CHLORHEXIDINE AND THE PREVENTION OF SURGICAL SITE INFECTION

CYNTHIA T CROSBY

Doctor of Philosophy

ASTON UNIVERSITY

September 2009

Aston University

CHLORHEXIDINE AND THE PREVENTION OF SURGICAL SITE INFECTION

Cynthia T Crosby

Doctor of Philosophy

September 2009

SUMMARY

Surgical site infections (SSI) are a prevalent healthcare-associated infection (HAI). Prior to the mid-19th century, surgical sites commonly developed postoperative wound complications. It was in the 1860's, after Joseph Lister introduced carbolic acid and the principles of antisepsis that postoperative wound infection significantly decreased. Today, patient preoperative skin preparation with an antiseptic agent prior to surgery is a standard of practice. Povidone-iodine and chlorhexidine gluconate are currently the most commonly used antimicrobial agents used to prep the patient's skin. In this current study, the epidemiology, diagnosis, surveillance and prevention of SSI with chlorhexidine were investigated. The antimicrobial activity of chlorhexidine was assessed. In *in-vitro* and *in-vivo* studies the antimicrobial efficacy of 2% (w/v) chlorhexidine gluconate (CHG) in 70% isopropyl alcohol (IPA) and 10% povidone-iodine (PVP-I) in the presence of 0.9% normal saline or blood were examined. The 2% CHG in 70% IPA solutions antimicrobial activity was not diminished in the presence of 0.9% normal saline or blood. In comparison, the traditional patient preoperative skin preparation, 10% PVP-I antimicrobial activity was not diminished in the presence of 0.9% normal saline, but was diminished in the presence of blood. In an *in-vivo* human volunteer study the potential for reduction of the antimicrobial efficacy of aqueous patient preoperative skin preparations compromised by mechanical removal of wet product from the application site (blot) was assessed. In this evaluation, 2% CHG and 10% povidone-iodine (PVP-I) were blotted from the patient's skin after application to the test site. The blotting, or mechanical removal, of the wet antiseptic from the application site did not produce a significant difference in product efficacy. In a clinical trial to compare 2% CHG in 70% IPA and PVP-I scrub and paint patient preoperative skin preparation for the prevention of SSI, there were 849 patients randomly assigned to the study groups (409 in the chlorhexidine-alcohol and 440 in the povidone-iodine group) in the intention-to-treat analysis. The overall surgical site infection was significantly lower in the 2% CHG in 70% IPA group than in the PVP-I group (9.5% versus 16.1%, p=0.004; relative risk, 0.59 with 95% confidence interval of 0.41 to 0.85). Preoperative cleansing of the patient's skin with chlorhexidine-alcohol is superior to povidone-iodine in preventing surgical site infection after clean-contaminated surgery.

Keywords: Antiseptic, Povidone-iodine, Wound Infection, Antisepsis, Healthcare Associate Infections

DEDICATION

To my husband Keith, my daughters Casey, Whitney, and Sydney,
my son Cory and my grand-daughter, Ms. Olive Crosby-Phipps.

and

To my parents

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Professor Tom Elliott for encouraging and allowing me this opportunity to undertake my Ph.D. Without his support, guidance, and encouragement, I would not ever have envisioned or completed this project.

I would like to thank Professor Peter Lambert for his advice, encouragement, and passion for education. Thank goodness for individuals like him that put a smile on your face every time you are with them.

I am grateful to BioScience Laboratories for their laboratory and technician support and allowing me to "hang-out" in their labs.

