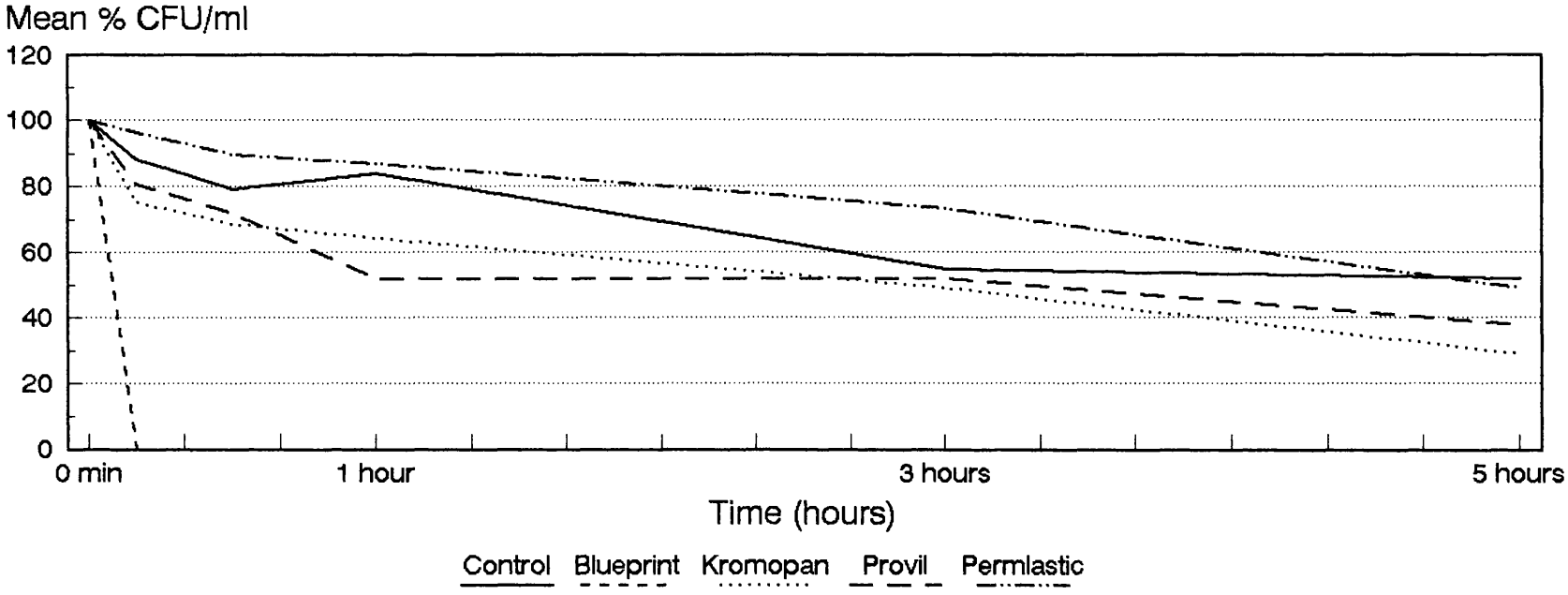


Figure 2.12 (Experiment 1)

Control suspensions : Microbial survival
(5 Microorganisms)

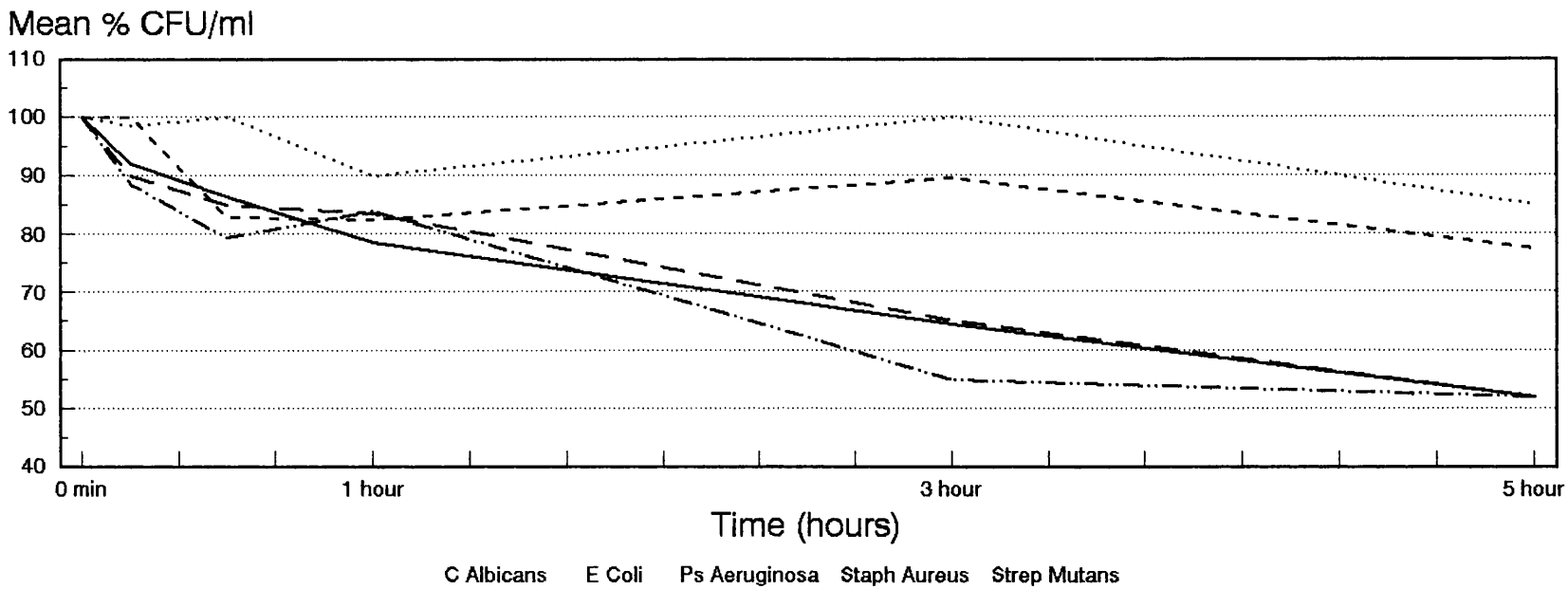


Figure 2.13 (Experiment 1)

Blueprint suspensions : Microbial survival (5 Microorganisms)

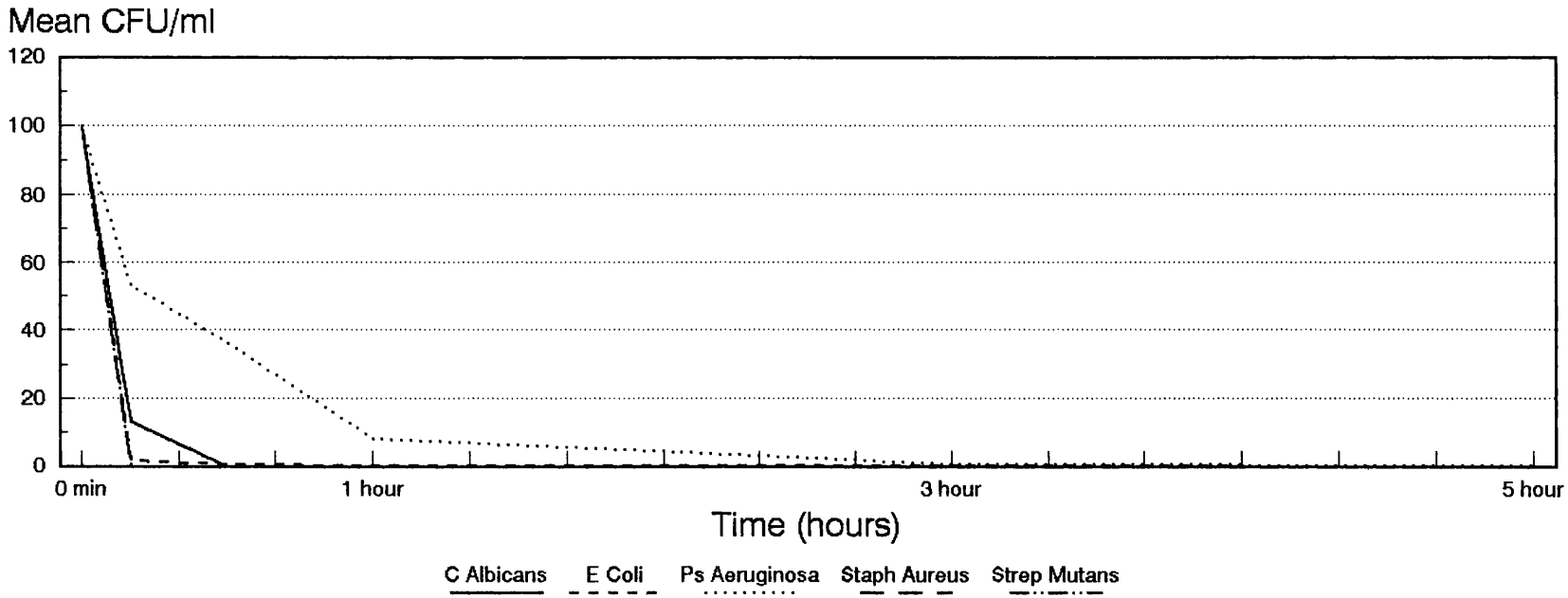


Figure 2.14 (Experiment 1)

Kromopan suspensions : Microbial survival
(5 Microorganisms)

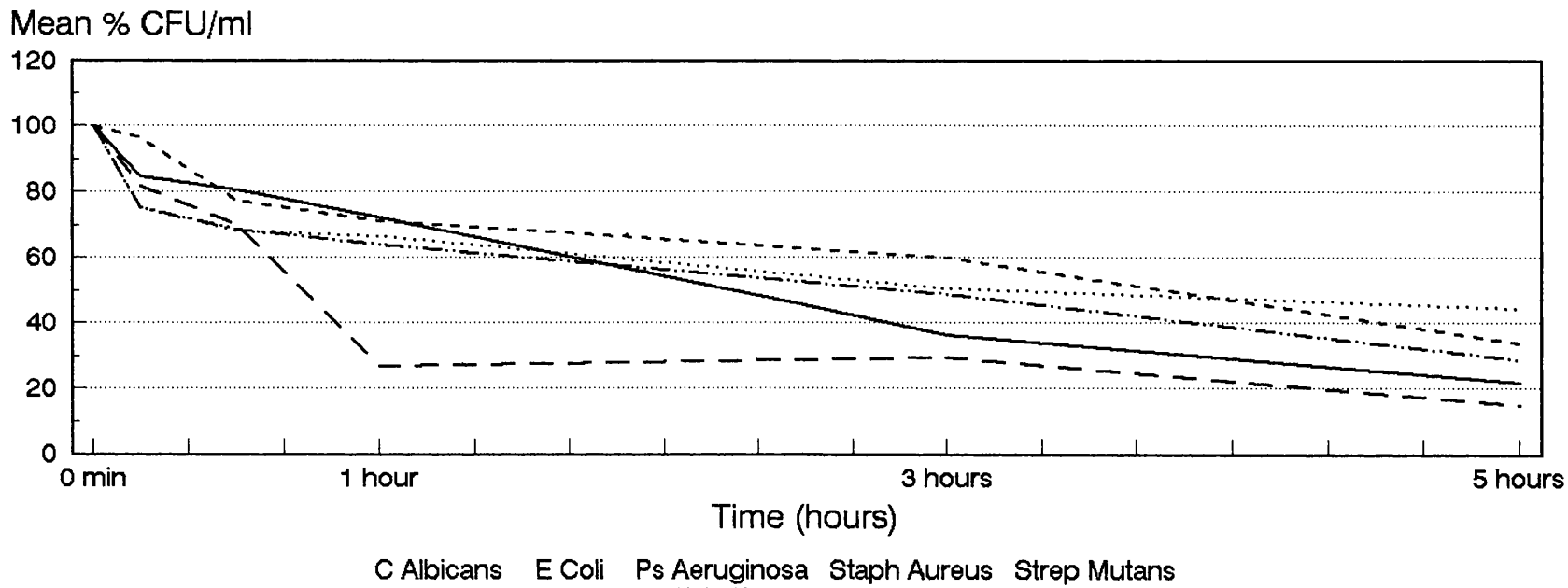


Figure 2.15 (Experiment 1)

Provil suspensions : Microbial survival
(5 Microorganisms)

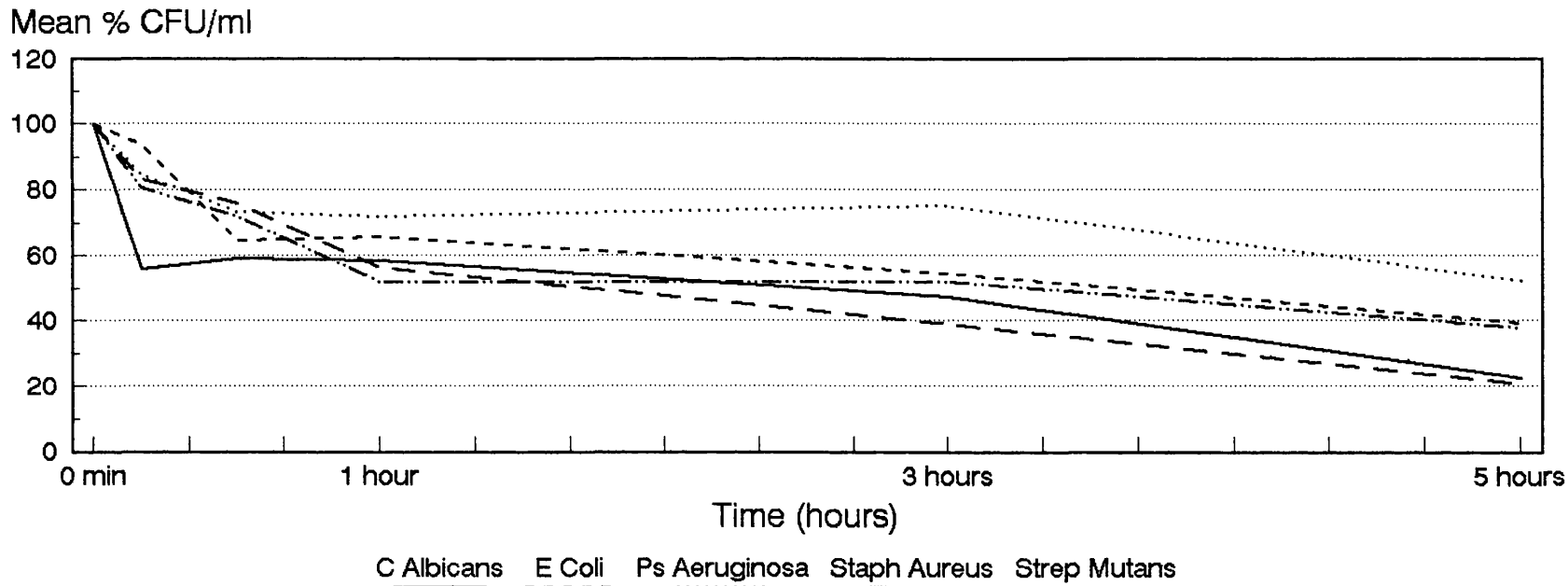


Figure 2.16 (Experiment 1)

Permlastic suspensions : Microbial survival (5 Microorganisms)

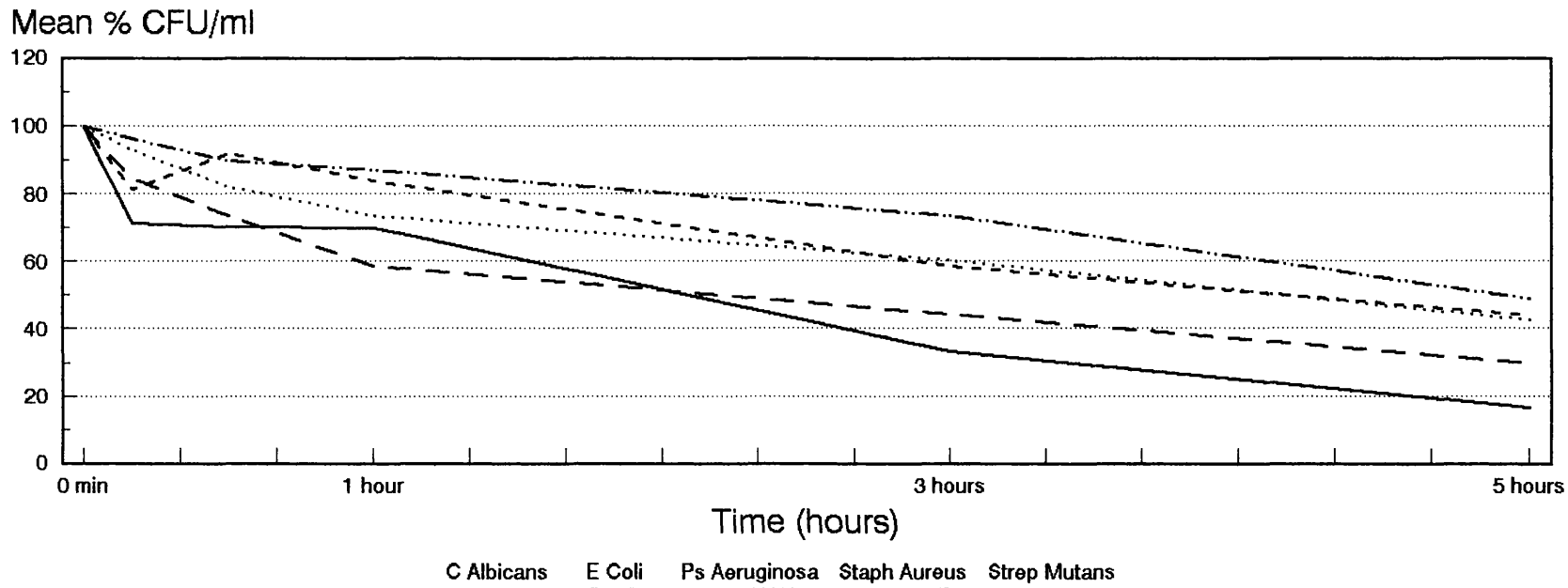


Figure 2.17 (Experiment 2)

Control suspensions : Microbial survival (5 Microorganisms)

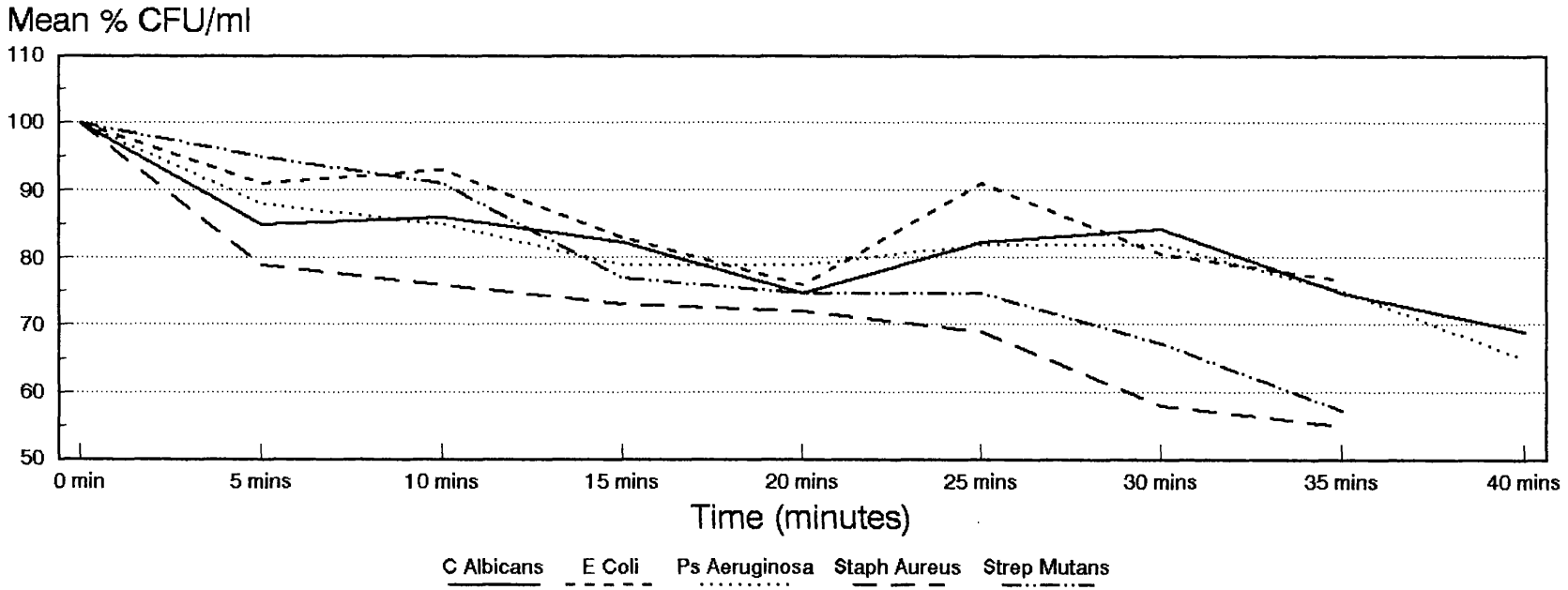


Figure 2.18 (Experiment 2)

Blueprint suspensions : Microbial survival (5 Microorganisms)

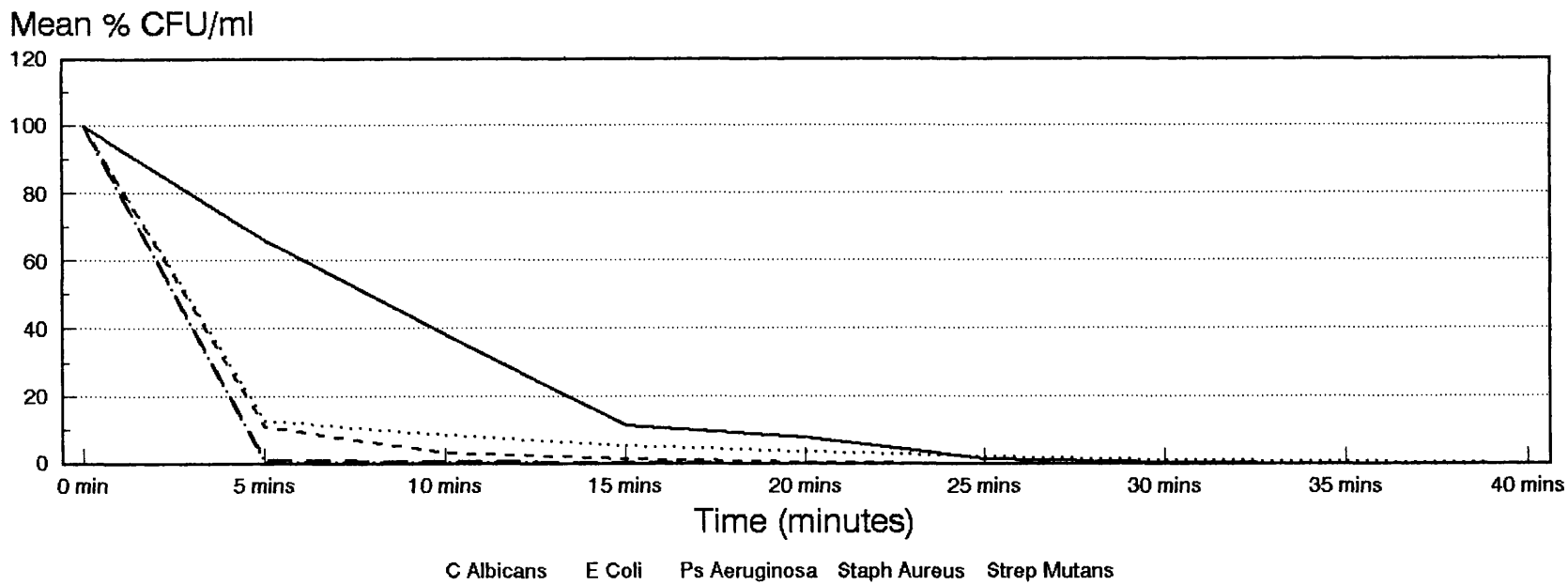


FIGURE 2.19

Impression moulds for each material were made in 10cm diameter petri-dishes.

Figure 2.20 (Experiment 3)

Persistence of Microorganisms (% CFU)

Control Suspensions

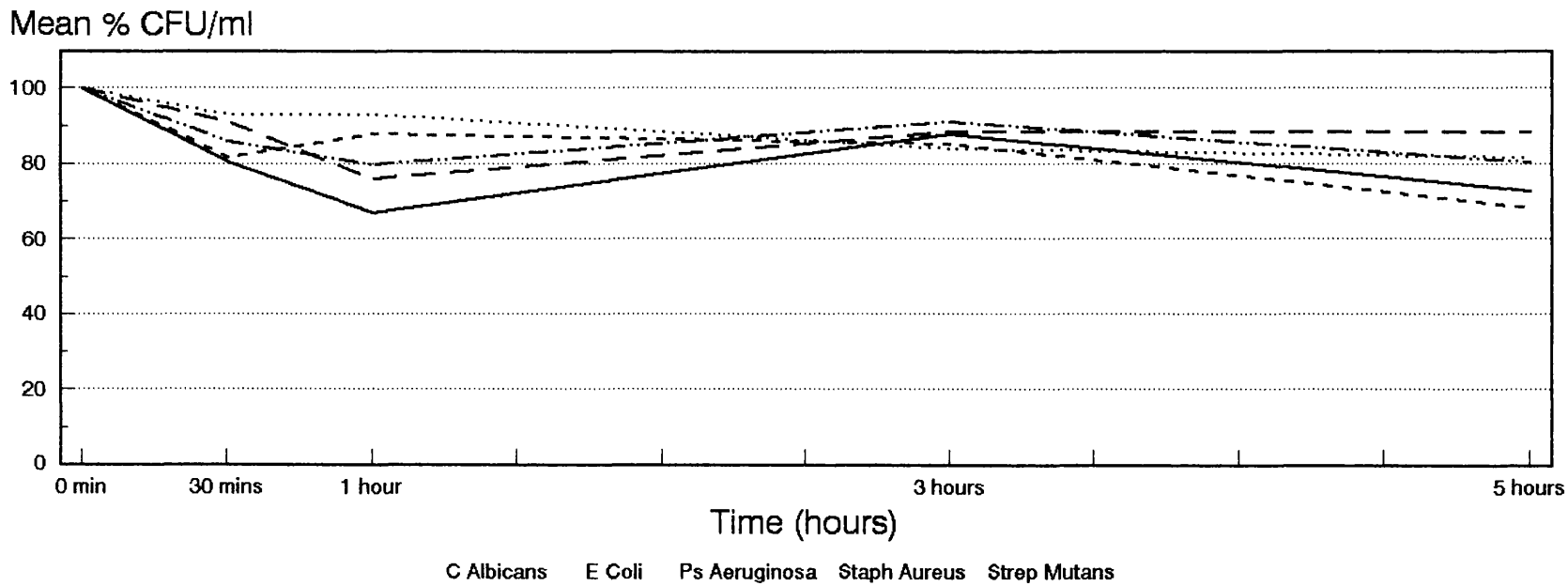
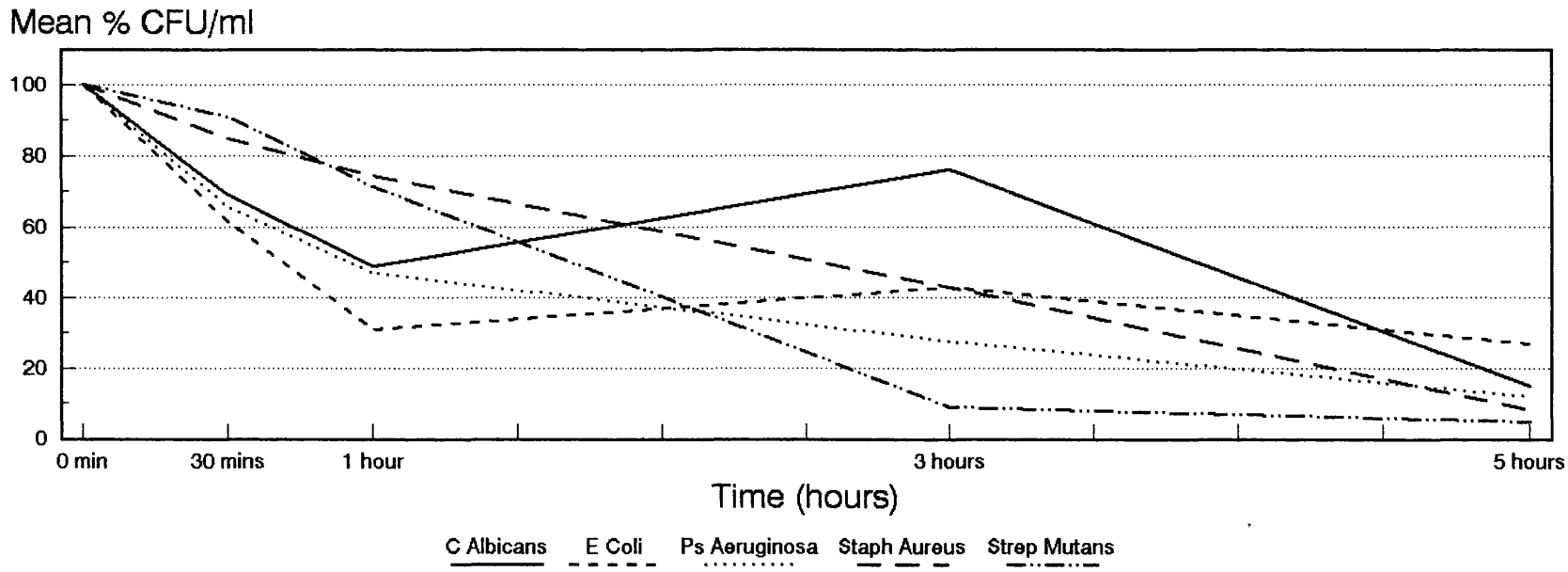


Figure 2.21 (Experiment 3)

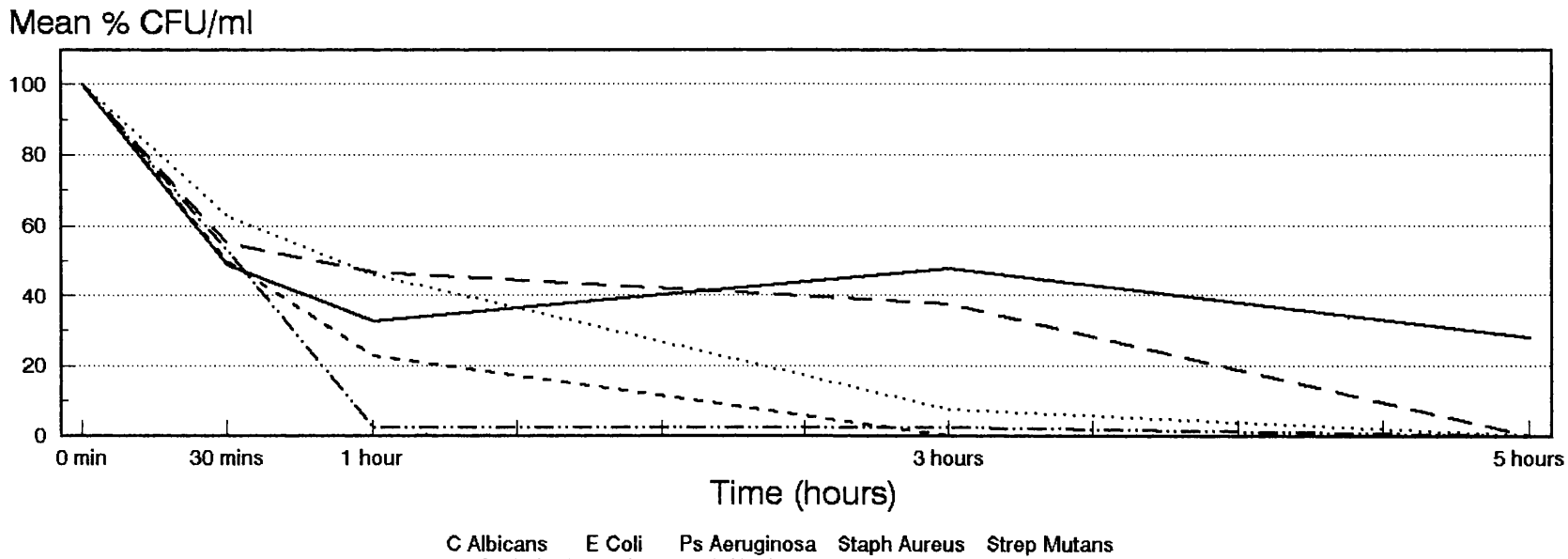
Persistence of Microorganisms (% CFU) Suspensions in Contact with Alginate (Kromopan)



KROMOPAN

Figure 2.22 (Experiment 3)

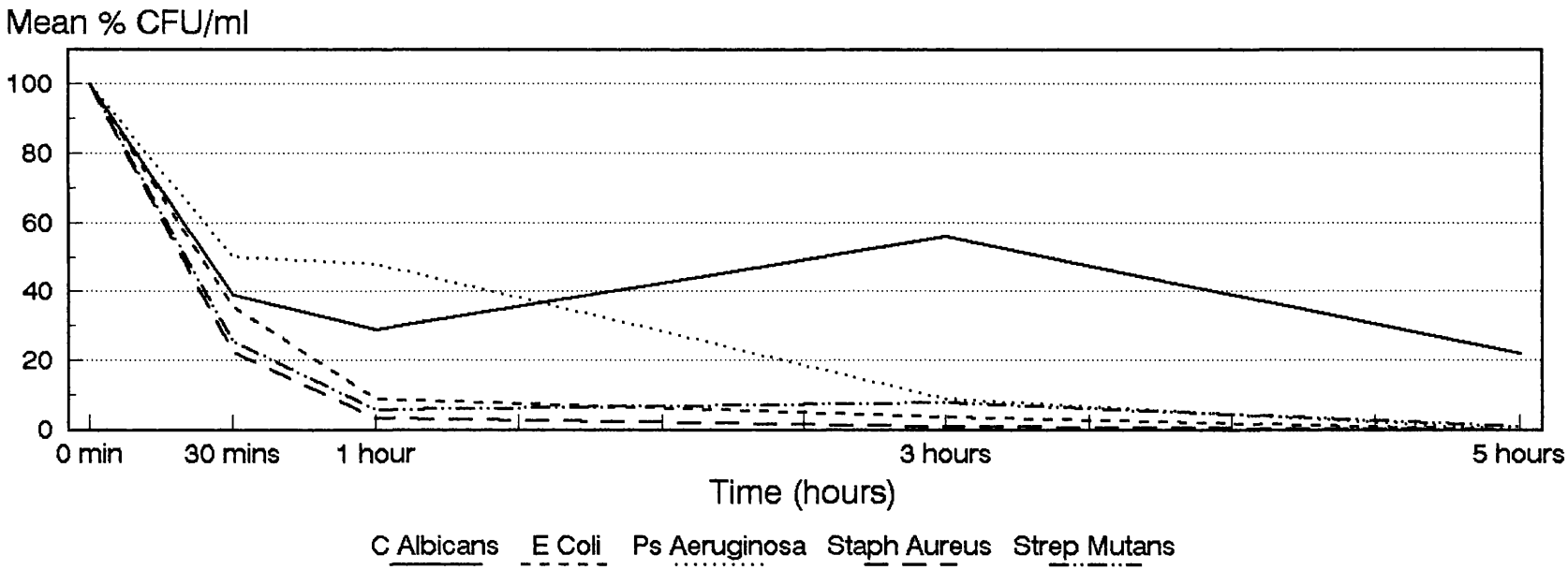
Persistence of Microorganisms (% CFU) Suspensions in Contact with Poly(Vinyl Siloxane)



PROVIL

Figure 2.23 (Experiment 3)

Persistence of Microorganisms (% CFU)
Suspensions in Contact with Polysulphide Rubber



PERMLASTIC

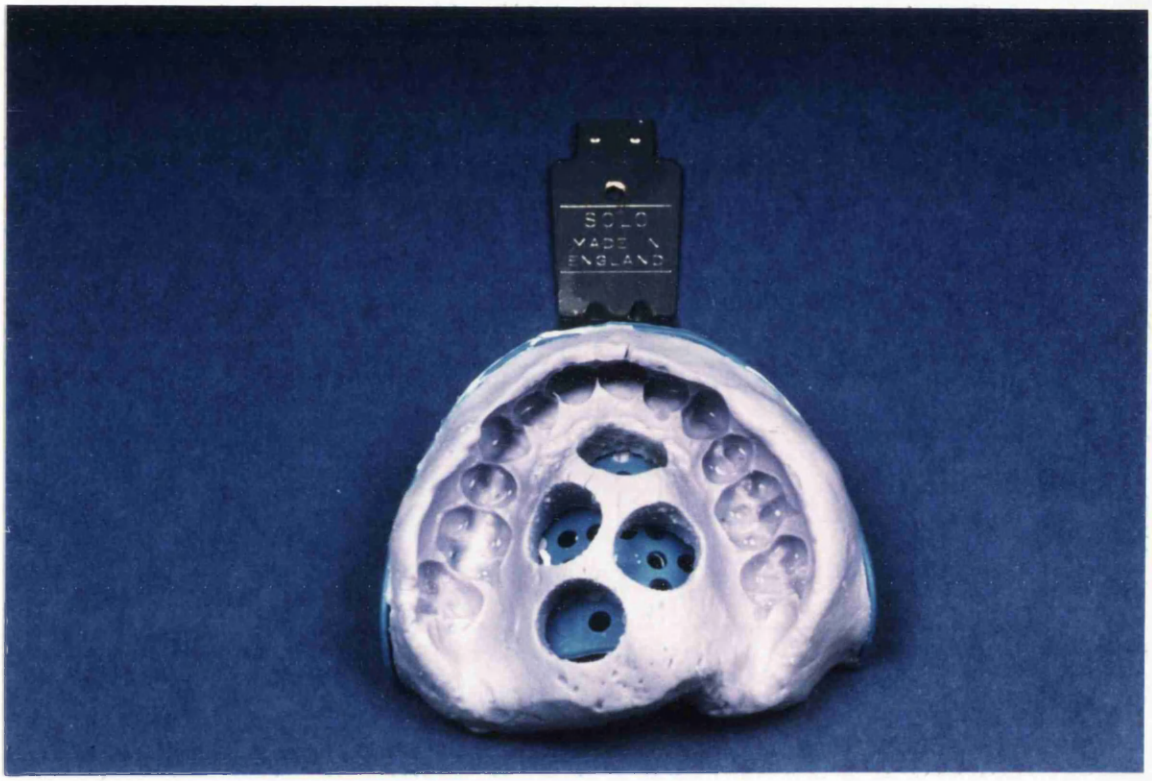


FIGURE 2.24

Experiment 4.

Impression samples were removed

from the palatal area of maxillary impressions.

TABLE 2.1

IMPRESSION MATERIALS EXAMINED IN *IN-VITRO* STUDIES

Material	Type	Manufacturer
Blueprint Asept	Alginate	DeTray Dentsply, England
New Kromopan	Alginate	Wright Dental, Scotland
Permlatic regular	Polysulphide rubber	Bayer Dental, Leverkusen, Germany
Provil	Poly(vinyl siloxane)	Kerr Europe, Basel, Switzerland

TABLE 2.2

MICROORGANISMS USED IN *IN-VITRO* STUDIES

Microorganism.....Strain
Candida albicans..... MRL 3153
Escherichia coli.....NCTC 10418
Pseudomonas aeruginosa.....NCTC 10662
Staphylococcus aureus.....NCTC 6571
Streptococcus mutans.....NCTC 11063

MRL - Mycological Reference Laboratory, London, England.
NCTC - National Collection of Type Cultures, USA.