I am especially grateful to Rabih Darouiche MD for his mentorship and friendship. His continuous support and guidance has allowed me to become well educated in device-related and surgical site infections. I would like to thank him for encouraging the undertaking and completion of the prospective, randomized, controlled, multi-center surgical study comparing two antiseptic agents in the prevention of surgical site infections. In addition, I would like to thank Kamal Itani MD, Matt Wall MD, Mary Otterson MD, Matt Carrick MD, Harold Miller MD, Sam Awad MD, David Berger MD, and their staff for the past 4 years, without their help and dedication this work would not have been possible. I acknowledge gratefully Atef Alsharif MD for his endless support in patient enrollment and his passion for medicine. I kindly would like to recognize CareFusion (formerly Medi-Flex, and Enturia, and Cardinal Health) for their unrestricted educational grant that made this study possible.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	arterial catheter
ANOVA	analysis of variance
ANTT	aseptic non-touch technique
ASA	American Society of Anesthesiology
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
AORN	Association of Perioperative Registered Nurses
BSI	blood stream infections
CDC	Centers for Disease Control and Prevention
CHG	chlorhexidine gluconate
CI	confidence interval
CMS	Centers for Medicare & Medicaid services
CoNS	coagulase-negative staphylococci
CVC	central venous catheter
DoH	Department of Health
DSN	Dialysis Surveillance Network
GEE	generalized estimating equation
GIRB	Gallatin Institutional Review Board
HAI	healthcare-associated infections
HICPAC	Healthcare Infection Control Practices Advisory Committee
ICU	intensive care unit
IPA	isopropyl alcohol
ITU	intensive therapy unit
IVD	intravascular-device
JCAHO	Joint Commission Association Healthcare Organization
NICE	National Institute for Health and Clinical Excellence
mL	milliliter
MRSA	meticillin-resistant Staphylococcus aureus
MIC	minimum inhibitory concentration
min	minimum
NaSH	National Surveillance System for Health Care Workers

NHNS	National Healthcare Safety Network
NNIS	National Nosocomial Infection Surveillance System
PHLS	Public Health Laboratory Service
PICC	peripherally inserted central venous catheter
PVP-I	povidone-iodine
SAE	serious adverse event
SIPP	surgical infection prevention project
SSI	surgical site infections
TFM	Tentative Final Monograph
UK	United Kingdom
US	United States
USP	United States Pharmacopeia
UTI	urinary tract infection
VRE	vancomycin-resistant entrococci
v/v	volume per volume
w/v	weight per volume

CHAPTER 1: INTRODUCTION

1.1. Healthcare Associated Infections

Healthcare-associated infections (HAI), also known as nosocomial infections, are infections that patients acquire while receiving treatment for medical or surgical conditions. HAI occur in all healthcare settings, including in acute care hospitals and same day surgical centers, ambulatory outpatient care clinics, and in long-term care facilities, such as nursing homes and rehabilitation facilities. HAI are associated with a variety of causes, including (but not limited to) the use of medical devices, such as catheters and ventilators, complications following a surgical procedure, transmission between patients and healthcare workers, or the result of antibiotic overuse.

Hospital-associated infections contribute significantly to medical complications, encourage the emergence of antibiotic resistant microorganisms and result in increased healthcare costs worldwide. Given the wide range of microbial pathogens and different healthcare settings involved, reliable, consistent figures on HAI at national and international levels are not widely available.

In the United States (US), an estimated 2 million patients acquire healthcare-associated infections annually and account for between 44,000 and 98,000 deaths (Kohn 1999, CDC 2001, Gaynes 2001). The incidence of hospital-associated infections in the US is tracked by the National Nosocomial Infection Surveillance System (NNIS) of the Centers of Disease Control (CDC). According to NNIS data, the most common infections include pneumonia, urinary tract infections (UTI), surgical site infections (SSI) and bloodstream infections (BSI) (Emori and Gaynes, 1993; Richards, *et al.*, 2000). The rate of infection, based on hospital surveillance data, has been reported to be 5%, which it is claimed equates to 5 infections per 1,000 hospital days. (Wenzel and Edmond, 2001). The resulting extended hospital stay and treatment for infection-related illnesses add significant cost to patient care. Approximately one in ten patients in acute care hospitals in the United Kingdom (UK) has a hospital-associated infection (DoH, PHLS 1995). It is also estimated that approximately 5,000 deaths related to hospital-associated infections occur annually in the UK. Their cost to the NHS is approximately £1 billion, mainly due to an increased length of inpatient stay (Plowman, *et al.*, 2000). Over the past 20 years, European countries have reported an overall HAI rate in hospitalized patients which ranges from 3.5% to 14.8% (Pittet, *et al.*, 2005). It has also been estimated that between two to three million

people in the EU acquire a HAI with an associated annual economic burden of €800 million (Fabry 2004, Ganter 2004).