EXPERIMENT 1

TABLE 2.3 (a)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

C albicans : Data from first procedure
CFU/ml 10^3

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	9.90	9.90	9.90	9.90	9.90
10 mins	9.70	0.36	7.00	5.10	6.70
30 mins	8.80	0.00	7.00	4.80	6.50
1 hour	8.10	0.00	7.60	4.80	6.40
2 hour	8.10	0.00	4.80	3.20	4.20
3 hour	6.80	0.00	3.50	2.40	1.40
5 hour	5.10	0.00	1.40	2.20	1.00

TABLE 2.3 (b)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

C albicans : Data from second procedure
CFU/ml 10^3

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	7.80	7.80	7.80	7.80	7.80
10 mins	6.60	1.80	7.80	4.80	6.00
30 mins	6.60	0.00	7.20	5.40	6.00
1 hour	6.00	0.00	5.40	4.80	4.20
3 hour	4.80	0.00	3.00	5.40	2.40
5 hour	4.20	0.00	2.40	1.80	1.80

TABLE 2.3 (c)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

***C albicans* : Percentage suvival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100.00	100.00	100.00	100.00
10 mins	92.00	13.00	85.00	56.00	71.50
30 mins	86.50	00.00	81.00	59.50	70.50
1 hour	78.50	00.00	72.50	58.50	70.00
3 hour	64.50	00.00	36.50	47.50	33.50
5 hour	52.00	00.00	22.00	22.50	16.50

EXPERIMENT 1

TABLE 2.4 (a)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

E coli : Data from first procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	4.50	4.50	4.50	4.50	4.50
10 mins	4.50	0.07	4.20	3.40	3.20
30 mins	3.70	0.04	3.40	2.10	4.00
1 hour	3.40	0.03	3.20	2.70	4.00
3 hour	4.50	0.02	2.70	2.40	2.60
5 hour	3.40	0.00	2.10	1.80	1.80

TABLE 2.4 (b)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

E coli : data from second procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	6.60	6.60	6.60	6.60	6.60
10 mins	6.60	1.40	6.60	6.30	6.10
30 mins	5.60	0.00	5.30	5.60	6.40
1 hour	6.00	0.00	4.80	4.80	5.30
3 hour	4.80	0.00	4.00	3.70	4.00
5 hour	5.30	0.00	1.50	2.60	3.20

TABLE 2.4 (c)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

***E coli* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100.00	100.00	100.00	100.00
10 mins	100.00	1.85	96.50	94.00	81.50
30 mins	83.00	0.53	77.50	65.00	92.00
1 hour	82.50	0.40	71.50	66.00	84.00
3 hour	89.50	0.30	60.00	54.50	58.50
5 hour	77.50	0.00	34.00	39.50	44.00

EXPERIMENT 1

TABLE 2.5 (a)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

Ps aeruginosa : Data from first procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	4.80	4.80	4.80	4.80	4.80
10 mins	4.80	3.20	3.70	4.00	4.50
30 mins	4.80	2.60	3.40	3.40	3.70
1 hour	4.50	0.80	3.20	3.40	2.90
3 hour	4.80	0.04	3.20	2.60	2.60
5 hour	4.00	0.02	3.40	2.60	2.40

TABLE 2.5 (b)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

Ps aeruginosa : Data from second procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	3.10	3.10	3.10	3.10	3.10
10 mins	3.00	1.30	2.30	2.70	2.90
30 mins	3.10	0.60	2.10	2.40	2.70
1 hour	2.70	0.02	2.10	2.30	2.70
3 hour	3.10	0.01	1.10	2.40	2.10
5 hour	2.70	0.00	0.60	1.60	1.10

TABLE 2.5 (c)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

***Ps aeruginosa* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100.00	100.00	100.00	100.00
10 mins	98.50	53.50	75.50	85.00	93.00
30 mins	100.00	36.50	68.50	73.50	82.00
1 hour	90.00	8.30	66.50	72.00	73.50
3 hour	100.00	0.56	50.50	75.50	60.50
5 hour	85.00	0.20	44.50	52.50	42.50

EXPERIMENT 1

TABLE 2.6 (a)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

Staph areus : Data from first procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	3.70	3.70	3.70	3.70	3.70
10 mins	3.20	0.00	3.40	3.10	3.70
30 mins	2.70	0.00	2.90	2.70	2.90
1 hour	2.70	0.00	1.10	2.00	2.30
3 hour	1.50	0.00	1.50	2.10	1.90
5 hour	1.30	0.00	0.60	1.00	1.60

TABLE 2.6 (b)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

Staph aueus : Data from second procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	7.60	7.60	7.60	7.60	7.60
10 mins	7.20	0.00	5.60	6.40	6.10
30 mins	7.40	0.00	4.80	6.10	5.30
1 hour	7.20	0.00	1.90	4.50	4.20
3 hour	6.90	0.00	1.50	1.70	2.90
5 hour	5.30	0.00	1.10	1.20	1.30

TABLE 2.6 (c)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

***Staph areus* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100.00	100.00	100.00	100.00
10 mins	90.00	0.00	82.00	83.50	84.50
30 mins	85.00	0.00	70.50	76.00	73.50
1 hour	83.50	0.00	27.00	56.50	58.50
3 hour	65.00	0.00	29.50	39.00	44.50
5 hour	52.00	0.00	15.00	21.00	30.00

EXPERIMENT 1

TABLE 2.7 (a)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

Strep mutans : Data from first procedure
CFU/ml 10^4

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	3.30	3.30	3.30	3.30	3.30
10 mins	3.00	0.00	2.30	2.30	3.30
30 mins	2.80	0.00	2.10	2.10	2.90
1 hour	2.90	0.00	1.80	1.80	2.90
3 hour	1.80	0.00	1.60	1.60	2.40
5 hour	1.80	0.00	1.30	1.50	1.60

TABLE 2.7 (b)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

Strep mutans : Data from second procedure
CFU/ml 10^3

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	9.60	9.60	9.60	9.60	9.60
10 mins	8.40	0.00	7.80	9.00	9.09
30 mins	7.20	0.00	7.20	7.80	9.00
1 hour	7.80	0.00	7.20	4.80	8.40
3 hour	5.40	0.00	4.80	5.40	7.30
5 hour	4.80	0.00	1.80	3.00	4.80

TABLE 2.7 (c)

PERSISTENCE OF MICROORGANISMS IN IMPRESSION MOULDS

***Strep mutans* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100.00	100.00	100.00	100.00
10 mins	88.50	0.00	75.50	81.00	96.50
30 mins	79.50	0.00	69.00	72.00	90.00
1 hour	84.00	0.00	64.50	52.00	87.00
3 hour	55.00	0.00	49.00	52.00	73.50
5 hour	52.00	0.00	29.00	38.00	49.00

EXPERIMENT 2

TABLE 2.8 (a)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS
C albicans : CFU/ml

Time	I		II		III	
	Control (10^4)	Blueprint	Control (10^3)	Blueprint	Control (10^3)	Blueprint
0 min	1.60		9.10		6.00	
5 min	1.40	7400	9.00	7272	4.20	4480
10 min	1.30	1200	9.00	4848	4.80	3340
15 min	1.20	400	8.40	1960	4.80	580
20 min	1.20	360	7.20	300	4.20	140
25 min	1.20	80	8.40	260	4.80	20
30 min	1.20	40	9.00	20	4.80	20
35 min	1.30	40	7.20	0	4.20	0
40 min	1.20	0	6.60	0	3.60	0

TABLE 2.8 (b)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS

***C albicans* : Mean %CFU/ml**

Time	Control	Blueprint
0 min	100.00	100.00
5 min	85.00	66.00
10 min	86.00	38.30
15 min	82.30	11.20
20 min	74.60	7.75
25 min	82.30	1.20
30 min	84.30	0.26
35 min	74.60	0.08
40 min	69.00	0.00

EXPERIMENT 2

TABLE 2.9 (a)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS
E Coli : CFU/ml

Time	I		II		III	
	Control (10^3)	Blueprint (10^3)	Control (10^4)	Blueprint (10^4)	Control (10^4)	Blueprint (10^4)
0 min	1.40		4.50		3.20	
5 min	1.40	0.22	3.70	0.33	2.90	0.35
10 min	1.30	0.02	4.50	0.15	2.70	0.14
15 min	1.00	0.00	4.20	0.13	2.70	0.05
20 min	1.00	0.00	3.40	0.06	2.60	0.01
25 min	1.40	0.00	4.20	0.06	2.56	0.00
30 min	1.30	0.00	3.40	0.00	2.40	0.00
35 min	1.20	0.00	3.40	0.00	2.24	0.00

TABLE 2.9 (b)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS

***E Coli* : Mean %CFU/ml**

Time	Control	Blueprint
0 min	100.00	100.00
5 min	91.00	11.10
10 min	93.00	3.07
15 min	83.00	1.53
20 min	76.00	0.17
25 min	91.00	0.11
30 min	80.60	0.00
35 min	76.70	0.00

EXPERIMENT 2

TABLE 2.10 (a)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS
Ps Aeruginosa : CFU/ml

Time	I		II		III	
	Control (10^4)	Blueprint (10^4)	Control (10^4)	Blueprint (10^4)	Control (10^4)	Blueprint (10^4)
0 min	6.90		8.00		4.80	
5 min	6.40	0.89	6.60	0.88	4.10	0.67
10 min	6.40	0.57	6.10	0.66	4.10	0.48
15 min	6.40	0.49	5.10	0.29	3.90	0.26
20 min	6.40	0.33	6.40	0.14	3.80	0.19
25 min	6.40	0.16	6.40	0.11	3.50	0.14
30 min	6.40	0.05	6.90	0.08	3.30	0.07
35 min	5.60	0.03	6.60	0.06	3.00	0.04
40 min	4.00	0.00	6.10	0.00	3.00	0.00

TABLE 2.10(b)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS

***Ps Aeruginosa* : Mean %CFU/ml**

Time	Control	Blueprint
0 min	100.00	100.00
5 min	88.00	12.60
10 min	85.00	8.60
15 min	79.00	5.30
20 min	79.00	3.40
25 min	82.00	2.20
30 min	82.00	1.00
35 min	75.60	0.77
40 min	65.00	0.00

EXPERIMENT 2

TABLE 2.11 (a)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS
Staph Aureus : CFU/ml

Time	I		II		III	
	Control	Blueprint	Control (10^4)	Blueprint	Control (10^4)	Blueprint
0 min	380		8.50		3.20	
5 min	300	0.00	7.20	2200	2.40	0.00
10 min	300	0.00	6.90	1600	2.30	0.00
15 min	260	0.00	6.90	1100	2.30	0.00
20 min	260	0.00	6.10	160	2.20	0.00
25 min	260	0.00	6.10	120	2.20	0.00
30 min	200	0.00	6.00	0	1.70	0.00
35 min	200	0.00	5.30	0	1.70	0.00

TABLE 2.11 (b)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS

***Staph Aureus* : Mean %CFU/ml**

Time	Control	Blueprint
0 min	100.00	100.00
5 min	79.00	0.83
10 min	76.00	0.60
15 min	73.00	0.40
20 min	72.00	0.06
25 min	69.00	0.04
30 min	58.00	0.00
35 min	55.00	0.00

EXPERIMENT 2

TABLE 2.12 (a)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS
Strep Mutans : CFU/ml

Time	I		II		III	
	Control (10^4)	Blueprint	Control (10^4)	Blueprint (10^4)	Control (10^4)	Blueprint (10^4)
0 min	3.00		4.50		1.80	
5 min	2.80	0.00	4.40	0.02	1.71	0.01
10 min	2.60	0.00	4.40	0.01	1.63	0.00
15 min	2.30	0.00	3.40	0.00	1.47	0.00
20 min	2.60	0.00	2.90	0.00	1.33	0.00
25 min	2.20	0.00	3.40	0.00	1.36	0.00
30 min	2.00	0.00	2.90	0.00	1.79	0.00
35 min	1.80	0.00	2.00	0.00	1.77	0.00

TABLE 2.12 (b)

PERSISTENCE OF MICROORGANISMS IN BLUEPRINT ASEPT MOULDS

***Strp Mutans* : Mean %CFU/ml**

Time	Control	Blueprint
0 min	100.00	100.00
5 min	95.00	0.36
10 min	91.00	0.17
15 min	77.00	0.00
20 min	74.60	0.00
25 min	74.60	0.00
30 min	67.30	0.00
35 min	57.30	0.00

EXPERIMENT 3

TABLE 2.13 (a)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

C Albicans : Data from first procedure
CFU/ml

Time	Control (10^4)	Blueprint	Kromopan	Provil	Permlastic
0 mins	8.00	0.00	1580	180	40
30 mins	6.90	0.00	1260	120	200
1 hour	5.30	0.00	800	80	80
3 hour	8.00	0.00	1520	120	400
5 hour	6.40	0.00	280	100	140

TABLE 2.13 (b)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

C Albicans : Data from second procedure
CFU/ml

Time	Control (10^4)	Blueprint	Kromopan	Provil	Permlastic
0 mins	2.40	0.00	2120	1460	1000
30 mins	1.80	0.00	1240	460	280
1 hour	1.60	0.00	1000	300	380
3 hour	1.80	0.00	1180	300	120
5 hour	1.60	0.00	300	0	100

TABLE 2.13(c)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

***C Albicans* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	0.00	100.00	100.00	100.00
30 mins	80.50	0.00	69.00	49.00	39.00
1 hour	67.00	0.00	49.00	33.00	29.00
3 hour	88.00	0.00	76.00	48.00	56.00
5 hour	73.00	0.00	15.00	28.00	22.00

EXPERIMENT 3

TABLE 2.14 (a)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

E Coli : Data from first procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	1.80	80	2540	300	260
30 mins	1.60	0	1800	180	100
1 hour	1.60	0	3000	20	0
3 hour	1.40	0	1460	0	20
5 hour	1.20,	0	800	0	0

TABLE 2.14 (b)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

E Coli : Data from second procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	6.40	100	5200	1240	1800
30 mins	4.80	0	2700	500	600
1 hour	5.60	0	1640	480	320
3 hour	6.00	0	1500	0	0
5 hour	4.50	0	1200	0	0

TABLE 2.14(c)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

***E Coli* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100	100.00	100.00	100.00
30 mins	82.00	0	61.50	50.00	35.50
1 hour	88.00	0	31.00	22.80	9.00
3 hour	85.50	0	43.00	0.00	3.80
5 hour	68.50	0	27.00	0.00	0.00

EXPERIMENT 3

TABLE 2.15 (a)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

Ps Aeruginosa : Data from first procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	2.10	100	10000	1970	1320
30 mins	1.90	0	7600	1700	1400
1 hour	1.80	0	4247	1580	1080
3 hour	1.80	0	3030	0	0
5 hour	1.50	0	1200	0	0

TABLE 2.15 (b)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

Ps Aeruginosa : Data from second procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	3.00	140	5600	2000	2200
30 mins	2.90	0	3100	800	1100
1 hour	3.00	0	2900	240	320
3 hour	2.50	0	1400	300	400
5 hour	2.80	0	700	0	0

TABLE 2.15(c)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

***Ps Aeruginosa* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	100	100.00	100.00	100.00
30 mins	80.50	0	65.50	63.00	50.00
1 hour	67.00	0	47.00	46.00	48.00
3 hour	88.00	0	27.50	7.50	9.00
5 hour	73.00	0	12.00	0.00	0.00

EXPERIMENT 3

TABLE 2.16 (a)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

Staph Aureus : Data from first procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	7.40	0.00	6840	2240	5760
30 mins	6.80	0.00	7000	860	1200
1 hour	5.70	0.00	4100	80	100
3 hour	6.50	0.00	1100	80	120
5 hour	6.50	0.00	440	0	0

TABLE 2.16 (b)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

Staph Aureus : Data from second procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	3.20	0.00	3400	1680	2160
30 mins	2.90	0.00	2380	1200	520
1 hour	2.40	0.00	3100	100	120
3 hour	2.90	0.00	2400	80	0
5 hour	2.90	0.00	800	0	0

TABLE 2.16(c)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

***Staph Aureus* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	0.00	100.00	100.00	100.00
30 mins	91.00	0.00	85.00	55.00	22.50
1 hour	76.00	0.00	74.50	47.00	3.50
3 hour	88.50	0.00	43.00	37.50	1.00
5 hour	88.50	0.00	8.50	0.00	0.00

EXPERIMENT 3

TABLE 2.17 (a)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

Strep Mutans : Data from first procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	1.70	0.00	2700	760	1040
30 mins	1.40	0.00	2500	560	500
1 hour	1.20	0.00	2280	20	120
3 hour	1.60	0.00	260	40	140
5 hour	1.40	0.00	140	0	20

TABLE 2.17 (b)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

Strep Mutans : Data from second procedure
CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
	(10^4)				
0 mins	3.30	0.00	4100	1860	2700
30 mins	3.00	0.00	3700	600	780
1 hour	3.00	0.00	2420	40	12
3 hour	2.90	0.00	380	0	80
5 hour	2.60	0.00	200	0	0

TABLE 2.17(c)

PERSISTENCE OF MICROORGANISMS ON IMPRESSION SURFACES

***Strep Mutans* : Percentage survival with time**
Mean : %CFU/ml

Time	Control	Blueprint	Kromopan	Provil	Permlastic
0 mins	100.00	0.00	100.00	100.00	100.00
30 mins	86.00	0.00	91.00	53.00	25.50
1 hour	80.00	0.00	71.50	2.50	5.50
3 hour	91.00	0.00	9.00	2.50	8.00
5 hour	80.50	0.00	5.00	0.00	1.00

EXPERIMENT 4

TABLE 2.18 (a)
Carriage of Microorganisms - Dentate Subjects
Blueprint : CFU/ml

Subject	0 mins	10 mins	20 mins	30 mins
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
Mean	**0**	**0**	**0**	**0**

TABLE 2.18 (b)
Carriage of Microorganisms - Dentate Subjects.
KROMOPAN : CFU/ml

Subject	0 mins	30 mins	1 hour	3 hour	5 hour
8	1920	2560	540	500	0
9	760	540	340	640	80
10	4600	6666	2300	3600	900
11	3840	3800	3260	1515	2400
12	6880	4640	1540	1520	1180
13	1700	1420	1620	1340	1300
14	4800	1260	2280	180	1120
Mean	**3500**	**2983**	**1697**	**1327**	**997**
+/- SE	**811**	**824**	**389**	**428**	**306**

TABLE 2.18(c)
Carriage of Microorganisms - Dentate Subjects
Provil : CFU/ml

Subject	0 mins	30 mins	1 hour	3 hour	5 hour
15	940	20	120	180	0
16	500	240	0	60	20
17	360	80	40	60	60
18	600	360	360	60	20
19	1380	1380	1280	40	0
20	1280	880	80	20	0
21	680	40	380	0	0
Mean	**820**	**428**	**322**	**60**	**14**
+/- SE	**148**	**194**	**169**	**21**	**8**

EXPERIMENT 4

TABLE 2.18 (d)

Carriage of Microorganisms - Dentate Subjects
Mean CFU/ml

Material	0 mins	30 mins	1 hour	3 hour	5 hour
Blueprint	0	0	0	0	0
Kromopan	3500	2983	1697	1327	997
Provil	820	428	322	60	14

TABLE 2.18 (e)

Carriage of Microorganisms - Dentate Subjects
Mean %CFU/ml

Material	0 mins	30 mins	1 hour	3 hour	5 hour
Blueprint	0	0	0	0	0
Kromopan	100	85	48	38	28
Provil	100	52	39	7	2

EXPERIMENT 4

TABLE 2.19 (a)

Carriage of Microorganisms - Edentulous Subjects
Blueprint : CFU/ml

Subject	0 mins	10 mins	20 mins	30 mins
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
Mean	**0**	**0**	**0**	**0**

TABLE 2.19 (b)

Carriage of Microorganisms - Edentulous Subjects.
KROMOPAN : CFU/ml

Subject	0 mins	30 mins	1 hour	3 hour	5 hour
5	2860	400	200	2120	460
6	1440	3080	1800	1720	1520
7	1540	480	280	220	420
8	3460	2280	2760	960	1020
Mean	**2325**	**1560**	**1260**	**1255**	**855**
+/- SE	**497**	**667**	**620**	**420**	**260**

TABLE 2.19(c)

Carriage of Microorganisms - Edentulous Subjects

Time	Blueprint	Kromopan	Kromopan
	Mean %CFU/ml	Mean CFU/ml	Mean %CFU/ml
0 mins	**0**	**2325**	**100**
30 mins	**0**	**1560**	**67**
1 hour	**0**	**1260**	**54**
3 hour	**0**	**1255**	**53**
5 hour	**0**	**855**	**36**

CHAPTER 3

THE PERSISTENCE OF MICROORGANISMS ON IMPRESSION MATERIALS FOLLOWING DISINFECTION

INTRODUCTION

While the importance of cross-infection control has been highlighted in the dental literature [4,22], few data exist regarding the efficacy of procedures recommended to remove microorganisms from the surface of impression materials. In addition, there is little published information comparing different types of impression materials with respect to ease of disinfection, or the efficacy of different commercially available disinfectant agents. In this study, findings reported in Chapter 2 were developed, by examination of the ease of removal of microorganisms from different impression materials by disinfection, and by the examination of the efficacy of three commonly used disinfectant agents.

AIMS OF STUDY

The aims of the study were to assess the persistence of two strains of microorganism (which had been artificially inoculated on three types of dental impression material) following disinfection procedures, and to examine and compare the efficacy of three commercially available agents in the disinfection of one of the test impression materials, irreversible hydrocolloid.

3.1 EXPERIMENT 1 - MATERIALS AND METHODS

3.1.1 IMPRESSION MATERIALS AND MOULD PREPARATION

The persistence of two species of microorganism on the surface of impression materials following the application of mild disinfectant agents was examined. Irreversible hydrocolloid, poly (vinyl siloxane) and polysulphide rubber materials, representative of the main types in common use in dental practice, were selected for investigation (Table 3.1). Flat impression moulds were made for each material (Fig 3.1), as described in Section 2.5.1, prior to contamination, disinfection and sampling procedures.

3.1.2 MICROORGANISMS AND CULTURE CONDITIONS

The microorganisms selected for artificial inoculation of the impression materials were *Pseudomonas aeruginosa* and *Candida albicans* (Table 3.2). They were selected because in the investigations described in Chapter 2, it was found that *Ps aeruginosa* was the most persistent of the bacteria on irreversible hydrocolloid over a 30 minute period (the time interval proposed for this experiment), and *C albicans* was the only microorganism to survive in substantial numbers on the rubber base materials.

Ps aeruginosa was grown aerobically on blood agar, at 37^{o}C for 24 hours, and *C albicans* was grown aerobically on Sabouraud's dextrose agar (Oxoid Ltd, Basingstoke, England) at 37^{o}C for 48 hours. Suspensions of each micro-organism were prepared in low and high concentrations. Suspensions of low concentration (Suspension A) were prepared by harvesting two loopfulls of the appropriate microorganism, from either blood agar or Sabourauds's agar, and inoculating this into 20ml phosphate buffered saline (0.1M, pH7.2). The inoculum was dispersed by vortex mixing for 60 seconds and the resultant suspension serially diluted (1:10) with sterile saline. Spiral plate counts (2.1.5) indicated these suspensions yielded approximately 2 x 10^5 CFU/ml of *Ps aeruginosa* and 3 x 10^5 CFU/ml of *C albicans*. The high concentration suspensions (Suspension B) were prepared by dispersing four loopfulls of the microorganism in question (in 20ml of phosphate buffered saline), and mixing to ensure even distribution. Spiral plate counts indicated that suspensions yielded approximately 4 x 10^5 CFU/ml *Ps aeruginosa*, and 3.5 x 10^5 CFU/ml *C albicans*.

3.1.3 INOCULATION OF IMPRESSION MOULDS

To assess microbial retention for both concentrations of each microorganism, 20ml samples of inoculum were delivered aseptically on to flat moulds of the impression materials under test.

The suspensions covered the entire impression surface which was left submerged for three minutes. After this time the inoculum was discarded and the materials sampled using a 13mm diameter sterile cork borer (Fig 3.2), as described in Section 2.5.4. This was pre-disinfection Sample A.

3.1.4 DISINFECTION AND SAMPLE COLLECTION

After removal of the microbial inoculum and initial sampling, impression moulds were either left as non-disinfected control specimens, or disinfected for 30 seconds using aqueous solutions of 0.1% or 0.02% chlorhexidine gluconate. Relatively weak solutions of disinfectant agent were used so that total elimination of microorganisms did not occur, thus allowing comparison to be made between the impression materials tested.

Samples of impression material were collected from random sites using a 13mm diameter cork borer, as previously described. In the non-disinfected control specimens, the second sample (Sample B) was collected 60 seconds after discarding the microbial inoculum. Sampling was repeated 10 minutes thereafter (Sample C), and again after 30 minutes (Sample D).

In the experimental groups, after discarding inoculum, one of the test disinfectant agents was applied for 30 seconds. Sampling was repeated (Sample B) after discarding the disinfectant, approximately 60 seconds after the inoculum was discarded, was repeated 10 minutes later (Sample C), and finally at 30 minutes (Sample D).

3.1.5 VIABLE MICROORGANISMS AFTER DISINFECTION

Each impression material sample was deposited in 10ml of sterile saline in a sterile universal container and vortex mixed for 60 seconds to dislodge microorganisms from the sample surface. Immediately after mixing, 5ml of each suspension was decanted into a sterile bijou container, before any re-settling of microorganisms could occur on the impression material. To quantify the number of viable microorganisms in each suspension, a 50ul sample from the bijou container was distributed on the surface of a 10cm diameter culture plate using spiral plating apparatus. The number of colony forming units was counted following culture (2.1.7). For each sample obtained by vortex mixing an impression sample in saline, spiral plating was carried out in duplicate. Suspensions of *C albicans* were plated on to Sabouraud's dextrose agar plates and incubated aerobically at 37^{o}C for 48 hours. *Ps aeruginosa* suspensions were plated on to blood agar and incubated aerobically at 37^{o}C for 24 hours.

Each experiment was repeated on two separate occasions for both high and low concentrations of each microbial species, and for each dilution of chlorhexidine gluconate. The results were derived from the mean number of colony forming units obtained from four spiral plate counts, two from each of two different microbial suspensions obtained in separate procedures.

In total 576 culture plates were examined :

a) Each of three impression materials was contaminated with both *C albicans* and *Ps aeruginosa* **(3 x 2 = 6).**
b) Two disinfection regimes, and a control regime were examined **(6 x 3 = 18).**
c) Each impression mould was sampled four times at the chosen time intervals **(18 x 4 = 72).**
d) Two 50ul samples from suspensions obtained after vortex mixing of each of the impression samples were plated for culture and quantification of the colony forming units **(72 x 2 = 144).**
e) The procedure was repeated **(144 x 2 = 288).**
f) The experiment was undertaken for both high and low concentrations of microbial inocula **(288 x 2 = 576).**

A summary of the protocol for EXPERIMENT 1 is shown in Table 3.3.

3.2 EXPERIMENT 1 - RESULTS

3.2.1 PERSISTENCE OF LOW CONCENTRATIONS OF *PS AERUGINOSA* AND *C ALBICANS* ON IMPRESSION MATERIALS FOLLOWING DISINFECTION WITH CHLORHEXIDINE GLUCONATE.

Initial microbial loading was negligible on poly (vinyl siloxane), with both *Ps aeruginosa* and *C albicans* (Table 3.5, 3.6). No growth was evident after disinfection procedures were carried out.

Substantial loading with *Ps aeruginosa* was evident on initial sampling of the polysulphide rubber impression material. *C albicans* was evident on initial sampling but the number of colony forming units was considerably less than with *Ps aeruginosa* (Table 3.7, 3.8). The number of colony forming units from the samples which had been inoculated with *Ps aeruginosa* diminished rapidly with time on the control specimens, with a small percentage (1.5%) evident after 30 minutes. The application of either strength of disinfectant agent rapidly resulted in the virtual elimination of *Ps Aeruginosa*. In contrast, *C albicans* persisted to a considerable extent on the polysulphide rubber control specimens over the 30 minute experimental period, although disinfection again produced immediate elimination.

With irreversible hydrocolloid, there was substantial initial colonisation of the impression moulds with both microorganisms, and numbers of microorganisms cultured from control samples increased with time (Table 3.9, 3.10). Disinfection procedures were only partially successful, and the stronger of the two solutions of chlorhexidine gluconate (0.1%) was found to be the more effective. Enumeration of colony forming units cultured from samples collected 30 minutes after disinfection with the weaker solution of chlorhexidine gluconate (0.02%), showed a colony forming unit value which was 73% of that found in initial pre-disinfection samples with *Ps aeruginosa*, and 43% of that found in pre-disinfection samples with *C albicans*.

This contrasted with polysulphide rubber and poly (vinyl siloxane), where all microorganisms were eliminated following this disinfection regime.

From comparison of the data from pre-disinfection samples of the polysulphide rubber and irreversible hydrocolloid materials, it was evident that initial colonisation with *C albicans* was considerably less profuse than with *Ps aeruginosa*. Neither microorganism showed appreciable colonisation of poly (vinyl siloxane) on any samples.

3.2.2 PERSISTENCE OF HIGH CONCENTRATION OF *PS AERUGINOSA* AND *C ALBICANS* ON IMPRESSION MATERIALS FOLLOWING DISINFECTION WITH CHLORHEXIDINE GLUCONATE.

With poly (vinyl siloxane), initial loading following contamination with the high concentration of *C albicans* was negligible on both control and experimental samples, and no growth was evident after disinfection (Table 3.11). Appreciable growth of *Ps aeruginosa* on the poly (vinyl siloxane) material was evident on initial sampling (Table 3.12). *Ps aeruginosa* showed rapid reduction in control samples and was immediately eliminated following the application of the weak disinfectant solutions. In control specimens, only 9.6% of the initial loading with *Ps aeruginosa* was evident in samples collected after 60 seconds, as was 2.3% of the initial inoculum after 30 minutes.

With polysulphide rubber, a substantial presence of both *Ps aeruginosa* and *C albicans* was noted on the pre-disinfection samples of impression moulds (Table 3.13, 3.14). Both micro-organisms persisted on the control specimens over the test period. After 30 minutes the number of colony forming units from samples inoculated with *Ps aeruginosa* was 25.4% of the initial CFU value, and with *C albicans*, the number of colony forming units found after 30 minutes was 53.8% of the initial CFU value.

Disinfection was effective with immersion in the 0.1% solution of chlorhexidine gluconate for 30 seconds, which caused virtual elimination of both microorganisms within 10 minutes. Immersion in 0.02% chlorhexidine gluconate resulted in a substantial reduction in colony forming units for both *Ps aeruginosa* and *C albicans*, although a few colony forming units were still cultured from samples removed 30 minutes after disinfection.

With respect to alginate, there was no reduction in the number of colony forming units on the control samples for either *Ps aeruginosa* or *C albicans*, over the 30 minute experimental period, and neither disinfection regime was effective in totally eliminating microorganisms (Table 3.15, 3.16). After 30 minutes from disinfection with an aqueous solution of 0.02% chlorhexidine gluconate, the number of colony forming units from samples which had been inoculated with *Ps aeruginosa*, was 60% of the pre-disinfection sample values. By comparison, at the same interval after disinfection with an aqueous solution of 0.1% chlorhexidine gluconate, the number of colony forming units was 20% of the original value. Following disinfection of the *C albicans* inoculated samples with 0.02% chlorhexidine gluconate, after the 30 minute experimental period the number of colony forming units was 29.5% of the CFU value of the pre-disinfection samples, and 30 minutes after disinfection with 0.1%

chlorhexidine gluconate, the number of colony forming units was 20% of the original CFU value. This contrasted with poly (vinyl siloxane), where all the microorganisms were eliminated, and with polysulphide rubber where very few remained after these disinfection regimes.

It is evident from examination of the data from the pre-disinfection samples, that initial colonisation with *C albicans*, on all three impression materials, was considerably less marked than with *Ps aeruginosa*.

3.3 EXPERIMENT 2 - MATERIALS AND METHODS

In this investigation, the persistence of the same two species of microorganism on the surface of irreversible hydrocolloid impression material, following application of three commercially available agents at concentrations recommended by the manufacturers, was assessed. The materials and methods employed were similar to those described for EXPERIMENT 1, with minor modifications. The material used to form impression moulds was restricted to the alginate material Kromogel, and only the high concentrations of *C albicans* and *Ps aeruginosa* were used. To disinfect the impression moulds following inoculation, 0.2% chlorhexidine gluconate (Corsodyl, ICI, Macclesfield, Cheshire, England); 2% glutaraldehyde

(Cidex, Johnson and Johnson, Slough, England), and 0.0125% sodium hypochlorite (Milton, Richardson-Vicks Ltd, Egham, Surrey, England) were applied. The sampling methods, the intervals between removal of impression samples, and the techniques for assessment of microbial persistence were as described in Sections 3.1.4 and 3.1.5. Each experiment was repeated on two separate occasions, and the number of microorganisms in each test suspension was verified by using duplicate spiral plate samples for culture. The quantitative assessment for microbial contamination of irreversible hydrocolloid for each microorganism, after a given time interval with a given disinfection regime, was therefore calculated and expressed as the mean value for the colony forming units from four spiral plates.

A total of 96 culture plates were examined:

a) Irreversible hydrocolloid impression material was contaminated with both *C albicans* and *Ps aeruginosa*, in high concentration **(1 x 2 = 2)**.

b) Three disinfection regimes were examined **(2 x 3 = 6)**.

c) Each mould of impression material was sampled four times at the chosen time intervals **(6 x 4 = 24)**.

d) Two 50 ul samples, from suspensions obtained by vortex mixing each of the impression samples, were plated for culture and CFU quantification **(24 x 2 = 48)**, and the entire procedure was repeated **(48 x 2 = 96)**,

A summary of the protocol being shown in Table 3.4.

3.4 EXPERIMENT 2 - RESULTS

THE MICROBICIDAL EFFECTS OF THREE COMMERCIALLY AVAILABLE DISINFECTANTS ON AN ALGINATE IMPRESSION MATERIAL.

From examination of the pre-disinfection samples, it was evident that considerable numbers of microorganisms were retained on the alginate impression samples, and initial loading with *Ps aeruginosa* was considerably more profuse than with *C albicans*.

It had previously been noted in control samples (Section 3.3.2) that colonisation of alginate did not diminish over the experimental period. All three disinfectant agents used in this part of the study were effective in producing a substantial reduction in colonisation of the alginate.

Following disinfection with 2% glutaraldehyde, there was a large reduction in the number of colony forming units from the impression samples. With *Ps aeruginosa*, the number of colony forming units formed on culture of samples collected immediately after disinfection, was 2.7% of the pre-disinfection CFU value. For *C albicans*, the number of colony forming units evident immediately after disinfection was 3% of the pre-disinfection CFU value. There was virtually complete elimination of

alginate microbial colonisation 30 minutes after disinfection with 2% glutaraldehyde.

Following disinfection with 0.0125% sodium hypochlorite, the reduction in numbers of microorganisms followed a similar pattern to that described for 2% glutaraldehyde. With *Ps aeruginosa*, the number of colony forming units from samples collected immediately after disinfection, was 1.8% of the pre-disinfection CFU value.
With *C albicans* the number of colony forming units evident immediately after disinfection was 2.4% of the pre-disinfection CFU value. There was virtually complete elimination of alginate microbial colonisation 30 minutes after disinfection with sodium hypochlorite.

With 0.2% chlorhexidine gluconate disinfection there was a reduction in microbial colonisation of alginate materials, but this was less marked than with sodium hypochlorite or glutaraldehyde. Immediately following disinfection, there was a considerable reduction in the number of colony forming units cultured from samples inoculated with *Ps aeruginosa*, only 2.9% of the pre-disinfection CFU value remained. In samples collected 10 minutes after disinfection, the number of colony forming units was 8% of the pre-disinfection CFU value, and after 30 minutes it was 12% of the pre-disinfection CFU value.

Immediately following disinfection of impression samples which had been inoculated with *C albicans*, the number of colony forming units cultured was 31% of the original CFU value. In samples collected 10 minutes after disinfection, the number of colony forming units was 15% of the pre-disinfection CFU value, and after 30 minutes it was 5% of the pre-disinfection CFU value.

3.5 DISCUSSION

The microbial contamination of dental materials and prostheses has been documented by a number of workers [124,125,126]. Such contaminants include opportunistic oral pathogens such as *Ps aeruginosa* and *C albicans*, robust microorganisms, already shown to be persistent on the impression materials tested in Chapter 2. These were considered to be suitable indicator microorganisms for microbial retention assessment on the surface of impression materials following disinfection.

Weak solutions of the disinfectant agents were used in EXPERIMENT 1 to allow the survival of a proportion of the contaminating microorganisms and permit comparison between the three impression materials tested. The materials exhibited different properties following contamination and disinfection, and the data suggest that

considerably greater numbers of microorganisms settled on polysulphide rubber and irreversible hydrocolloid than on poly (vinyl siloxane), and that irreversible hydrocolloid is considerably more resistant to disinfection procedures than polysulphide rubber. This variation in microbial colonisation may have reflected a difference in the surface morphology of the materials; it may have been due to the differential hydrophobic nature of the materials, or it may have been due to a variation in the anti-microbial effect of the impression materials.

From EXPERIMENT 2, even with the high concentration of microorganisms used, two of the disinfectant agents tested produced almost complete destruction of the contaminant microbes. As the disinfectants are compatible with the impression materials used, and are inexpensive, it seems reasonable that consideration should be given to the routine use of such techniques in alginate impression material disinfection. However of chlorhexidine gluconate in the concentration tested does not appear to be an effective measure in the control of cross-infection by immersion of dental impressions.

3.6 CONCLUSIONS

With respect to the materials and microorganisms tested in the study, it was concluded that:

- the carriage of microorganisms on poly (vinyl siloxane) material is limited, and is significantly less than with polysulphide rubber ($P<.001$), or irreversible hydrocolloid ($P<.001$).
- the number of microorganisms found on the surface of polysulphide rubber diminishes significantly after 30 minutes ($P<.05$) without disinfection, and following disinfection of polysulphide rubber with 0.1% or 0.02% chlorhexidine gluconate, virtual elimination of micro-organisms results within 30 minutes.
- there is no reduction in the number of microorganisms found on the surface of non-disinfected irreversible hydrocolloid after a 30 minute period and, in contrast with the other impression materials tested, elimination of microorganisms from irreversible hydrocolloid is not facilitated by the use of mild disinfectant agents (0.1% and 0.02% chlorhexidine gluconate).
- while with 0.2% chlorhexidine gluconate (Corsodyl) substantial numbers of microorganisms still persisted, disinfection of all three impression materials for 30 minutes with 2% glutaraldehyde (Cidex), or 0.0125% sodium hypochlorite (Milton), resulted in the virtual elimination of the microorganisms tested, and a simple disinfection regime of 30 minutes with these two commonly available disinfectants may be effective for almost complete destruction of microbes contaminating impression materials.

FIGURE 3.1

Impression moulds,

three impression materials in petri-dishes.

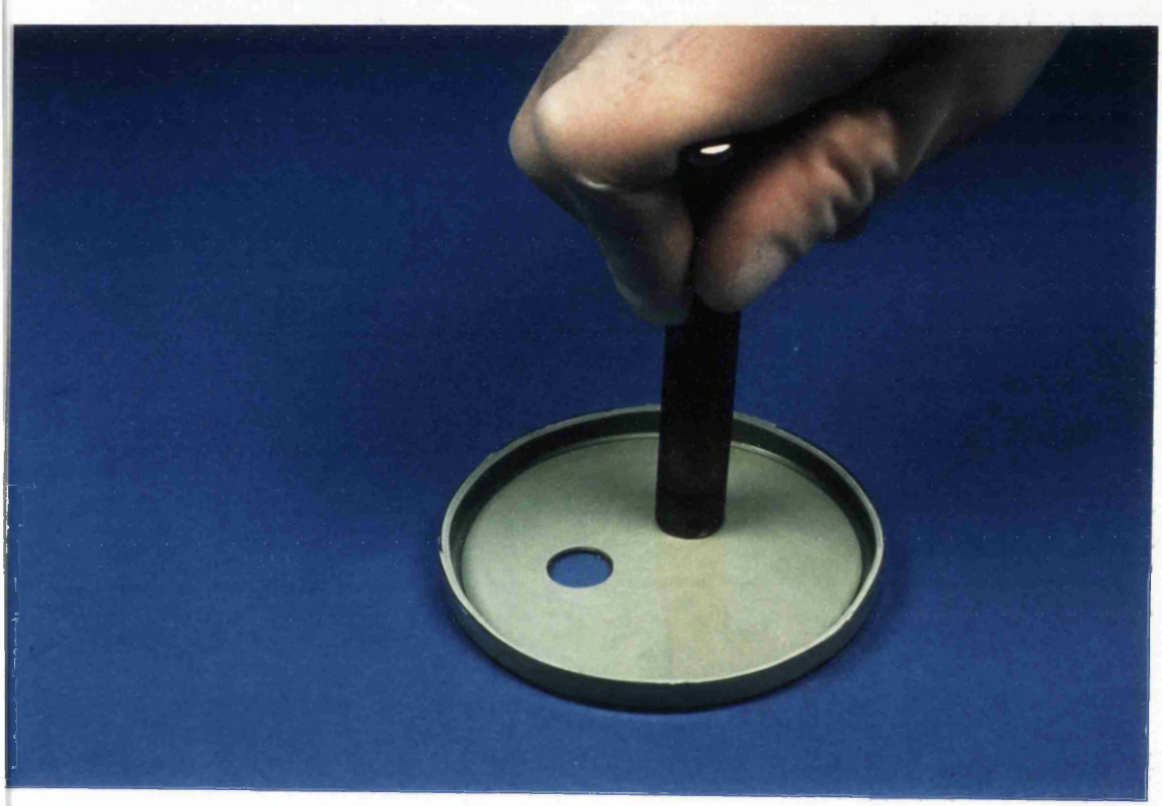


FIGURE 3.2

Cork borer (13mm diameter),
used in collection of samples.

TABLE 3.1

IMPRESSION MATERIALS EXAMINED IN EXPERIMENT 1

Material	Type	Manufacturer
Kromogel	Alginate	Wright, Scotland
Permlastic	Polysulphide rubber	Kerr, Switzerland
President	Poly (vinyl siloxane)	Coltene, Switzerland

TABLE 3.2

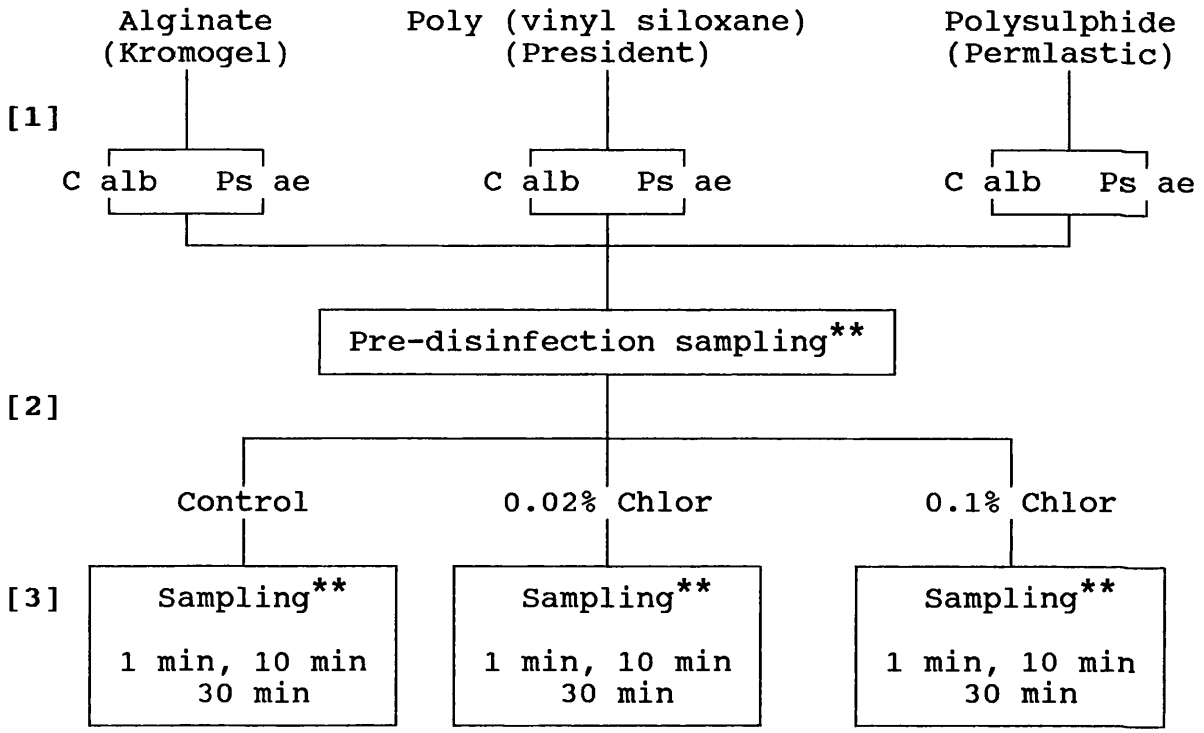
MICROORGANISMS SELECTED FOR ARTIFICIAL INOCULATION

Microorganism.....Strain
Candida albicans.....MRL 3153 *Pseudomonas aeruginosa*.....NCTC 2131

MRL - Mycological Reference Laboratory, London, England.
NCTC - National Collection of Type Cultures, USA.

TABLE 3.3

PROTOCOL FOR EXPERIMENT I*



C alb - *Candida albicans*.
Ps ae - *Pseudomonas aeruginosa*.
Chlor - Chlorhexidine gluconate.

* Protocol followed for high and low concentrations of both microorganisms.

** The microbial suspension obtained from each sample plated and cultured in duplicate.

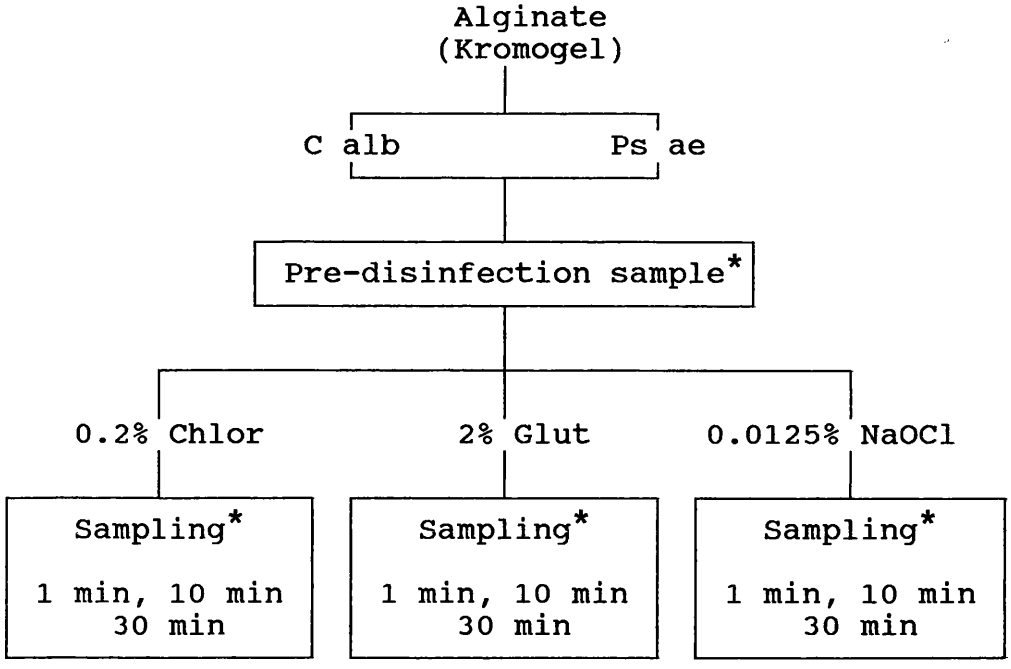
[1] Moulds of each impression material inoculated with either *C albicans* or *Ps aeruginosa*.

[2] After discarding the inoculum, each material sampled and then left as control specimins or disinfected with 0.02% or 0.1% chlorhexidine gluconate for 30 seconds.

[3] Further sampling carried out at 1 minute, 10 minute and 30 minute intervals after the inoculum was discarded from the impression moulds. Experimental groups disinfected with 0.02% or 0.1% chlorhexidine gluconate for 30 seconds in the interval between the initial sampling (Sample A) and the removal of second sample one minute after discarding the inoculum (Sample B).

TABLE 3.4

PROTOCOL FOR EXPERIMENT 2



C alb - *Candida albicans*.
Ps ae - *Pseudomonas aeruginosa*.
Chlor - Chlorhexidine gluconate.
Glut - Glutaraldehyde.
NaOCl - Sodium hypochlorite.

* Microbial suspension obtained from each sample, plated and cultured in duplicate.

[1] Irreversible hydrocolloid impression moulds inoculated with high concentration suspensions of either *C albicans* or *Ps aeruginosa*.

[2] After discarding inoculum, impression moulds sampled, and disinfected with 0.2% chlorhexidine, 2% glutaraldehyde or 0.0125% sodium hypochlorite, for 30 seconds.

[3] Further sampling of materials carried out at 1 minute, 10 minute and 30 minute intervals after inoculum discarded from impression moulds.