The HAI rates are however likely to be underestimated. This is because many HAI occur in facilities including long-term care facilities, private clinics and nursing homes which have a low awareness or non-existent infection control programmes resulting in under reporting.

1.2 Consequences of Healthcare-Associated Infections

Patients who acquire hospital-associated infections are more likely to experience health complications with a recognized increase in mortality (Emori and Gaynes 1993). The crude mortality rate associated with HAI has been estimated at 27%, varying by type of infection, underlying disease, and pathogen (Edmond, *et al.*, 1999, Wenzel and Edmond 2001). Depending on the infection rate, healthcare-associated infections may results in an estimated 87,500 to 350,000 years of life lost annually in the United States (Wenzel and Edmond 2001). An analysis of vital statistics data in the United States reported that septicaemia alone accounted for 1% of all deaths, following heart disease, malignancies, cerebrovascular disease, and pneumonia and influenza (Wenzel and Edmond 2001). In addition, septicaemia accounts for 15% of all healthcare-associated infections (Richards, *et al.*, 2000; Hugonnet, *et al.*, 2004). Among surgical patients, SSI is the most common healthcare-associated infection, accounting for 38% of all infections, and was associated with 77% of all post surgical deaths (Mangram, *et al.*, 1999).

The negative impact of healthcare-associated infections on health and survival is substantial and represents a challenge to health care institutions. As expected, the costs of health care are also negatively affected, with annual total costs attributed to infections widely reported at over $4.5 billion in the US alone (Weinstein, 1998). Of concern is that, more recent studies suggest that costs are now between $17 to 29 billion in additional US healthcare costs each year (Kohn, *et al.*, 1999; Gaynes, *et al.*, 2001). The infection-related financial burden includes the direct cost of treatment and longer hospital stays. According to a retrospective epidemiological study, the average length of stay for patients who acquired healthcare-associated infections increased by 18.2 days and cost an additional $3,306 per patient (Chen, *et al.*, 2005). In the US, approximately 5 to 10 billion dollars are spent annually in the treatment of healthcare-associated infections (Wenzel and Pfallar, 1991).

Since 1970, the National Nosocomial Infection Surveillance System (NNIS) of the Centers of Disease Control (CDC) has tracked the incidence of hospital-associated infections. Publication of NNIS data has resulted in wider recognition of negative outcomes associated with

healthcare-associated infections and has encouraged efforts to reduce infection rates. Based on data collected by the NNIS, the CDC issued several evidence-based guidelines designed to address sources of infectious contamination. These include Guidelines for Hand Hygiene in Health-Care Settings and Guidelines for the Prevention of Intravascular Catheter-Related Infections which were published by the CDC in 2002 and the Guidelines for the Prevention of Surgical Site Infections published in 1999. The guidelines facilitate the education of healthcare providers in the important areas of infection control (Boyce, *et al.*, 2002; O'Grady, *et al.*, 2002).

The source of many infections including intravascular catheter related sepsis and surgical site infections is considered to be predominantly the patients endogenous skin flora. Sub-optimal skin antisepsis is therefore considered to be a primary cause of Healthcare-Associated Infections (Crosby, *et al.*, 2009). Indeed adequate skin cleansing and protection is essential to pre- and post-care in regards to invasive medical procedures in minimising the subsequent risk of HAI. Examples of important HAI are presented below. The role of skin antisepsis is reviewed in chapter 2 of this thesis.

1.2.1 Intravascular Catheter Related Infections

Intravascular catheters are essential devices in current medical practice, especially in the intensive care unit (ICU). They are used for the administration of medication including antibiotics and chemotherapy, fluids, nutrition and for haemodialysis. Arterial catheters (AC) are used for the continuous monitoring of patients vital parameters in the intensive care unit (ICU). Each year in the US, more than 250 million intravascular catheters are purchased. Most of these are peripheral venous catheters. Of these 5 million are central venous catheters (CVC) which are more commonly associated with infection (O'Grady 2002). In the UK, it has been estimated that approximately 200,000 CVC are used per year and these have also been recognised as a major cause of sepsis, in particular catheter related blood stream infection (Elliott and Worthington 2005).

Although intravascular catheters provide essential vascular access, their use increases the patient's risk of developing local and systemic infections. Local infections may occur at the catheter insertion site or along a subcutaneous track if the device is tunnelled. Symptoms and signs of these infections include the presence of erythema, oedema, and a purulent exudate at the insertion site. The patient may not be systemically unwell. Systemic intravascular catheter infections in comparison are more complex to diagnose and a definition derived from the CDC is outlined in Figure 1.1.

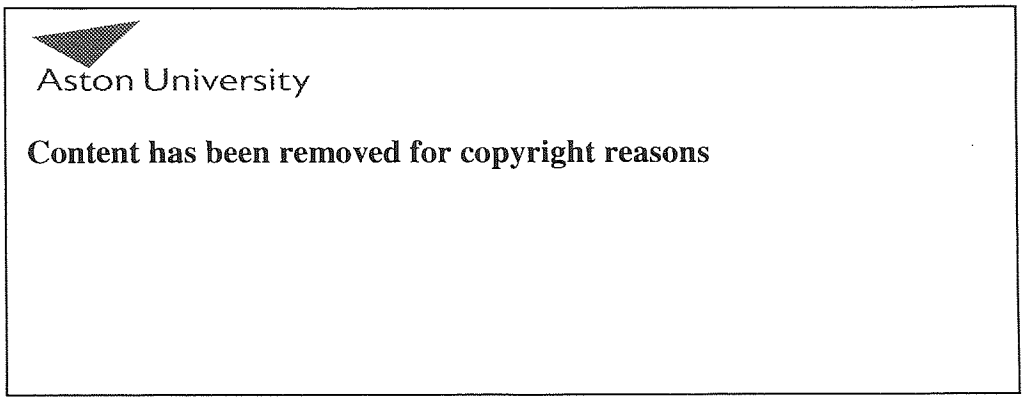


Figure 1.1: Catheter-related bloodstream infections in adults, as defined by the CDC - National Nosocomial Infections Surveillance System.

The incidence of intravascular device-related bloodstream infection (IVD-BSI) varies according to the type of intravascular catheter, frequency of catheter manipulation, and additional patient-related dynamics such as site of catheter placement and the patients underlying condition. Intravascular devices are the number one cause of healthcare-associated bloodstream infections, resulting in 250,000 to 500,000 intravascular device related blood stream infections annually in the United States (Maki, 1994; Crinch and Maki 2002). Although there has been debate in recent years as to the true morbidity of IVD-BSI (Pittet, *et al.,* 1994; Renaud and Brun-Buisson 2001; Orsi, *et al.,* 2002; Blot, *et al.,* 2005), there is worldwide consensus that IVD-BSI are associated with increase length of hospital stay of between 10 to 20 days per patient infected, with increased hospital costs reaching $4,000 to $56,000 per incident (Pittet, *et al.,* 1994; Renaud and Brun-Buisson 2001; Orsi, *et al.,* 2002; Crinch and Maki 2002; Blot, *et al.,* 2005).

Peripheral venous catheters are the most frequently used device for vascular access (Maki, *et al.,* 2006). Although the incidence of local or bloodstream infections (BSI) associated with peripheral venous catheters is low as compared to CVC (DoH PHLS, 2002; Maki, *et al.,* 2006) serious infections do arise and also result in significant morbidity each year.