TABLE 3.5

PERSISTENCE OF LOW CONCENTRATION SUSPENSIONS OF *CANDIDA ALBICANS* ON POLY (VINYL SILOXANE) (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	0	0	3
pre-	i	0	0	3
disinfection	ii	0	0	1
	ii	0	0	1
	Mean	**0**	**0**	**2**
Sample B	i	0	0	0
post-	i	0	0	0
disinfection	ii	0	0	0
	ii	0	0	0
	Mean	**0**	**0**	**0**
Sample C	i	0	0	0
10 minutes	i	0	0	0
	ii	0	0	0
	ii	0	0	0
	Mean	**0**	**0**	**0**
Sample D	i	3	0	0
30 minutes	i	3	0	0
	ii	2	0	0
	ii	3	0	0
	Mean	**3**	**0**	**0**

TABLE 3.6

PERSISTENCE OF A LOW CONCENTRATION SUSPENSIONS OF *PSEUDOMONAS AERUGINOSA* ON POLY (VINYL SILOXANE) (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	5	2	3
pre-	i	7	2	3
disinfection	ii	6	4	1
	ii	7	3	1
	Mean	**6**	**3**	**2**
Sample B	i	2	0	0
post-	i	3	0	0
disinfection	ii	2	0	0
	ii	4	0	0
	Mean	**3**	**0**	**0**
Sample C	i	0	0	0
10 minutes	i	0	0	0
	ii	1	0	0
	ii	1	0	0
	Mean	**1**	**0**	**0**
Sample D	i	6	0	0
30 minutes	i	6	0	0
	ii	9	0	0
	ii	13	0	0
	Mean	**9**	**0**	**0**

TABLE 3.7

PERSISTENCE OF A LOW CONCENTRATION OF *CANDIDA ALBICANS* ON POLYSULPHIDE RUBBER (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	20	14	6
pre-	i	15	12	5
disinfection	ii	29	25	11
	ii	23	23	12
	Mean	**22**	**19**	**9**
	SD	**6**	**6**	**/**
Sample B	i	27	1	0
post-	i	22	3	0
disinfection	ii	20	0	0
	ii	28	0	0
	Mean	**24**	**1**	**0**
	SD	**4**	**/**	**/**
Sample C	i	13	0	0
10 minutes	i	22	0	0
	ii	22	0	0
	ii	30	0	0
	Mean	**22**	**0**	**0**
	SD	**7**	**/**	**/**
Sample D	i	14	1	0
30 minutes	i	22	1	0
	ii	16	0	0
	ii	12	0	0
	Mean	**16**	**1**	**0**
	SD	**4**	**/**	**/**

TABLE 3.8

PERSISTENCE OF A LOW CONCENTRATION OF *PSEUDOMONAS AERUGINOSA* ON POLYSULPHIDE RUBBER (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	1853	1127	1185
pre-	i	1962	1000	1367
disinfection	ii	2539	930	1027
	ii	2101	807	1209
	Mean	**2214**	**966**	**1197**
	SD	**301**	**134**	**139**
Sample B	i	1509	1	2
post-	i	1588	0	0
disinfection	ii	1711	0	0
	ii	1707	2	0
	Mean	**1629**	**1**	**1**
	SD	**98**	**/**	**/**
Sample C	i	124	0	0
10 minutes	i	117	0	0
	ii	136	0	0
	ii	130	0	0
	Mean	**127**	**0**	**0**
	SD	**8**	**/**	**/**
Sample D	i	41	0	0
30 minutes	i	40	0	0
	ii	21	0	0
	ii	21	0	0
	Mean	**31**	**0**	**0**
	SD	**11**	**/**	**/**

TABLE 3.9

PERSISTENCE OF A LOW CONCENTRATION OF *CANDIDA ALBICANS* ON IRREVERSIBLE HYDROCOLLOID (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	79	106	127
pre-	i	78	108	112
disinfection	ii	72	86	84
	ii	65	104	72
	Mean	**74**	**101**	**99**
	SD	**7**	**10**	**25**
Sample B	i	88	71	47
post-	i	80	51	42
disinfection	ii	88	1	46
	ii	87	3	32
	Mean	**86**	**32**	**42**
	SD	**4**	**37**	**7**
Sample C	i	78	36	86
10 minutes	i	84	44	86
	ii	93	22	96
	ii	104	14	80
	Mean	**90**	**29**	**87**
	SD	**11**	**14**	**7**
Sample D	i	69	58	7
30 minutes	i	91	57	6
	ii	124	30	1
	ii	143	26	0
	Mean	**107**	**43**	**4**
	SD	**33**	**17**	**/**

TABLE 3.10

PERSISTENCE OF A LOW CONCENTRATION OF *PSEUDOMONAS AERUGINOSA* ON IRREVERSIBLE HYDROCOLLOID (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	1454	1520	1450
pre-	i	1572	1995	1236
disinfection	ii	1216	1842	1742
	ii	1273	1886	2152
	Mean	**1379**	**1811**	**1645**
	SD	**164**	**204**	**397**
Sample B	i	1323	1063	378
post-	i	1145	1277	184
disinfection	ii	1084	1298	422
	ii	832	1454	496
	Mean	**1096**	**1273**	**370**
	SD	**203**	**161**	**133**
Sample C	i	1879	977	285
10 minutes	i	1727	1050	303
	ii	1545	1162	341
	ii	1151	1689	300
	Mean	**1576**	**1220**	**307**
	SD	**314**	**322**	**24**
Sample D	i	1727	1298	43
30 minutes	i	1636	1188	49
	ii	1364	1472	60
	ii	1364	1313	73
	Mean	**1523**	**1318**	**56**
	SD	**187**	**117**	**13**

TABLE 3.11

PERSISTENCE OF A HIGH CONCENTRATION OF *CANDIDA ALBICANS* ON POLY (VINYL SILOXANE) (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	0	1	0
pre-	i	0	1	0
disinfection	ii	0	0	0
	ii	0	0	0
	Mean	**0**	**1**	**0**
Sample B	i	7	0	0
post-	i	8	0	0
disinfection	ii	8	0	0
	ii	12	0	0
	Mean	**9**	**0**	**0**
Sample C	i	0	0	0
10 minutes	i	0	0	0
	ii	5	0	0
	ii	10	0	0
	Mean	**4**	**0**	**0**
Sample D	i	10	0	0
30 minutes	i	7	0	0
	ii	8	0	0
	ii	9	0	0
	Mean	**9**	**0**	**0**

TABLE 3.12

THE PERSISTENCE OF A HIGH CONCENTRATION OF *PSEUDOMONAS AERUGINOSA* ON POLY (VINYL SILOXANE) (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	688	434	664
pre-	i	616	392	613
disinfection	ii	584	384	667
	ii	576	325	656
	Mean	**616**	**384**	**650**
	SD	**51**	**45**	**25**
Sample B	i	67	2	0
post-	i	42	0	0
disinfection	ii	74	0	0
	ii	54	0	0
	Mean	**59**	**1**	**0**
	SD	**14**	**/**	**/**
Sample C	i	43	0	0
10 minutes	i	25	0	0
	ii	4	0	0
	ii	6	0	0
	Mean	**20**	**0**	**0**
	SD	**18**	**/**	**/**
Sample D	i	36	0	0
30 minutes	i	15	0	0
	ii	5	0	0
	ii	1	0	0
	Mean	**14**	**0**	**0**
	SD	**16**	**/**	**/**

TABLE 3.13

PERSISTENCE OF A HIGH CONCENTRATION OF *CANDIDA ALBICANS* ON POLYSULPHIDE RUBBER (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	1110	940	1076
pre-	i	1010	890	1011
disinfection	ii	1121	992	1083
	ii	1224	947	1042
	Mean	**1116**	**942**	**1053**
	SD	**88**	**42**	**33**
Sample B	i	984	0	2
post-	i	1053	1	0
disinfection	ii	1156	17	5
	ii	1306	13	16
	Mean	**1125**	**8**	**6**
	SD	**140**	**/**	**/**
Sample C	i	594	2	2
10 minutes	i	688	1	0
	ii	768	7	0
	ii	931	12	0
	Mean	**745**	**6**	**1**
	SD	**143**	**/**	**/**
Sample D	i	482	0	0
30 minutes	i	552	1	0
	ii	746	4	0
	ii	620	5	0
	Mean	**600**	**3**	**0**
	SD	**113**	**/**	**/**

TABLE 3.14

THE PERSISTENCE OF A HIGH CONCENTRATION OF *PSEUDOMONAS AERUGINOSA* ON POLYSULPHIDE RUBBER (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	4655	4302	2502
pre-	i	4346	3964	3633
disinfection	ii	5029	3392	4062
	ii	4770	3808	5003
	Mean	**4700**	**3867**	**3800**
	SD	**283**	**378**	**1037**
Sample B	i	4600	170	3
post-	i	3467	210	4
disinfection	ii	6026	156	18
	ii	5040	128	45
	Mean	**4783**	**166**	**18**
	SD	**1061**	**34**	**20**
Sample C	i	2600	46	0
10 minutes	i	3006	24	0
	ii	3724	11	0
	ii	3200	6	0
	Mean	**3133**	**22**	**0**
	SD	**467**	**18**	**/**
Sample D	i	710	4	0
30 minutes	i	850	5	0
	ii	2020	10	0
	ii	1200	7	0
	Mean	**1195**	**7**	**0**
	SD	**587**	**/**	**/**

TABLE 3.15

THE PERSISTENCE OF THE HIGH CONCENTRATION OF *CANDIDA ALBICANS* ON IRREVERSIBLE HYDROCOLLOID (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	1503	1342	1473
pre-	i	2117	1589	1662
disinfection	ii	885	1246	1304
	ii	1279	1011	1409
	Mean	**1446**	**1297**	**1462**
	SD	**515**	**239**	**150**
Sample B	i	1873	849	504
post-	i	2472	1277	735
disinfection	ii	1603	697	326
	ii	1523	425	403
	Mean	**1868**	**812**	**492**
	SD	**430**	**356**	**178**
Sample C	i	1787	85	92
10 minutes	i	1909	100	54
	ii	1681	52	42
	ii	1744	68	19
	Mean	**1780**	**76**	**52**
	SD	**96**	**21**	**31**
Sample D	i	1793	401	365
30 minutes	i	1858	714	527
	ii	1499	131	300
	ii	1713	283	247
	Mean	**1716**	**382**	**360**
	SD	**156**	**247**	**122**

TABLE 3.16

PERSISTENCE OF THE HIGH CONCENTRATION OF *PSEUDOMONAS AERUGINOSA* ON IRREVERSIBLE HYDROCOLLOID (CFU/50ul)

		Control	0.02% Chlor	0.1% Chlor
Sample A	i	2651	3239	3449
pre-	i	3283	3427	2417
disinfection	ii	3888	3698	3826
	ii	3512	3502	4508
	Mean	**3334**	**3467**	**3550**
	SD	**519**	**190**	**873**
Sample B	i	2778	2508	2316
post-	i	2889	2387	1881
disinfection	ii	2934	2651	2486
	ii	3066	2549	2621
	Mean	**2917**	**2524**	**2326**
	SD	**119**	**109**	**322**
Sample C	i	3431	2034	622
10 minutes	i	4036	2433	770
	ii	4914	2796	1433
	ii	4286	2578	2901
	Mean	**4167**	**2461**	**1432**
	SD	**614**	**320**	**1041**
Sample D	i	3214	1790	145
30 minutes	i	3315	2011	179
	ii	3457	2412	713
	ii	3347	2121	1807
	Mean	**3333**	**2084**	**711**
	SD	**100**	**259**	**776**

TABLE 3.17

THE MICROBICIDAL EFFECT OF THREE DISINFECTANT AGENTS ON IRREVERSIBLE HYDROCOLLOID INOCULATED WITH *C ALBICANS* (CFU/50ul)

		Control	0.2% Chlor	2% Glut	0.0125%NaOCl
Sample A	i	1503	1077	2124	1828
pre-	i	2117	1552	2085	1686
disinfection	ii	885	2294	2231	1710
	ii	1279	1821	2435	2497
	Mean	**1446**	**1686**	**2219**	**1930**
	SD	**515**	**509**	**157**	**383**
Sample B	i	1873	455	54	46
post-	i	2472	403	57	78
disinfection	ii	1603	664	77	20
	ii	1523	583	81	41
	Mean	**1868**	**526**	**67**	**46**
	SD	**430**	**119**	**14**	**24**
Sample C	i	1787	204	4	6
10 minutes	i	1909	195	5	13
	ii	1681	336	30	7
	ii	1744	254	22	7
	Mean	**1780**	**247**	**15**	**8**
	SD	**96**	**65**	**13**	**/**
Sample D	i	1793	58	6	7
30 minutes	i	1858	66	2	9
	ii	1499	141	12	6
	ii	1713	72	15	14
	Mean	**1716**	**84**	**9**	**9**
	SD	**156**	**38**	**/**	**/**

TABLE 3.18

THE MICROBICIDAL EFFECT OF THREE DISINFECTANT AGENTS ON IRREVERSIBLE HYDROCOLLOID INOCULATED WITH *PS AERUGINOSA* (CFU/50ul)

		Control	0.2% Chlor	2% Glut	0.0125%NaOCl
Sample A	i	2651	1928	3468	4188
pre-	i	3283	2939	3577	3998
disinfection	ii	3888	4697	3322	3587
	ii	3512	3953	2901	4105
	Mean	**3334**	**3379**	**3317**	**3970**
	SD	**519**	**1206**	**296**	**267**
Sample B	i	2778	100	117	107
post-	i	2889	109	99	56
disinfection	ii	2934	92	87	79
	ii	3066	86	58	46
	Mean	**2917**	**97**	**90**	**72**
	SD	**119**	**10**	**25**	**27**
Sample C	i	3431	293	33	26
10 minutes	i	4036	305	39	17
	ii	4914	236	26	5
	ii	4286	253	18	3
	Mean	**4167**	**272**	**29**	**13**
	SD	**614**	**33**	**10**	**11**
Sample D	i	3214	723	1	2
30 minutes	i	3315	629	0	1
	ii	3457	124	0	0
	ii	3347	153	0	0
	Mean	**3333**	**407**	**0**	**1**
	SD	**100**	**313**	**/**	**/**

CHAPTER 4

IN-VITRO INVESTIGATIONS OF THE USE OF CHLORHEXIDINE GLUCONATE IN THE DISINFECTION OF AN ALGINATE IMPRESSION MATERIAL

INTRODUCTION

The risk of cross-infection from contaminated dental impressions may be reduced by immersion of impressions in suitable disinfectants, and an aqueous solution of chlorhexidine gluconate can be employed for this purpose. The use of chlorhexidine in this way was investigated in Chapter 3, and it was found to be less effective as an immersion disinfectant than commercially available preparations of glutaraldehyde or sodium hypochlorite. In this chapter an alternative way of using chlorhexidine to reduce the risk of contamination from dental impressions was examined in four *in-vitro* investigations. The aim was to verify the antimicrobial effect of a chlorhexidine-containing material, under experimental conditions reflecting its use in the clinical situation.

4.1 EXPERIMENT 1 [i] - AIMS OF STUDY

The purpose of the investigation was to examine the antimicrobial effect of chlorhexidine incorporated within the powder of a commercially available alginate impression material, by contamination of experimental and control samples of alginate with standardised laboratory cultured microorganisms, followed by microbiological testing of the impression materials after various time-intervals.

4.2 EXPERIMENT 1 [i] - MATERIALS AND METHODS

Three species of microorganism were used to contaminate the impression materials, and quantitative methods were used in the assessment of microbial carriage. Findings for the chlorhexidine-containing material (Hydrogum) were compared with standard alginate material (Kromogel), and material containing the quaternary ammonium compound, sodium didecyldimethyl ammonium chloride (Blueprint with Antibac). The aim was to establish whether the use of chlorhexidine incorporated within the powder of an alginate impression material constitutes an effective antimicrobial measure.

4.2.1 MICROORGANISMS AND CULTURE CONDITIONS

Here, three different types of alginate impression material were selected for investigation (Table 4.1), and flat impression moulds were made for each, as previously described (2.5.1). Microorganisms chosen for artificial inoculation of impression materials were *Staph aureus* (NCTC 7447), *Ps aeruginosa* (NCTC 10662) and *C albicans* (MRL 3153). These microorganisms were selected because, as described in Chapters 2 and 3, they had been found to persist on the surface of alginate impression materials. *Staph aureus* and *Ps aeruginosa* were grown on blood agar at 37^{o}C for 24 hours, and *C albicans* grown on Sabouraud's

dextrose agar (Oxoid, Basingstoke, England) at 37°C for 48 hours. For each strain, a suspension was prepared by dispersing four inoculating loops of the microorganism in 20ml of phosphate buffered saline and vortex mixing for 60 seconds to ensure even distribution.

4.2.2 INOCULATION OF IMPRESSION MOULDS

To assess microbial retention for each microorganism, 20ml samples of inoculum were delivered aseptically on to moulds of the three impression materials under test. The impression surface was covered and left submerged for three minutes. The inoculum was discarded, and impression materials sampled using a sterile 13mm diameter cork borer (Sample A). Sampling was repeated 10 minutes after discarding the inoculum (Sample B), and again 30 minutes after discarding the inoculum (Sample C).

4.2.3 VIABLE MICROORGANISMS

Each impression sample was deposited in 10ml of sterile saline in a universal container and vortex mixing carried out for 60 seconds to dislodge microorganisms from the sample surface. Immediately after mixing, 5ml of the suspension was decanted into a sterile bijoux container, before any resettling of microorganisms on the impression material. To quantify the number of viable microorganisms

in each suspension, a 50ul sample from this container was distributed on the surface of a 10cm culture plate using a spiral plating apparatus (2.1.5) and the colony forming units were counted following culture (2.1.7). Suspensions of *C albicans* were plated on Sabouraud's dextrose agar and incubated aerobically at 37°C for 48 hours. *Staph aureus* and *Ps aeruginosa* were plated on blood agar and incubated aerobically at 37°C for 24 hours. Each experiment was repeated on three separate occasions for each microbial species, and triplicate samples for spiral plating were taken from each suspension. Thus, results were derived from the number of colony forming units obtained from three spiral plate counts from each of three different microbial suspensions, from separate procedures. A total of 243 culture plates were examined :

- Each of the impression materials was contaminated with *Staph aureus*, *Ps aeruginosa* and *C albicans* **(3 x 3 : 9)**.
- Each mould of impression material for examination was sampled three times (once at each of the chosen time-intervals **(9 x 3 : 27)**.
- Three 50 ul samples from the microbial suspension obtained after vortex mixing of each of the four samples from each impression mould, were plated for culture and quantification of colony forming units **(27 x 3 : 81)**.
- This entire procedure carried out on three occasions **(81 x 3 : 243)**.

4.3 EXPERIMENT 1 [i] - RESULTS

The number of colony forming units (CFU) cultured from impression samples contaminated with *C albicans* , *Staph aureus* and *Ps aeruginosa* in EXPERIMENT 1 [i], are shown in Appendices 4.1 to 4.9. The summary data are presented in Tables 4.2 to 4.4.

With respect to *C albicans* (Table 4.2), considerable growth was found on Kromogel and Hydrogum at initial sampling. The CFU values on the conventional alginate (Kromogel) diminished slightly over the 30 minute test period, and on the chlorhexidine-containing material (Hydrogum) there was no reduction of the CFU count over the test interval. There was substantially less evidence of *C albicans* on Blueprint than on the other materials on initial sampling, with virtual elimination of the fungus after 10 minutes. For *Staph aureus* (Table 4.3), there was also considerable growth on Kromogel and Hydrogum at initial sampling. The CFU values on the conventional alginate (Kromogel) again diminished slightly over the 30 minute period. Although with the chlorhexidine-containing material (Hydrogum) there was a reduction in CFU counts over the test interval, this was less marked than was the case for Kromogel. There was no evidence of *Staph aureus* colonisation on Blueprint on any of the samples tested.

With respect to *Ps aeruginosa* (Table 4.4), substantial growth was found on Kromogel and Hydrogum at initial sampling, *ie* considerably greater than was found on these materials following inoculation with the other micro-organisms. The CFU values for the conventional alginate (Kromogel) increased slightly over the 30 minute test period, and on the chlorhexidine-containing material there was a small reduction of the CFU count over the test interval. There was substantially less evidence of *Ps aeruginosa* colonisation of Blueprint, than on the other impression materials at initial sampling, with a marked reduction in colonisation after 30 minutes.

Statistical analysis was undertaken to assess differences between the impression materials with respect to carriage of microorganisms. The Mann-Whitney U test was used in comparison of values for the carriage of each of the microorganisms on Kromogel, Hydrogum and Blueprint, at each of the time intervals tested.

It was found that for microbial loading (CFU values) at each time interval tested, with one exception, there was no statistically significant difference between the conventional alginate (Kromogel) and the chlorhexidine-containing alginate (Hydrogum). The higher CFU counts for initial loading of Hydrogum with *Ps aeruginosa*, as compared with the conventional alginate (Kromogel), was

found to be of statistical significance ($p<0.05$). Otherwise no difference was apparent between these materials with respect to carriage of the microorganisms tested.

In all cases the microbial colonisation of Blueprint was found consistently to be significantly less ($p<0.05$) than for either of the other two materials.

4.4 EXPERIMENT 1 [ii] - MATERIALS AND METHODS

Further investigation of differences in the carriage of microorganisms on Kromogel and Hydrogum was undertaken using more dilute solutions of *Staph aureus*, *C albicans* and *Ps aeruginosa*. The same three species of microorganism (culture and preparation as described in Section 4.1.1), were used to inoculate flat impression moulds of Kromogel and Hydrogum. The dilute suspensions for contamination of materials were prepared for each strain by dispersing two inoculating loops of microorganism in 20ml phosphate buffered saline, followed by vortex mixing for 60 seconds to ensure even distribution.

Inoculation of impression moulds, sampling of impression materials, and culture and counting of colonies of viable microorganisms, was carried out as described in Section 4.1.1.

A total of 162 culture plates were examined :

- Each of the impression materials was contaminated with *Staph aureus*, *Ps aeruginosa* and *C albicans* **(2 x 3 : 6).**
- Each mould of impression material for examination was sampled three times (once at each of the chosen time intervals **(6 x 3 : 18).**
- Three 50 ul samples from the microbial suspension obtained after vortex mixing of each of the four samples from each impression mould were plated for culture and quantification of colony forming units **(18 x 3 : 54).**
- This entire procedure was carried out on three occasions **(54 x 3 : 162).**

4.5 EXPERIMENT 1 [ii] - RESULTS

The number of colony forming units (CFU) cultured from impression samples contaminated with *C albicans , Staph aureus and Ps aeruginosa* in EXPERIMENT 1 [ii], are shown in Appendices 4.10 to 4.15. Summary data are presented in Tables 4.5 to 4.7.

With respect to *C albicans* (Table 4.5), considerable growth was found on both materials on initial sampling. The CFU values on the conventional alginate (Kromogel) diminished only slightly over the 30 minute period, and

on the chlorhexidine-containing material (Hydrogum), there was a greater reduction in the CFU count over the test interval. For *Staph aureus* (Table 4.6), there was less growth than with *C albicans*, on both materials at initial sampling. The CFU values on the conventional alginate (Kromogel) increased slightly over the 30 minute period, while Hydrogum showed a reduction in CFU counts over the test interval. With respect to *Ps aeruginosa* (Table 4.7), substantially more growth was found on both materials at initial sampling than was found following inoculation with the other microorganisms. There was a slight reduction in CFU values for both materials over the 30 minute test period.

The Mann-Whitney U test was used to compare values for the carriage of each microorganism on Kromogel and Hydrogum, at each of the time intervals tested. It was found that *C albicans'* colonisation of the conventional alginate (Kromogel) was significantly greater than the colonisation of the chlorhexidine-containing Hydrogum, at each time interval tested ($p<0.05$). *Ps aeruginosa* colonisation of Hydrogum was significantly greater than colonisation of Kromogel at each time interval ($p<0.05$). There was no clear distinction between the two materials for the carriage of *Staph aureus*.

4.6 EXPERIMENT 1 [i & ii] - DISCUSSION

A clear and statistically significant difference between Blueprint Asept and the other impression materials is obvious from the first experiment (EXPERIMENT 1 [i]). The relative resistance of *Ps aeruginosa*, and the susceptibility of *C albicans* and *Staph aureus* were not unexpected in view of the findings reported in Chapter 2. What is of interest is the limited antimicrobial effect of Hydrogum in this experiment, particularly the finding that Kromogel appeared to have a slightly greater antimicrobial effect against *Staph aureus* and *C albicans* over the duration of the experimental period. There seems little doubt from the study, that sodium didecyldimethyl ammonium chloride is considerably more effective than chlorhexidine in these impression materials.

The second experiment was undertaken to compare further any differences in antimicrobial effect of the chlorhexidine-containing material and the conventional material, using a weaker concentration of inoculated microorganisms, and there was confirmation of the initial finding that there was no evidence of any substantial antimicrobial effect of the chlorhexidine-containing impression material.

4.7 EXPERIMENT 2 - AIMS OF STUDY

The aim of this experiment was to use an alternative method of assessment of the antimicrobial effectiveness of chlorhexidine-containing Hydrogum, and to compare it with the standard alginate, Kromogel, and with Blueprint with Antibac (containing sodium didecyldimethyl ammonium chloride), using a cylinder assay plate method diffusion test. This test is based on the principle of diffusion of disinfectant from discs of impression material on to surrounding culture media, causing inhibition of growth of inoculated microorganisms.

4.8 EXPERIMENT 2 - MATERIALS AND METHODS

4.8.1 PREPARATION OF IMPRESSION MATERIAL DISCS

For each of the impression materials, alginate powder was mixed with tap water in accordance with manufacturer's instructions, placed in wells in a sterile polytetrafluoroethylene (PTFE) mould and flattened with a sterile blade (Fig 4.1). Care was taken to minimise incorporation of air so that a solid disc of alginate without air blows was produced. Each PTFE well was 10mm in diameter and 2mm deep, and identical discs of impression material were produced for application on to the surface of inoculated culture plates.

Before use, rubber mixing bowls and spatulae were cleaned with a 70% solution of iso-propyl alcohol. The PTFE mould was sterilised in an autoclave before each impression material application and, to prevent any risk of chemical carry-over, it was divided into three sections (using a waterproof marker pen), each section being used to prepare discs of only one impression material type.

4.8.2 INOCULATION AND CULTURE OF MICROORGANISMS

In this study, six microorganisms, representative of normal oral flora were used. *Strep sanguis* (NCTC 7863), *Strep mutans* (NCTC 10449), *Strep milleri* (NCTC 10713) and *Strep salivarius* (NCTC 8618) were grown on blood agar plates and incubated in a carbon dioxide chamber. *Actino viscous* (NCTC 10951) and *Porph gingivalis* (NCTC 11834) were grown anaerobically on blood agar for 48 hours at 37^{0}C.

A sample (one culture loop) of each microorganism was dispersed individually in 5ml of peptone water, and two samples of the resultant suspension were each inoculated on to a culture plate containing the appropriate media for incubation, to give a confluent lawn of growth. A disc of alginate impression material was then applied to the surface of each inoculated plate, which was incubated under the appropriate conditions.

After incubation, the zone of growth inhibition around each disc (Fig 4.2) was determined using a ruler. The size of this area was taken as a measure of the susceptibility of each microorganism to the disc of impression material on the surface of the culture plate. This procedure was undertaken for each of the impression materials against each of the microorganisms, and the entire process was repeated on three separate occasions.

4.9 EXPERIMENT 2 - RESULTS

As care was taken to minimise the incorporation of air within the alginate discs, relatively uniform rings of growth inhibition were found around the alginate materials following incubation. The inhibition zones were measured to the nearest 0.5mm, using a ruler (Table 4.8).

For each of the test microorganisms, a similar degree of inhibition occurred with both Hydrogum and Blueprint. Statistical analysis (Mann-Whitney U Test) of the inhibition values, showed no significant difference between the materials for growth of *Strep milleri*, *Strep Mutans*, *Strep salivarius* or *Actino viscous*. For *Strep sanguis*, inhibition of growth with Hydrogum (6.1mm) was greater, to a statistically significantly degree, than with Blueprint (4mm), and for *Porph Gingivalis* inhibition

of growth with Blueprint (4.8mm) was significantly greater than with Hydrogum (3.5mm).

With the exception of *Strep mutans*, there no evidence of growth inhibition around the Kromogel samples, and inhibition of *Strep mutans* around Kromogel (1.1mm) was considerably less than occurred with Hydrogum (7mm) and Blueprint (7.2mm).

4.10 EXPERIMENT 2 - DISCUSSION

The above findings were in contrast to the observations of EXPERIMENT 1. The antimicrobial effect of Hydrogum was more marked than the conventional alginate (Kromogel), and the antimicrobial action of Hydrogum and Blueprint were comparable. In order to examine the action of the alginate impression materials against a wide range of microbial agents, the group of microorganisms selected for inoculation of the materials differed from EXPERIMENT 1, and it may be that the choice of inoculating agent was an important factor in influencing antimicrobial activity of the impression materials. However it seems more likely that prolonged exposure of microorganisms to the impression samples was responsible for the apparent increased activity of chlorhexidine in Hydrogum.

It was possible to conclude that in all cases the anti-microbial alginates were more effective than the conventional alginate (Kromogel) in inhibiting growth of the test microorganisms. It was also evident that, in most cases, the Blueprint alginate was slightly more effective than Hydrogum against the chosen micro-organisms, with the exception of *Strep sanguis*, against which Hydrogum proved to be superior. Kromogel appeared to have no detrimental effect on the growth of any microorganism, other than *Strep Mutans*, but it did not seem to enhance growth. *Strep mutans* appeared to be the most susceptible of the microorganisms, to all three alginate materials tested.

4.11 EXPERIMENT 3 - AIMS OF STUDY

The aim of this study was to determine the time killing period for Hydrogum (containing chlorhexidine) against two test microorganisms, *Staph aureus* (NCTC 7447) and *Strep sanguis* (NCTC 7863). A conventional alginate material (Kromogel) was tested as a control. Culture media containing neutralisers for chlorhexidine were used to allow assessment of the time course killing period, and to allow determination of a time interval, after which chlorhexidine-containing impressions could be considered decontaminated.

4.12 EXPERIMENT 3 - PRELIMINARY INVESTIGATION

Staph aureus was grown on blood agar at 37^0C for 24 hours, and *Strep sanguis* was grown on blood agar incubated in a carbon dioxide chamber for 24 hours. Both microorganisms were inoculated on to the culture media used in the study (Table 4.9) and it was found that growth on each of the test media was sustained.

A test was undertaken to ensure that the neutralisation media adequately overcame the disinfectant properties of chlorhexidine. Here, two sets of test tubes were prepared, one set containing 9ml sterile D/E Neutralising Broth and the second containing 9ml of sterile D/E Neutralising Broth Base. To each test tube one millilitre of 2% aqueous chlorhexidine gluconate was added, mixed thoroughly and left to stand for 15 minutes. The test tubes were inoculated with 0.1ml of a 1:100,000 dilution of an overnight broth culture of *Strep sanguis* and incubated at 37^0C for 48 hours. After incubation the test tubes were observed for growth, indicated by a colour change from purple to yellow, or by formation of a pellicle. Whereas no growth was observed in the test tubes containing the Broth Base, microbial growth was found in the test tubes containing Bacto D/E Neutralising Broth, indicating satisfactory neutralisation of chlorhexidine.

4.13 EXPERIMENT 3 - MATERIALS AND METHODS

Standard blocks (20mm x 10mm x 10mm) of both impression materials were made in a sterile PTFE mould. The mould was autoclaved between applications and the alginate was cast around a sterile plastic inoculating loop to allow easy handling (Figs 4.3, 4.4). Impression blocks were immersed momentarily in a brain heart infusion broth culture of either *Staph aureus* or *Strep sanguis*. Thereafter four individual alginate blocks (of the same impression material) were simultaneously dipped into one of the microbial broth cultures and post-contamination microbial sampling undertaken; one of the blocks was sampled immediately, and the other three sampled after 10 minutes, 30 minutes and 60 minutes respectively.

After the appropriate time interval, each inoculated alginate block was placed into 10ml sterile brain heart infusion broth and swirled vigorously for 30 seconds. Aliquots (0.1mlx2) from each of these suspensions were removed and added to 10ml Bacto D/E Neutralising Broth, and to 10ml Bacto Neutralising Broth Base respectively. The test-tubes were incubated for 48 hours and observed for microbial growth, which was indicated by a colour change from purple to yellow, or by the formation of a pellicle. The impression blocks were sealed in plastic bags and stored at room temperature until sampling.

All broth cultures which tested negative were then plated on to Bacto D/E Neutralising Agar and incubated to ensure that complete killing had occurred. These procedures were carried out for both impression materials with each test microorganism in duplicate, and the protocol was repeated on three separate occasions.

4.14 EXPERIMENT 3 - RESULTS

The experiment incorporated the use of neutralising media to allow an assessment of the time-course of killing of microorganisms applied to the surface of standardised blocks of conventional and chlorhexidine-containing impression materials. After incubation, microbial growth was indicated by a colour change (from purple to yellow). If the alginate material had successfully eliminated all microorganisms, there was no colour change and the media remained purple. Of the three media used, only the Bacto D/E Neutralising Broth Base did not contain neutralisers and, if there was no growth on this media alone it could be assumed that the killing was due to the carry-over of chemicals from the alginate and hence did not occur within the designated time period. The experiment tested two chosen organisms on two brands of alginate over a given time period, and measured growth in both the Neutralising Broth and the Neutralising Broth Base.

The results are shown in Tables 4.10 and 4.11. To ensure that total killing of the microorganisms had occurred, each negative culture (where Neutralising Broth or Neutralising Broth Base remained purple) was plated on to Neutralising Agar and incubated for a further 48 hours. For samples of Kromogel it was found that all the negative cultures obtained showed subsequent growth on the Neutralising Agar.

With Hydrogum, in all but two of the samples showing no growth, no additional growth was obtained on further culture on Neutralising Agar. In two specimens with negative culture on the first procedure, growth was found following incubation on Neutralising Agar:

- the negative culture of Staph aureus (after 10 minutes on Hydrogum) on Neutralising Broth Base gave growth following incubation on Neutralising Agar.
- the negative culture of Strep sanguis (after 30 minutes on Hydrogum) on Neutralising Broth Base gave growth following incubation on Neutralising Agar.

4.15 EXPERIMENT 3 - DISCUSSION

It was possible to conclude that even after 60 minutes contact, the control alginate (Kromogel) failed to eradicate the test microorganisms, and that there was no

significant difference between its effect on *Staph aureus* and *Strep sanguis*. With the antimicrobial alginate all microorganisms were eliminated after 60 minutes, and in some cases there was no evidence of microbial growth after 30 minutes. Again there appeared to be no significant difference between the effect on *Staph aureus* or *Strep sanguis*.

From these results it would seem likely that in the clinical situation, most impressions taken using Hydrogum would be free of bacterial contamination within 60 minutes. In order to determine with more accuracy the minimum time required for complete decontamination of Hydrogum impressions, as with all experiments described in this chapter, it would be of value to use whole mouth saliva as the inoculum, or to examine these factors in an *in-vivo* study, to ensure that the materials are effective against a typically mixed oral flora.

4.16 CONCLUSIONS

The alginate material containing the quaternary ammonium compound, sodium didecyldimethyl ammonium chloride, appeared more effective in the immediate elimination of microorganisms following short contact times, while the chlorhexidine-containing material appeared effective

following more prolonged contact. Therefore, from these investigations, there is some doubt about the antimicrobial efficacy of Hydrogum during contact times which would typically occur during the recording of dental impressions. Examination of its antimicrobial action against whole saliva, in the *in-vivo* situation, would be of value in the further assessment of this chlorhexidine-containing alginate.

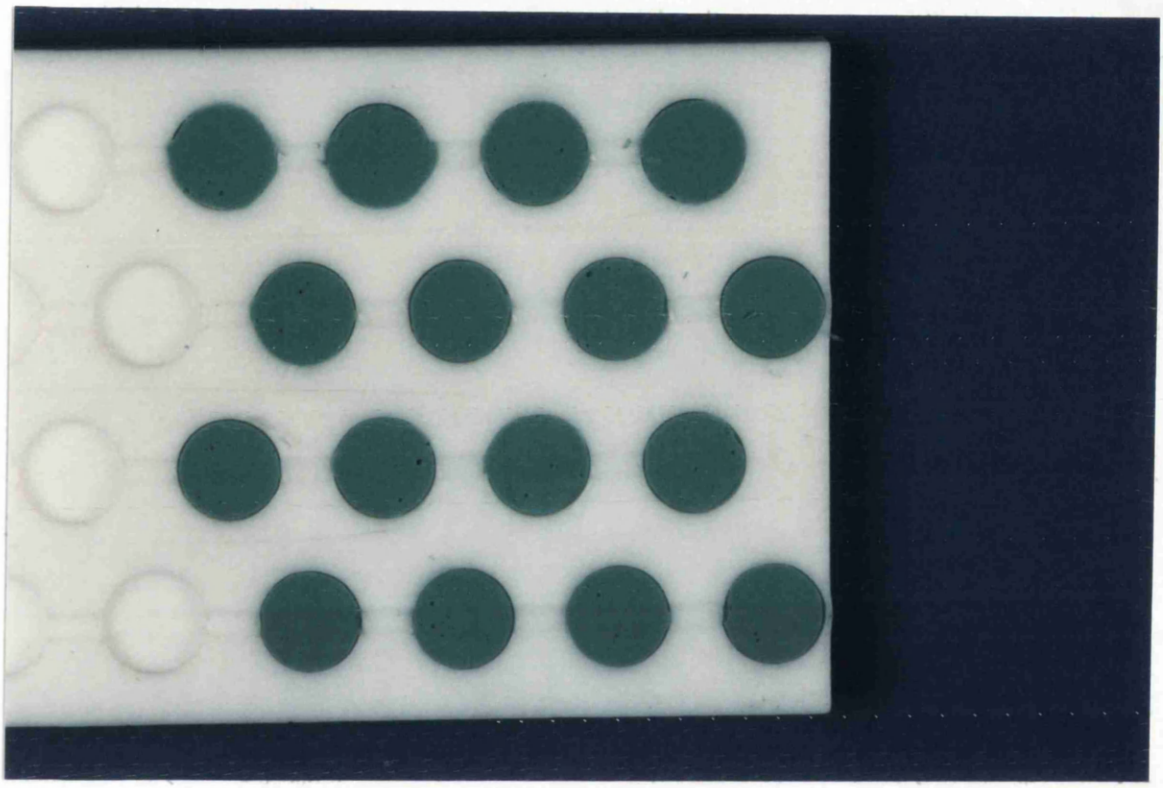


FIGURE 4.1

Hydrogum impression material in wells of PFTE mould.

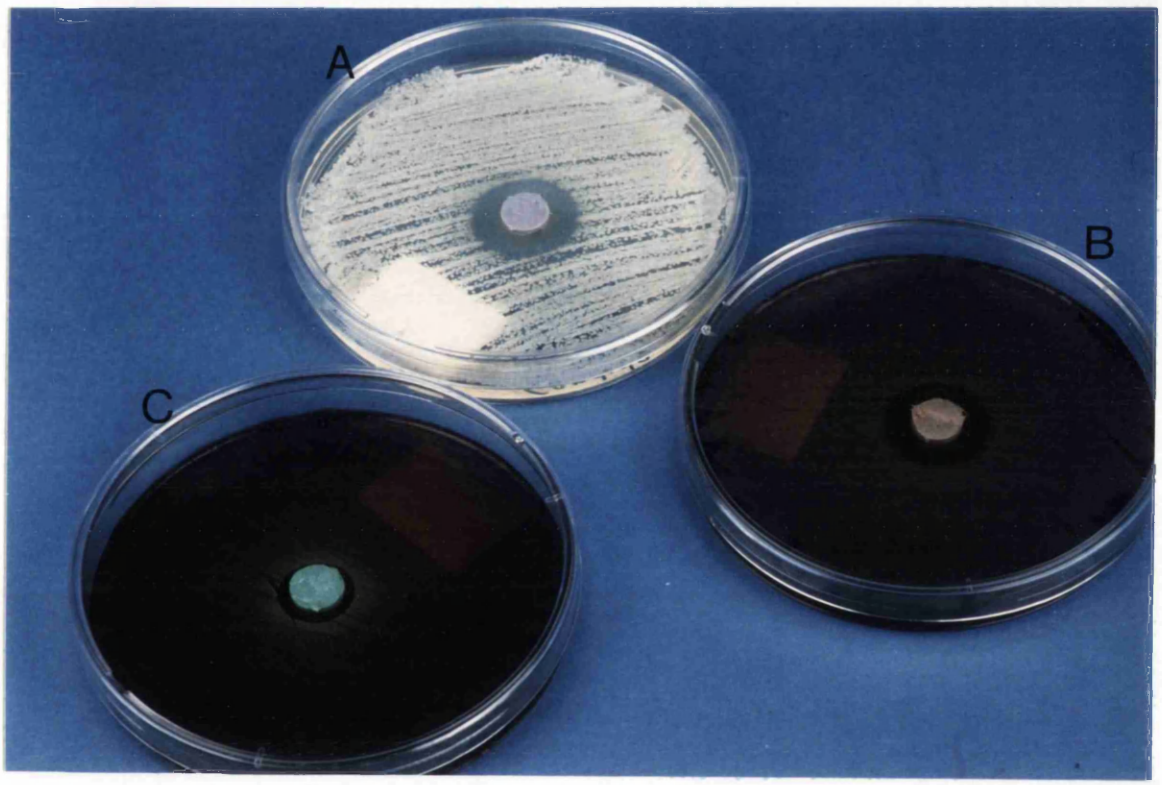


FIGURE 4.2

Inhibition of microbial growth around discs of Blueprint (with Antibac) impression material (A & B). Considerably less inhibition is evident around sample of Hydrogum (C).

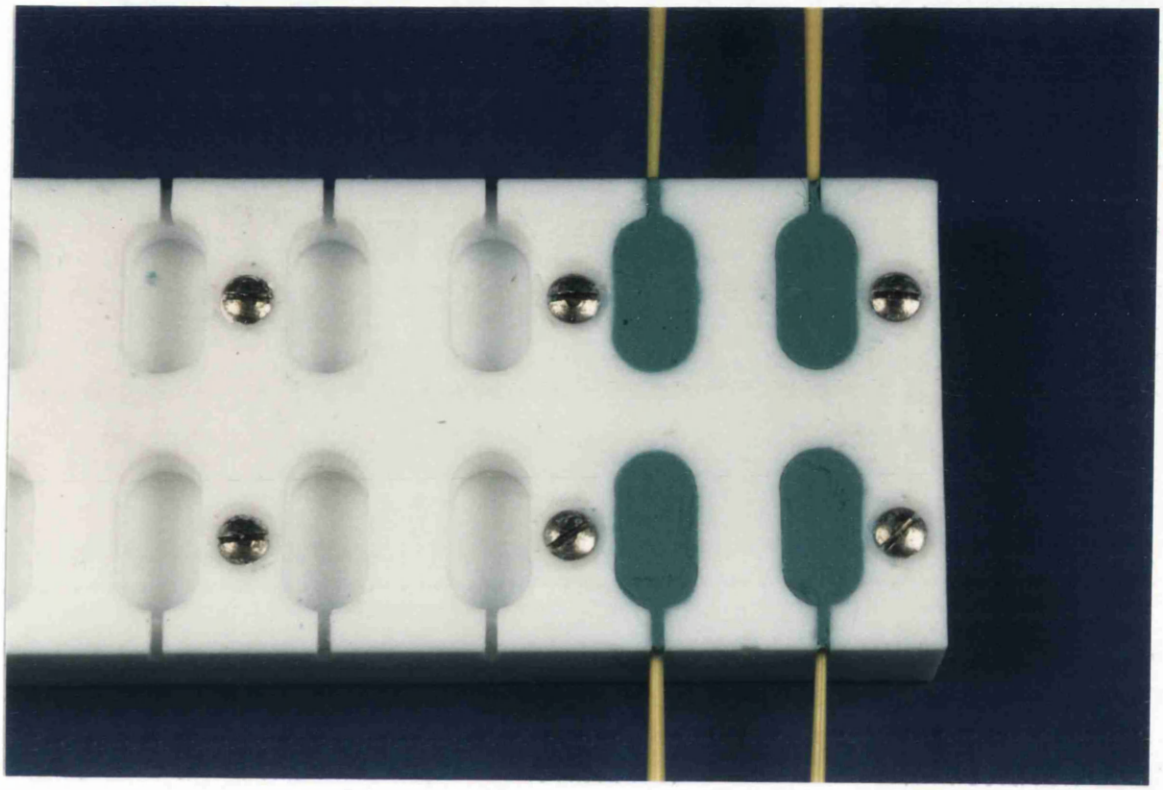


FIGURE 4.3

Hydrogum impression samples (in PTFE mould)
cast around sterile plastic inoculation loops.

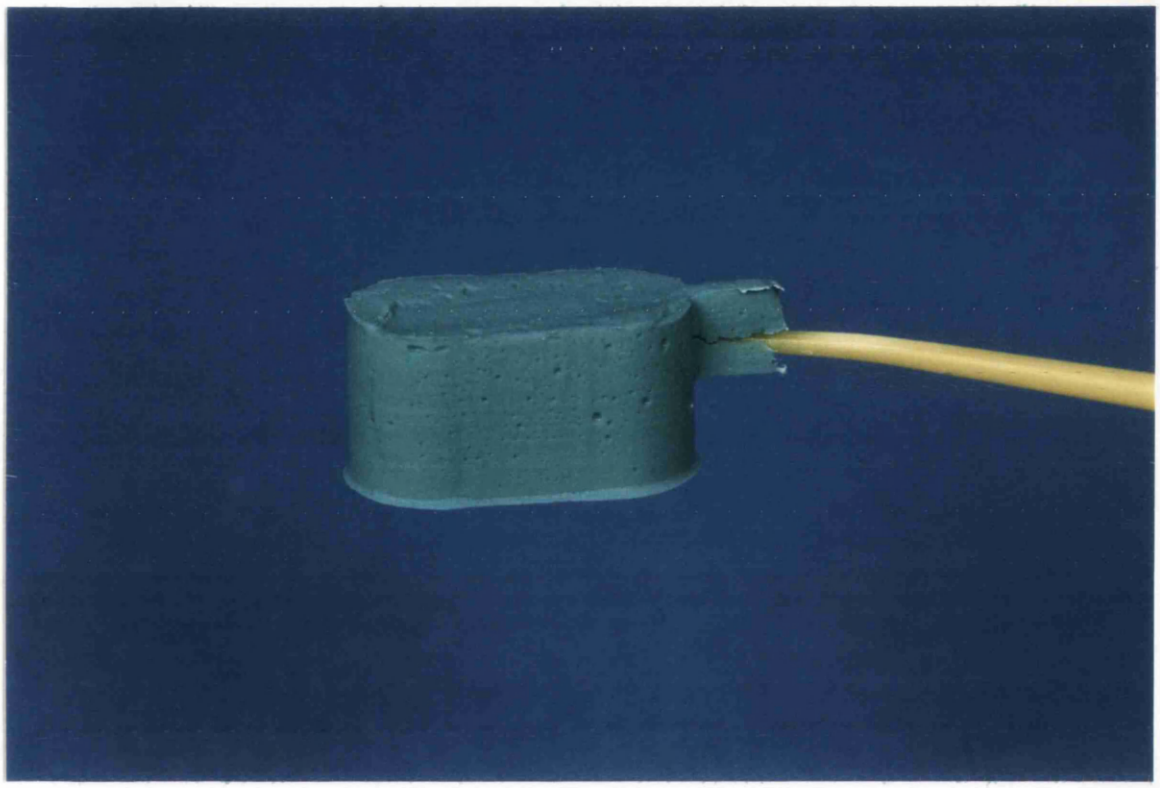


FIGURE 4.4

Impression sample, on inoculation loop, for immersion in broth culture of *Staph aureus* or *Strep sanguis*.

TABLE 4.1

IMPRESSION MATERIALS EXAMINED *IN-VITRO* STUDIES

Material	Type	Manufacturer
Kromogel	Alginate	Wright Dental, Scotland
Blueprint with Antibac	Alginate	DeTray Dentsply England
Hydrogum	Alginate	Zhermack, Italy

TABLE 4.2

EXPERIMENT 1 (i)
GROWTH OF *C ALBICANS* ON TEST IMPRESSION MATERIALS
(Mean CFU/50ul)

	0 min	10 mins	30 mins
KROMOGEL			
MEAN	2686	2259	1936
SD	866	578	859
HYDROGUM			
MEAN	2411	2488	2478
SD	357	445	424
BLUEPRINT			
MEAN	443	5	0
SD	164	4	0

TABLE 4.3

EXPERIMENT 1 (i)
GROWTH OF *STAPH AUREUS* ON TEST IMPRESSION MATERIALS
(Mean CFU/50ul)

	0 min	10 mins	30 mins
KROMOGEL			
MEAN	4411	3306	3074
SD	1092	1002	949
HYDROGUM			
MEAN	3960	2973	3647
SD	1637	977	1494
BLUEPRINT			
MEAN	0	0	0
SD	0	0	0

TABLE 4.4

EXPERIMENT 1 (i)
GROWTH OF *PS AERUGINOSA* ON TEST IMPRESSION MATERIALS
(Mean CFU/50ul)

	0 min	10 mins	30 mins
KROMOGEL			
MEAN	6306	8124	7421
SD	558	752	1627
HYDROGUM			
MEAN	8525	7987	7737
SD	708	988	729
BLUEPRINT			
MEAN	3168	2643	946
SD	1635	1545	761

TABLE 4.5

EXPERIMENT 1 (ii)
GROWTH OF *C ALBICANS* ON TEST IMPRESSION MATERIALS
(Dilute suspension : Mean CFU/50ul)

	0 min	10 mins	30 mins
KROMOGEL			
MEAN	3236	2976	3061
SD	281	411	191
HYDROGUM			
MEAN	2252	1781	1586
SD	257	650	410

TABLE 4.6

EXPERIMENT 1 (ii)
GROWTH OF *STAPH AUREUS* ON TEST IMPRESSION MATERIALS
(Dilute suspensions : Mean CFU/50ul)

	0 min	10 mins	30 mins
KROMOGEL			
MEAN	1646	1791	1852
SD	448	414	243
HYDROGUM			
MEAN	1943	1963	1266
SD	508	774	162

TABLE 4.7

EXPERIMENT 1 (ii)
GROWTH OF *PS AERUGINOSA* ON TEST IMPRESSION MATERIALS
(Dilute solutions : Mean CFU/50ul)

	0 min	10 mins	30 mins
KROMOGEL			
MEAN	6916	6117	6003
SD	1006	722	866
HYDROGUM			
MEAN	8414	8438	7290
SD	351	226	920

TABLE 4.8

EXPERIMENT 2

Inhibition of Growth (mm) around Alginate Discs

Streptococcus milleri NCTC 10713

Hydrogum	2	2	2.5	2	2	2	**Mean**	2.1mm
Blueprint	2	2	3	3	3	2	**Mean**	2.5mm
Kromogel	0	0	0	0	0	0	**Mean**	0 mm

Streptococcus sanguis NCTC 7863

Hydrogum	6	6	6	6.5	6	6	**Mean**	6.1mm
Blueprint	4	4.5	4	3.5	4	4	**Mean**	4 mm
Kromogel	0	0	0	0	0	0	**Mean**	0 mm

Streptococcus salivarius NCTC 8618

Hydrogum	2	1	1.5	1.5	2	2	**Mean**	1.7mm
Blueprint	2	2	2	2	2	2	**Mean**	2 mm
Kromogel	0	0	0	0	0	0	**Mean**	0 mm

TABLE 4.8 (continued)

EXPERIMENT 2

Inhibition of Growth (mm) around Alginate Discs

Actinomyces viscous NCTC 1095

Hydrogum	2	2	2	1.5	2	1.5	**Mean**	1.8mm
Blueprint	2	2	3	2	3	2	**Mean**	2.3mm
Kromogel	0	0	0	0	0	0	**Mean**	0 mm

Streptococcus mutans NCTC 10449

Hydrogum	7	7	6	7	8	7	**Mean**	7 mm
Blueprint	8	7	6	6	9	7	**Mean**	7.2mm
Kromogel	1.5	0	2	1	1	1	**Mean**	1.1mm

Porphyromonas gingivalis NCTC 11834

Hydrogum	3	3	4	3	4	4	**Mean**	3.5mm
Blueprint	4	5	5	5	5	5	**Mean**	4.8mm
Kromogel	0	0	0	0	0	0	**Mean**	0 mm

TABLE 4.9

EXPERIMENT 3

Culture Media used in Experiment 3

Bacto D/E Neutralising Agar*
Bacto D/E Neutralising Broth*
Bacto D/E Neutralising Broth Base*

* Difco Laboratories, East Molesey, Surrey, England

TABLE 4.10 (a)

MICROBIAL GROWTH (HYDROGUM/*STREP SANGUIS*)

EXPERIMENT 3*

	Hydrogum/*Strep sanguis*						Hydrogum/*Strep sanguis*					
	Bacto D/E Neutralising Broth						Bacto D/E Neutralising Broth Base					
0 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
30 min	Y	Y	Y	Y	Y	P	Y	P	Y	Y	Y	Y
60 min	P	P	P	P	P	P	P	P	P	P	P	P

TABLE 4.10 (b)

MICROBIAL GROWTH (KROMOGEL/*STREP SANGUIS*)

EXPERIMENT 3*

	Kromogel/*Strep sanguis*						Kromogel/*Strep sanguis*					
	Bacto D/E Neutralising Broth						Bacto D/E Neutralising Broth Base					
0 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	P	Y	Y
30 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
60 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	P	Y

* Culture and testing with two impression samples for each brain heart infusion culture, and the entire protocol repeated on three occasions.

* Y (Yellow), microbial growth from impression sample.
P (Purple), no microbial growth from impression sample.

TABLE 4.11 (a)

MICROBIAL GROWTH (HYDROGUM/*STAPH AUREUS*)

EXPERIMENT 4*

	Hydrogum/*Staph aureus*						**Hydrogum/*Staph aureus***					
	Bacto D/E Neutralising Broth						Bacto D/E Neutralising Broth Base					
0 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10 min	Y	Y	Y	Y	Y	Y	Y	Y	P	Y	Y	Y
30 min	Y	Y	Y	P	Y	Y	Y	Y	Y	Y	P	Y
60 min	P	P	P	P	P	P	P	P	P	P	P	P

TABLE 4.11 (b)

MICROBIAL GROWTH (KROMOGEL/*STAPH AUREUS*)

EXPERIMENT 4*

	Kromogel/*Staph aureus*						**Kromogel/*Staph aureus***					
	Bacto D/E Neutralising Broth						Bacto D/E Neutralising Broth Base					
0 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
30 min	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
60 min	Y	Y	P	Y	Y	Y	Y	Y	Y	Y	Y	Y

* Culture and testing with two impression samples for each brain heart infusion culture, and the entire protocol repeated on three occasions.

* Y (Yellow), microbial growth from impression sample.
P (Purple), no microbial growth from impression sample.

APPENDIX 4.1

EXPERIMENT 1 (i)
GROWTH OF *C ALBICANS* ON KROMOGEL SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	4091	2939	2152
	3818	2879	2333
	3364	2788	2333
Impression 2	1727	1485	848
	1970	1606	939
	1939	1576	697
Impression 3	2212	2212	2545
	2606	2424	2727
	2424	2424	2848
MEAN	**2683**	**2259**	**1936**
SD	**866**	**578**	**859**

APPENDIX 4.2

EXPERIMENT 1 (i)
GROWTH OF *STAPH AUREUS* ON KROMOGEL SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	5545	2848	1970
	5364	4152	4879
	4515	3879	4242
Impression 2	4061	2727	2364
	5727	4758	2667
	5091	4424	3515
Impression 3	3212	2606	2545
	3303	2364	2788
	2879	2000	2697
MEAN	**4411**	**3306**	**3074**
SD	**1092**	**1002**	**949**

APPENDIX 4.3

EXPERIMENT 1 (i)
GROWTH OF *PS AERUGINOSA* ON KROMOGEL SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	5879	7848	6970
	5818	7515	6758
	5788	7909	5909
Impression 2	7242	7636	5939
	6424	7455	6000
	5970	7636	6727
Impression 3	6970	8424	9939
	6758	9455	9697
	5909	9242	8848
MEAN	**6306**	**8124**	**7421**
SD	**558**	**752**	**1627**

APPENDIX 4.4

EXPERIMENT 1 (i)
GROWTH OF *C ALBICANS* ON HYDROGUM SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	2333	2697	2758
	2485	2788	2061
	2788	2545	2273
Impression 2	2515	3091	3030
	2667	2636	3212
	2909	2515	2364
Impression 3	1909	1909	2030
	2152	1697	2212
	1939	2152	2364
MEAN	**2411**	**2448**	**2478**
SD	**357**	**445**	**424**

APPENDIX 4.5

EXPERIMENT 1 (i)
GROWTH OF *STAPH AUREUS* ON HYDROGUM SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	5939	3909	5333
	6091	3121	4758
	5606	3273	5394
Impression 2	4273	3667	4394
	3545	3939	4242
	3727	3273	2909
Impression 3	2455	1091	1667
	2061	1727	1667
	1939	2758	2455
MEAN	**3960**	**2973**	**3647**
SD	**1637**	**977**	**1494**

APPENDIX 4.6

EXPERIMENT 1 (i)
GROWTH OF *PS AERUGINOSA* ON HYDROGUM SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	8818	7818	8303
	8212	8970	7636
	8152	8242	7000
Impression 2	9818	9091	8606
	9121	8909	7182
	8788	8394	9000
Impression 3	7970	6455	7364
	8455	6667	7000
	7394	7333	7545
MEAN	**8525**	**7987**	**7737**
SD	**708**	**988**	**729**

APPENDIX 4.7

EXPERIMENT 1 (i)
GROWTH OF *C ALBICANS* ON BLUEPRINT SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	414	1	0
	402	0	0
	430	1	0
Impression 2	664	11	0
	702	8	0
	540	9	0
Impression 3	336	6	0
	260	3	0
	236	3	0
MEAN	**443**	**5**	**0**
SD	**164**	**4**	**0**

APPENDIX 4.8

EXPERIMENT 1 (i)
GROWTH OF *STAPH AUREUS* ON BLUEPRINT SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	0	0	0
	0	0	0
	0	0	0
Impression 2	0	0	0
	0	0	0
	0	0	0
Impression 3	0	0	0
	0	0	0
	0	0	0
MEAN	**0**	**0**	**0**
SD	**0**	**0**	**0**

APPENDIX 4.9

EXPERIMENT 1 (i)
GROWTH OF *PS AERUGINOSA* ON BLUEPRINT SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	3485	4576	2000
	5394	4545	2152
	6273	4909	1667
Impression 2	3091	1636	504
	1909	1848	544
	2485	1606	544
Impression 3	2303	1273	218
	1606	2030	446
	1970	1364	442
MEAN	**3168**	**2643**	**946**
SD	**1635**	**1545**	**761**

APPENDIX 4.10

EXPERIMENT 1 (ii - dilute suspension)
GROWTH OF *C ALBICANS* ON KROMOGEL SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	3152	2394	2970
	3303	2515	3091
	3424	2970	3182
Impression 2	3212	3121	3030
	3758	2758	3152
	2818	3545	2939
Impression 3	3333	3485	3333
	2879	3273	2667
	3242	2727	3182
MEAN	**3236**	**2976**	**3061**
SD	**281**	**411**	**191**

APPENDIX 4.11

EXPERIMENT 1 (ii - dilute suspension)
GROWTH OF *STAPH AUREUS* ON KROMOGEL SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	970	1242	1485
	1061	1424	1848
	1333	1485	1667
Impression 2	1758	1758	2091
	1879	1424	1939
	1545	2030	2303
Impression 3	2212	2333	1879
	1939	2242	1758
	2121	2182	1697
MEAN	**1646**	**1791**	**1852**
SD	**448**	**414**	**243**

APPENDIX 4.12

EXPERIMENT 1 (ii - dilute suspension)
GROWTH OF *PS AERUGINOSA* ON KROMOGEL SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	5667	5636	7121
	5515	5394	6727
	5848	5061	6242
Impression 2	7333	6030	4606
	6727	5818	5030
	8091	6424	5182
Impression 3	7515	6515	6394
	7697	6901	6667
	7848	7273	6061
MEAN	**6916**	**6117**	**6003**
SD	**1006**	**722**	**866**

APPENDIX 4.13

EXPERIMENT 1 (ii - dilute suspension)
GROWTH OF *C ALBICANS* ON HYDROGUM SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	2061	1000	1182
	2333	848	970
	1939	1121	1485
Impression 2	2242	2061	1606
	2000	1758	1364
	2091	1939	1455
Impression 3	2364	2303	1970
	2697	2667	2091
	2545	2333	2152
MEAN	**2252**	**1781**	**1586**
SD	**257**	**650**	**410**

APPENDIX 4.14

EXPERIMENT 1 (ii - dilute suspension)
GROWTH OF *STAPH AUREUS* ON HYDROGUM SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	1545	3030	1455
	1394	2394	1061
	1212	1758	1121
Impression 2	2515	1242	1091
	2333	1061	1364
	2697	818	1394
Impression 3	2000	2273	1242
	1818	2485	1182
	1970	2606	1485
MEAN	**1943**	**1963**	**1266**
SD	**508**	**774**	**162**

APPENDIX 4.15

EXPERIMENT 1 (ii - dilute suspension)
GROWTH OF *PS AERUGINOSA* ON HYDROGUM SPECIMENS (CFU/50ul)

	0 min	10 mins	30 mins
Impression 1	8636	8455	6667
	8788	8727	7212
	8182	8333	7485
Impression 2	8061	8030	8424
	8182	8333	8182
	8788	8788	8485
Impression 3	8788	8485	6091
	7879	8455	6182
	8424	8333	6879
MEAN	**8414**	**8438**	**7290**
SD	**351**	**226**	**920**

CHAPTER 5

IN-VIVO INVESTIGATIONS OF THE ANTIMICROBIAL EFFECT OF CHLORHEXIDINE ON ALGINATE IMPRESSION MATERIALS

INTRODUCTION

An aqueous solution of chlorhexidine gluconate (0.2%), used as a mouthwash, appears to prevent the formation of intra-oral bacterial plaque [140], and in Chapters 3 & 4 the use of chlorhexidine as an immersion disinfectant and incorporated within an alginate impression material (to reduce the risk of contamination from dental impressions) was examined in *in-vitro* investigations. Rinsing with aqueous chlorhexidine prior to recording impressions has also been advocated to reduce microbial contamination of impression surfaces [117], but the effect of this measure in the clinical situation is not well documented.

AIMS OF STUDY

In this study, use of chlorhexidine to reduce the risk of contamination from dental impressions was examined in three *in-vivo* experiments. The objective was to monitor the effect of chlorhexidine as a disinfectant agent incorporated within an impression material or as a mouth-rinse prior to the recording of impressions, using quantitative methods of microbiological assessment.

In a preliminary investigation with a group of dental students as the experimental population, the carriage of

microorganisms on impression surfaces was assessed to give an indication of an appropriate study design, and with a view to determining the minimum interval required between impressions to allow assessment of microorganism carriage. Impressions were recorded on different occasions to help define an effective experimental protocol for the major investigation.

The main study, utilising a group of dental hygienists, was in two parts. In the first phase, numbers of micro-organisms present on impression surfaces was determined to establish whether the incorporation of chlorhexidine within alginate materials might constitute an effective antimicrobial measure. In control procedures, the use of standard alginate and alginate containing sodium didecyl-dimethyl ammonium chloride, was examined. In the second phase, the effect of a pre-impression mouth-rinse with a 0.2% aqueous solution of chlorhexidine gluconate was assessed. As a control mouth-rinse tap water was used.

5.1 EXPERIMENT 1 - MATERIALS AND METHODS

Here 12 subjects, senior dental students with an age range of 20-23 years, participated in this study by agreeing to have maxillary alginate impressions recorded

on three occasions. Prior to starting experimental procedures, bowls and spatulae to be used in mixing impression materials were cleaned via a bactericidal wipe saturated with a 70% solution of isopropyl alcohol (Azowipes, Vernon-Carus Ltd, Preston, England).

For each subject 3 alginate impressions were recorded in stock impression trays; the first impression on Day 0, the second on Day 2 and the third on Day 3. The impression material used was Kromogel (Table 5.1), which was quantified and manipulated in accordance with manufacturer's instructions. Spray adhesive (Redifix, Wright, Dundee) was applied to stock trays prior to recording the impressions, all of which were taken in mid-morning with subjects having been instructed to follow normal oral hygiene practices. Immediately on removal from the mouth, a sample of impression material was taken from each impression using a 13mm diameter cork borer. Before sampling, the cork borer was sterilised by passing it through the flame of a Bunsen burner, and cooled in sterile phosphate buffered saline.

The impression material samples were taken from the mid-line, 3mm posterior to the gingival margin of the central incisor teeth (Figure 5.1). Each of the impression samples was placed in a universal container, holding 20ml of sterile PBS, and mechanically vortex- mixed for 60

seconds. Approximately 5ml of the resultant suspension was decanted into a sterile bijou container and a 50ul sample from this suspension was plated on to blood agar in 10cm culture plates, using a spiral plating apparatus (as described in Section 2.1.5). The plates were incubated for 24 hours, and following incubation the number of colony forming units on the culture plates was counted by an electronic colony counter under good lighting conditions, with the aid of a magnifying glass (x10).

5.2 EXPERIMENT 1 - RESULTS

Quantitative values for the colonies cultured from the microbial suspensions obtained by vortex-mixing samples of impression material obtained on Days 0, 2 and 3, are shown in Table 5.2.

5.2.1 NUMBERS OF COLONIES COUNTED

There was considerable variation between subjects in the number of colonies counted over the study period. Mean values for colony counts for the entire group show a slight increase on Day 2 compared with Day 0, and a sharp decrease on Day 3. The variation between subjects was most marked in samples collected on Day 2.

On Day 0, the range was 48-325 CFU, with a standard deviation of 93 (Figure 5.2), whereas two days later the range was 12-606 (±SD=196; Figure 5.3). Finally by Day 3 equivalent data were 8-282 (±SD=73; Figure 5.4).

5.2.2 PATTERNS OF CHANGE IN COLONY COUNTS OVER FOUR DAYS

There was considerable variation between subjects in the pattern of colony counts on the three days samples were taken (Figure 5.5). The most consistent values for colony counts were found in Subject 4 (SD=13.75), Subject 11 (SD=37) and Subject 1 (SD=38.4). In Subjects 4 and 11, the values for all three colony counts were below 90, while in subject 1, values for CFU counts on Day 0 (107) and Day 2 (110) were particularly close.

Subjects 1, 2, 3, 6, 7, and 12 showed the most common pattern of distribution for colony counts, in that higher counts were recorded on Day 2 than on Day 0, and the count on Day 3 was the lowest of the three. However there was a considerable variation in the magnitude of colony counts for these subjects.

Subject 3 was distinctive as all three colony counts were in excess of 250. The largest variation between counts for the three days was found for subject 12 (SD=269). This was due to a large value for colony forming units on

Day 2 (606), compared with Day 0 (225) and Day 3 (87). In subjects 5 and 9, colony counts on Day 0 were considerably greater than in the two subsequent samples. In only one individual (Subject 11) was the colony count on Day 3 the largest of the three values. In this case all counts were relatively small, and the pattern was similar to that occurring with Subject 4. Here values for Day 0 and Day 3 were similar and the colony count for Day 2 was lowest.

5.3 EXPERIMENT 1 - DISCUSSION

For almost all subjects examined in this preliminary study, there was a considerable variation between the number of colonies cultured on each day. This may have been a reflection of normal quantitative daily variation in the oral flora, or may have been a response to the taking of impressions.

However, variables in experimental technique may also have had an influence on the results, eg the time-interval between impressions, or the lack of a uniform oral hygiene regime may have been important, and a more controlled experimental technique may have given more consistent values for each subject.

With respect to the numbers of colony forming units, it was apparent from the large variation between individuals that comparison between subjects on a quantitative basis was not particularly meaningful. Repeat measurements, to allow changes to be determined for each subject in a more detailed research protocol, would be of more value in showing trends within the experimental group as a whole. In addition, it seems likely that the abnormally high colony count for Subject 12 on Day 2 is a 'rogue' value, not representative of the general colonisation level of the impression surface, perhaps caused by adherence of a large amount of plaque on to the impression surface in the area sampled. Multiple sampling for each subject would have been helpful in compensating for this and any other sampling errors.

5.4 EXPERIMENT 2 - MATERIALS AND METHODS

In this investigation, the efficacy of chlorhexidine as an anti-microbial agent incorporated within an impression material was examined in the clinical situation. The experimental population consisted of ten female student dental hygienists who were caries-free and who presented with excellent standards of oral hygiene. The age range of the group was from 19 to 27 years. In all cases the dental arches were intact, other than where third molar

teeth were missing or there had been dental extractions for orthodontic purposes. The use of this well-controlled and co-operative group allowed various experimental protocols to be examined over an extended period.

Multiple impression samples were collected for each subject so that the values obtained could be identified with confidence as being representative for each individual, and to allow appropriate statistical analysis to be undertaken. The time interval between sample collection was increased, such that the minimum period between sampling was five days, and oral hygiene practices within the experimental group were standardised as much as was possible.

5.4.1 INDIVIDUAL TRAYS

Prior to starting experimental procedures, maxillary dental impressions were made for all 10 subjects; stone casts were poured; 1.2mm thick wax spacers laid down on the casts, and individual trays (with wire handles) made in heat-cured acrylic resin. To ensure a consistent thickness of material when impressions were recorded, each individual tray was made with occlusal stops on the central incisors and on the first molar tooth bilaterally.

5.4.2 ORAL HYGIENE PROTOCOL

The required oral hygiene protocol was explained, and the volunteers agreed to follow this prior to impression taking, *ie* no tooth-brushing was to be performed for the 12 hour period before this event (Appendix 5.1).

5.4.3 PREPARATION FOR IMPRESSION SAMPLING

Prior to impressions being taken, 20ml of sterile phosphate buffered saline was pipetted into each of twenty sterile universal containers; two for each subject. Containers were marked with a number to identify from which subject the impression samples had been taken. For each universal container, two small sterile bijoux bottles were marked to indicate from which universal container samples for analysis had come. The universal and bijoux containers, and apparatus for vortex-mixing of the collected samples, were gathered in the clinical area where impression samples were collected. Hence there was a minimal time-interval between recording of impressions and preparation of microbial suspensions for micro-biological assessment. A separate mixing bowl and spatula were prepared for each subject by cleaning with a bacter-icidal wipe saturated with a 70% solution of isopropyl alcohol.

Rubber gloves were worn by the operator while recording impressions, and prior to use the impression trays were immersed in activated 2% glutaraldehyde (Cidex, Johnson and Johnson, Slough, England) for five minutes, rinsed and dried. Spray adhesive was applied to the trays, and a Bunsen burner, two universal containers with sterile PBS as coolant, a 13mm diameter cork borer, and a pair of tweezers were laid out for impression sampling.

5.4.4 IMPRESSION PROCEDURES

Alginate powder and room temperature water were used in manufacturers' recommended proportions, and impressions were recorded in a uniform manner for each subject. To ensure an even and standardised thickness of impression material, the individual trays were seated fully on to the occlusal stops on the incisor and molar teeth during the recording of impressions. The use of sufficient material, positive seating of trays and functional border moulding ensured that high quality impressions were obtained. The three impression materials examined in the *in-vitro* study in Chapter 4, were tested (Table 5.1). Thus, alginate material containing chlorhexidine (Hydrogum) was compared with standard alginate material (Kromogel), and material containing sodium didecyl-dimethyl ammonium chloride (Blueprint with Antibac).

5.4.5 SAMPLING OF IMPRESSION SPECIMENS

Immediately after removal from the mouth, impressions were sampled in two sites, using a 13mm diameter cork borer to remove two discs of impression material. The cork borer was sterilised prior to sampling by passing it through the Bunsen flame, and cooled in sterile PBS. Impression samples were taken from the palatal aspect of the molar teeth (Figure 5.6). The discs of impression material were transferred immediately to a universal container with 20ml of phosphate buffered saline. Each container was agitated mechanically for 60 seconds on a vortex-mixer, and two 5ml samples of the resultant microbial suspension were decanted, before any settlement could occur, into two of the sterile bijoux containers. Therefore, from each impression, two 'contaminated' samples were taken, and from each sample two 5ml microbial suspensions were produced for analysis. For each impression material, this protocol was followed on three occasions.

5.4.6 LABORATORY PROCEDURE

The microbial contamination of each suspension (produced by mechanical vortex mixing of the impression discs) was assessed by plating measured small volumes (50ul) of the suspension on to growth media on 10cm culture plates, and

counting the number of colonies present after culture for the appropriate time interval. The spiral plating apparatus (2.1.5) was used to draw up and distribute samples on to culture plates.

In this phase three culture media were used. Incubation on blood agar (BA) gave the overall level of bacterial growth; mitis salivarius agar (MSA) yielded streptococcal growth, and Sabouraud's dextrose agar (SDA) was used to isolate candidal species. Samples plated on to blood agar and mitis salivarius agar were cultured for 48 hours before quantitative assessment was undertaken. Samples plated on to Sabouraud's dextrose agar were cultured for a minimum of 24 hours before being assessed.

In any case where no growth was apparent, culture plates were returned to the incubator for a further 24 hours, then reassessed. Good direct light, an illuminated screen, a magnifying glass and an electronic colony counter were used to maximise the efficiency of the colony counting process.

5.5 EXPERIMENT 2 - RESULTS

The data obtained (for subjects 1-10) from each of the three impression materials, after assessment of microbial

growth on culture plates, are shown in Appendices 5.2 to 5.11, and summary data are presented in Table 5.3. Each of the mean values in Table 5.3 represents the collation of values from the culture of 12 microbial suspensions (three impressions for each subject, each giving two samples of impression material, and two microbial suspensions from each of the impression samples). The standard deviation values were calculated from the mean CFU counts for each of the impression samples (six values, one from each impression sample). The data were collated for each of the three different types of impression material, and microbial growth was assessed on three different culture media.

5.5.1 BLUEPRINT WITH ANTIBAC

There was no detectable growth on any culture medium from microbial suspensions made from Blueprint impressions, confirming the previously described antimicrobial effect of this material, and providing an effective control for assessment of the chlorhexidine-containing preparation.

5.5.2 CANDIDAL SPECIES

There was no detectable growth on Sabouraud's dextrose agar from microbial suspensions obtained from any of the impressions.

5.5.3 CARRIAGE OF MICROORGANISMS ON KROMOGEL AND HYDROGUM

There was evidence of microorganism carriage on all samples obtained from these two materials. However, there was considerable variation in the numbers of colony forming units cultured, both within samples obtained for each subject, and between subjects. At one extreme, no growth was observed from one sample of Hydrogum (Subject 3, Sample 1) following culture on mitis salivarius agar, although a small number of colonies was evident following culture on blood agar. At the other extreme the largest colony count (458) was obtained after culture of a microbial suspension from Kromogel (Subject 6, Sample 3), on blood agar.

Examination of mean values for microbial growth (CFU values) on Kromogel and Hydrogum, with both BA and MSA culture media, showed there to be wide variations (Fig 5.7), with neither impression material being consistently more effective in eliminating microorganisms. Greater growth was seen from samples of Kromogel in 5 subjects (Nos 1, 2, 3, 5 and 6); greater growth from Hydrogum in 2 subjects (Nos 4 and 8), with approximately equal growth from both materials in three subjects (Nos 7, 9 and 10).

5.5.4 BLOOD AGAR AND MITIS SALIVARIUS AGAR AS INDICATORS OF MICROBIAL GROWTH

As expected, growth on blood agar was heavier than on mitis salivarius agar, which is specific for streptococcal species growth. Analysis to examine any relation between growth on BA and growth on MSA was carried out for Kromogel and Hydrogum. The correlation coefficient between overall growth on the two media was particularly high for samples obtained from Hydrogum ($r=0.952$). For samples of Kromogel, the correlation coefficient between the two culture media was 0.844. With a similarity in the pattern, if not the amount, of growth occurring for samples plated on each of the culture media, it seemed likely that the findings with respect to antimicrobial characteristics of the materials would be reflected in the results from both data sets.

5.5.5 STATISTICAL ANALYSIS (MANN-WHITNEY TEST)

Statistical analysis was undertaken to assess differences between the impression materials with respect to micro-organism carriage. The Mann-Whitney test was used in comparing values from Kromogel and Hydrogum, for six specimens of each material, for each subject. Growth on mitis salivarius agar and blood agar was assessed.

In three subjects, the growth from Kromogel samples was greater than from samples of Hydrogum, by a degree that was statistically significant:

Subject 1 - growth on blood agar from samples of Kromogel was statistically greater than from samples of Hydrogum ($p<0.05$).

Subject 3 - growth on blood agar and mitis salivarius agar was statistically greater from samples of Kromogel than from samples of Hydrogum ($p<0.01$).

Subject 6 - growth on blood agar was greater from samples of Kromogel than samples of Hydrogum ($p<0.01$) and on mitis salivarius agar ($p<0.05$).

In no case was the growth on Hydrogum significantly greater than growth on Kromogel.

5.5.6 STATISTICAL ANALYSIS (WILCOXON SIGNED-RANK TEST).

Further analysis of results for the group as a whole, was carried out by paired comparison for each subject, of the mean value for CFU growth from the six specimens obtained from Kromogel, with the mean value for CFU growth from the six specimens obtained from Hydrogum. The paired analysis was carried out using the Wilcoxon signed-rank test, and growth on blood agar and mitis salivarius agar

was examined. There was no statistically significant difference between growth from samples of Kromogel and growth from Hydrogum samples, on either growth medium.

5.6 EXPERIMENT 2 - DISCUSSION

There was no consistent effect on the carriage of micro-organisms from the use of an alginate impression material containing chlorhexidine (Hydrogum), as compared with the use of standard alginate impression material (Kromogel). There was a considerable variation in mean CFU values between the 10 subjects with both Kromogel and Hydrogum (Fig 5.7), and no consistent pattern of colonisation was evident. In one case (Subject 6), growth from Kromogel samples was substantially greater than from Hydrogum samples, and considerably greater than growth seen from the other subjects. However in one case (Subject 7), growth on both media from samples of both impression materials was minimal. Between the extremes, a range of patterns and values for CFU counts was evident. Multiple sampling was undertaken with a view to providing mean CFU values more likely to be representative for each subject than the single values obtained in EXPERIMENT 1; variability within the different samples for individual subjects, with both Kromogel and Hydrogum, is seen clearly seen in Figures 5.8 to 5.11.

The CFU values for individual impression samples for each of the subjects, for Kromogel, are shown in Figure 5.8 (test suspensions cultured on blood agar) and Figure 5.9 (test suspensions cultured on mitis salivarius agar). The need for multiple sampling was apparent in subjects where some individual CFU values were high, (eg Subjects 2 and 5, and particularly Subject 6). It is clear that most of the individual CFU values for these subjects would give a misleading representation of the mean CFU value. More consistent patterns of culture from different samples were apparent where the individual CFU values were lower (eg Subjects 1 and 7). The close correlation ($r=0.84$) between growth on these two culture media is evident from comparison of Figures 5.8 and 5.9; subjects with high and variable CFU values are common to both culture media, as are subjects with more consistent colonisation patterns.

Individual CFU values (for each of the ten subjects) for Hydrogum are shown in Figure 5.10 (test suspensions cultured on blood agar) and Figure 5.11 (test suspensions cultured on mitis salivarius agar). The value of multiple sampling was apparent, particularly for subjects 4 and 8, where the standard deviations of the CFU values were highest, and in subjects 2,8,10, where one or two individual values differed substantially from the others. Close correlation ($r=0.95$) between growth on the media is evident from comparison of Figures 5.10 and 5.11.

It is noteworthy that subjects showing most variability in CFU values from the Kromogel specimens (ie subjects 2,5,6) were not those who showed greatest variability in CFU values from the Hydrogum specimens (subjects 4,8), underlining the need for multiple sampling to minimise the effect of sampling error on the overall results.

5.7 EXPERIMENT 3

In this study the effect of oral rinsing, immediately prior to taking impressions, on the carriage of micro-organisms was investigated *in-vivo*. A single type of alginate impression material was used (Kromogel, Table 5.1), and oral rinsing with tap water (10ml) or with 0.2% chlorhexidine gluconate (10ml), was carried out for 60 seconds immediately prior to recording impressions.

5.8 EXPERIMENT 3 - MATERIALS AND METHODS

The experimental population consisted of the same group of student dental hygienists involved in EXPERIMENT 2. Multiple impression samples were collected for each of the subjects with a minimum interval of five days between sampling. Oral hygiene measures were standardised (Appendix 5.1), individual trays constructed, and

impression and sampling procedures carried out as described previously in EXPERIMENT 2 (5.4.1, 5.4.2, 5.4.3, 5.4.4, 5.4,5) with two modifications;

1. Kromogel was the only impression material used.

- and -

2. prior to recording the impressions, subjects rinsed with either tap water or 0.2% aqueous chlorhexidine gluconate (Corsodyl, SmithKline Beecham, Brentford, England), for 60 seconds.

The procedures for EXPERIMENT 2 and EXPERIMENT 3 were carried out simultaneously, and the findings for the use of pre-impression oral rinses were compared with results for the contamination of standard Kromogel impressions, described in EXPERIMENT 2. The various protocols, using differing impression materials and with different pre-impression rinsing procedures, were carried out in a random order to limit the influence of external factors on the results. Immediately after removal of impressions from the mouth, they were sampled at two sites (Fig 5.6), and microbial samples prepared for analysis as described previously (5.4.6) using spiral plating apparatus (2.1.5) and three culture media (BA, MSA, SDA). Samples plated on to blood agar and mitis salivarius agar were cultured for 48 hours before quantitative assessment was carried out. Samples plated on to Sabouraud's agar were cultured

for a minimum of 24 hours before being assessed. In situations where no growth was apparent, culture plates were incubated for a further 24 hours, then reassessed. Good direct light, an illuminated screen, a magnifying glass (x10) and an electronic colony counter were used to maximise the efficiency of the colony counting process. This protocol was followed on three occasions for both pre-impression oral rinse regimes.

5.9 EXPERIMENT 3 - RESULTS

The data obtained (for Subjects 1-10) from the two oral rinse regimes (and for the standard Kromogel impressions described in EXPERIMENT 2), after assessment of microbial growth on culture plates, are shown in Appendices 5.12 to 5.21. The summary data are presented in Table 5.4. Each of the mean values in Table 5.4 represents the collation of values from the culture of 12 microbial suspensions (3 impressions for each subject, each giving two samples of impression material, and two microbial suspensions from each of the impression samples). The standard deviation values were calculated from the mean CFU counts for each of the impression samples (six values, one from each impression sample). The data were collated for each oral rinse regime, and microbial growth was assessed on three different culture media.

5.9.1 CANDIDAL SPECIES

With respect to candidal species, there was no detectable growth on SDA from microbial suspensions obtained from any of the impressions.

5.9.2 CARRIAGE OF MICROORGANISMS ON KROMOGEL FOLLOWING A PRE-IMPRESSION ORAL-RINSE WITH 0.2% AQUEOUS CHLORHEXIDINE GLUCONATE, OR WITH TAP WATER

Mean CFU values for standard Kromogel impressions, for Kromogel impressions made after subjects rinsed with 10ml 0.2% chlorhexidine gluconate, and for impressions made after subjects rinsed with 10ml tap water, are shown in Table 5.4. There was considerable variation in the mean numbers of colony forming units cultured, within samples for different experimental regimes for each subject, and between subjects for each of the experimental regimes.

In all but one instance, the lowest mean CFU counts were found following the pre-impression aqueous chlorhexidine oral rinse, but for Subject 2 the lowest mean CFU values were found after pre-impression rinsing with water. These were the findings after culture on both blood agar (Fig 5.12) and mitis salivarius agar (Fig 5.13).

In four instances, following culture on blood agar, mean values obtained for growth of microorganisms were greater following a pre-impression oral-rinse with tap water, than were seen following sampling of standard Kromogel impressions (Subjects 7, 8, 9 and 10 : Fig 5.12). In two cases (Subjects 7 and 8) the level of colonisation of the standard impressions was very low, and it seems likely that the degree of tap water microbial contamination was greater than levels of salivary contamination with oral commensal microorganisms. However, for Subject 8, the increase following rinsing with tap water was so high that it seems likely this may, in part, be due to an extreme reaction, or a sampling irregularity.

In two cases (Subjects 2 and 6), rinsing with tap water produced an appreciable reduction in the mean values for the amount of growth on impression samples, in comparison with the standard Kromogel samples. In Subjects 1, 3, 4 and 5, there was a slight reduction in the colonisation of impression samples following rinsing with tap water, as compared to the standard Kromogel samples.

The CFU values for individual samples following growth on blood agar after the pre-impression chlorhexidine rinse (Fig 5.14) showed that almost no growth occurred on any of the samples, with the exception of a moderate amount of growth on three samples for Subject 2 and a single sample for Subject 6.

Only one sample (for Subject 6) showed appreciable growth on mitis salivarius agar, after pre-impression rinsing with chlorhexidine (Fig 5.15). In contrast, in a number of instances (Subjects 5,6,8,9,10) there was appreciable colonisation following culture on blood agar, when tap water was used as a 60 second pre-impression rinse (Fig 5.16). With the exception of Subject 8, there was little growth on any samples after MSA culturing when tap water was used as a pre-impression rinse (Fig 5.17).

5.9.3 STATISTICAL ANALYSIS (MANN-WHITNEY TEST)

The Mann-Whitney test was used to analyse the significance of the values for microorganism carriage on the six impression specimens obtained for each subject following pre-impression rinsing with chlorhexidine. Growth on blood agar and mitis salivarius agar was assessed.

In all subjects, following culture on both media, there was a statistically significant reduction in microbial growth on samples from the impressions taken after pre-impression rinsing with chlorhexidine, when compared with standard Kromogel impression samples ($p<0.05$).

There was a small difference between findings for the respective culture media, when microbial growth following a pre-impression oral-rinse with chlorhexidine was

compared with a pre-impression water rinse. After culture of microbial samples on blood agar, the number of colony forming units was reduced with chlorhexidine rinsing as compared to water rinsing ($p<0.05$) in eight subjects (nos 1,3,4,5,7,8,9,10). It is noteworthy that, although CFU values for different rinsing regimes for Subjects 7 and 8 were significant in the non-parametric statistical test used, in mathematical terms, contamination levels as indicated by CFU values, and differences between the test regimes, were small. In Subject 6 there was also substantially less growth following pre-impression rinsing with chlorhexidine, but due to some considerable variation between samples, this difference was not of statistical significance. Unexpectedly, for Subject 2 profuse growth was evident from three of the six impression samples after pre-impression rinsing with chlorhexidine, when the mean CFU value was greater than the equivalent value following rinsing with tap water, although the difference was not statistically significant. Following growth of microbial samples on MSA, although the mean CFU value for Subject 2 was less after a pre-impression rinse with chlorhexidine than with tap water, the difference did not attain significance. Otherwise, results from MSA growth were similar to those for blood agar.

5.9.4 STATISTICAL ANALYSIS (WILCOXON SIGNED-RANK TEST).

Analysis of the results for the entire group was carried out by paired comparison for each subject, of the mean value (from six specimens) for CFU growth after pre-impression rinsing with chlorhexidine, and the mean CFU values from standard Kromogel impressions (ten paired CFU values in total). Analysis was carried out using the Wilcoxon signed-rank test, and growth on blood agar and mitis salivarius agar was examined. It was confirmed that CFU values from impression samples collected after pre-impression rinsing with chlorhexidine were reduced with respect to standard Kromogel impressions, to a degree that was statistically significant (from samples cultured on both media). The Wilcoxon sign-ranked test also confirmed a significant reduction in colonisation following rinsing with chlorhexidine, in comparison with pre-impression rinsing with tap water (samples cultured on both media). However no difference was evident between standard Kromogel impressions, and those recorded after rinsing with tap water.

5.10 EXPERIMENT 3 - DISCUSSION

There was a consistent effect on the carriage of micro-organisms from the use of a pre-impression chlorhexidine

oral rinse for 60 seconds, as compared with the use of a standard alginate impression material, and also with standard alginate material following an oral rinse with tap water. There was considerable variation in mean CFU values between the ten subjects with both pre-impression rinse regimes. In one case (Subject 2) growth following rinsing with chlorhexidine was greater than that following an oral rinse with tap water, although consistently reduced CFU values after chlorhexidine rinsing by others must call the finding for Subject 2 into question. Following pre-impression rinsing with tap water, a wide range of CFU count values was evident. Multiple sampling was undertaken in order to provide representative mean CFU values for each subject, and the variability between samples for most subjects after rinsing with tap water, is clearly seen in Figure 5.16, underlining the value of multiple sampling.

5.11 EXPERIMENTS 1,2,3 - CONCLUSIONS

The alginate material containing sodium didecyldimethyl ammonium chloride (Blueprint Plus Antibac) was clearly and consistently more effective in eliminating the test microorganisms than standard alginate impression material (Kromogel) and alginate material containing chlorhexidine (Hydrogum). There was a trend for the chlorhexidine-

containing material to be more effective in eliminating the test microorganisms than the standard material, but this microbicidal effect was not consistent and, in some instances, chlorhexidine-containing alginate appeared to be less effective than standard alginate.

The use of 0.2% aqueous chlorhexidine gluconate as a pre-impression mouth rinse seemed to be a worthwhile anti-microbial measure, and was substantially more effective than the incorporation of chlorhexidine within the alginate material tested. The elimination of most micro-organisms in almost all samples tested suggests that the use of 0.2% aqueous chlorhexidine gluconate as a pre-impression mouthwash should be considered as a routine measure in clinical dental practice. The effect of chlorhexidine as a disinfectant in the clinical situation, underlines the importance of dental impression disinfection by immersion in either 2% glutaraldehyde or 0.0125% sodium hypochlorite, as these agents were found (in Chapter 3) to be considerably more effective than 0.2% chlorhexidine gluconate in removing microorganisms from impression materials after removal from the mouth.

While the effect of pre-impression rinsing with tap water seemed to be dependant upon the pre-existing levels of microbial colonisation of the oral cavity, there was no doubt that this measure does not have an antimicrobial

action comparable with the use of chlorhexidine. Rinsing with tap water was undertaken mainly to act as a control procedure, but from the results it is clear that in any further investigation of the efficacy of pre-impression oral rinsing, the use of sterile water would be preferable in such control studies.

From the main investigations, involving a well-controlled population of student dental hygienists, it is clear there are considerable variations between individuals with respect to the contamination of impression surfaces. Although this was suggested from the preliminary study, in which a single microbial suspension from a single impression sample was examined, it is evident from the main studies that even with a well-controlled experimental population, the use of multiple samples for each subject is essential to allow for sampling variability. It is also apparent that assessment of experimentally-induced changes in microorganism carriage on impression materials, must be undertaken on an individual basis, with subjects acting as their own control, as inter-subject comparison is of no value. With respect to further *in-vivo* investigations it seems reasonable to suggest that the collection of multiple samples from individuals demonstrating well-controlled oral hygiene practices, and with a minimum of five days between sampling procedures, was an acceptable protocol.

The findings of these *in-vivo* experiments coincide with those of the first phase of the *in-vitro* study described in Chapter 4, in as much as no clear benefit is apparent from use of an impression material incorporating chlorhexidine. This suggests that the experimental procedures described in Chapter 2 (2.5.4), and repeated in Chapters 3 and 4, in which the contaminating microbial inoculum was discarded after three minutes' contact with the impression materials and no neutralising agents were used, are valid with respect to representing clinical conditions.

With respect to an effective method of protection against contamination from dental impressions, from the preceding work it would seem that prior to recording impressions, patients should rinse with 0.2% aqueous chlorhexidine. In addition, following use of a rubber base impression material, or alginate containing sodium didecyldimethyl ammonium chloride, impressions should be immersed in 2% activated glutaraldehyde, or 0.0125% sodium hypochlorite, for a minimum of 3 minutes.

Examination of the dimensional stability, and the effect of immersion disinfection under conditions representative of general dental practice, for the different types of alginate impression materials considered in Chapters 2, 3, 4 and 5, is undertaken in Chapter 6.

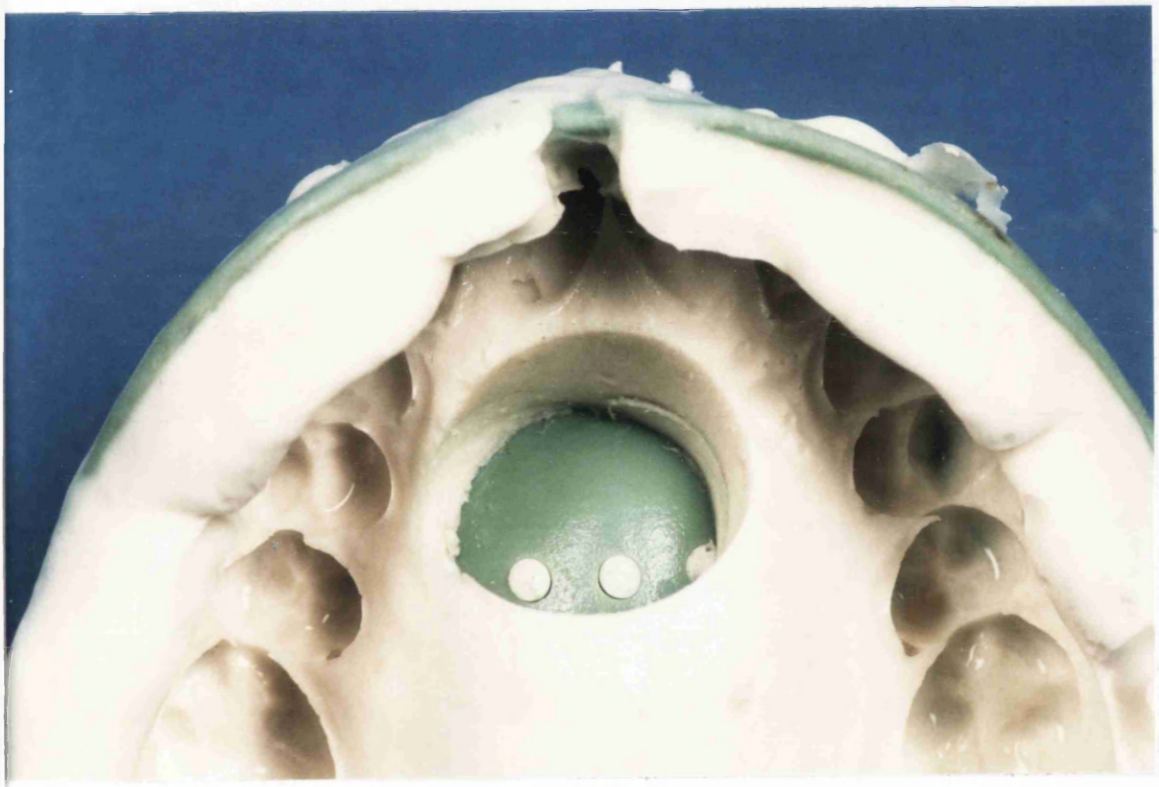


FIGURE 5.1

Kromogel alginate impression.

Sample site used in Experiment 1.