The sequel of intravascular device -related infection is primarily associated with central venous catheters (CVC). The rate of infection is highest amongst the intensive care unit (ICU) or intensive therapy unit (ITU) patient population. This is due to the patients' underlying severity of illness, possible colonization with drug resistant microorganisms, constant access and

manipulation of the catheter with for example the administration of fluids, antimicrobial agents, chemotherapy agents, and blood products. The NNIS data reported the CVC - associated bloodstream infection (BSI) rate in the ICU at 5.3 per 1,000 catheter days (NNIS, 1997) resulting in 80,000 CVC-associated BSI in the ICU setting annually in the US. Mortality rates are however controversial, ranging from zero to 35% annually (Collignon, 1994; Pittet, *et al.*, 1994; Digiovine, *et al.*, 1999; Soufir, *et al.*, 1999).

The cost per CVC-associated BSI in the ICU ranges from $34,500 to $56,000 (Rello, *et al.*, 2000; Dimick, *et al.*, 2001). Additional care for ICU patients with CVC-related BSI in the US costs from $296 million to $2.3 billion (Mermel 2000).

If all hospital settings are considered, it has been estimated that in the US there would be approximately 250,000 to 400,000 (Darouiche, *et al.*, 1999; Mermel 2000) cases of CVC-associated BSI with a mortality of approximately 12% to 25% at a predicted cost of $25,000 per episode (Kluger and Maki, 2000).

1.2.1.1 Sources of Catheter-related Bloodstream Infection

Microorganisms which cause catheter-related bloodstream infection (Figure 1.2) are derived from several sources (Maki, 1986; Maki, 1994; Elliott, *et al.*, 1997):

1. Patient's skin at procedure site
2. Impaction on insertion
3. Contamination of the catheter hub
4. Haematogenous colonization
5. Contaminated infusate

Evidence suggests that the most prevalent source of catheter-related bloodstream infection (CRBSI) is the resident microorganisms associated with the patient's skin. The microorganisms can be located on the surface of the skin and also in sub-epidermal layers and the hair follicles. The primary causative microorganisms are the coagulase-negative staphylococci (CoNS) that reside on skin. CoNS is now recognized as a pathogenic microorganism (Maki 1994, Elliott, *et al.*, 1997, Livesley, *et al.*, 1998, Jeske, *et al.*, 2003). A list of the microorganisms associated with catheter related blood stream infections and their relative incidence is shown in Table 1.1.

1.2.1.2 Laboratory Diagnostic methods for Catheter-related Bloodstream Infection

Diagnosis of catheter-related infections can be difficult. Approximately half of all patients do not exhibit clinical signs of inflammation at the catheter insertion site. If exudate is

Contaminated

Figure 1.2: Diagram illustrating potential sources of microbial contamination of intravascular devices

Table 1.1: Common pathogens isolated from bloodstream infections and percentage of the overall infections they cause

Pathogen	Infections (%)
Coagulase-negative staphylococci	37.3

All other pathogens	17.2

Note: Data from National Nosocomial Infections Surveillance System (NNIS 1999)

present at the catheter site, a swab should be sent for culture, ideally accompanied by two sets of blood cultures. The following methods can be used for suspected infections when no exudate is present:

1. Paired sets of cultures of blood drawn percutaneously and through the catheter. This method does not require catheter removal for diagnosis; although, interpretation of the results requires clinical judgment. In general, evidence of *Staphylococcus aureus*, gram-negative bacilli, or *Candida species* from either a percutaneous culture or a catheter-drawn culture, or both, represents a true bacteraemia. Common skin contaminants, such as coagulase-negative staphylococci, viridans streptococci, diphtheroids, *Bacillus species, Micrococcus species, and Propionibacterium species*, are likely to be contaminants if cultured from only one of the paired sets of cultures (Worthington and Elliott 2005).
2. Culture of catheter segment. A common technique is the roll plate method. It is a semi-quantitative technique where the catheter tip is cultured and the colony-forming units (CFU) are counted. A count of 15 CFU or higher, with accompanying signs of local or systemic infection, suggests infection. The sensitivity of the roll plate method is approximately 60%. The roll plate method does not detect intraluminal infections. Less commonly used methods are where the catheter segment is either flushed with broth or sonicated in broth, followed by serial dilutions and surface plating on blood agar. A yield of 100 CFU or higher indicates infection. The sensitivities of sonication have been reported at 80%; and flush culture, 40% to 50% (Worthington and Elliott 2005).
3. Quantitative cultures of peripheral and catheter-drawn blood samples. Catheter-related bloodstream infection is suggested when blood drawn from the catheter yields a colony count at least five times greater than that of blood obtained percutaneously (Worthington and Elliott 2005).
4. Differential time to positivity for catheter-drawn versus peripheral blood cultures. If laboratories have the capability for continuous blood culture monitoring, the length of time from blood draw to positive result for catheter-drawn versus percutaneously obtained cultures is tracked. If the time to positivity of the catheter-drawn culture is at least 2 hours less than that of the peripheral culture, catheter infection is suggested.