Figure 5.2

EXPERIMENT 1

CFU - Day 0 (Subjects 1-12)

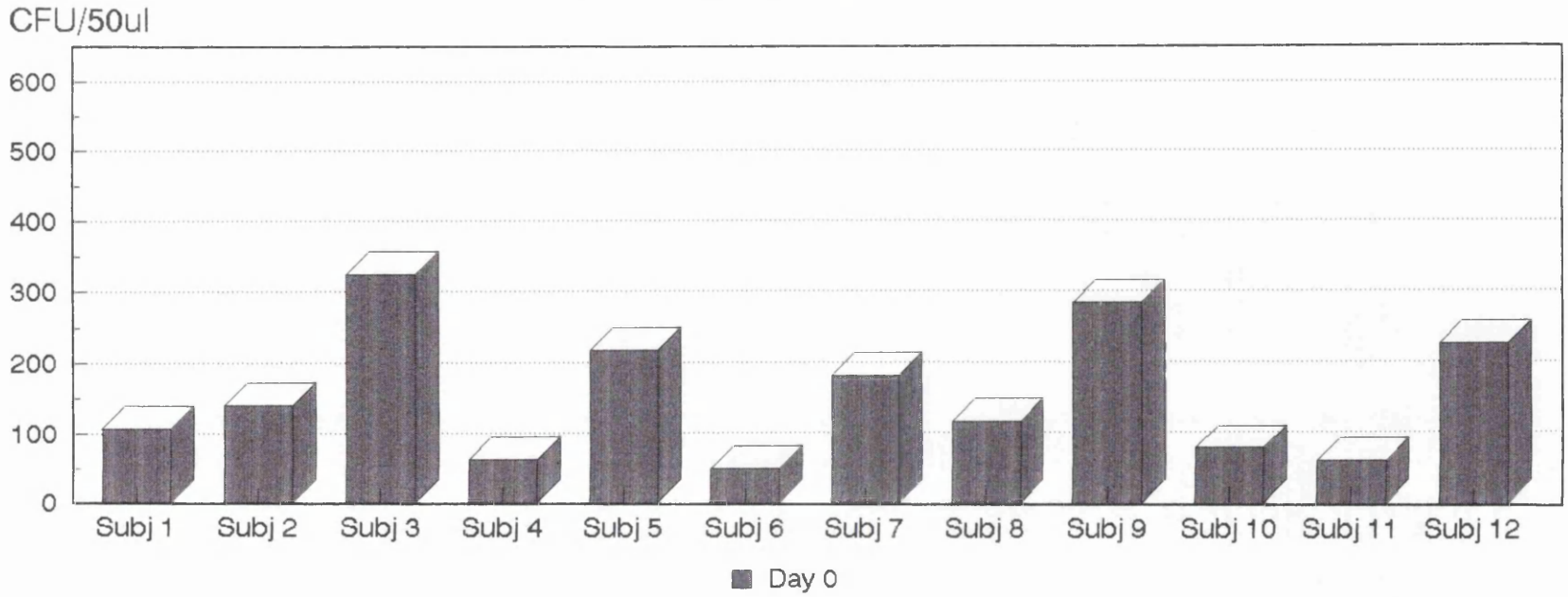


Figure 5.3

EXPERIMENT 1

CFU - Day 2 (Subjects 1-12)

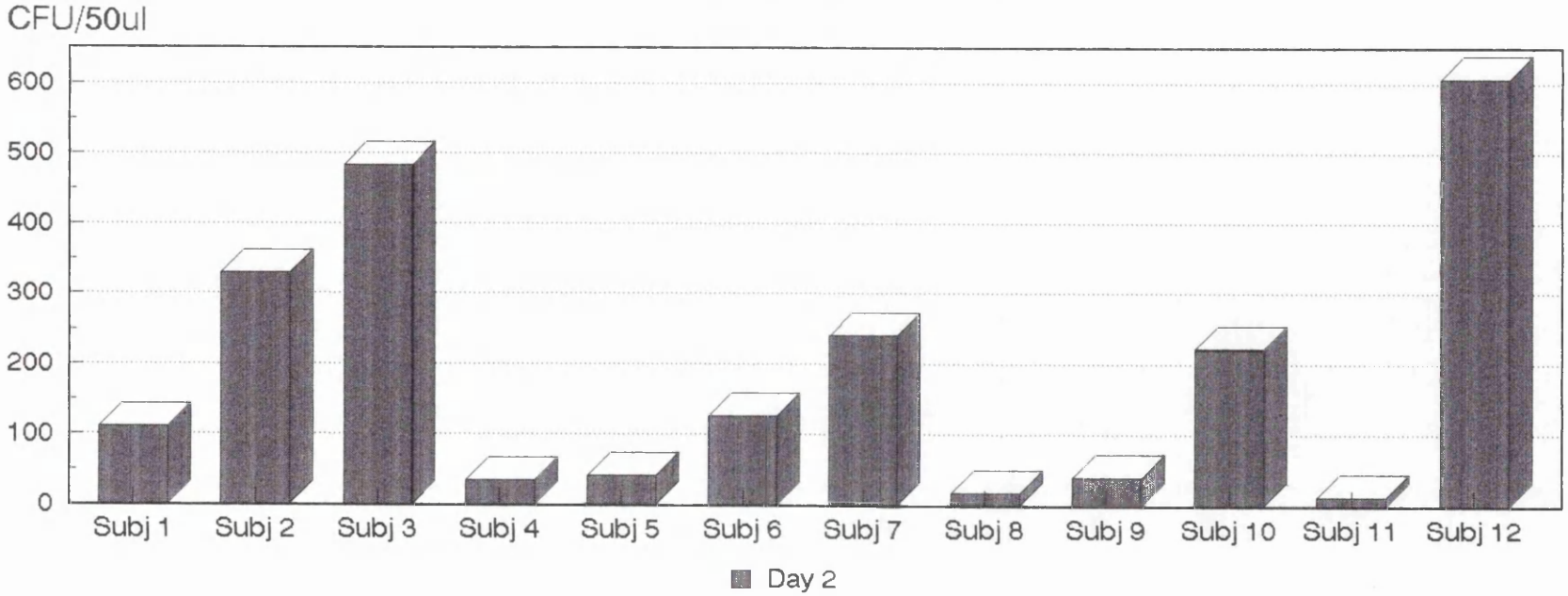


Figure 5.4

EXPERIMENT 1

CFU - Day 3 (Subjects 1-12)

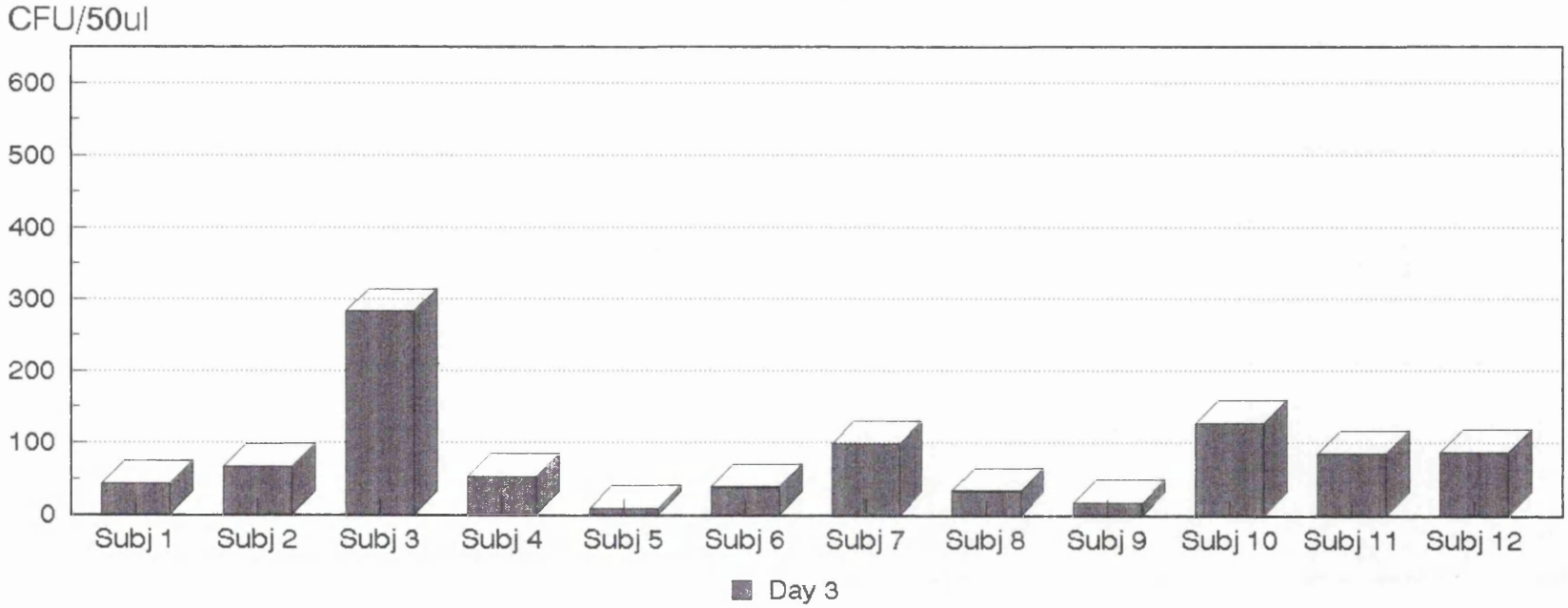
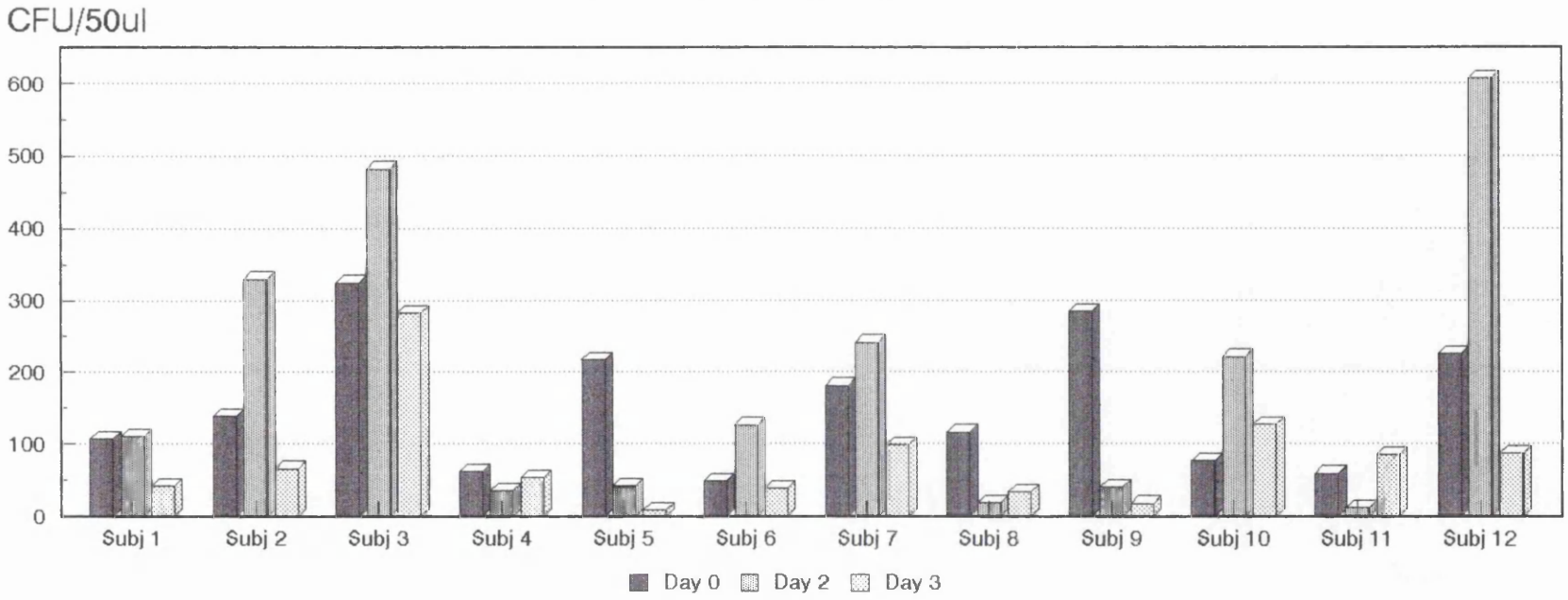


Figure 5.5

EXPERIMENT 1

CFU - Day 0, Day 2, Day 3 (Subjects 1-12)



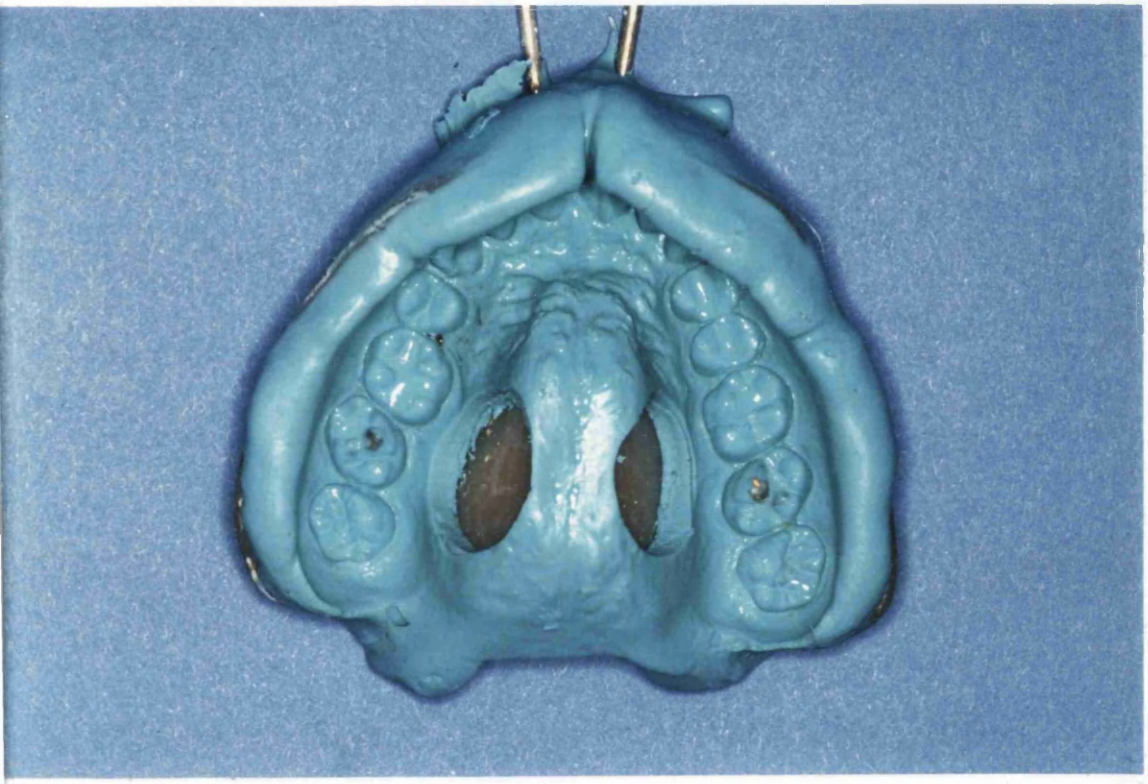


FIGURE 5.6

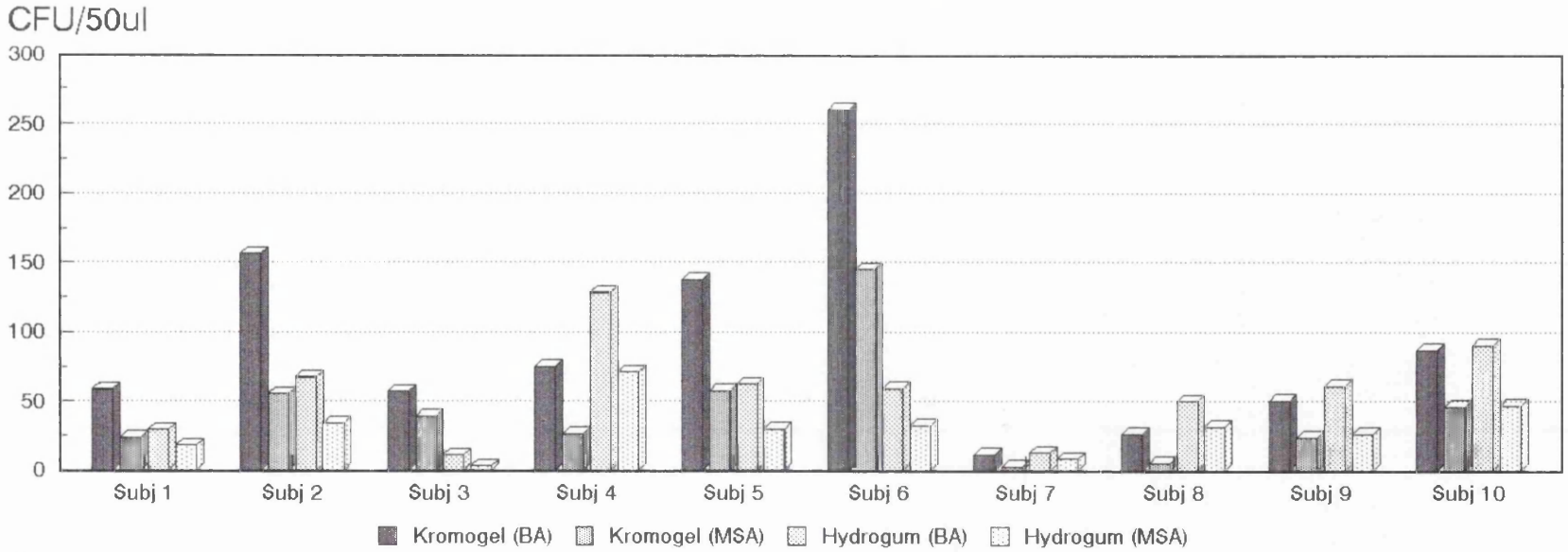
Hydrogum alginate impression.

Sample sites used in Experiments 2 & 3.

Figure 5.7

EXPERIMENT 2 (Subjects 1-10)

Mean CFU Values - Kromogel and Hydrogum



BA - Blood agar
MSA - Mitis salivarius agar

Figure 5.8

EXPERIMENT 2 (Subjects 1-10)
CFU Values - Kromogel (Blood Agar)

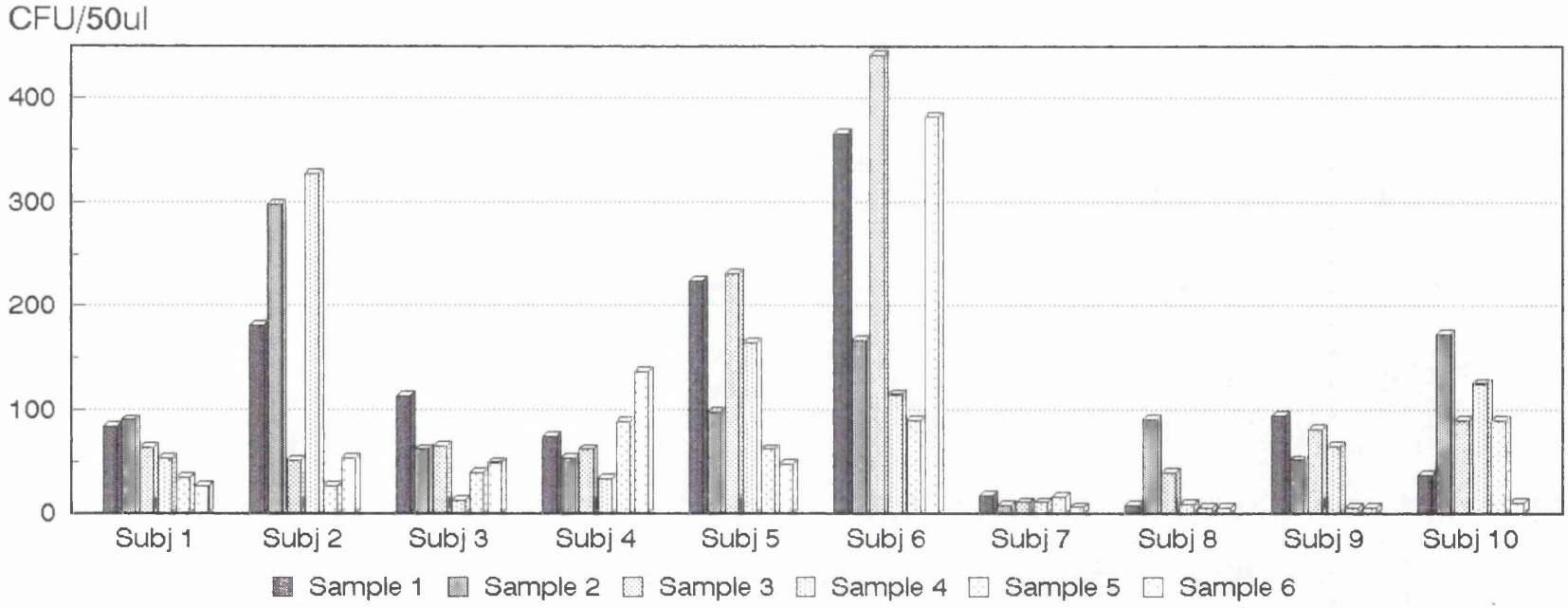


Figure 5.9

EXPERIMENT 2 (Subjects 1-10)

CFU Values - Kromogel (Mitis Salivarius Agar)

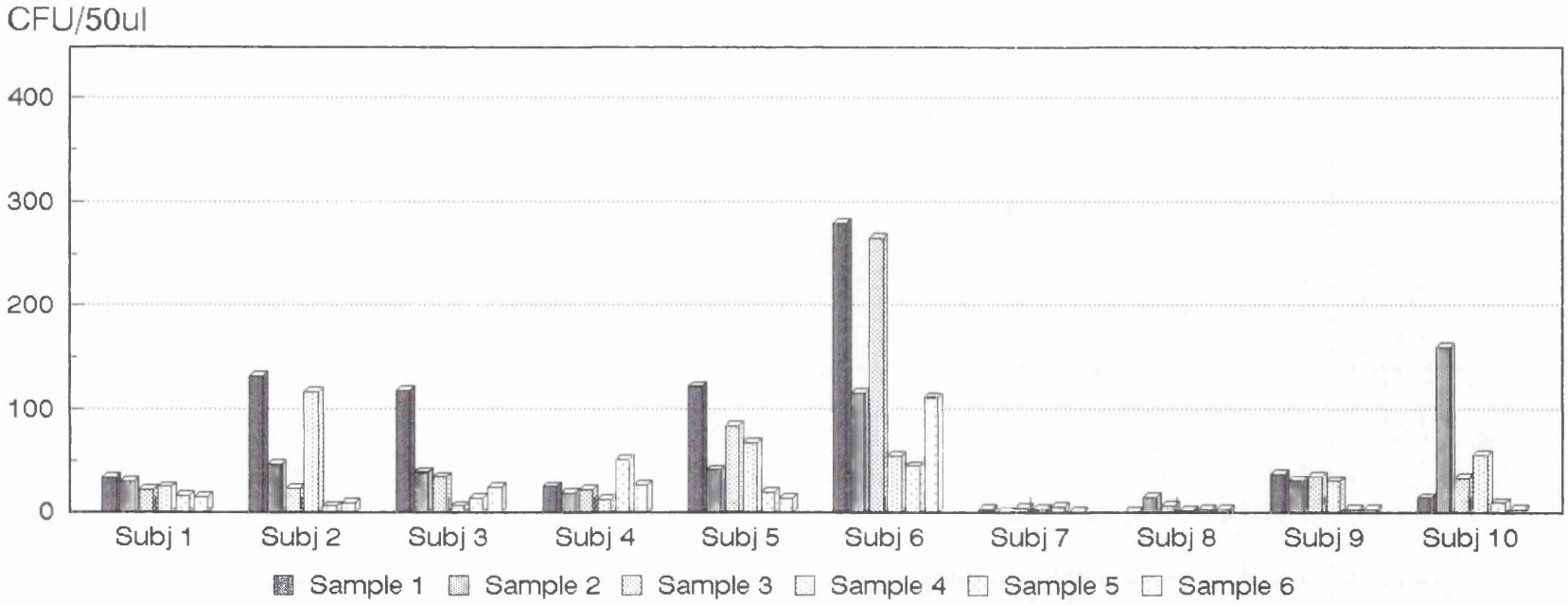


Figure 5.10

EXPERIMENT 2 (Subjects 1-10)

CFU Values - Hydrogum (Blood Agar)

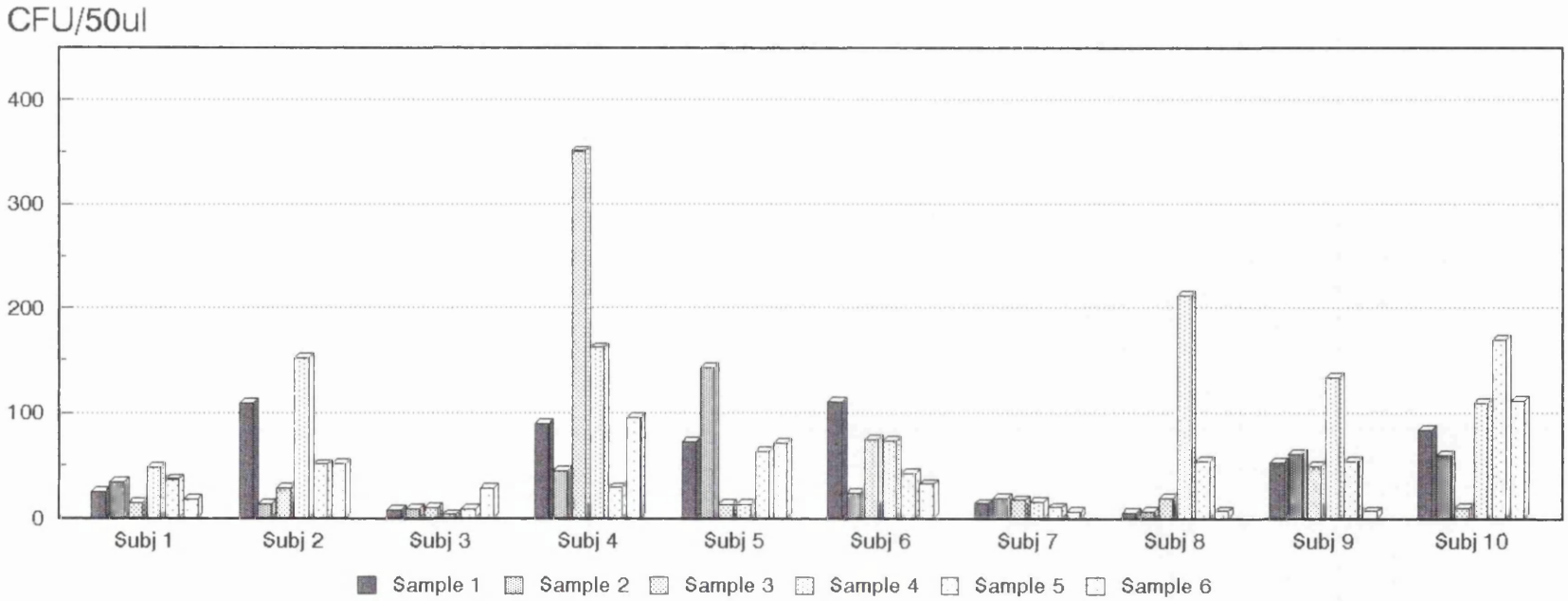


Figure 5.11

EXPERIMENT 2 (Subjects 1-10)

CFU Values - Hydrogum (Mitis Salivarius Agar)

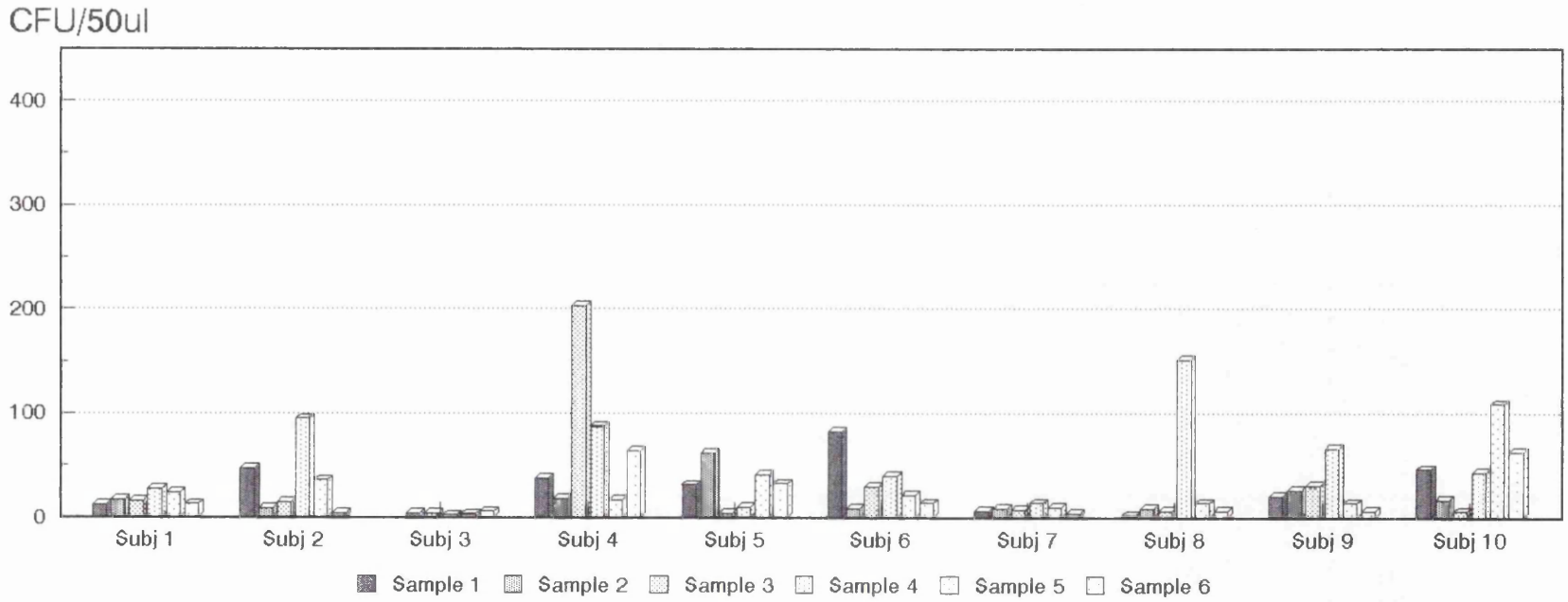


Figure 5.12

EXPERIMENT 3 (Subjects 1-10)

Mean CFU Values - Oral Rinse (Blood Agar)

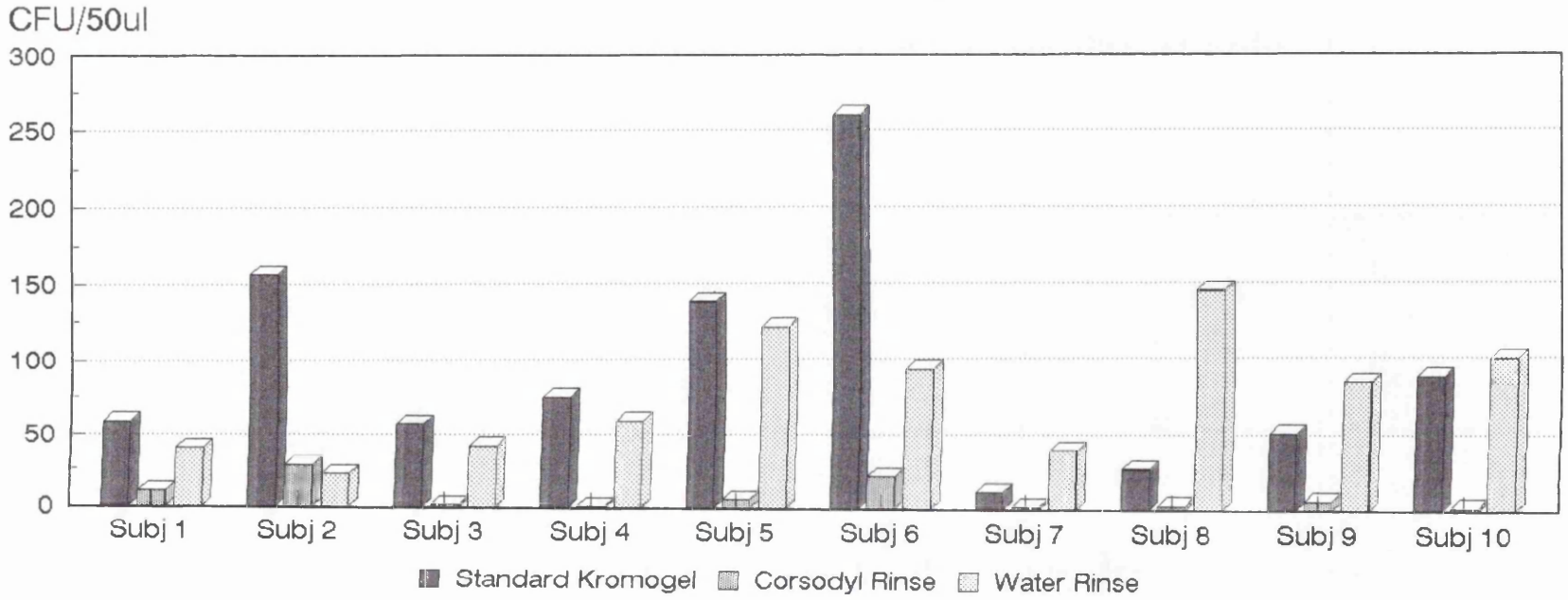


Figure 5.13

EXPERIMENT 3 (Subjects 1-10)
Mean CFU Values - Oral Rinse (MSA)

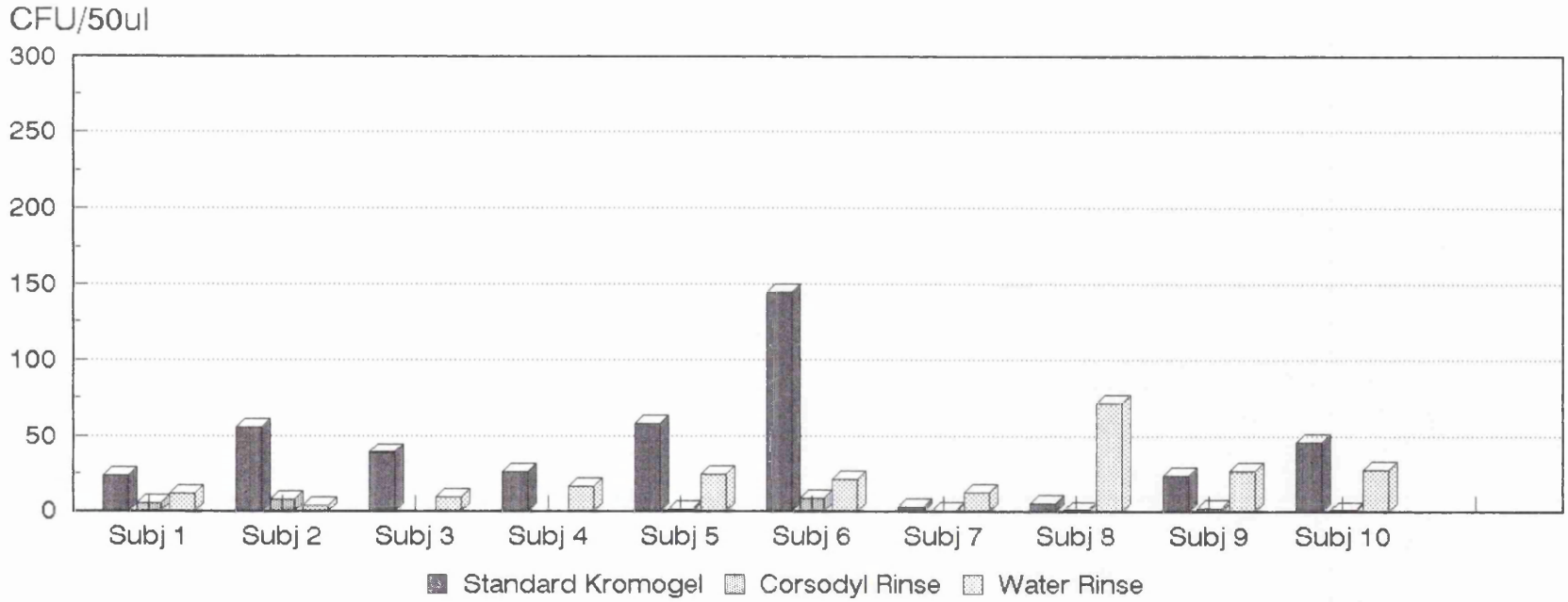


Figure 5.14

EXPERIMENT 3 (Kromogel : Subjects 1-10)

CFU Values - Corsodyl Rinse (Blood Agar)

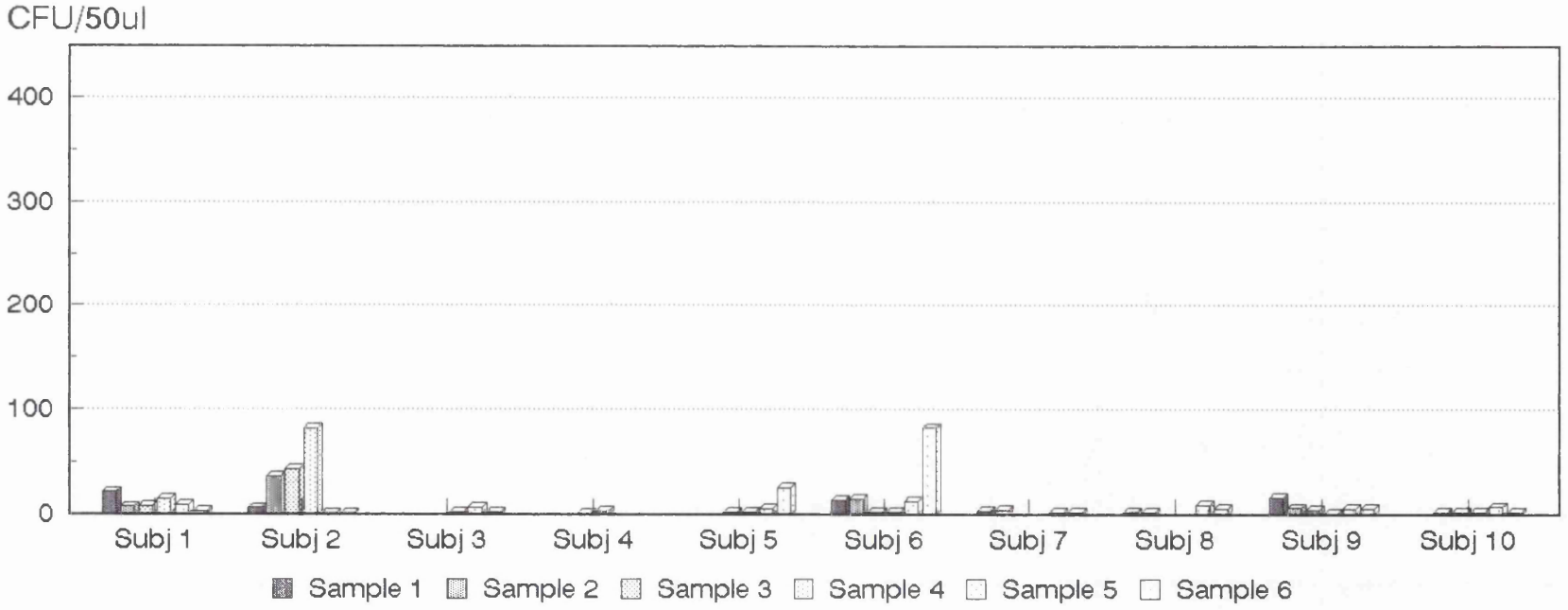


Figure 5.15

EXPERIMENT 3 (Kromogel : Subjects 1-10)

CFU Values - Corsodyl Rinse (MSA)

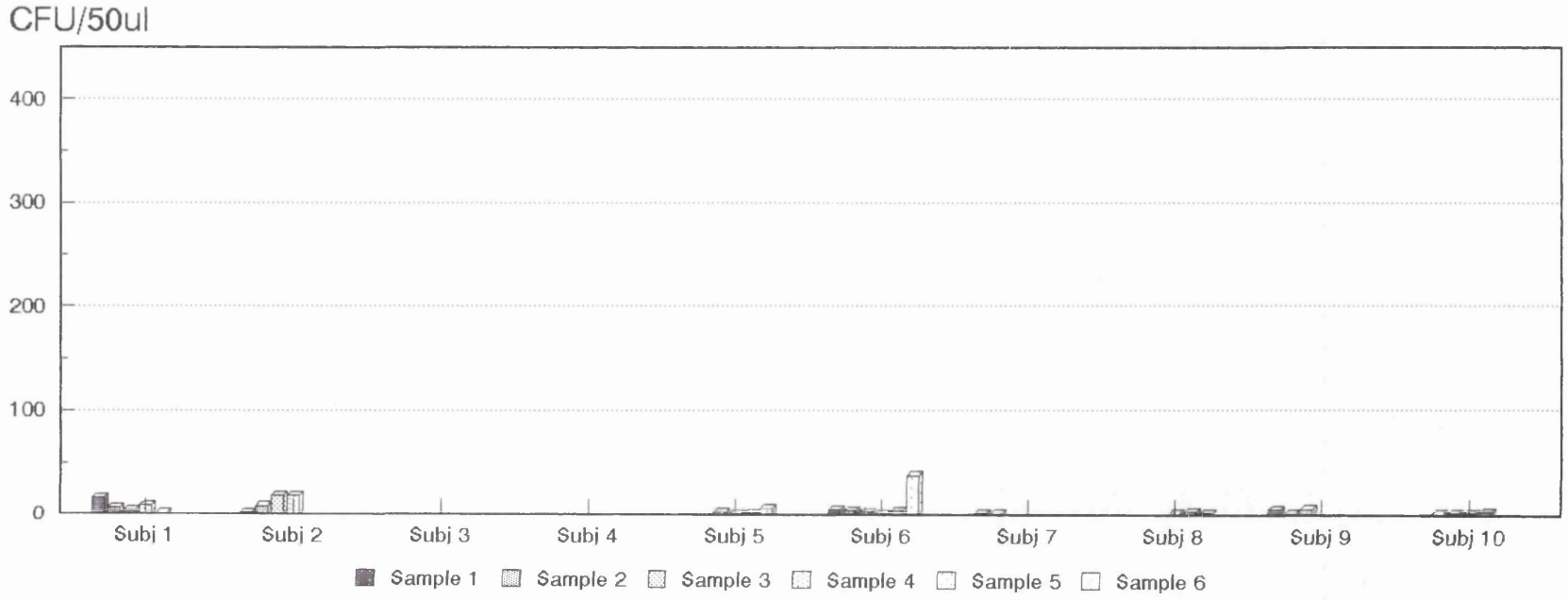


Figure 5.16

EXPERIMENT 3 (Kromogel : Subjects 1-10)
CFU Values - Water Rinse (Blood Agar)

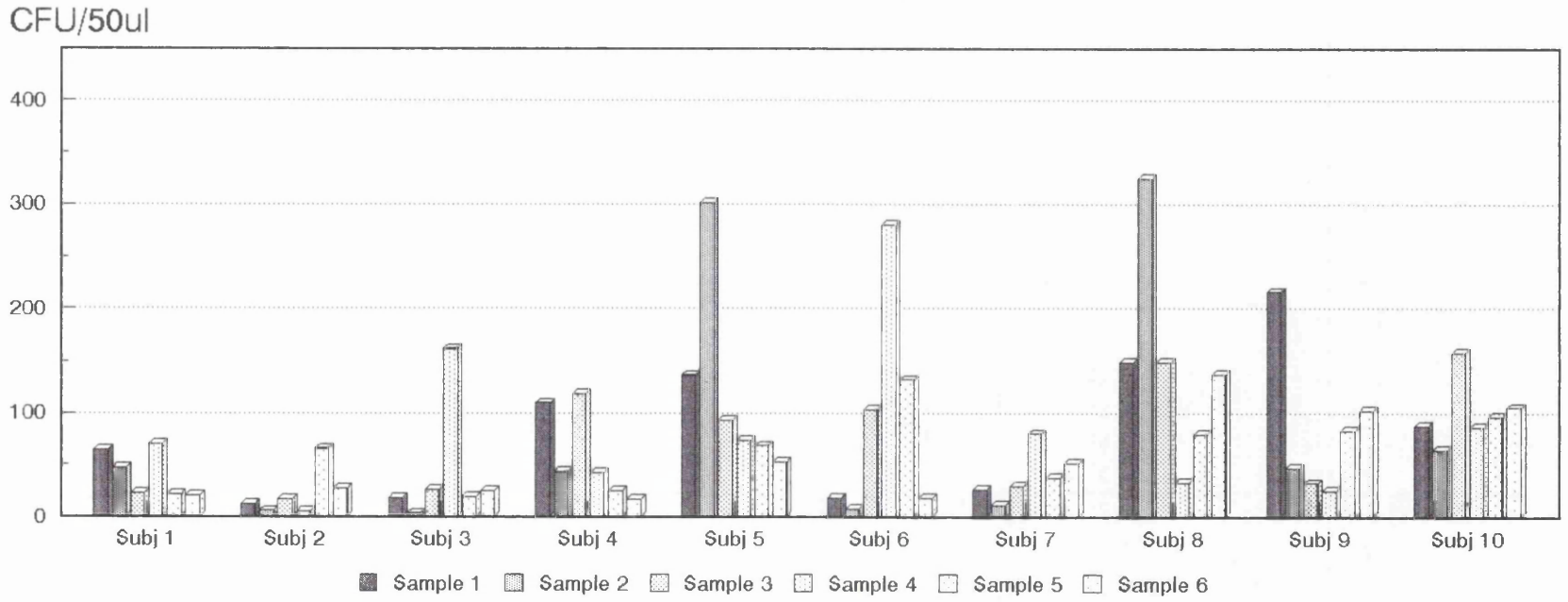


Figure 5.17

EXPERIMENT 3 (Kromogel : Subjects 1-10)
CFU Values - Water Rinse (Mitis Salivarius Agar)

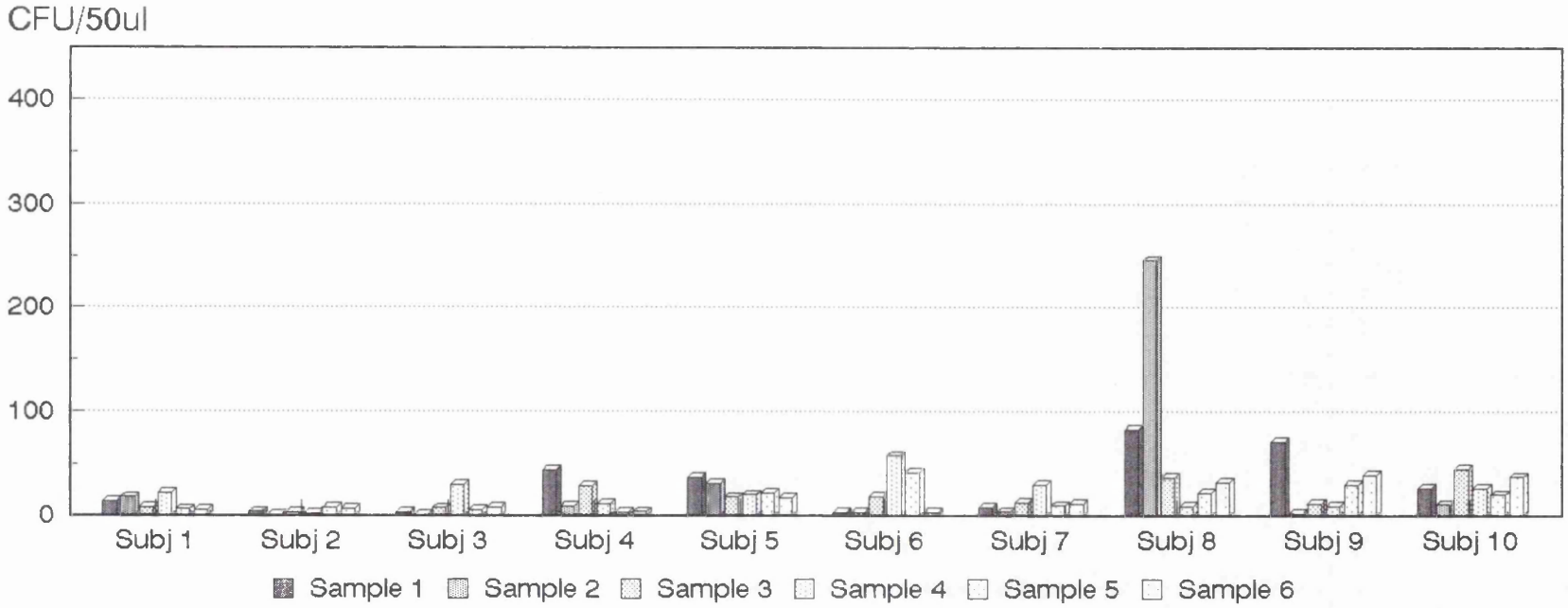


TABLE 5.1

IMPRESSION MATERIALS EXAMINED IN *IN-VIVO* STUDIES

Material	Type	Manufacturer
Kromogel	Alginate	Wright Dental, Scotland
Blueprint with Antibac	Alginate	DeTray Dentsply, England
Hydrogum	Alginate	Zhermack, Italy

TABLE 5.2

EXPERIMENT 1

MEAN CFU/50ul VALUES - ALGINATE IMPRESSION MATERIALS

SUBJECTS 1-12

	Day 0	Day 2	Day 3	SD
Subject 1	106	110	44	**38**
Subject 2	139	330	66	**136**
Subject 3	325	482	282	**105**
Subject 4	62	35	53	**14**
Subject 5	217	41	8	**112**
Subject 6	48	126	38	**48**
Subject 7	181	241	98	**72**
Subject 8	116	18	33	**53**
Subject 9	284	40	17	**148**
Subject 10	77	221	127	**73**
Subject 11	59	12	85	**37**
Subject 12	225	606	87	**269**
Mean	**153**	**189**	**78**	
SD	**93**	**196**	**73**	

TABLE 5.3

EXPERIMENT 2

MEAN CFU/50ul VALUES - ALGINATE IMPRESSION MATERIALS

SUBJECTS 1-10

	Kromogel			Hydrogum			Blueprint		
Subject	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 Mean	58.5	23.3	0	29.1	17.7	0	0	0	0
SD	25.6	7.4	0	12.8	6.2	0	0	0	0
2 Mean	156.0	55.2	0	67.2	33.8	0	0	0	0
SD	132.8	55.2	0	52.9	34.0	0	0	0	0
3 Mean	56.8	38.9	0	10.8	3.0	0	0	0	0
SD	33.5	40.4	0	8.7	1.9	0	0	0	0
4 Mean	74.7	25.8	0	128.0	70.9	0	0	0	0
SD	35.5	13.4	0	118.3	70.3	0	0	0	0
5 Mean	137.6	57.5	0	62.1	29.8	0	0	0	0
SD	80.3	41.5	0	47.8	21.0	0	0	0	0
6 Mean	259.9	144.7	0	59.0	32.3	0	0	0	0
SD	153.6	102.6	0	32.8	26.9	0	0	0	0
7 Mean	11.3	2.5	0	12.7	8.2	0	0	0	0
SD	4.6	1.7	0	4.9	3.7	0	0	0	0
8 Mean	26.2	4.9	0	49.9	31.2	0	0	0	0
SD	34.2	5.3	0	81.3	58.8	0	0	0	0
9 Mean	50.4	23.2	0	60.8	26.1	0	0	0	0
SD	37.8	16.0	0	40.7	20.9	0	0	0	0
10Mean	87.1	45.8	0	90.3	47.1	0	0	0	0
SD	58.6	59.0	0	54.1	36.7	0	0	0	0

TABLE 5.4

EXPERIMENT 3

CFU/50ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECTS 1 -10

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Subj	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
No 1									
Mean	58.5	23.3	0	10.4	5.3	0	40.8	11.8	0
SD	25.6	7.4	0	6.4	5.6	0	22.5	6.7	0
No 2									
Mean	156.0	55.2	0	27.8	7.25	0	21.8	3.8	0
SD	132.8	55.2	0	31.8	8.42	0	22.9	2.8	0
No 3									
Mean	56.8	38.9	0	1.6	0	0	41.8	8.8	0
SD	33.5	40.4	0	2.5	0	0	59.2	10.8	0
No 4									
Mean	74.7	25.8	0	0.7	0	0	58.8	16.5	0
SD	35.5	13.4	0	1.2	0	0	43.9	16.4	0
No 5									
Mean	137.6	57.5	0	5.7	1.5	0	120.6	24.1	0
SD	80.3	41.5	0	9.7	2.1	0	93.0	8.1	0
No 6									
Mean	259.9	144.7	0	21.0	8.6	0	92.8	21.0	0
SD	153.6	102.6	0	30.5	14.2	0	105.1	23.5	0
No 7									
Mean	11.2	2.8	0	1.7	0.3	0	39.0	12.4	0
SD	4.1	1.7	0	1.5	0.4	0	24.0	9.2	0
No 8									
Mean	26.2	4.9	0	2.8	0.7	0	144.8	71.3	0
SD	34.2	5.3	0	3.4	1.0	0	99.2	89.0	0
No 9									
Mean	50.4	23.2	0	6.2	1.7	0	83.8	26.3	0
SD	37.8	16.0	0	4.9	2.4	0	71.3	25.6	0
No 10									
Mean	87.1	45.8	0	2.3	0.8	0	99.3	27.6	0
SD	58.6	59.0	0	2.4	0.8	0	31.8	12.3	0

APPENDIX 5.1

Instructions to Subjects (Dental Hygienists)

Impression contamination study : Prosthodontic Clinic

Once a week for approximately 15 visits, impressions will be taken of each of your mouths. This will take place at lunch time on Wednesdays and individual time-slots have been allocated. To help with mixing materials in the clinic, I have asked that you attend in pairs.

The aim of the study is to see if disinfectants within alginate impression materials are effective in removing microorganisms from the surface of the impressions, and to compare 'disinfectant' materials with those which do not contain disinfectants. In addition, I wish to test the effect of a pre-impression mouth rinse with Corsodyl.

To help standardise the regime, no tooth brushing should be carried out on the day that impressions are recorded. If brushing is undertaken by mistake, impressions can be postponed until the following day, or the first suitable occasion.

APPENDIX 5.2

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 1

	Kromogel			Hydrogum			Blueprint		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	78	35	0	20	9	0	0	0	0
ii	89	32	0	29	15	0	0	0	0
Mean	**83.5**	**33.5**	**0**	**24.5**	**12**	**0**	**0**	**0**	**0**
2 i	81	20	0	27	17	0	0	0	0
ii	99	39	0	41	15	0	0	0	0
Mean	**90**	**29.5**	**0**	**34**	**16**	**0**	**0**	**0**	**0**
3 i	62	19	0	14	10	0	0	0	0
ii	65	25	0	14	20	0	0	0	0
Mean	**63.5**	**22**	**0**	**14**	**15**	**0**	**0**	**0**	**0**
4 i	48	26	0	49	23	0	0	0	0
ii	58	23	0	47	31	0	0	0	0
Mean	**53**	**24.5**	**0**	**48**	**27**	**0**	**0**	**0**	**0**
5 i	39	18	0	38	26	0	0	0	0
ii	31	14	0	35	21	0	0	0	0
Mean	**35**	**16**	**0**	**36.5**	**23.5**	**0**	**0**	**0**	**0**
6 i	30	15	0	17	13	0	0	0	0
ii	22	14	0	18	12	0	0	0	0
Mean	**26**	**14.5**	**0**	**17.5**	**12.5**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 1

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**58.5**	**23.3**	**0**	**29.1**	**17.7**	**0**	**0**	**0**	**0**
SD**	**25.6**	**7.4**	**0**	**12.8**	**6.2**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.3

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 2

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	171	173	0	113	35	0	0	0	0
ii	190	90	0	105	58	0	0	0	0
Mean	**180.5**	**131.5**	**0**	**109**	**46.5**	**0**	**0**	**0**	**0**
2 i	312	23	0	11	11	0	0	0	0
ii	283	69	0	15	6	0	0	0	0
Mean	**297.5**	**46**	**0**	**13**	**8.5**	**0**	**0**	**0**	**0**
3 i	50	22	0	24	12	0	0	0	0
ii	52	24	0	31	16	0	0	0	0
Mean	**51**	**23**	**0**	**27.5**	**14**	**0**	**0**	**0**	**0**
4 i	344	112	0	142	89	0	0	0	0
ii	310	120	0	162	100	0	0	0	0
Mean	**327**	**116**	**0**	**152**	**94.5**	**0**	**0**	**0**	**0**
5 i	25	6	0	50	41	0	0	0	0
ii	28	6	0	51	30	0	0	0	0
Mean	**26.5**	**6**	**0**	**50.5**	**35.5**	**0**	**0**	**0**	**0**
6 i	59	7	0	46	3	0	0	0	0
ii	48	10	0	56	5	0	0	0	0
Mean	**53.5**	**8.5**	**0**	**51**	**4**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 2

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**156.0**	**55.2**	**0**	**67.2**	**33.8**	**0**	**0**	**0**	**0**
SD**	**132.8**	**55.2**	**0**	**52.9**	**34.0**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.4

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 3

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	105	121	0	9	0	0	0	0	0
ii	121	114	0	6	0	0	0	0	0
Mean	**113**	**117.5**	**0**	**7.5**	**0**	**0**	**0**	**0**	**0**
2 i	56	35	0	7	5	0	0	0	0
ii	68	42	0	10	2	0	0	0	0
Mean	**62**	**38.5**	**0**	**8.5**	**3.5**	**0**	**0**	**0**	**0**
3 i	72	37	0	9	6	0	0	0	0
ii	58	31	0	10	2	0	0	0	0
Mean	**65**	**34**	**0**	**9.5**	**4**	**0**	**0**	**0**	**0**
4 i	12	7	0	0	2	0	0	0	0
ii	12	5	0	6	2	0	0	0	0
Mean	**12**	**6**	**0**	**3**	**2**	**0**	**0**	**0**	**0**
5 i	44	17	0	7	5	0	0	0	0
ii	35	10	0	9	1	0	0	0	0
Mean	**39.5**	**13.5**	**0**	**8**	**3**	**0**	**0**	**0**	**0**
6 i	55	23	0	32	6	0	0	0	0
ii	43	25	0	24	5	0	0	0	0
Mean	**49**	**24**	**0**	**28**	**5.5**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 3

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**56.8**	**38.9**	**0**	**10.8**	**3.0**	**0**	**0**	**0**	**0**
SD**	**33.5**	**40.4**	**0**	**8.7**	**1.9**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.5

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 4

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	79	25	0	82	46	0	0	0	0
ii	69	24	0	96	28	0	0	0	0
Mean	**74**	**24.5**	**0**	**89**	**37**	**0**	**0**	**0**	**0**
2 i	61	22	0	39	21	0	0	0	0
ii	46	15	0	49	15	0	0	0	0
Mean	**53.5**	**18.5**	**0**	**44**	**18**	**0**	**0**	**0**	**0**
3 i	59	16	0	318	206	0	0	0	0
ii	65	28	0	382	200	0	0	0	0
Mean	**62**	**22**	**0**	**350**	**203**	**0**	**0**	**0**	**0**
4 i	32	15	0	164	109	0	0	0	0
ii	35	9	0	159	66	0	0	0	0
Mean	**33.5**	**12**	**0**	**161.5**	**87.5**	**0**	**0**	**0**	**0**
5 i	91	47	0	25	14	0	0	0	0
ii	86	55	0	32	18	0	0	0	0
Mean	**88.5**	**51**	**0**	**28.5**	**16**	**0**	**0**	**0**	**0**
6 i	126	16	0	111	73	0	0	0	0
ii	147	37	0	79	55	0	0	0	0
Mean	**136.5**	**26.5**	**0**	**95**	**64**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 4

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**74.7**	**25.8**	**0**	**128.0**	**70.9**	**0**	**0**	**0**	**0**
SD**	**35.5**	**13.4**	**0**	**118.3**	**70.3**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.6

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 5

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	232	117	0	66	31	0	0	0	0
ii	216	126	0	78	31	0	0	0	0
Mean	**224**	**121.5**	**0**	**72**	**31**	**0**	**0**	**0**	**0**
2 i	97	46	0	126	46	0	0	0	0
ii	98	36	0	158	77	0	0	0	0
Mean	**97.5**	**41**	**0**	**142**	**61.5**	**0**	**0**	**0**	**0**
3 i	221	82	0	12	5	0	0	0	0
ii	240	85	0	13	3	0	0	0	0
Mean	**230.5**	**83.5**	**0**	**12.5**	**4**	**0**	**0**	**0**	**0**
4 i	168	72	0	13	9	0	0	0	0
ii	160	62	0	13	10	0	0	0	0
Mean	**164**	**67**	**0**	**13**	**9.5**	**0**	**0**	**0**	**0**
5 i	77	16	0	58	40	0	0	0	0
ii	47	22	0	67	41	0	0	0	0
Mean	**62**	**19**	**0**	**62.5**	**40.5**	**0**	**0**	**0**	**0**
6 i	49	13	0	72	32	0	0	0	0
ii	46	13	0	69	32	0	0	0	0
Mean	**47.5**	**13**	**0**	**70.5**	**32**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 5

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**137.6**	**57.5**	**0**	**62.1**	**29.8**	**0**	**0**	**0**	**0**
SD**	**80.3**	**41.5**	**0**	**47.8**	**21.0**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.7

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 6

Sample	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	356	226	0	110	60	0	0	0	0
ii	375	332	0	110	105	0	0	0	0
Mean	**365.5**	**279**	**0**	**110**	**82.5**	**0**	**0**	**0**	**0**
2 i	164	109	0	29	9	0	0	0	0
ii	170	121	0	17	8	0	0	0	0
Mean	**167**	**115**	**0**	**23**	**8.5**	**0**	**0**	**0**	**0**
3 i	425	242	0	75	24	0	0	0	0
ii	458	287	0	74	34	0	0	0	0
Mean	**441.5**	**264.5**	**0**	**74.5**	**29**	**0**	**0**	**0**	**0**
4 i	123	57	0	68	34	0	0	0	0
ii	106	51	0	78	45	0	0	0	0
Mean	**114.5**	**54**	**0**	**73**	**39.5**	**0**	**0**	**0**	**0**
5 i	97	42	0	48	15	0	0	0	0
ii	81	47	0	35	27	0	0	0	0
Mean	**89**	**44.5**	**0**	**41.5**	**21**	**0**	**0**	**0**	**0**
6 i	371	54	0	26	17	0	0	0	0
ii	393	168	0	38	10	0	0	0	0
Mean	**382**	**111**	**0**	**32**	**13.5**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 6

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**259.9**	**144.7**	**0**	**59.0**	**32.3**	**0**	**0**	**0**	**0**
SD**	**153.6**	**102.6**	**0**	**32.8**	**26.9**	**0**	**0**	**0**	**0**

* Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.8

CFU/ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 7

Sample	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	19	4	0	15	3	0	0	0	0
ii	14	2	0	12	10	0	0	0	0
Mean	**16.5**	**3**	**0**	**13.5**	**6.5**	**0**	**0**	**0**	**0**
2 i	12	1	0	25	8	0	0	0	0
ii	4	1	0	13	9	0	0	0	0
Mean	**8**	**1**	**0**	**19**	**8.5**	**0**	**0**	**0**	**0**
3 i	9	4	0	18	10	0	0	0	0
ii	12	5	0	16	4	0	0	0	0
Mean	**10.5**	**4.5**	**0**	**17**	**7**	**0**	**0**	**0**	**0**
4 i	11	3	0	14	11	0	0	0	0
ii	10	2	0	16	16	0	0	0	0
Mean	**10.5**	**2.5**	**0**	**15**	**13.5**	**0**	**0**	**0**	**0**
5 i	15	6	0	13	11	0	0	0	0
ii	16	4	0	7	7	0	0	0	0
Mean	**15.5**	**5**	**0**	**10**	**9**	**0**	**0**	**0**	**0**
6 i	4	0	0	3	5	0	0	0	0
ii	8	2	0	9	2	0	0	0	0
Mean	**6**	**1**	**0**	**6**	**3.5**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 7

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**11.3**	**2.5**	**0**	**12.7**	**8.2**	**0**	**0**	**0**	**0**
SD**	**4.6**	**1.7**	**0**	**4.9**	**3.7**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.9

CFU/ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 8

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	6	0	0	5	2	0	0	0	0
ii	9	2	0	4	2	0	0	0	0
Mean	**7.5**	**1**	**0**	**4.5**	**2**	**0**	**0**	**0**	**0**
2 i	93	15	0	4	4	0	0	0	0
ii	88	15	0	7	4	0	0	0	0
Mean	**90.5**	**15**	**0**	**5.5**	**8**	**0**	**0**	**0**	**0**
3 i	42	7	0	16	8	0	0	0	0
ii	37	6	0	21	5	0	0	0	0
Mean	**39.5**	**6.5**	**0**	**18.5**	**6.5**	**0**	**0**	**0**	**0**
4 i	7	1	0	198	153	0	0	0	0
ii	11	2	0	225	149	0	0	0	0
Mean	**9**	**1.5**	**0**	**211.5**	**151**	**0**	**0**	**0**	**0**
5 i	6	5	0	53	18	0	0	0	0
ii	4	0	0	53	9	0	0	0	0
Mean	**5**	**2.5**	**0**	**53**	**13.5**	**0**	**0**	**0**	**0**
6 i	8	2	0	10	5	0	0	0	0
ii	3	4	0	3	7	0	0	0	0
Mean	**5.5**	**3**	**0**	**6.5**	**6**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 8

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**26.2**	**4.9**	**0**	**49.9**	**31.2**	**0**	**0**	**0**	**0**
SD**	**34.2**	**5.3**	**0**	**81.3**	**58.8**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.10

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 9

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	66	33	0	55	20	0	0	0	0
ii	122	41	0	49	20	0	0	0	0
Mean	**94**	**37**	**0**	**52**	**20**	**0**	**0**	**0**	**0**
2 i	60	37	0	61	26	0	0	0	0
ii	43	25	0	59	25	0	0	0	0
Mean	**51.5**	**31**	**0**	**60**	**25.5**	**0**	**0**	**0**	**0**
3 i	79	45	0	62	32	0	0	0	0
ii	84	25	0	36	29	0	0	0	0
Mean	**81.5**	**35**	**0**	**49**	**30.5**	**0**	**0**	**0**	**0**
4 i	67	33	0	141	71	0	0	0	0
ii	63	28	0	125	61	0	0	0	0
Mean	**65**	**30.5**	**0**	**133**	**66**	**0**	**0**	**0**	**0**
5 i	6	5	0	53	18	0	0	0	0
ii	4	0	0	53	9	0	0	0	0
Mean	**5**	**2.5**	**0**	**53**	**13.5**	**0**	**0**	**0**	**0**
6 i	8	2	0	10	5	0	0	0	0
ii	3	4	0	3	7	0	0	0	0
Mean	**5.5**	**3**	**0**	**6.5**	**6**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 9

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**50.4**	**23.2**	**0**	**60.8**	**26.1**	**0**	**0**	**0**	**0**
SD**	**37.8**	**16.0**	**0**	**40.7**	**20.9**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.11

CFU/50ul for ALGINATE IMPRESSION MATERIALS

SUBJECT 10

	Kromogel			Hydrogum			Blueprint		
Sample	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	38	13	0	88	30	0	0	0	0
ii	36	15	0	79	62	0	0	0	0
Mean	**37**	**14**	**0**	**83.5**	**46**	**0**	**0**	**0**	**0**
2 i	181	168	0	57	18	0	0	0	0
ii	164	151	0	61	14	0	0	0	0
Mean	**172.5**	**159.5**	**0**	**59**	**16**	**0**	**0**	**0**	**0**
3 i	89	34	0	7	7	0	0	0	0
ii	89	33	0	12	4	0	0	0	0
Mean	**89**	**33.5**	**0**	**9.5**	**5.5**	**0**	**0**	**0**	**0**
4 i	110	70	0	117	34	0	0	0	0
ii	130	42	0	101	53	0	0	0	0
Mean	**125**	**56**	**0**	**109**	**43.5**	**0**	**0**	**0**	**0**
5 i	92	2	0	174	112	0	0	0	0
ii	86	17	0	165	105	0	0	0	0
Mean	**89**	**9.5**	**0**	**169.5**	**108.5**	**0**	**0**	**0**	**0**
6 i	13	0	0	105	58	0	0	0	0
ii	7	5	0	118	68	0	0	0	0
Mean	**10**	**2.5**	**0**	**111.5**	**63**	**0**	**0**	**0**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 10

	Kromogel			Hydrogum			Blueprint		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**87.1**	**45.8**	**0**	**90.3**	**47.1**	**0**	**0**	**0**	**0**
SD*	**58.6**	**59.0**	**0**	**54.1**	**36.7**	**0**	**0**	**0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.12

CFU/50ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 1

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	78	35	0	18	15	0	73	13	0
ii	89	32	0	24	15	0	55	13	0
Mean	**83.5**	**33.5**	**0**	**21**	**15**	**0**	**64**	**13**	**0**
2 i	81	20	0	9	4	0	42	16	0
ii	99	39	0	5	7	0	52	19	0
Mean	**90**	**29.5**	**0**	**7**	**5.5**	**0**	**47**	**17.5**	**0**
3 i	62	19	0	9	6	0	30	9	0
ii	65	25	0	7	0	0	15	7	0
Mean	**63.5**	**22**	**0**	**8**	**3**	**0**	**22.5**	**8**	**0**
4 i	48	26	0	10	6	0	68	24	0
ii	58	23	0	19	10	0	71	19	0
Mean	**53**	**24.5**	**0**	**14.5**	**8**	**0**	**69.5**	**21.5**	**0**
5 i	39	18	0	8	1	0	24	8	0
ii	31	14	0	10	0	0	19	4	0
Mean	**35**	**16**	**0**	**9**	**0.5**	**0**	**21.5**	**6**	**0**
6 i	30	15	0	2	0	0	21	5	0
ii	22	14	0	4	0	0	19	5	0
Mean	**26**	**14.5**	**0**	**3**	**0**	**0**	**20**	**5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 1

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**58.5**	**23.3**	**0**	**10.4**	**5.3**	**0**	**40.8**	**11.8**	**0**
SD**	**25.6**	**7.4**	**0**	**6.4**	**5.6**	**0**	**22.5**	**6.7**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.13

CFU/50ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 2

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	171	173	0	4	2	0	17	3	0
ii	190	90	0	8	0	0	6	4	0
Mean	**180.5**	**131.5**	**0**	**6**	**1**	**0**	**11.5**	**3.5**	**0**
2 i	312	23	0	29	9	0	6	1	0
ii	283	69	0	42	6	0	5	0	0
Mean	**297.5**	**46**	**0**	**35.5**	**7.5**	**0**	**5.5**	**0.5**	**0**
3 i	50	22	0	34	15	0	17	4	0
ii	52	24	0	52	20	0	15	2	0
Mean	**51**	**23**	**0**	**43**	**17.5**	**0**	**16**	**3**	**0**
4 i	344	112	0	76	12	0	2	0	0
ii	310	120	0	86	22	0	8	1	0
Mean	**327**	**116**	**0**	**81**	**17.5**	**0**	**5**	**1.5**	**0**
5 i	25	6	0	0	0	0	65	10	0
ii	28	6	0	1	0	0	66	15	0
Mean	**26.5**	**6**	**0**	**0.5**	**0**	**0**	**65.5**	**7.5**	**0**
6 i	59	7	0	1	0	0	35	9	0
ii	48	10	0	1	0	0	19	4	0
Mean	**53.5**	**8.5**	**0**	**1**	**0**	**0**	**27**	**6.5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 2

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**156.0**	**55.2**	**0**	**27.8**	**7.25**	**0**	**21.8**	**3.8**	**0**
SD**	**132.8**	**55.2**	**0**	**31.8**	**8.42**	**0**	**22.9**	**2.8**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.14

CFU/50ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 3

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	105	121	0	0	0	0	13	0	0
ii	121	114	0	0	0	0	23	6	0
Mean	**113**	**117.5**	**0**	**0**	**0**	**0**	**18**	**3**	**0**
2 i	56	35	0	0	0	0	4	0	0
ii	68	42	0	0	0	0	2	1	0
Mean	**62**	**38.5**	**0**	**0**	**0**	**0**	**3**	**0.5**	**0**
3 i	72	37	0	0	0	0	28	8	0
ii	58	31	0	0	0	0	23	6	0
Mean	**65**	**34**	**0**	**0**	**0**	**0**	**25.5**	**7**	**0**
4 i	12	7	0	2	0	0	149	24	0
ii	12	5	0	1	0	0	174	35	0
Mean	**12**	**6**	**0**	**1.5**	**0**	**0**	**161.5**	**29.5**	**0**
5 i	44	17	0	13	0	0	21	3	0
ii	35	10	0	0	0	0	17	7	0
Mean	**39.5**	**13.5**	**0**	**6.5**	**0**	**0**	**19**	**5**	**0**
6 i	55	23	0	1	0	0	23	7	0
ii	43	25	0	2	0	0	25	8	0
Mean	**49**	**24**	**0**	**1.5**	**0**	**0**	**24**	**7.5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 3

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**56.8**	**38.9**	**0**	**1.6**	**0**	**0**	**41.8**	**8.8**	**0**
SD**	**33.5**	**40.4**	**0**	**2.5**	**0**	**0**	**59.2**	**10.8**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.15

CFU/50ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 4

Sample*	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	79	25	0	0	0	0	82	27	0
ii	69	24	0	0	0	0	137	61	0
Mean	**74**	**24.5**	**0**	**0**	**0**	**0**	**109.5**	**44**	**0**
2 i	61	22	0	0	0	0	45	9	0
ii	46	15	0	0	0	0	42	9	0
Mean	**53.5**	**18.5**	**0**	**0**	**0**	**0**	**43.5**	**9**	**0**
3 i	59	16	0	1	0	0	135	25	0
ii	65	28	0	1	0	0	101	32	0
Mean	**62**	**22**	**0**	**1**	**0**	**0**	**118**	**28.5**	**0**
4 i	32	15	0	3	0	0	36	10	0
ii	35	9	0	3	0	0	48	13	0
Mean	**33.5**	**12**	**0**	**3**	**0**	**0**	**42**	**11.5**	**0**
5 i	91	47	0	0	0	0	27	2	0
ii	86	55	0	0	0	0	21	3	0
Mean	**88.5**	**51**	**0**	**0**	**0**	**0**	**24**	**2.5**	**0**
6 i	126	16	0	0	0	0	16	4	0
ii	147	37	0	0	0	0	16	3	0
Mean	**136.5**	**26.5**	**0**	**0**	**0**	**0**	**16**	**3.5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 4

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**74.7**	**25.8**	**0**	**0.7**	**0**	**0**	**58.8**	**16.5**	**0**
SD**	**35.5**	**13.4**	**0**	**1.2**	**0**	**0**	**43.9**	**16.4**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.16

CFU/ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 5

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	232	117	0	0	0	0	145	41	0
ii	216	126	0	0	0	0	127	33	0
Mean	**224**	**121.5**	**0**	**0**	**0**	**0**	**136**	**37**	**0**
2 i	97	46	0	0	0	0	315	38	0
ii	98	36	0	0	0	0	287	24	0
Mean	**97.5**	**41**	**0**	**0**	**0**	**0**	**301**	**31**	**0**
3 i	221	82	0	2	3	0	106	22	0
ii	240	85	0	2	1	0	80	13	0
Mean	**230.5**	**83.5**	**0**	**2**	**2**	**0**	**93**	**17.5**	**0**
4 i	168	72	0	0	0	0	72	24	0
ii	160	62	0	1	1	0	74	16	0
Mean	**164**	**67**	**0**	**1.5**	**0.5**	**0**	**73**	**20**	**0**
5 i	77	16	0	6	0	0	74	29	0
ii	47	22	0	5	2	0	62	15	0
Mean	**62**	**19**	**0**	**5.5**	**1**	**0**	**68**	**22**	**0**
6 i	49	13	0	18	2	0	69	19	0
ii	46	13	0	32	9	0	36	15	0
Mean	**47.5**	**13**	**0**	**25**	**5.5**	**0**	**52.5**	**17**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 5

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	137.6	57.5	0	5.7	1.5	0	120.6	24.1	0
SD**	80.3	41.5	0	9.7	2.1	0	93.0	8.1	0

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.17

CFU/ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 6

Sample*	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	356	226	0	14	5	0	15	2	0
ii	375	332	0	13	4	0	21	4	0
Mean	**365.5**	**279**	**0**	**13.5**	**4.5**	**0**	**18**	**3**	**0**
2 i	164	109	0	11	5	0	11	2	0
ii	170	121	0	19	2	0	3	3	0
Mean	**167**	**115**	**0**	**15**	**3.5**	**0**	**7**	**2.5**	**0**
3 i	425	242	0	2	1	0	92	16	0
ii	458	287	0	1	3	0	114	21	0
Mean	**441.5**	**264.5**	**0**	**1.5**	**2**	**0**	**103**	**18.5**	**0**
4 i	123	57	0	2	0	0	264	62	0
ii	106	51	0	0	1	0	296	53	0
Mean	**114.5**	**54**	**0**	**1.5**	**0.5**	**0**	**280**	**57.5**	**0**
5 i	97	42	0	16	3	0	147	37	0
ii	81	47	0	9	4	0	116	46	0
Mean	**89**	**44.5**	**0**	**12.5**	**3.5**	**0**	**131.5**	**41.5**	**0**
6 i	371	54	0	75	32	0	16	2	0
ii	393	168	0	89	43	0	19	4	0
Mean	**382**	**111**	**0**	**82**	**37.5**	**0**	**17.5**	**3**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 6

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	259.9	144.7	0	21.0	8.6	0	92.8	21.0	0
SD**	153.6	102.6	0	30.5	14.2	0	105.1	23.5	0

* Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.18

CFU/50ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 7

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	19	4	0	2	1	0	29	10	0
ii	14	2	0	3	1	0	23	5	0
Mean	**16.5**	**3**	**0**	**2.5**	**1**	**0**	**26**	**7.5**	**0**
2 i	12	1	0	4	0	0	14	4	0
ii	4	1	0	4	1	0	7	3	0
Mean	**8**	**1**	**0**	**4**	**0.5**	**0**	**10.5**	**3.5**	**0**
3 i	9	4	0	0	0	0	24	9	0
ii	12	5	0	0	0	0	34	16	0
Mean	**10.5**	**4.5**	**0**	**0**	**0**	**0**	**29**	**12.5**	**0**
4 i	11	3	0	0	0	0	80	22	0
ii	10	2	0	0	0	0	79	38	0
Mean	**10.5**	**2.5**	**0**	**0**	**0**	**0**	**79.5**	**30**	**0**
5 i	15	6	0	2	0	0	34	7	0
ii	16	4	0	1	0	0	41	12	0
Mean	**15.5**	**5**	**0**	**1.5**	**0**	**0**	**37.5**	**9.5**	**0**
6 i	4	0	0	2	0	0	51	14	0
ii	8	2	0	2	0	0	52	9	0
Mean	**6**	**1**	**0**	**2**	**0**	**0**	**51.5**	**11.5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 7

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**11.2**	**2.8**	**0**	**1.7**	**0.3**	**0**	**39.0**	**12.4**	**0**
SD**	**4.1**	**1.7**	**0**	**1.5**	**0.4**	**0**	**24.0**	**9.2**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.19

CFU/ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 8

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	6	0	0	1	0	0	146	70	0
ii	9	2	0	0	0	0	151	95	0
Mean	**7.5**	**1**	**0**	**1.5**	**0**	**0**	**148.5**	**82.5**	**0**
2 i	93	15	0	1	0	0	307	236	0
ii	88	15	0	2	0	0	341	255	0
Mean	**90.5**	**15**	**0**	**1.5**	**0**	**0**	**324**	**245.5**	**0**
3 i	42	7	0	0	0	0	134	33	0
ii	37	6	0	0	0	0	163	40	0
Mean	**39.5**	**6.5**	**0**	**0**	**0**	**0**	**148.5**	**36.5**	**0**
4 i	7	1	0	0	1	0	39	11	0
ii	11	2	0	0	0	0	26	6	0
Mean	**9**	**1.5**	**0**	**0**	**0.5**	**0**	**32.5**	**8.5**	**0**
5 i	6	5	0	7	4	0	77	19	0
ii	4	0	0	10	1	0	80	25	0
Mean	**5**	**2.5**	**0**	**8.5**	**2.5**	**0**	**78.5**	**22**	**0**
6 i	8	2	0	8	1	0	131	27	0
ii	3	4	0	3	1	0	143	38	0
Mean	**5.5**	**3**	**0**	**5.5**	**1**	**0**	**137**	**32.5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 8

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**26.2**	**4.9**	**0**	**2.8**	**0.7**	**0**	**144.8**	**71.3**	**0**
SD**	**34.2**	**5.3**	**0**	**3.4**	**1.0**	**0**	**99.2**	**89.0**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.20

CFU/ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 9

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	66	33	0	15	4	0	274	56	0
ii	122	41	0	16	5	0	158	85	0
Mean	**94**	**37**	**0**	**15.5**	**4.5**	**0**	**216**	**70.5**	**0**
2 i	60	37	0	6	0	0	39	0	0
ii	43	25	0	6	1	0	54	3	0
Mean	**51.5**	**31**	**0**	**6**	**0.5**	**0**	**46.5**	**1.5**	**0**
3 i	79	45	0	4	5	0	33	15	0
ii	84	25	0	5	5	0	31	7	0
Mean	**81.5**	**35**	**0**	**4.5**	**5**	**0**	**32**	**11**	**0**
4 i	67	33	0	2	0	0	20	8	0
ii	63	28	0	0	0	0	29	10	0
Mean	**65**	**30.5**	**0**	**1**	**0**	**0**	**24.5**	**9**	**0**
5 i	6	5	0	7	0	0	68	31	0
ii	4	0	0	3	0	0	96	29	0
Mean	**5**	**2.5**	**0**	**5**	**0**	**0**	**82**	**30**	**0**
6 i	8	2	0	2	0	0	108	35	0
ii	3	4	0	8	0	0	96	43	0
Mean	**5.5**	**3**	**0**	**5**	**0**	**0**	**102**	**39**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 9

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	**50.4**	**23.2**	**0**	**6.2**	**1.7**	**0**	**83.8**	**26.8**	**0**
SD**	**37.8**	**16.0**	**0**	**4.9**	**2.4**	**0**	**71.3**	**25.6**	**0**

** Standard deviation - from mean CFU counts for each of the six impression samples.

APPENDIX 5.21

CFU/ul FOLLOWING PRE-IMPRESSION RINSE (KROMOGEL)

SUBJECT 10

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
Sample*	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
1 i	38	13	0	0	0	0	76	22	0
ii	36	15	0	0	0	0	98	31	0
Mean	**37**	**14**	**0**	**0**	**0**	**0**	**87**	**26.5**	**0**
2 i	181	168	0	2	1	0	63	7	0
ii	164	151	0	1	0	0	65	14	0
Mean	**172.5**	**159.5**	**0**	**1.5**	**0.5**	**0**	**64**	**10.5**	**0**
3 i	89	34	0	1	2	0	160	42	0
ii	89	33	0	2	0	0	156	48	0
Mean	**89**	**33.5**	**0**	**1.5**	**1**	**0**	**158**	**45**	**0**
4 i	110	70	0	3	1	0	78	21	0
ii	130	42	0	1	1	0	94	31	0
Mean	**125**	**56**	**0**	**2**	**1**	**0**	**86**	**26**	**0**
5 i	92	2	0	1	2	0	87	17	0
ii	86	17	0	13	2	0	105	23	0
Mean	**89**	**9.5**	**0**	**7**	**2**	**0**	**96**	**20**	**0**
6 i	13	0	0	3	0	0	112	40	0
ii	7	5	0	1	0	0	98	35	0
Mean	**10**	**2.5**	**0**	**2**	**0**	**0**	**105**	**37.5**	**0**

* Two microbial suspensions (i and ii) were prepared from each of the six impression samples.

MEAN AND STANDARD DEVIATION VALUES - SUBJECT 10

	Kromogel Standard			Kromogel Corsodyl Rinse			Kromogel Water Rinse		
	BA	MSA	SDA	BA	MSA	SDA	BA	MSA	SDA
Mean	87.1	45.8	0	2.3	0.8	0	99.3	27.6	0
SD*	58.6	59.0	0	2.4	0.8	0	31.8	12.3	0

** Standard deviation - from mean CFU counts for each of the six impression samples.

CHAPTER 6

THE DIMENSIONAL STABILITY OF FOUR CONTEMPORARY ALGINATE IMPRESSION MATERIALS AND THE EFFECT OF IMMERSION DISINFECTION

INTRODUCTION

Although it has been reported that dimensional accuracy of alginate impression materials can be comparable with the best of the elastomeric materials [158], it is widely accepted that they are subject to dimensional change with time [156,157]. On storage, alginate materials exhibit the phenomena of imbibition and syneresis, and it has been recommended that casts are poured within twenty minutes of alginate impressions being recorded, in order to limit dimensional change [155,157]. However, it is an increasing trend for technical work to be undertaken by laboratories distant from the dental surgery and a considerable time interval may elapse between recording impressions and pouring casts from them. There is little published information on the dimensional stability properties of contemporary alginate impression materials following storage, or the effect of storage following recommended immersion disinfection procedures.

AIMS OF STUDY

The aim of this study was to measure the linear accuracy, relative to time, of four contemporary alginate materials (including two containing disinfection agents), and to examine the effects of disinfection by immersion, followed by storage, on linear dimensional accuracy.

6.1 MATERIALS AND METHODS

6.1.1 MEASURING STANDARD

A cast steel standard with abutments in the canine and first molar regions (Fig 1), representative of a partially dentate arch, was used for recording distance measurements. Intersecting lines in the form of a cross, were cut on the flat occlusal surface of each of the four abutments (Fig 2), and the most clearly defined of the intersection points on each abutment was identified and used as a reference location.

6.1.2 IMPRESSIONS

For each of the materials under test, eight impressions were recorded and analysed (Fig 3). Customised impression trays with palatal stops, designed to seat on the die in a stable and reproducible position, and to give a uniform thickness (1.2mm) of impression material between the die and the impression tray (Fig 4), were made in heat-cured acrylic resin. Trays were perforated, and handles were incorporated to help remove the impressions from the die. The steel standard was held in a stable position on a template, and the impression trays were seated fully on the palatal stops during the recording of impressions (Figs 5 and 6).

In all, four alginate impression materials were tested (Table 4.1). Xantalgin and Palgat were chosen because they are materials which have been found to be satisfactory on clinical use within the Department of Prosthodontics at Glasgow Dental School, over a number of years. Hydrogum and Blueprint with Antibac, which contain antimicrobial agents (Chapters 4 and 5), have recently been marketed as self-disinfecting alginates. The materials were mixed according to the manufacturers' instructions, loaded into an impression tray and seated on the steel standard for seven minutes to allow complete setting, before removal.

The alginate impression material Kromogel (Wright Dental, Scotland), which was used in previous studies examining the carriage and elimination of microorganisms from impression surfaces, was found to be unsuitable for use in this experiment. Because of its white colour, it was difficult to identify the fine detail of the impression surface under the lighting conditions used in analysis of specimens on the microscope stage (6.1.6).

6.1.3 DISINFECTION

For each of the four materials tested, impressions were divided into two groups such that measurement was carried out on four standard impression specimens without prior

immersion, and the other four impression specimens were immersed in disinfectant solution before analysis, as follows:

Group A (16 impressions : 4 of each material)

- Impression specimens were measured immediately after they were made, and again after 24 and 48 hours.

Group B (16 impressions : 4 of each material)

- Impression specimens were immediately immersed for 30 minutes in a disinfectant solution made by dissolving 4.6mg sodium dichloroisocyanurate (HAZ Tablet, Guest Medical, Sevenoaks, England) in one litre of water (giving 1,000 ppm available chlorine), before the test dimensions were measured. These were repeated after 24 and 48 hours.

6.1.4 STORAGE

Between measurements all impression specimens were stored under standardised conditions, representative of those found in general dental practice, by covering them with a damp gauze swab and sealing in a plastic bag.

6.1.5 DIMENSION MEASUREMENTS

Measurements of the dimensions between test points were carried out using a Reflex microscope (Reflex Measurement

Ltd, Butleigh, England) at a magnification of x40. The apparatus claimed measurement accuracy of 0.002mm in the X-axis and Y-axis, and 0.004mm in the Z-axis. The microscope was linked to a microcomputer, programmed to allow data gathering and calculation of distances between test points. Thus the dimensional accuracy, and the stability characteristics of the impression materials, were measured without the additional step of pouring casts.

6.1.6 MEASUREMENT PROCEDURES

As the spatial orientation of the impression specimens on the microscope stand could not be exactly similar in each case, calibration of the measuring apparatus was carried out prior to recording distance measurements each time an impression was examined. A re-orientation programme was employed in order that distances measured would relate correctly to each other. After calibration, the location of each test point was recorded, in three axes, by optically aligning a 5um spot (from a light emitting diode) on to the point. This procedure was repeated for each of the four reference points in turn, in a fixed sequence, to give values for the six distances between the reference points (Fig 6.7). Using these procedures, each impression sample was examined on three occasions. The measuring standard was similarly assessed three times. Data were collected on the microcomputer linked to the microscope.

6.1.7 STATISTICAL ANALYSIS

For each of the six measured distances (A-F), nine groups of data were compared, *ie* the die standard plus four impression materials, disinfected and non-disinfected. Analysis of variance, contrasting variance within each of the groups with variance between the groups, was used in statistical evaluation of the data.

Analysis of values for each of the six distance measurements (A-F) for each impression sample allowed:

a) Examination of the accuracy and the effects of storage time on the dimensional characteristics of each material, and comparison between materials.
b) Examination of the effects of immersion in a powerful chlorinating disinfectant agent on the dimensional characteristics of each material, and a comparison between materials for this effect.

In the statistical analysis, each measurement obtained for each impression was considered. Mean values were calculated using all 12 observations (4 impressions x 3 repeat measurements) for each impression material.

6.2 RESULTS

Mean distances measured between the test points on the steel standard are shown in Table 6.2. Appendices 6.1a - 6.6d show the measured distances between the test points, on both disinfected and non-disinfected impressions. Mean values for distance A, for each impression material (non-immersed and immersed) at the time of making impressions, and after 24 and 48 hours, are shown in Table 6.3. Tables 6.4 - 6.8 show the corresponding values for distances B to F respectively.

In Figure 6.8a the measured mean values of distance A, plotted against time for the four standard materials (each plotted point represents the mean of twelve observations; 4 impressions x 3 measurements) are shown, while Figure 6.8b shows the measured mean values of distance A plotted against time, for the four materials following immersion. In Figures 6.9a - 6.13b the corresponding plots for distances B - F respectively are illustrated. Most of the observed distances on the impressions are less than the actual distances on the test standard, indicating a reduction for each material for almost all distances at each time point.

For Distance Measurement A, non-disinfected impressions of Xantalgin, Palgat and Hydrogum (Fig 6.8a) show

impressive accuracy and dimensional stability, and Blueprint, which appeared to be less dimensionally stable, had excellent initial accuracy and showed a mean dimensional change of less than 0.2mm over the 48 hour test period. Immersion disinfection reduced the initial accuracy of all four impression materials (Fig 6.8b), with Blueprint being the least accurate immediately after the impressions were made, and showing the biggest dimensional change over 48 hours. Palgat, which was the most accurate material, also showed the greatest dimensional stability, and after 48 hours there was less than 0.1mm difference between it and the test die measurement.

For Distance Measurement B, non-disinfected impressions of Xantalgin and Hydrogum were particularly accurate and stable; the other two materials performed less well (Fig 6.9a). In all cases, immersion adversely affected initial accuracy, which was found to have improved after 24 hours in Palgat, Hydrogum and Blueprint impressions (Fig 6.9b).

For Distance Measurement C, non-disinfected impressions of all four materials showed excellent initial accuracy (Fig 6.10a). There was little to choose between Palgat, Xantalgin and Hydrogum for dimensional stability over the 48 hour experimental period, and Blueprint, which was the

least stable, showed a change in dimension over 48 hours of less than 0.3mm. The initial accuracy of Palgat and Hydrogum were least affected by immersion disinfection (Fig 6.10b), and Palgat, in particular, retained a high level of dimensional stability over the 48 hour test period. Immersion was found to affect the dimensional stability of Blueprint to a greater degree than the other materials.

For Distance Measurement D, non-disinfected impressions of Palgat were particularly accurate and dimensionally stable (Fig 6.11a). The initial accuracy of the other materials was not as striking as for the other dimension measurements. Blueprint performed least well of the four impression materials although for all three measurements in the 48 hour test period, the accuracy of the material was within 0.1mm of the test standard. Immersion affected the initial accuracy of Palgat, although this was improved after 24 hours (Fig 6.11b). The other materials (with poorer initial accuracy) were less affected by immersion than Palgat, and Blueprint was the least accurate and least stable material.

For Distance Measurement E, non-disinfected impressions of Xantalgin, Palgat and Hydrogum showed good initial accuracy and dimensional stability over the 48 hour test period (Fig 6.12a). Blueprint was less accurate and less

stable than the other materials over the 48 hour test period. Disinfected impressions of Palgat (Fig 6.12b) were particularly accurate and stable, and Xantalgin and Hydrogum impressions, moderately so. Following immersion, impressions of Blueprint were found to be less accurate than the other materials at initial assessment, and after the test period of 48 hours there was a difference of more than 0.3mm between the mean value for Distance Measurement E with immersed Blueprint impressions, and the test standard.

In the values for Distance Measurement F (Fig 6.13a), the accuracy and dimensional stability of the non-disinfected Xantalgin, Palgat and Hydrogum impressions, with respect to Blueprint, were highlighted. Following disinfection, Palgat was again seen to be more accurate and stable than the other materials, and Blueprint was the least accurate and least dimensionally stable material (Fig 6.13b).

For each distance A-F separately, an analysis of variance was carried out with the type of material, time interval after the impressions were made (0hr, 24hr or 48hr), and immersion or non-immersion as fixed factors, and each impression specimen as the variable factor. For each material, the variability between the four impression specimens was assessed, and compared with the variability within replicate measurements for each impression.

The results were subjected to ANOVA analysis and the main findings were that:

- for each of the materials, a storage time interval was significant in producing significant dimensional change for each distance, except B.
- immersion produced significant dimensional change for all distances, except D.
- there was a significant material and time interaction for all distances, except B.
- there was a significant time and immersion interaction for distances A, C, E & F.

Material and immersion interaction was not significant for any of the distances, indicating there was no evidence that the effect of immersion is different for the four materials. Since the time effect, and the time/material and the time/immersion interactions were highly significant for most distances A-F, further analysis was carried out to compare the materials and to examine the effect of immersion at each time point separately. The results are shown in Table 6.9 for immersion, and Table 6.10 for materials.

Data in Table 6.9 show the size of the mean immersion effect for each distance at each time point. The significance of the immersion effect was tested at each

time point for each distance using ANOVA. All except one of these means are negative, indicating that additional dimensional change had occurred with immersion. The effect is most pronounced for distances A, B, E and F, and is generally larger at 48 hours than at 0 or 24 hours. Data in Table 6.10 allow comparison of the four materials for each distance at each time point. The significance of the difference between materials was tested at each time point for each distance using ANOVA. For all distances at time 24 and 48 hours, and for distances B, C & F at time 0, the difference between the materials was highly significant. For all 18 comparisons, Blueprint showed the greatest mean difference between the actual and measured distances, and was clearly the poorest. For 16 of the 18 comparisons, Palgat showed the smallest mean differences between actual and measured distances, and was clearly the best; there was little difference between Hydrogum and Xantalgin. There was no obvious trend over time for Xantalgin, Palgat or Hydrogum but Blueprint showed greater shrinkage at 24 and 48 hours for distances A, C, E and F.

The variability of the replicate measurements on each impression, and the variability between impressions, were established from the ANOVA for each material for each distance. There was no evidence that these differed consistently between materials, distances or time points.

The overall estimate of the standard deviation of the replicates was 0.047mm, and the overall estimate of the between-impressions standard deviation, was 0.062mm.

6.3 DISCUSSION

It was not the aim of this study to compare or contrast the clinical characteristics of the impression materials tested, but rather to examine each impression material under differing experimental conditions, and it is recognised that the clinical performance of materials may vary with different batches from the manufacturer, and that the performance of materials in the laboratory setting is not comparable with use in the treatment of patients. Nonetheless, it was intended in the design of the study, to ensure that the experimental conditions were representative of those found in general dental practice, and the data were gathered in such a way as to allow statistical assessment of the information obtained.

From the study it seemed that the accuracy of impressions was, to some extent, more dependant upon the choice of material, than on the length of storage time before casts were made. In almost every case, the dimensional change following storage of non-immersed impressions of Palgat, Xantalgin or Hydrogum, was less than the difference

between different materials and the dimensions of impressions made in these materials were affected by storage to a surprisingly small degree. The use of Blueprint was an important factor in producing a spread of values for reproduction of the dimensions of the die standard, particularly after storage for either 24 or 48 hours.

The cast steel test die used in the study was chosen to represent dimensions which may typically be encountered in the construction of a cast cobalt chromium base for a removable partial denture, master impressions for which are normally taken using alginate impression materials of the type used in this study. The conditions under which impressions were stored for up to 48 hours, were believed to represent those likely to be found in dental practice, rather than idealised conditions of the dental materials' laboratory. It seems likely that the clinical success of treatment would not be affected adversely by storage of non-immersed impressions of Xantalgin, Palgat or Hydrogum under the conditions described in the study, but there was a significantly greater likelihood that storage of Blueprint impressions would have a detrimental effect on the treatment outcome.

In non-disinfected impressions, there was greater variability between the impression materials for test

FIGURE 6.1

The cast steel die, with abutments in the canine
and first molar regions,
used for recording impressions,
and as the standard for distance measurements.

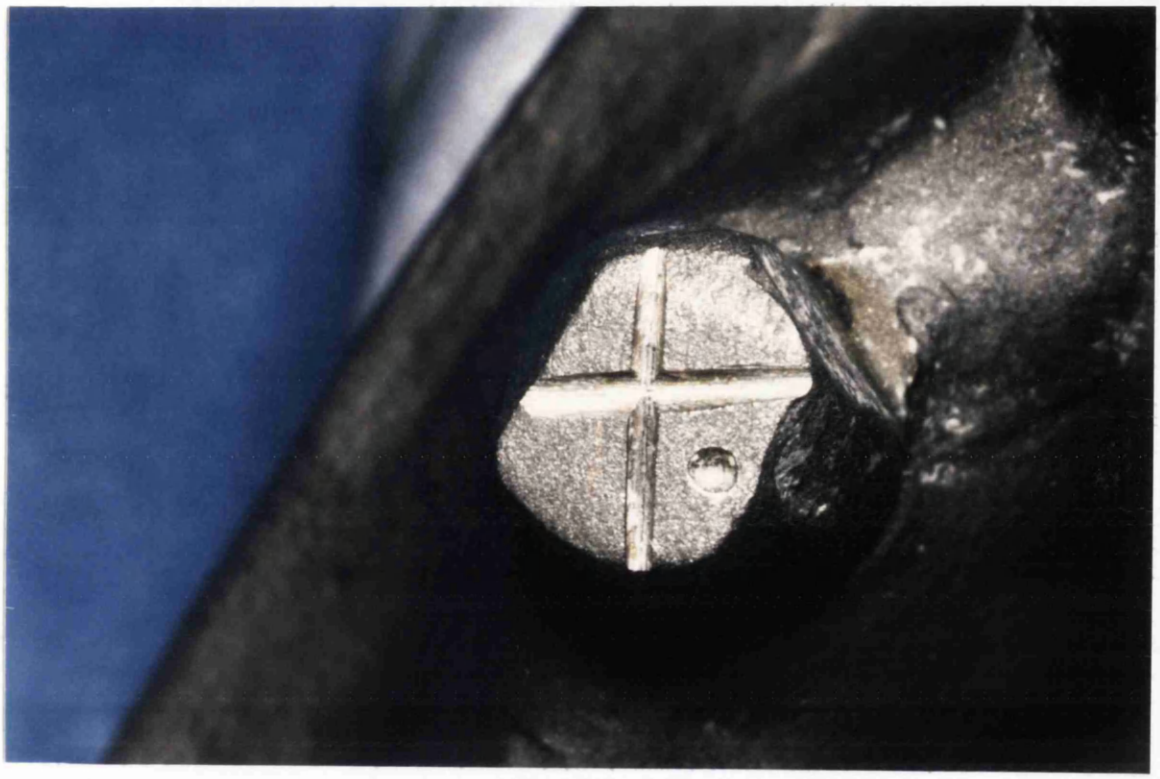


FIGURE 6.2

Intersecting lines were cut
on each abutment of the die standard
to provide a reference point for measurement.

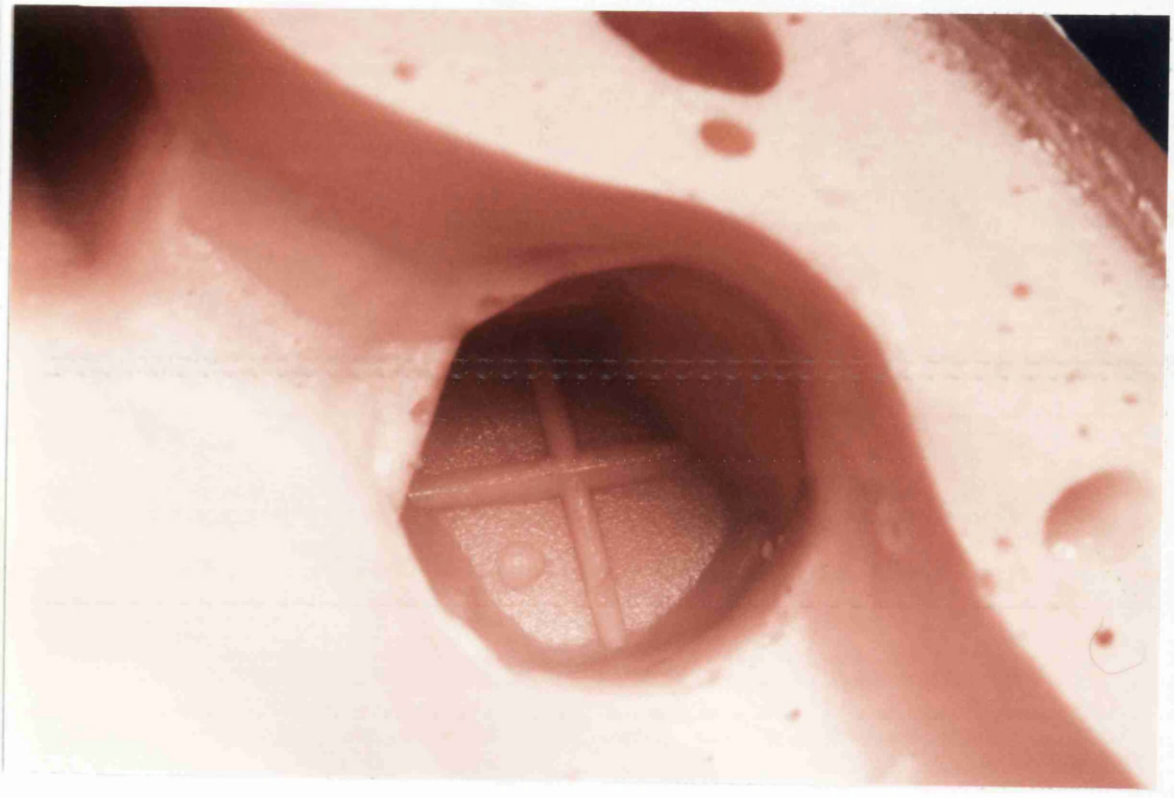


FIGURE 6.3

Detail of an alginate impression of the abutment shown in Fig 6.2.
Measurement was made from the point of intersection, identified as the most clearly defined on the abutment surface.

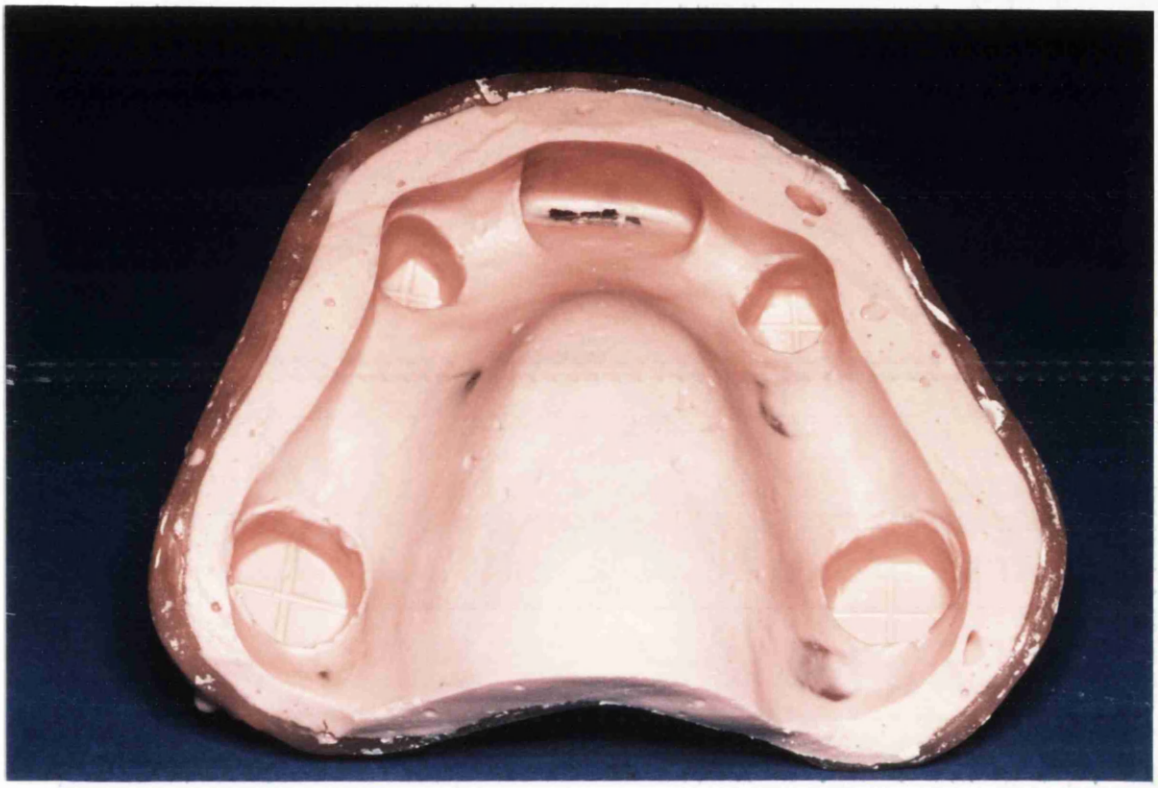


FIGURE 6.4

An alginate impression showing four abutments with markings for measurement. Acrylic resin stops, to facilitate positive seating of the impression tray on the steel die, are identifiable in the incisor, molar and premolar regions.

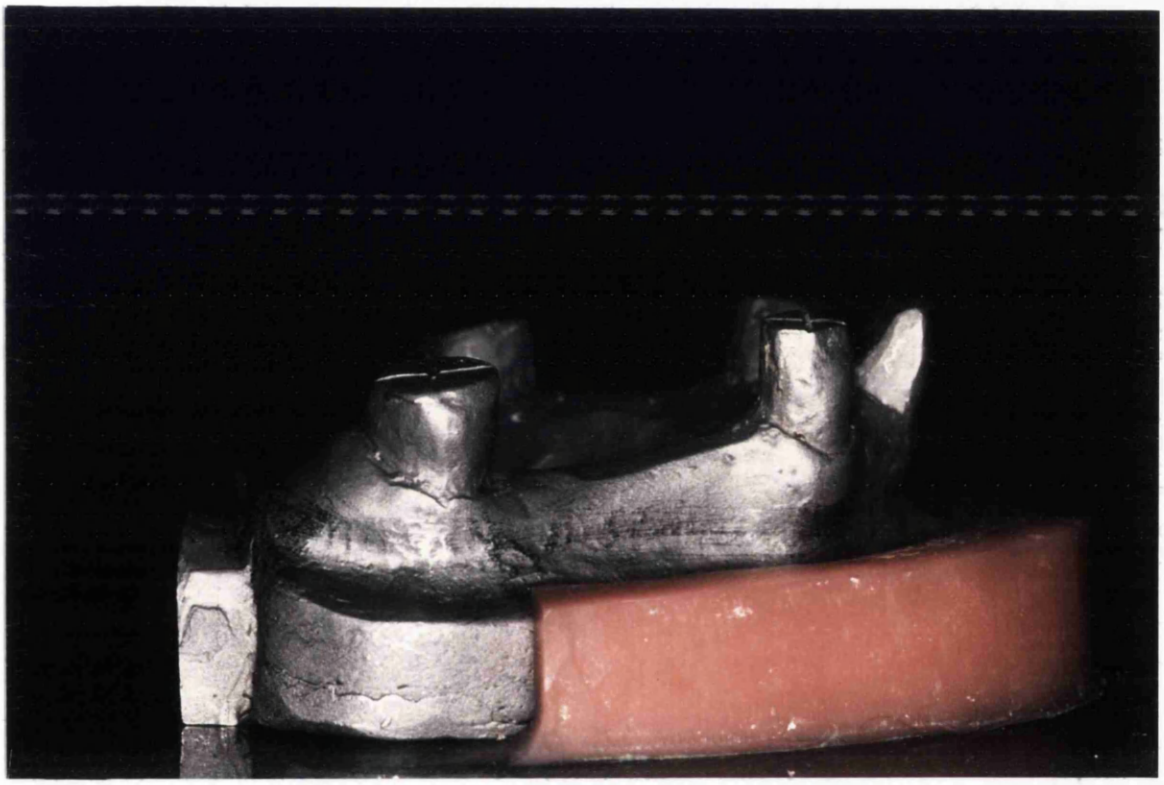


FIGURE 6.5

Steel standard held in position on template.

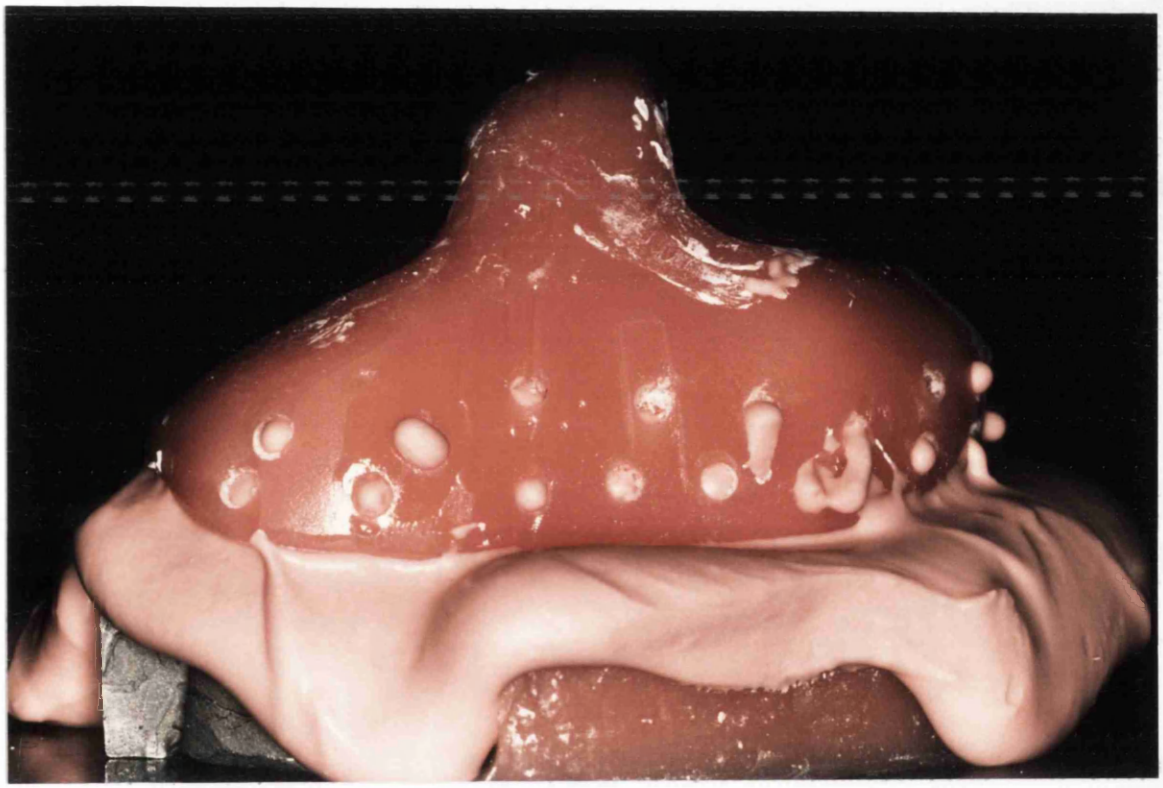


FIGURE 6.6

Impression tray seated on palatal stops during recording of impression.

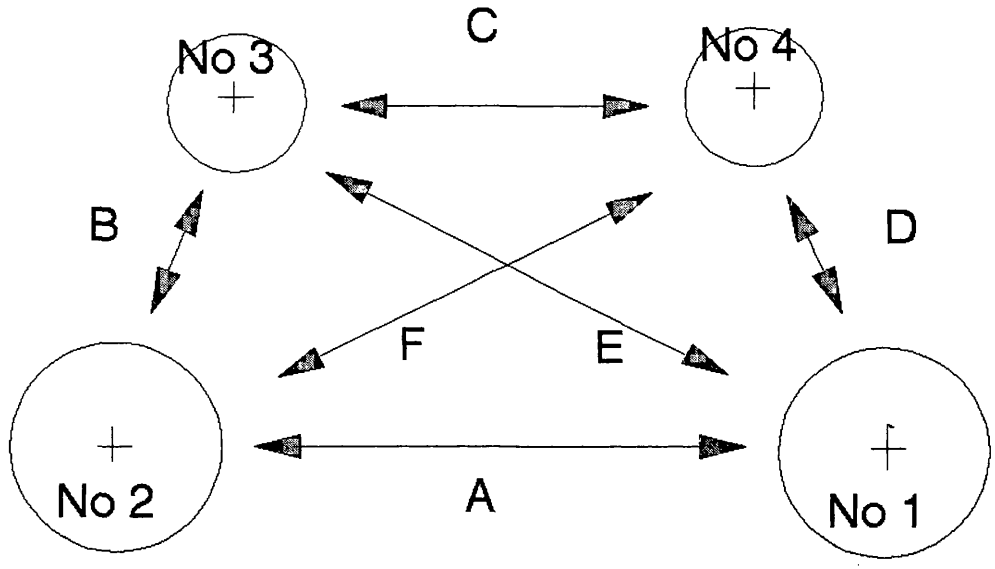


FIGURE 6.7

Diagrammatic representation of

reference points 1-4

distance measurements A-F

Figure 6.8a

Distance Measurement : A

Non-Disinfected Impressions

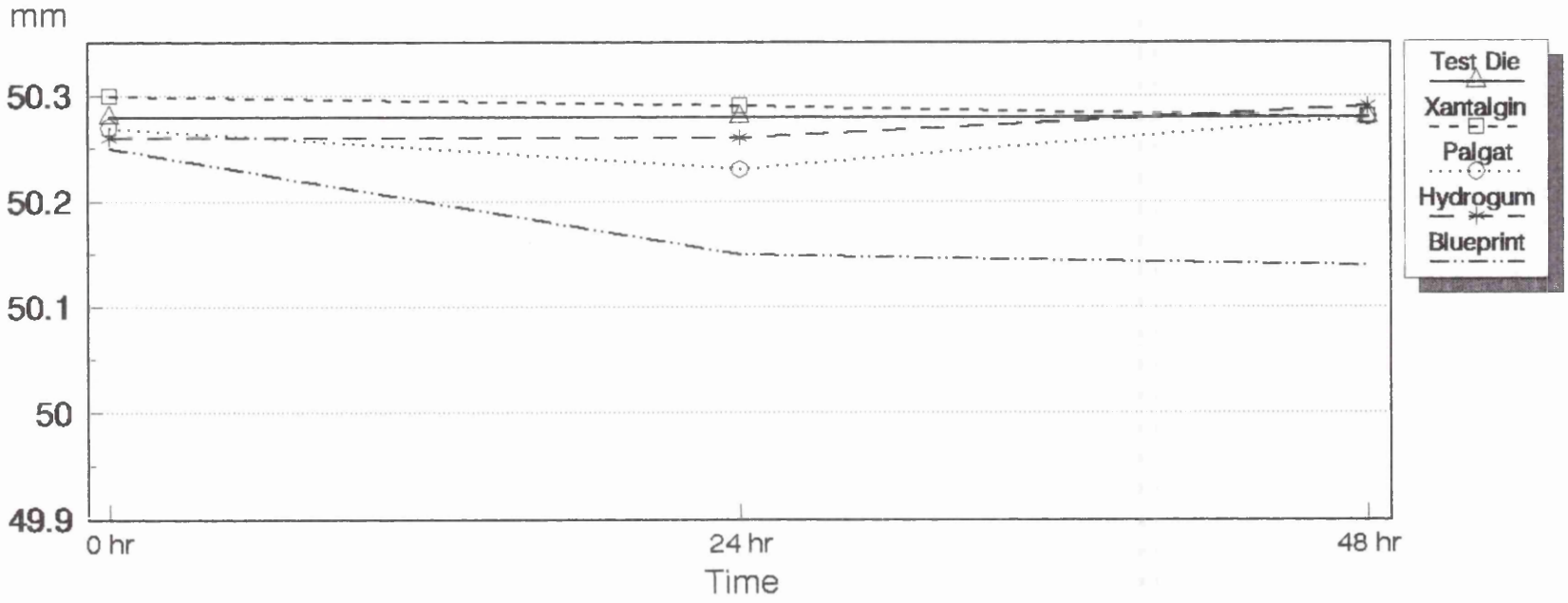


Figure 6.8b

Distance Measurement : A

Disinfected Impressions

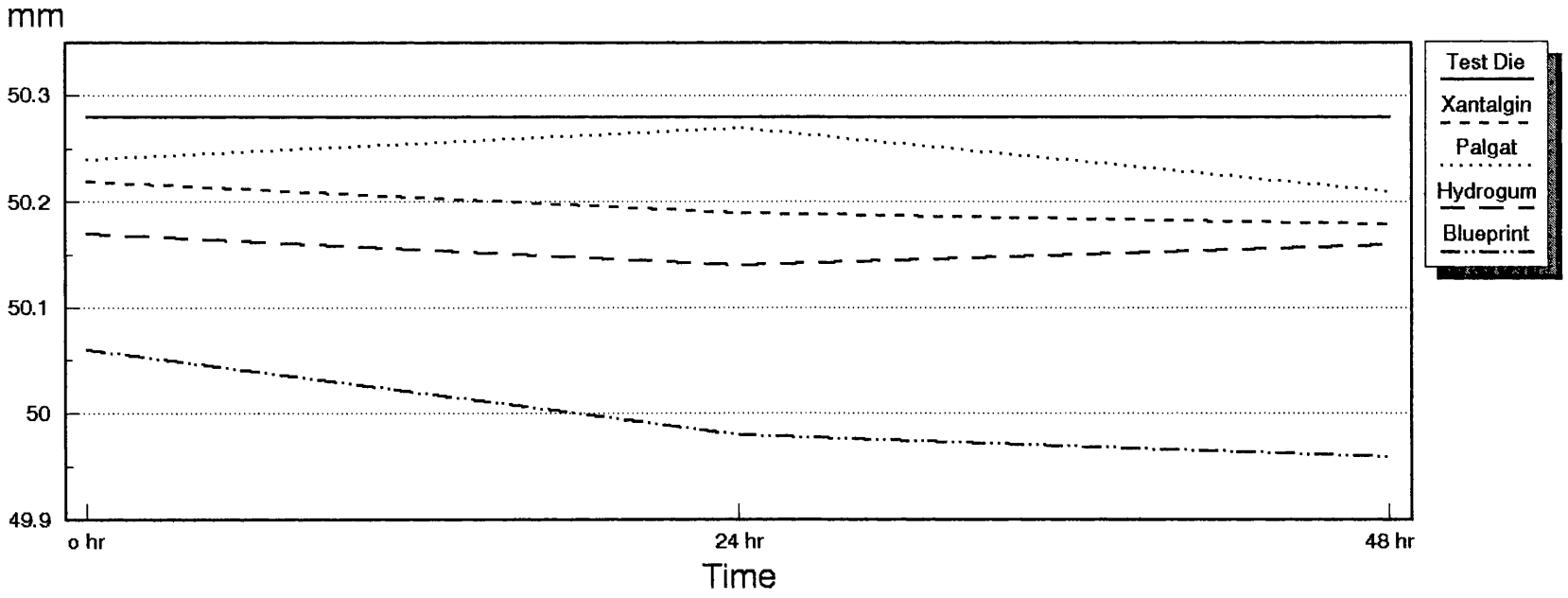


Figure 6.9a

Distance Measurement : B

Non-Disinfected Impressions

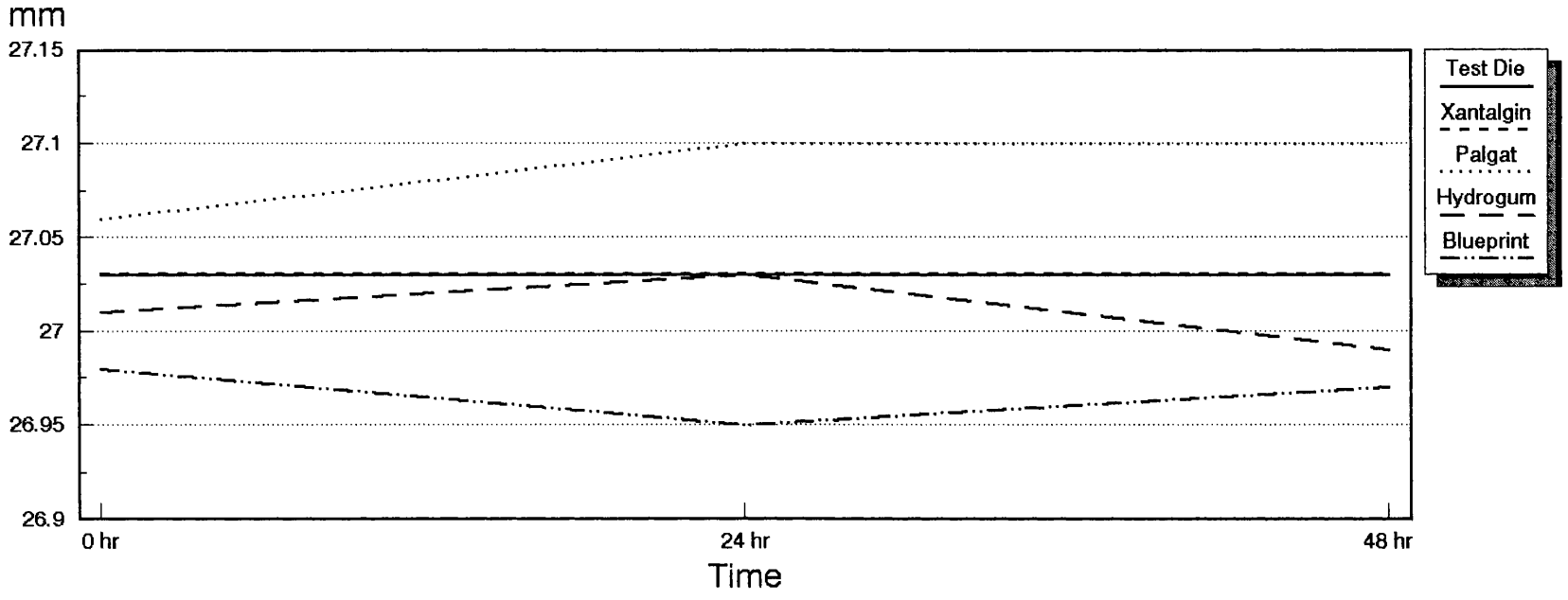


Figure 6.9b

Distance Measurement : B

Disinfected Impressions

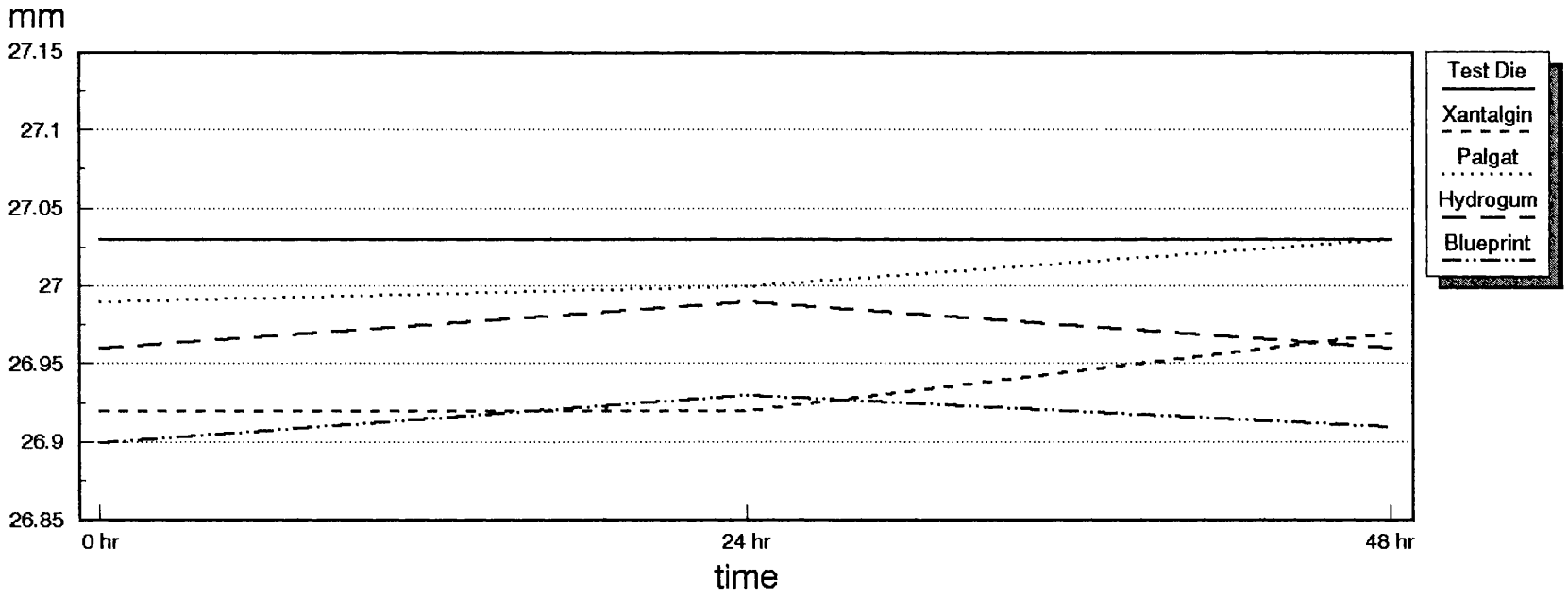


Figure 6.10a

Distance Measurement : C

Non-Disinfected Impressions

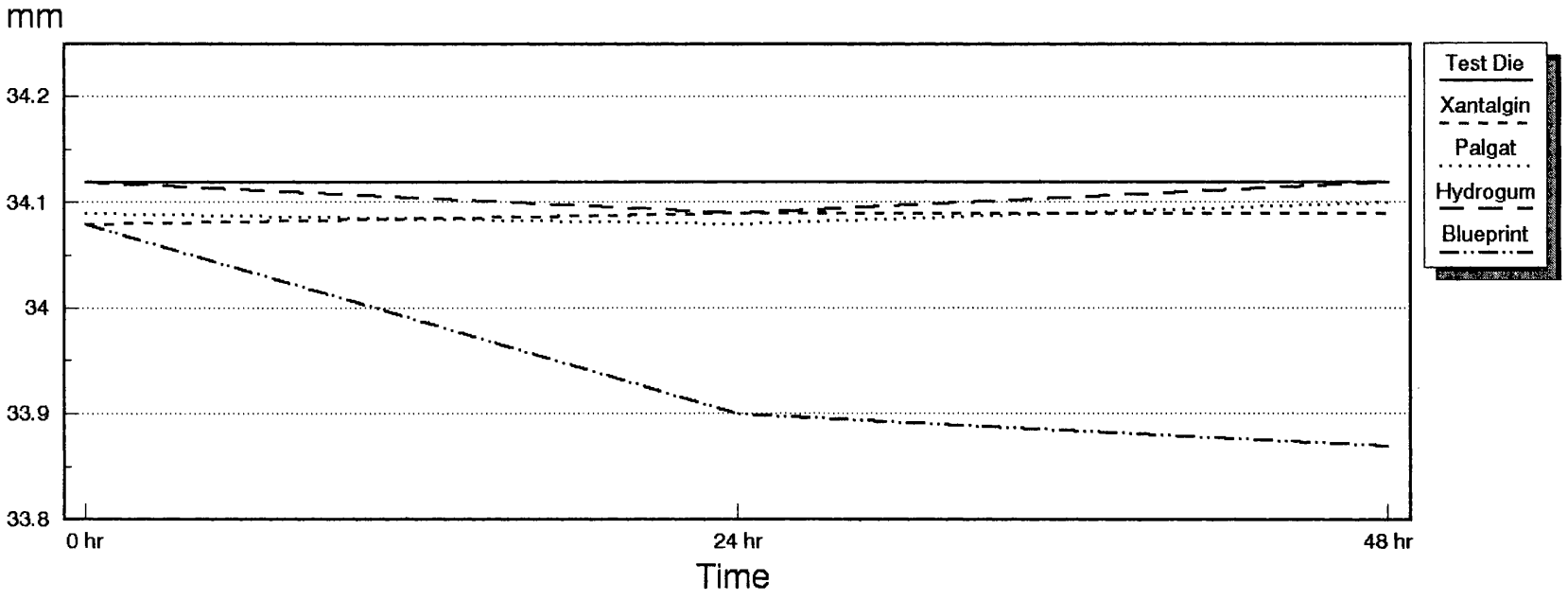


Figure 6.10b

Distance Measurement : C

Disinfected Impressions

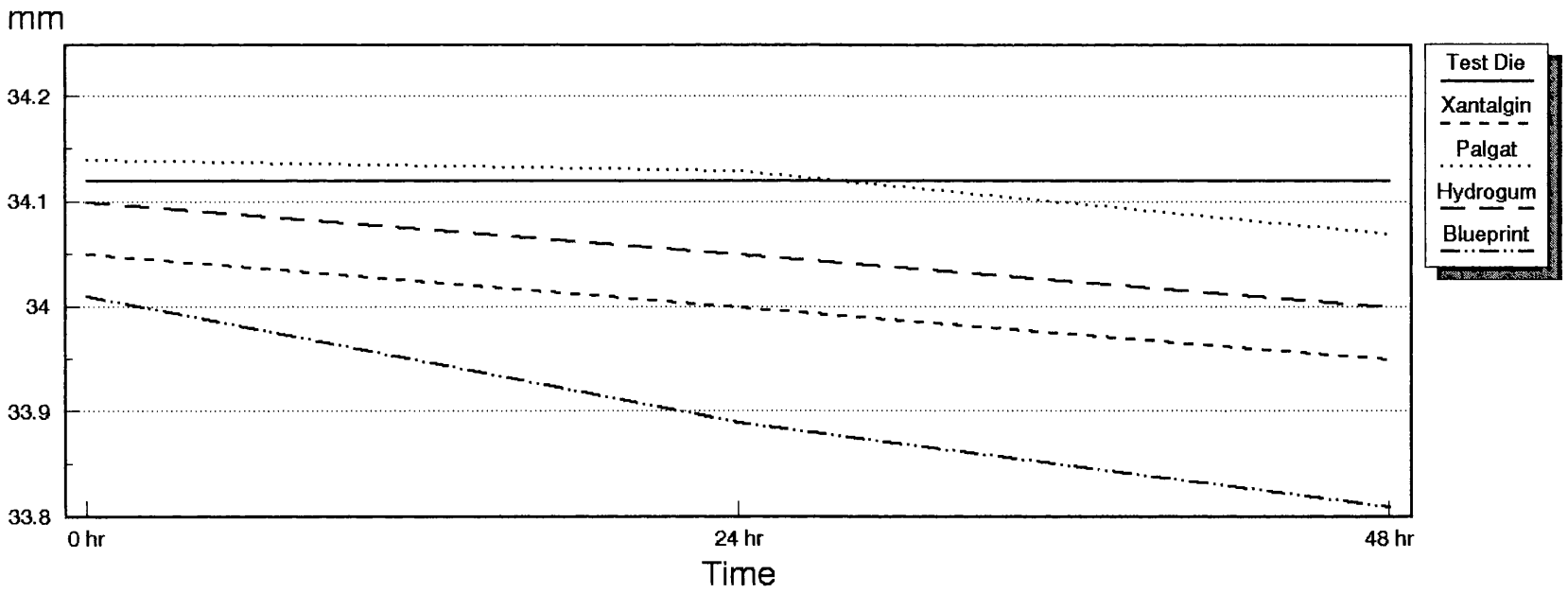


Figure 6.11a

Distance Measurement D
Non-Disinfected Impressions

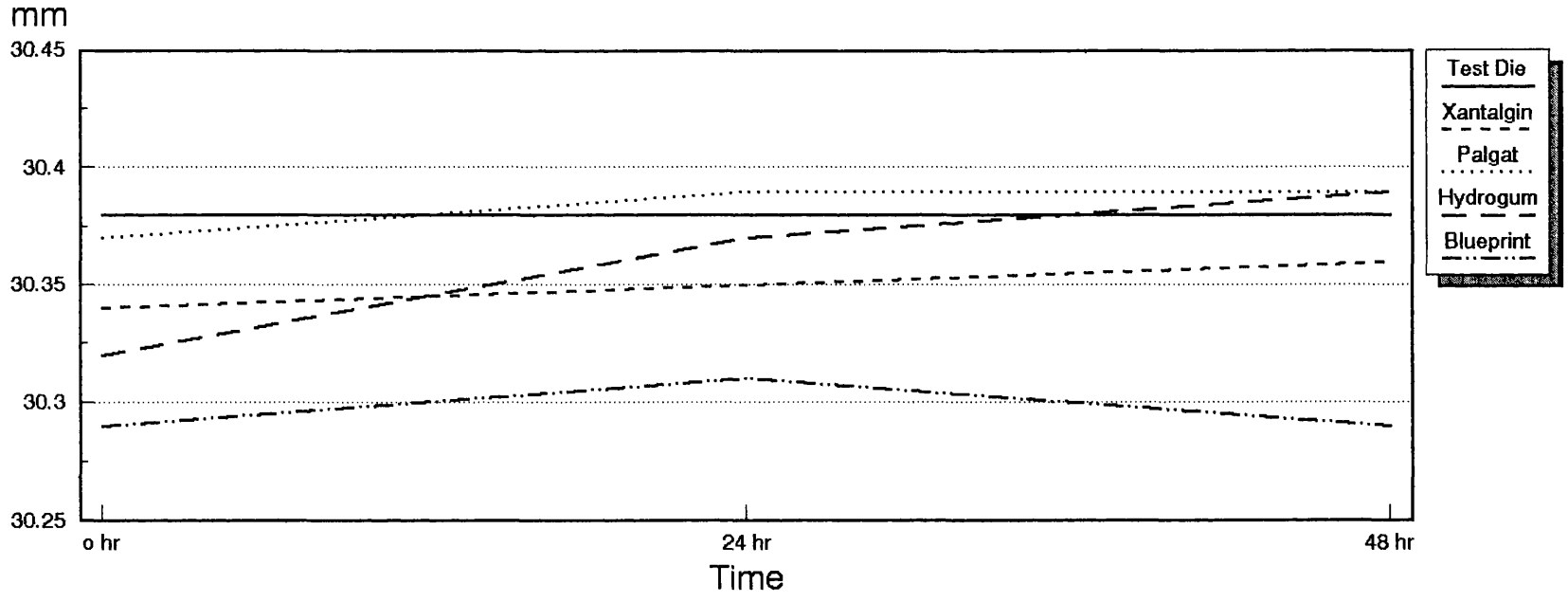


Figure 6.11b

Distance Measurement D
Disinfected Impressions

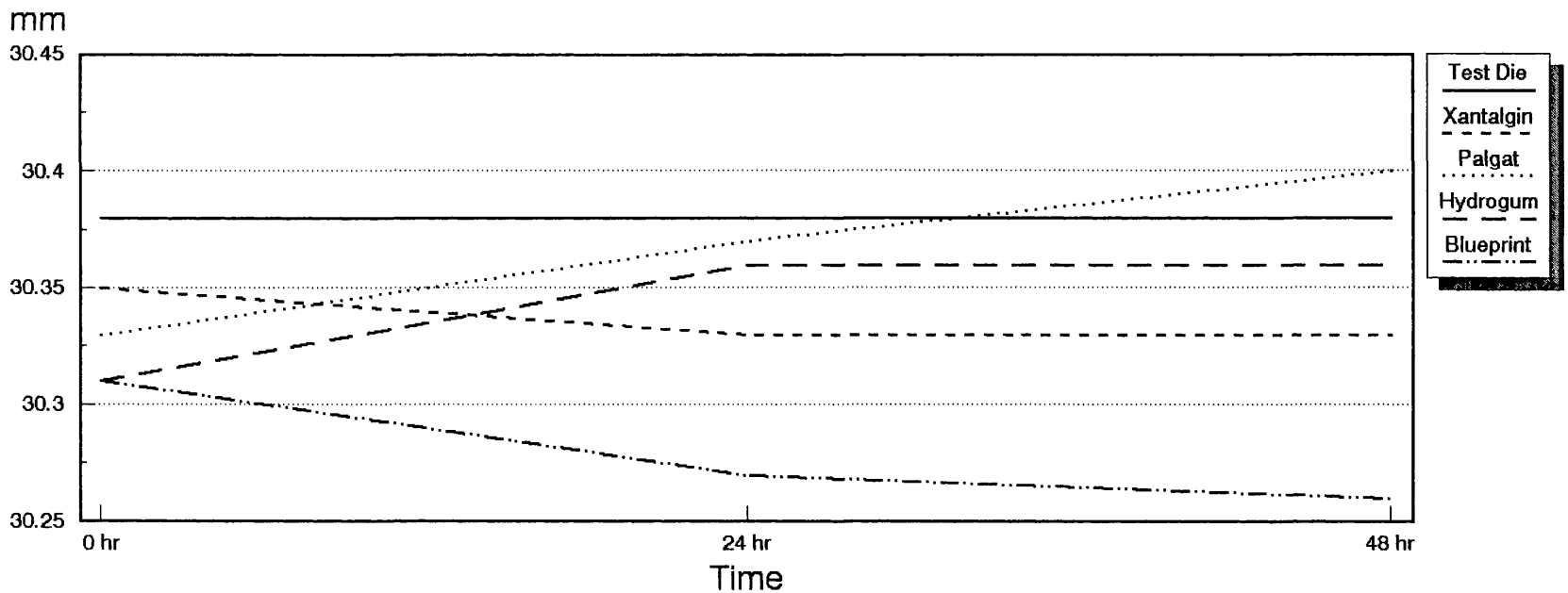


Figure 6.12a

Distance Measurement : E

Non-Disinfected Impressions

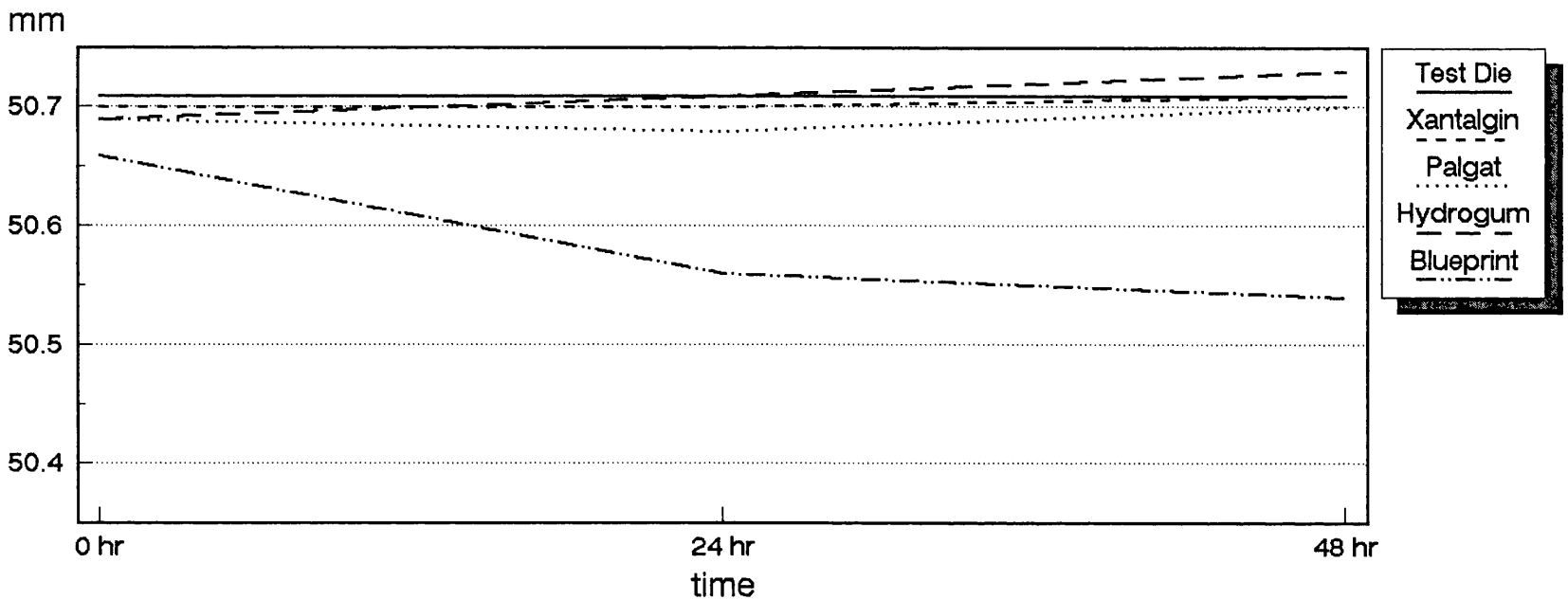


Figure 6.12b

Distance Measurement : E

Disinfected Impressions

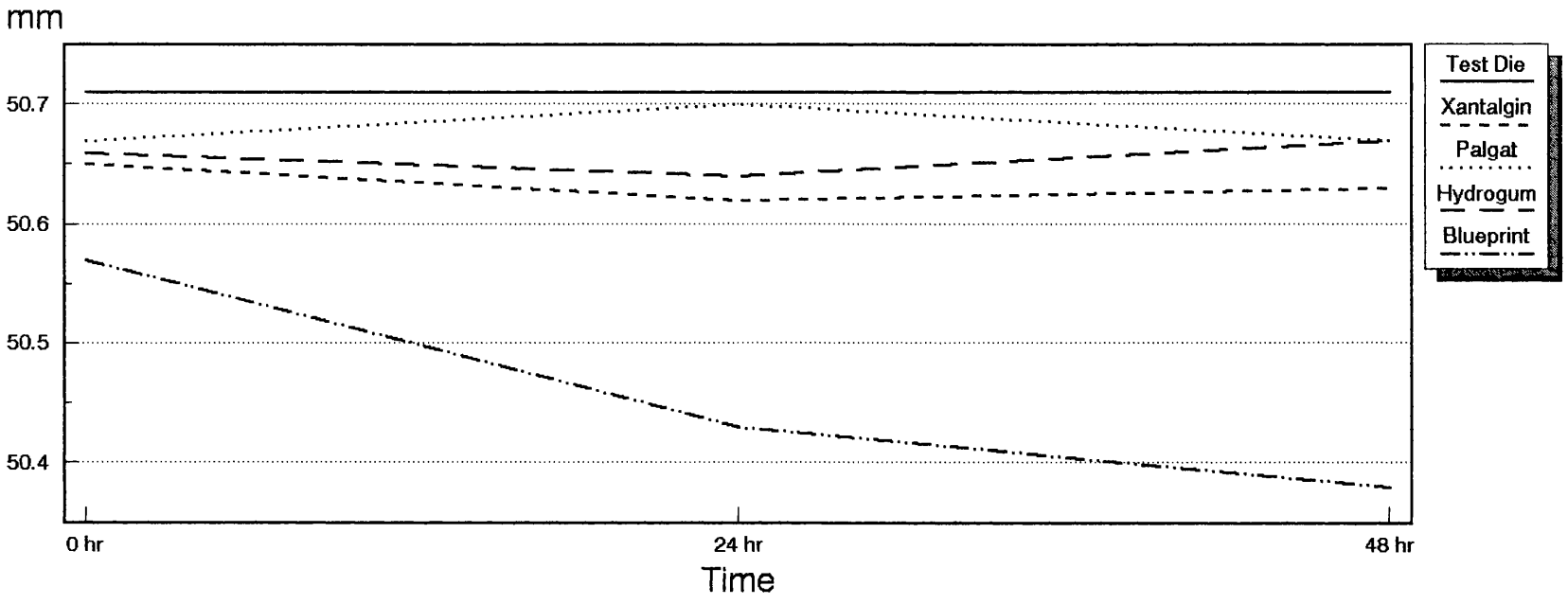


Figure 6.13a

Distance Measurement : F

Non-Disinfected Impressions

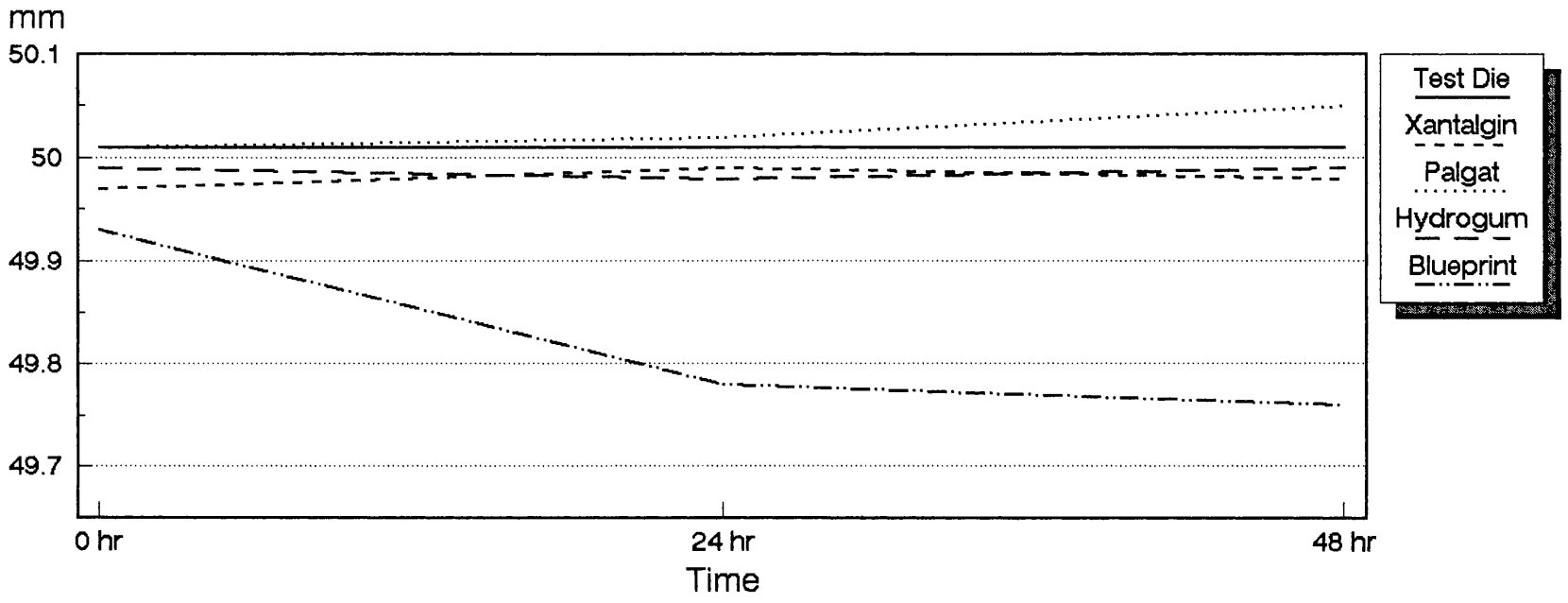


Figure 6.13b

Distance Measurement : F

Disinfected Impressions

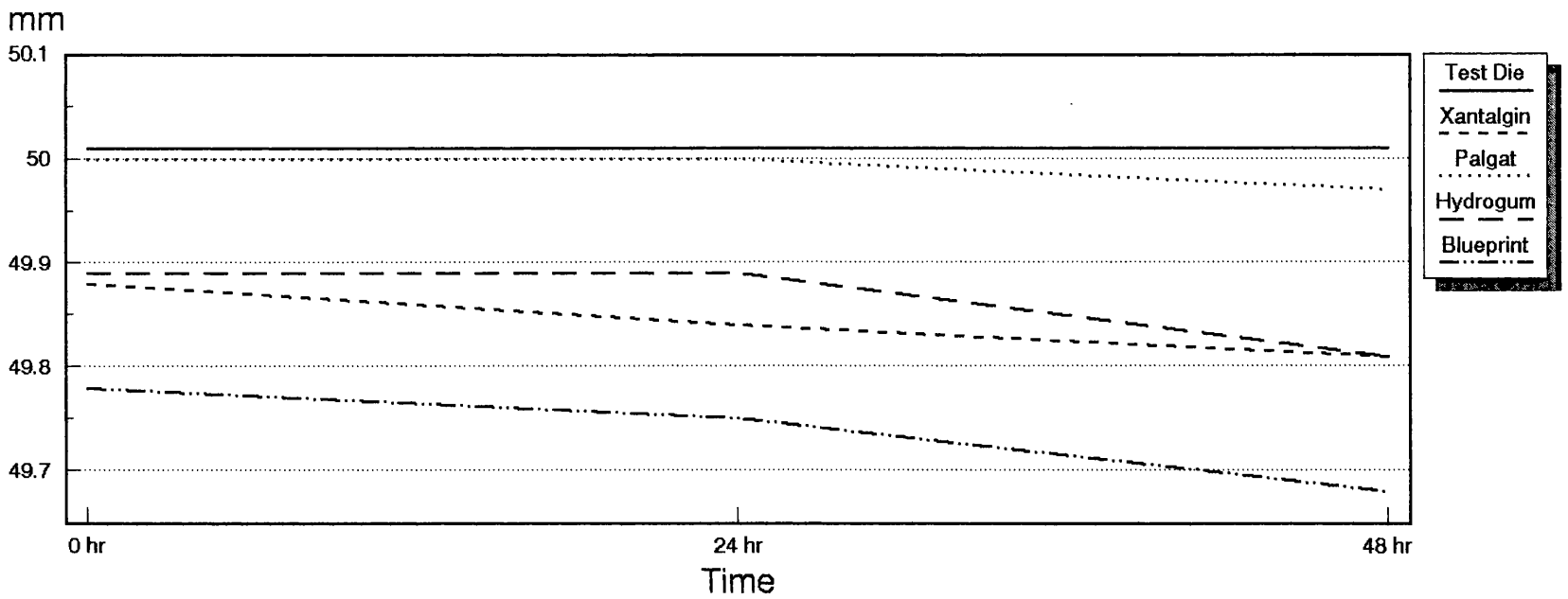


TABLE 6.1

ALGINATE IMPRESSION MATERIALS USED IN STUDY

Trade Name	Manufacturer
(a) Xantalgin	Bayer Dental, Leverkusen, Germany.
(b) Palgat	ESPE, Seefeld, Germany.
(c) Hydrogum	Zhermack, Badia Polesine, Italy.
(d) Blueprint with Antibac	De Trey Dentsply Co, Konstanz, Germany.

TABLE 6.2

DIMENSIONS OF TEST DIE

DISTANCE MEASUREMENT A Mean SD	 50.28 (0.01)
DISTANCE MEASUREMENT B Mean SD	 27.03 (0.03)
DISTANCE MEASUREMENT C Mean SD	 34.12 (0.025)
DISTANCE MEASUREMENT D Mean SD	 30.38 (0.01)
DISTANCE MEASUREMENT E Mean SD	 50.71 (0.01)
DISTANCE MEASUREMENT F Mean SD	 50.01 (0.02)

TABLE 6.3

ALGINATE IMPRESSION MATERIALS

DISTANCE MEASUREMENT : A (mm)

	0 hour	24 hours	48 hours
XANTALGIN (Standard)			
MEAN	50.30	50.29	50.28
SD	(0.05)	(0.03)	(0.03)
XANTALGIN (Disinfected)			
MEAN	50.22	50.19	50.18
SD	(0.05)	(0.05)	(0.06)
PALGAT (Standard)			
MEAN	50.27	50.23	50.28
SD	(0.02)	(0.02)	(0.03)
PALGAT (Disinfected)			
MEAN	50.24	50.27	50.21
SD	(0.03)	(0.04)	(0.07)
HYDROGUM (Standard)			
MEAN	50.26	50.26	50.29
SD	(0.06)	(0.04)	(0.05)
HYDROGUM (Disinfected)			
MEAN	50.17	50.14	50.16
SD	(0.09)	(0.16)	(0.18)
BLUEPRINT (Standard)			
MEAN	50.24	50.15	50.14
SD	(0.05)	(0.04)	(0.02)
BLUEPRINT (Disinfected)			
MEAN	50.06	49.98	49.96
SD	(0.22)	(0.23)	(0.21)

TABLE 6.4

ALGINATE IMPRESSION MATERIALS

DISTANCE MEASUREMENT : B (mm)

	0 hour	24 hours	48 hours
XANTALGIN (Standard)			
MEAN	27.03	27.03	27.03
SD	(0.06)	(0.05)	(0.04)
XANTALGIN (Disinfected)			
MEAN	26.92	26.92	26.97
SD	(0.08)	(0.07)	(0.10)
PALGAT (Standard)			
MEAN	27.06	27.10	27.10
SD	(0.03)	(0.05)	(0.03)
PALGAT (Disinfected)			
MEAN	26.99	27.00	27.030
SD	(0.09)	(0.07)	(0.07)
HYDROGUM (Standard)			
MEAN	27.01	27.03	26.99
SD	(0.07)	(0.04)	(0.05)
HYDROGUM (Disinfected)			
MEAN	26.96	26.99	26.96
SD	(0.11)	(0.07)	(0.09)
BLUEPRINT (Standard)			
MEAN	26.98	26.95	26.97
SD	(0.05)	(0.05)	(0.05)
BLUEPRINT (Disinfected)			
MEAN	26.90	26.93	26.91
SD	(0.05)	(0.05)	(0.05)

TABLE 6.5

ALGINATE IMPRESSION MATERIALS

DISTANCE MEASUREMENT : C (mm)

	0 hour	24 hours	48 hours
XANTALGIN (Standard)			
MEAN	34.08	34.09	34.09
SD	(0.03)	(0.06)	(0.03)
XANTALGIN (Disinfected)			
MEAN	34.05	34.00	33.95
SD	(0.04)	(0.04)	(0.06)
PALGAT (Standard)			
MEAN	34.09	34.08	34.10
SD	(0.03)	(0.03)	(0.04)
PALGAT (Disinfected)			
MEAN	34.14	34.13	34.07
SD	(0.04)	(0.05)	(0.08)
HYDROGUM (Standard)			
MEAN	34.12	34.09	34.12
SD	(0.02)	(0.04)	(0.02)
HYDROGUM (Disinfected)			
MEAN	34.10	34.05	34.00
SD	(0.07)	(0.10)	(0.13)
BLUEPRINT (Standard)			
MEAN	34.08	33.90	33.87
SD	(0.03)	(0.04)	(0.03)
BLUEPRINT (Disinfected)			
MEAN	34.01	33.89	33.81
SD	(0.04)	(0.03)	(0.04)

TABLE 6.6

ALGINATE IMPRESSION MATERIALS

DISTANCE MEASUREMENT : D (mm)

	0 hour	24 hours	48 hours
XANTALGIN (Standard)			
MEAN	30.34	30.35	30.36
SD	(0.05)	(0.06)	(0.03)
XANTALGIN (Disinfected)			
MEAN	30.35	30.33	30.33
SD	(0.07)	(0.05)	(0.06)
PALGAT (Standard)			
MEAN	30.37	30.39	30.39
SD	(0.02)	(0.05)	(0.05)
PALGAT (Disinfected)			
MEAN	30.33	30.37	30.40
SD	(0.03)	(0.06)	(0.08)
HYDROGUM (Standard)			
MEAN	30.32	30.37	30.39
SD	(0.06)	(0.03)	(0.04)
HYDROGUM (Disinfected)			
MEAN	30.31	30.36	30.36
SD	(0.06)	(0.06)	(0.09)
BLUEPRINT (Standard)			
MEAN	30.29	30.31	30.29
SD	(0.05)	(0.07)	(0.05)
BLUEPRINT (Disinfected)			
MEAN	30.31	30.27	30.26
SD	(0.11)	(0.06)	(0.07)

TABLE 6.7

ALGINATE IMPRESSION MATERIALS

DISTANCE MEASUREMENT : E (mm)

	0 hour	24 hours	48 hours
XANTALGIN (Standard)			
MEAN	50.70	50.70	50.71
SD	(0.04)	(0.02)	(0.01)
XANTALGIN (Disinfected)			
MEAN	50.65	50.62	50.63
SD	(0.06)	(0.06)	(0.07)
PALGAT (Standard)			
MEAN	50.69	50.68	50.70
SD	(0.03)	(0.02)	(0.02)
PALGAT (Disinfected)			
MEAN	50.67	50.70	50.67
SD	(0.02)	(0.04)	(0.12)
HYDROGUM (Standard)			
MEAN	50.68	50.71	50.73
SD	(0.03)	(0.02)	(0.03)
HYDROGUM (Disinfected)			
MEAN	50.66	50.64	50.67
SD	(0.05)	(0.12)	(0.16)
BLUEPRINT (Standard)			
MEAN	50.66	50.56	50.54
SD	(0.04)	(0.03)	(0.02)
BLUEPRINT (Disinfected)			
MEAN	50.57	50.43	50.38
SD	(0.14)	(0.14)	(0.13)

TABLE 6.8

ALGINATE IMPRESSION MATERIALS

DISTANCE MEASUREMENT : F (mm)

	0 hour	24 hours	48 hours
XANTALGIN (Standard)			
MEAN	49.97	49.99	49.98
SD	(0.03)	(0.05)	(0.03)
XANTALGIN (Disinfected)			
MEAN	49.88	49.84	49.81
SD	(0.07)	(0.07)	(0.09)
PALGAT (Standard)			
MEAN	50.01	50.02	50.05
SD	(0.04)	(0.06)	(0.05)
PALGAT (Disinfected)			
MEAN	50.00	50.00	49.97
SD	(0.06)	(0.05)	(0.07)
HYDROGUM (Standard)			
MEAN	49.99	49.98	49.99
SD	(0.06)	(0.05)	(0.04)
HYDROGUM (Disinfected)			
MEAN	49.89	49.89	49.81
SD	(0.11)	(0.15)	(0.16)
BLUEPRINT (Standard)			
MEAN	49.93	49.78	49.76
SD	(0.04)	(0.05)	(0.04)
BLUEPRINT (Disinfected)			
MEAN	49.78	49.75	49.68
SD	(0.08)	(0.10)	(0.06)

TABLE 6.9

DIFFERENCE IN MEAN DISTANCE (IMMERSED - NOT IMMERSED)

	TIME		
	0hr	24hr	48hr
Dist A	-0.008*	-0.09*	-0.12**
Dist B	-0.008***	-0.08***	-0.05*
Dist C	-0.002	-0.02	-0.09***
Dist D	0.000	-0.02	-0.02
Dist E	-0.004	-0.07*	-0.09*
Dist F	-0.009***	-0.07**	-0.13***

* $p<0.05$
** $p<0.01$
*** $p<0.001$

TABLE 6.10

MEAN DIFFERENCE BETWEEN ACTUAL AND OBSERVED DISTANCE FOR EACH MATERIAL AT EACH TIMEPOINT

Dist	Mat	0hr	24hr	48hr
A	XAN	(1.5) -0.03	(2) -0.04**	(2) -0.05**
	PAL	(1.5) -0.03	(1) -0.02	(1) -0.03
	HYD	(3) -0.06	(3) -0.08	(3) -0.06
	BLU	(4) -0.13	(4) -0.21	(4) -0.23
B	XAN	(3) -0.06***	(3) -0.06***	(2) -0.04**
	PAL	(1) 0.01	(1) 0.01	(1) -0.03
	HYD	(2) -0.05	(2) -0.02	(3) -0.06
	BLU	(4) -0.10	(4) -0.10	(4) -0.09
C	XAN	(3) -0.05***	(3) -0.07***	(3) -0.10***
	PAL	(1) 0.00	(1) -0.02	(1) -0.04
	HYD	(2) -0.01	(2) -0.04	(2) -0.06
	BLU	(4) -0.08	(4) -0.09	(4) -0.28
D	XAN	(1.5) -0.03	(3) -0.04**	(3) -0.03
	PAL	(1.5) -0.03	(1) 0.00	(2) 0.02
	HYD	(3) -0.06	(2) -0.01	(1) 0.00
	BLU	(4) -0.08	(4) -0.09	(4) -0.10
E	XAN	(2.5) -0.04	(3) -0.05***	(3) -0.05***
	PAL	(1) -0.03	(1) -0.03	(2) -0.03
	HYD	(2.5) -0.04	(2) -0.04	(1) -0.01
	BLU	(4) -0.11	(4) -0.22	(4) -0.25
F	XAN	(3) -0.09***	(3) -0.10***	(2.5) -0.12***
	PAL	(1) -0.01	(1) 0.00	(1) 0.00
	HYD	(2) -0.08	(2) -0.08	(2.5) -0.12
	BLU	(4) -0.16	(4) -0.25	(4) -0.29
Sum of Ranks		PAL 7.0 HYD 14.5 XAN 14.5 BLU 24.0	PAL 6.0 HYD 12.0 XAN 18.0 BLU 24.0	PAL 8.0 HYD 12.5 XAN 15.5 BLU 24.0

XAN - Xantalgin
PAL - Palgat
HYD - Hydrogum
BLU - Blueprint

APPENDIX 6.1a

XANTALGEN - DISTANCE MEASUREMENT: A (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.196	50.288	50.251	50.187	50.116	50.116
50.178	50.236	50.314	50.189	50.128	50.096
50.229	50.281	50.251	50.133	50.161	50.151
50.295	50.313	50.316	50.309	50.279	50.113
50.302	50.331	50.304	50.264	50.175	50.168
50.296	50.304	50.286	50.253	50.226	50.149
50.361	50.248	50.290	50.176	50.246	50.236
50.271	50.272	50.252	50.262	50.192	50.226
50.292	50.275	50.270	50.211	50.180	50.243
50.299	50.305	50.306	50.222	50.243	50.207
50.298	50.309	50.265	50.256	50.216	50.239
50.287	50.304	50.273	50.221	50.167	50.243

APPENDIX 6.1b

PALGAT - DISTANCE MEASUREMENT: A (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.301	50.269	50.274	50.309	50.239	50.266
50.276	50.223	50.280	50.262	50.234	50.247
50.248	50.254	50.295	50.228	50.241	50.252
50.271	50.252	50.287	50.240	50.254	50.243
50.264	50.244	50.299	50.229	50.223	50.293
50.287	50.257	50.351	50.208	50.302	50.249
50.230	50.223	50.272	50.255	50.316	50.278
50.277	50.233	50.291	50.186	50.279	50.221
50.288	50.224	50.282	50.215	50.301	50.187
50.245	50.186	50.250	50.245	50.306	50.134
50.263	50.243	50.246	50.225	50.256	50.090
50.249	50.199	50.255	50.258	50.335	50.081

APPENDIX 6.1c

HYDROGUM - DISTANCE MEASUREMENT: A (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.265	50.304	50.290	50.264	50.313	50.351
50.339	50.274	50.294	50.233	50.286	50.337
50.306	50.260	50.290	50.268	50.297	50.359
50.193	50.239	50.261	50.264	50.273	50.325
50.192	50.235	50.235	50.251	50.250	50.331
50.195	50.205	50.209	50.223	50.290	50.207
50.324	50.320	50.342	50.079	50.085	50.054
50.345	50.267	50.373	50.992	50.057	50.002
50.311	50.324	50.327	50.081	50.026	50.010
50.231	50.230	50.266	50.129	49.955	49.974
50.249	50.254	50.251	50.138	49.955	49.996
50.232	50.243	50.291	50.144	49.916	49.917

APPENDIX 6.1d

BLUEPRINT - DISTANCE MEASUREMENT: A (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.189	50.108	50.115	50.189	50.108	50.115
50.145	50.103	50.103	50.145	50.103	50.103
50.189	50.098	50.128	50.189	50.098	50.128
50.228	50.163	50.155	50.228	50.163	50.155
50.252	50.143	50.159	50.252	50.143	50.159
50.247	50.138	50.141	50.247	50.138	50.141
50.293	50.160	50.164	50.293	50.160	50.164
50.273	50.147	50.126	50.273	50.147	50.126
50.305	50.135	50.145	50.305	50.135	50.145
50.260	50.187	50.156	50.260	50.187	50.156
50.217	50.194	50.139	50.217	50.194	50.139
50.224	50.212	50.175	50.224	50.212	50.175

APPENDIX 6.2a

XANTALGIN - DISTANCE MEASUREMENT: B (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
27.044	26.976	27.018	27.090	27.008	26.967
27.030	26.932	27.005	27.011	26.971	27.090
27.061	27.017	26.975	26.939	26.978	26.975
27.005	27.084	27.011	26.883	26.933	26.797
27.007	27.076	27.090	26.924	26.824	26.809
26.972	27.007	27.103	26.902	26.887	26.839
27.172	27.053	26.962	26.922	26.837	27.037
27.032	26.973	26.997	26.857	26.904	27.036
27.077	27.024	27.062	27.001	26.838	27.008
26.939	27.062	27.029	26.846	26.985	27.065
27.004	27.061	27.049	26.864	27.020	26.946
26.989	27.079	27.007	26.804	26.882	27.075

APPENDIX 6.2b

PALGAT - DISTANCE MEASUREMENT: B (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
27.031	27.179	27.098	26.978	27.015	27.105
27.024	27.065	27.063	26.878	27.013	27.087
27.057	27.042	27.133	26.947	27.015	27.090
27.099	27.014	27.033	27.149	26.971	27.032
27.044	27.144	27.106	26.829	26.871	27.170
27.088	27.138	27.088	26.962	26.945	26.985
27.003	27.094	27.106	26.987	27.037	27.019
27.065	27.105	27.133	27.016	27.060	27.037
27.094	27.035	27.091	26.975	27.129	26.927
27.071	27.118	27.093	27.030	27.001	26.978
27.085	27.129	27.126	27.156	26.905	26.974
27.111	27.091	27.113	26.985	27.025	26.960

APPENDIX 6.2c

HYDROGUM - DISTANCE MEASUREMENT: B (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
26.879	27.106	26.949	27.074	27.028	27.073
27.022	27.021	27.036	26.993	27.041	27.058
26.930	27.011	26.958	26.811	27.027	27.063
26.959	26.990	27.004	27.032	27.046	27.070
26.992	27.036	26.945	27.065	27.001	26.911
27.102	26.999	26.939	26.795	27.094	26.952
27.007	27.051	26.969	26.940	27.003	26.915
27.083	27.058	26.948	26.803	26.958	26.980
27.072	27.040	27.003	26.934	26.966	26.887
26.966	26.953	27.064	26.924	26.869	26.883
27.005	27.059	26.976	27.107	26.935	26.921
27.120	27.065	27.063	27.023	26.903	26.826

APPENDIX 6.2d

BLUEPRINT - DISTANCE MEASUREMENT: B (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
26.961	27.028	27.031	26.961	27.028	27.031
26.991	26.924	26.942	26.991	26.924	26.942
27.011	26.938	27.021	27.011	26.938	27.021
26.988	26.920	26.887	26.988	26.920	26.887
26.943	26.943	26.939	26.943	26.943	26.939
26.908	26.887	26.904	26.908	26.887	26.904
26.987	26.954	26.950	26.987	26.954	26.950
26.933	26.962	27.016	26.933	26.962	27.016
26.987	26.928	27.016	26.987	26.928	27.016
27.054	26.877	26.995	27.054	26.877	26.995
26.917	26.978	26.963	26.917	26.978	26.963
27.027	27.024	26.947	27.027	27.024	26.947

APPENDIX 6.3a

XANTALGIN - DISTANCE MEASUREMENT: C (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
34.108	34.191	34.094	34.013	33.969	33.835
34.086	34.155	34.126	33.990	33.963	33.929
34.064	34.147	34.095	33.971	33.908	33.856
34.071	34.038	34.090	34.096	33.972	33.976
34.065	33.986	34.074	34.007	34.035	33.975
34.043	34.028	34.060	34.030	33.983	33.974
34.017	34.084	34.148	34.088	34.038	33.992
34.096	34.142	34.063	34.084	34.015	33.926
34.066	34.100	34.081	34.063	34.047	33.967
34.113	34.082	34.078	34.049	34.042	33.966
34.123	34.093	34.100	34.077	34.049	34.005
34.121	34.082	34.088	34.095	34.029	33.999

APPENDIX 6.3b

PALGAT - DISTANCE MEASUREMENT: C (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
34.123	34.083	34.143	34.211	34.151	34.093
34.131	34.097	34.103	34.151	34.159	34.116
34.132	34.111	34.121	34.176	34.165	34.143
34.075	34.071	34.116	34.094	34.125	34.110
34.082	34.081	34.066	34.197	34.176	34.045
34.097	34.097	34.086	34.108	34.131	34.132
34.127	34.104	34.104	34.161	34.151	34.109
34.072	34.082	34.103	34.187	34.079	34.123
34.069	34.065	34.166	34.139	34.165	34.106
34.083	34.028	34.038	34.108	34.021	33.938
34.050	34.038	34.050	34.104	34.139	33.964
34.058	34.062	34.048	34.135	34.078	33.931

APPENDIX 6.3c

HYDROGUM - DISTANCE MEASUREMENT: C (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
34.107	34.076	34.115	34.057	34.098	34.041
34.102	34.050	34.113	34.063	34.078	34.079
34.095	34.069	34.103	34.218	34.112	34.048
34.158	34.129	34.107	34.164	34.158	34.123
34.145	34.112	34.160	34.151	34.153	34.107
34.131	34.158	34.117	34.153	34.149	34.087
34.144	34.106	34.156	34.135	34.026	34.075
34.110	34.112	34.148	34.148	34.111	34.018
34.076	34.121	34.107	34.040	34.073	34.047
34.126	34.095	34.098	34.051	33.934	33.774
34.115	34.058	34.149	34.018	33.853	33.787
34.116	34.041	34.117	34.003	33.895	33.820

APPENDIX 6.3d

BLUEPRINT - DISTANCE MEASUREMENT: C (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
34.061	33.904	33.912	34.099	33.923	33.861
34.034	33.957	33.898	34.050	33.891	33.845
34.027	33.898	33.887	34.027	33.861	33.800
34.074	33.926	33.890	34.035	33.931	33.799
34.083	33.911	33.840	34.023	33.922	33.800
34.065	33.916	33.871	33.988	33.938	33.828
34.099	33.852	33.836	34.008	33.848	33.838
34.117	33.857	33.835	33.978	33.872	33.831
34.092	33.850	33.835	33.961	33.851	33.792
34.076	33.946	33.880	33.993	33.886	33.830
34.115	33.885	33.874	33.944	33.882	33.799
34.104	33.907	33.889	33.959	33.878	33.727

APPENDIX 6.4a

XANTALGIN - DISTANCE MEASUREMENT: D (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
30.347	30.334	30.370	30.398	30.349	30.345
30.345	30.365	30.309	30.375	30.372	30.401
30.379	30.331	30.326	30.416	30.419	30.271
30.314	30.341	30.358	30.443	30.311	30.362
30.415	30.224	30.332	30.357	30.295	30.378
30.344	30.265	30.348	30.327	30.352	30.367
30.212	30.450	30.418	30.373	30.241	30.344
30.320	30.398	30.362	30.422	30.366	30.299
30.309	30.392	30.385	30.300	30.334	30.378
30.330	30.383	30.374	30.204	30.351	30.267
30.409	30.387	30.376	30.299	30.305	30.357
30.380	30.361	30.332	30.316	30.300	30.219

APPENDIX 6.4b

PALGAT - DISTANCE MEASUREMENT: D (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
30.405	30.397	30.475	30.323	30.356	30.354
30.371	30.404	30.406	30.282	30.317	30.467
30.398	30.403	30.468	30.294	30.331	30.456
30.362	30.388	30.397	30.342	30.465	30.494
30.335	30.448	30.364	30.345	30.452	30.258
30.348	30.422	30.390	30.315	30.406	30.452
30.346	30.365	30.312	30.311	30.371	30.393
30.382	30.339	30.304	30.387	30.376	30.498
30.355	30.360	30.378	30.343	30.398	30.449
30.373	30.300	30.409	30.323	30.269	30.335
30.389	30.431	30.398	30.359	30.390	30.343
30.382	30.453	30.401	30.296	30.309	30.325

APPENDIX 6.4c

HYDROGUM - DISTANCE MEASUREMENT: D (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
30.266	30.362	30.410	30.390	30.347	30.522
30.235	30.361	30.436	30.323	30.355	30.471
30.224	30.371	30.350	30.411	30.404	30.453
30.391	30.367	30.357	30.293	30.441	30.338
30.413	30.372	30.409	30.339	30.460	30.406
30.387	30.367	30.373	30.270	30.337	30.285
30.327	30.382	30.425	30.315	30.314	30.385
30.320	30.397	30.348	30.316	30.385	30.388
30.319	30.412	30.413	30.279	30.376	30.335
30.370	30.290	30.376	30.253	30.312	30.217
30.261	30.385	30.463	30.332	30.264	30.260
30.299	30.372	30.349	30.204	30.342	30.282

APPENDIX 6.4d

BLUEPRINT - DISTANCE MEASUREMENT: D (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
30.277	30.292	30.305	30.247	30.401	30.298
30.338	30.264	30.247	30.261	30.235	30.287
30.203	30.171	30.245	30.241	30.210	30.312
30.229	30.375	30.295	30.443	30.333	30.241
30.234	30.402	30.344	30.429	30.260	30.237
30.261	30.357	30.339	30.279	30.311	30.389
30.272	30.246	30.183	30.215	30.218	30.185
30.331	30.288	30.282	30.086	30.218	30.204
30.314	30.245	30.289	30.336	30.209	30.171
30.299	30.347	30.319	30.429	30.344	30.354
30.336	30.330	30.334	30.390	30.291	30.289
30.322	30.407	30.316	30.395	30.261	30.176

APPENDIX 6.5a

XANTALGIN - DISTANCE MEASUREMENT: E (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.688	50.704	50.713	50.562	50.518	50.492
50.664	50.700	50.729	50.569	50.527	50.615
50.675	50.695	50.716	50.575	50.548	50.491
50.630	50.667	50.705	50.699	50.678	50.656
50.712	50.674	50.690	50.699	50.618	50.703
50.683	50.667	50.711	50.697	50.691	50.668
50.695	50.715	50.720	50.654	50.639	50.694
50.672	50.697	50.690	50.686	50.694	50.632
50.710	50.699	50.697	50.709	50.626	50.693
50.717	50.738	50.723	50.622	50.646	50.635
50.754	50.720	50.706	50.684	50.652	50.622
50.745	50.724	50.701	50.637	50.628	50.602

APPENDIX 6.5b

PALGAT - DISTANCE MEASUREMENT: E (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.705	50.662	50.710	50.692	50.725	50.714
50.679	50.672	50.694	50.685	50.713	50.765
50.653	50.662	50.712	50.670	50.729	50.712
50.693	50.646	50.677	50.624	50.716	50.776
50.636	50.681	50.661	50.659	50.688	50.723
50.664	50.678	50.695	50.650	50.733	50.714
50.698	50.695	50.713	50.699	50.729	50.704
50.723	50.684	50.690	50.662	50.718	50.717
50.717	50.694	50.730	50.678	50.718	50.717
50.698	50.663	50.713	50.656	50.640	50.512
50.712	50.674	50.685	50.659	50.649	50.453
50.704	50.703	50.696	50.653	50.630	50.473

APPENDIX 6.5c

HYDROGUM - DISTANCE MEASUREMENT: E (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.690	50.715	50.723	50.716	50.727	50.875
50.697	50.716	50.746	50.713	50.723	50.834
50.656	50.721	50.716	50.737	50.750	50.855
50.653	50.703	50.688	50.605	50.701	50.740
50.707	50.699	50.708	50.681	50.743	50.725
50.675	50.698	50.699	50.666	50.688	50.685
50.687	50.710	50.755	50.692	50.678	50.673
50.727	50.728	50.759	50.654	50.659	50.672
50.731	50.737	50.752	50.672	50.663	50.650
50.686	50.665	50.772	50.616	50.441	50.434
50.654	50.722	50.740	50.591	50.438	50.440
50.646	50.707	50.724	50.582	50.430	50.422

APPENDIX 6.5d

BLUEPRINT - DISTANCE MEASUREMENT: E (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.627	50.561	50.540	50.608	50.565	50.534
50.623	50.533	50.567	50.621	50.523	50.513
50.608	50.502	50.548	50.652	50.523	50.516
50.600	50.565	50.494	50.651	50.419	50.343
50.610	50.564	50.572	50.670	50.395	50.324
50.602	50.535	50.513	50.585	50.402	50.344
50.684	50.544	50.525	50.360	50.266	50.179
50.678	50.556	50.559	50.318	50.203	50.212
50.690	50.524	50.534	50.369	50.213	50.197
50.667	50.605	50.545	50.686	50.558	50.492
50.638	50.594	50.559	50.662	50.532	50.455
50.699	50.616	50.548	50.655	50.526	50.466

APPENDIX 6.6a

XANTALGIN - DISTANCE MEASUREMENT: F (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
49.964	50.040	49.960	50.038	49.872	49.785
49.942	49.959	49.978	49.949	49.884	49.850
49.984	50.026	49.909	49.853	49.927	49.797
50.005	50.013	49.989	49.937	49.806	49.654
49.970	49.905	50.018	49.822	49.770	49.667
49.920	49.909	49.989	49.810	49.758	49.701
49.981	50.007	50.016	49.898	49.794	49.866
49.996	50.018	49.936	49.896	49.784	49.832
49.968	50.010	50.010	49.850	49.801	49.847
49.933	49.994	49.980	49.784	49.932	49.856
49.984	50.026	50.002	49.837	49.913	49.896
49.964	50.006	49.953	49.833	49.788	49.926

APPENDIX 6.6b

PALGAT - DISTANCE MEASUREMENT: F (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
50.052	50.122	50.125	50.078	49.980	50.013
50.044	50.026	50.051	49.909	49.974	50.015
50.078	50.058	50.137	49.980	49.974	50.088
50.018	49.999	50.058	50.099	50.000	49.974
50.020	50.087	50.066	49.952	49.974	49.964
50.062	50.097	50.089	49.936	49.977	50.007
49.964	50.009	50.008	49.981	50.052	50.008
49.979	49.998	50.053	49.965	49.986	50.033
49.994	49.937	50.079	49.971	50.136	49.901
49.988	49.923	49.970	50.004	49.934	49.879
49.973	50.034	50.018	50.081	49.997	49.913
49.993	49.985	49.993	50.000	50.053	49.859

APPENDIX 6.6c

HYDROGUM - DISTANCE MEASUREMENT: F (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
49.863	50.025	49.969	49.965	49.984	49.930
49.974	49.928	50.019	49.875	49.963	49.962
49.924	49.931	49.944	49.928	49.989	49.919
50.005	49.972	49.986	50.091	50.090	50.021
49.973	49.993	49.985	50.058	50.012	49.951
50.053	49.981	49.915	49.844	50.089	49.861
50.050	50.047	50.034	49.832	49.779	49.791
50.038	50.011	49.995	49.731	49.868	49.745
49.972	50.047	49.998	49.736	49.810	49.714
49.967	49.909	49.949	49.814	49.733	49.567
49.972	49.951	50.007	49.963	49.684	49.635
50.059	49.938	50.016	49.842	49.723	49.578

APPENDIX 6.6d

BLUEPRINT - DISTANCE MEASUREMENT: F (mm)

NON-DISINFECTED			DISINFECTED		
0 hour	24 hours	48 hours	0 hour	24 hours	48 hours
49.884	49.791	49.831	49.897	49.812	49.713
49.880	49.785	49.700	49.790	49.718	49.735
49.864	49.720	49.772	49.787	49.635	49.680
49.942	49.819	49.789	49.787	49.884	49.622
49.931	49.818	49.725	49.786	49.712	49.597
49.910	49.791	49.775	49.728	49.759	49.764
49.949	49.719	49.691	49.769	49.630	49.686
49.948	49.731	49.723	49.633	49.678	49.649
49.963	49.704	49.764	49.670	49.615	49.601
49.978	49.767	49.807	49.876	49.877	49.741
49.947	49.786	49.761	49.791	49.815	49.747
49.947	49.867	49.796	49.826	49.807	49.644

CHAPTER 7

THE DISINFECTION OF DENTAL IMPRESSION MATERIALS

7.1 THE DISINFECTION OF DENTAL IMPRESSION MATERIALS

It is clear from the literature review carried out at the beginning of the thesis, that the potential for disease transmission within the dental surgery is a consideration of major, and increasing, importance in dentistry.

The experimental work undertaken in the thesis has been limited to aspects of infection control pertinent to the handling of dental impressions, with a view to providing information which may be of value in the development of sound clinical practice. From the laboratory experiments described in Chapter 2, it is evident that microorganisms can survive for some time on impressions, and alginate, in particular, appears to have considerable potential for microbial transmission. However, the anti-microbial effect of alginate containing didecyldimethyl ammonium chloride (Blueprint), was confirmed, even with limited direct contact with microbial suspensions. These laboratory findings were replicated in the preliminary clinical study described in Chapter 2, where the action of Blueprint in eliminating microorganisms, the low level of contamination of poly (vinyl siloxane), and high initial contamination of conventional alginate with retention of microorganisms over a five hour period, were observed.

In Chapter 3, although alginate was found to be more resistant to disinfection than the rubber base materials, hypochlorite and glutaraldehyde solutions produced almost complete removal of contaminants, even with inoculation of high concentrations of experimental microorganisms. Chlorhexidine, in the concentrations tested, was not as effective an agent for the immersion disinfection of dental impressions. When the efficacy of chlorhexidine, incorporated within alginate, was examined in laboratory investigations (Chapter 4), its antimicrobial effect was limited when the contact time was restricted, however, with more prolonged exposure, its microbicidal activity was comparable with the action of dimethyldidecyl ammonium chloride.

In-vivo investigation indicated that alginate, containing sodium didecyldimethyl ammonium chloride (Blueprint), was clearly and consistently more effective in eliminating test microorganisms than standard alginate impression material (Kromogel) and alginate containing chlorhexidine (Hydrogum), and, while there was a trend for the chlorhexidine-containing material to be more effective than standard alginate, the microbicidal effect of Hydrogum was not consistent. The use of 0.2% aqueous chlorhexidine gluconate, as a pre-impression mouth rinse, was more effective than the incorporation of chlorhexidine within alginate.

With respect to effective methods of protection against contamination from dental impressions, from the above, it would seem reasonable that, prior to having impressions recorded, patients should rinse with 0.2% aqueous chlorhexidine gluconate, and following the use of a rubber base impression material (or an alginate material containing sodium didecyldimethyl ammonium chloride), impressions should be immersed in 2% activated glutaraldehyde, or 0.0125% sodium hypochlorite, for a minimum of 3 minutes.

In Chapter 6, examination of the effect of immersion disinfection and storage, on dimensional stability, was undertaken for four different alginate materials. Accuracy was found to be dependant upon the choice of material, rather than on the length of storage before casts were made, and in most cases dimensional change following storage of non-immersed impressions was less than differences between materials. The dimensions of impressions were affected by storage (up to 48 hours) to a surprisingly small degree. In most instances, immersion produced a larger spread of values for the test dimensions, with greater dimensional change on subsequent storage. Blueprint performed less well than the other materials, on dry storage and after immersion, but whether it would be as acceptable in the clinical situation under similar storage and immersion conditions,

was not addressed in the study. Furthermore, it is not known whether the materials tested consistently behave in the manner outlined in this project.

With respect to further study, an important area for future investigation is the *in-vivo* evaluation of the carriage hepatitis and AIDS viruses on dental impression materials and the effect of the recommended measures for disinfection on these microorganisms. Clinical evaluation of the consequences of dimensional change induced by immersion disinfection of alginate materials, and the effect of immersion on surface detail reproduction of these materials, would also merit further investigation.

In addition, there is little data on the implementation of recommended cross infection control procedures, and attitudes towards their use, by practicing dentists; evaluation of these issues, and the financial implications of compliance, would be of value.

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