Sensitivity and specificity of 91% and 94%, respectively, have been reported for this method (Blot, *et al.*, 1999; Seifert, *et al.*, 2003)

5. Catheter exchange over a guidewire. With this method, the suspect CVC is removed, and the tip is sent for culture. If bacteraemia is suspected, replacement of CVC over a guidewire is not an acceptable strategy. With tunnelled haemodialysis catheters or long-term catheters, exchange over a guidewire with a combination of antibiotic therapy is an alternative (O'Grady, *et al.*, 2002).

1.2.2 Surgical Site Infections

The British surgeon Berkeley Moynihan stated that 'every operation in surgery is an experiment in bacteriology' and that the success of the experiment, in respect to the patient, depended not only on the skill, but also on the care exercised by the surgeon in the ritual of the operation (Klenerman, 2002). Prior to the mid-19th century, surgical sites commonly developed postoperative wound complications that included fever, purulent discharge, sepsis and often death. It was in the 1860's, after Joseph Lister introduced carbolic acid and the principles of antisepsis that postoperative wound infection significantly decreased.

Analysis of hospital data provides evidence of the negative consequences of SSI. Wound infections are second only to drug complications in causing adverse events among hospitalized patients. In a study of over 30,000 patients, 14% had complications associated with wound infections (Leape, *et al.*, 1991). Among patients with SSI who die, 77% of deaths are related to the infection, and 93% of these are serious infections involving organs or space accessed during the procedure (Mangram, *et al.*, 1999). In addition SSI also increase hospital length of stay by an average of 7 to 10 days and add over $3,000 per patient in extra costs (Mangram, *et al.*, 1999). In a comparative study of surgical patients with and without infections, the median direct costs of hospitalization were $7,531 for infected patients and $3,844 for uninfected patients. Patients who were readmitted after discharge had even higher costs of over $5,000. This study found that patients with SSI are twice as likely to die, 60% more likely to be admitted to the intensive care unit, and more than five times more likely to be readmitted to the hospital after discharge (Kirkland, *et al.*, 1999).

As of 1999, in the United States alone, an estimated 27 million surgical procedures are performed each year with up to 5% resulting in surgical site infections (SSI) (Martorell 2004). According to NNIS data, SSI are the third most frequently reported healthcare-associated infection (Mangram, *et al.*, 1999) accounting for 16% of the most commonly acquired infections

(Mangram, *et al.*, 1999, Richards, *et al.*, 2005). Surgical site infections are associated with substantial morbidity that can endanger a patient's life, increase the number of days in the hospital, and increase healthcare costs (Mangram, *et al.*, 1999, Kirkland, *et al.*, 1999).

A review of the incidence and economic burden of SSI in Europe estimated that the surgical site infection rate varied widely 1.5% to 20% with the type of surgical procedure and that the economic cost of SSI range from €1.47 – 19.1 billion (Leaper, *et al.*, 2004).

1.2.2.1 Defining Surgical Site Infection

The diagnosis of surgical site infections, involves the interpretation of both clinical and laboratory results. The CDC has developed standard definitions for SSI (Table 1.2). Surgical site infections are classified as either incisional or organ/space. Incisional SSI is further separated into skin and subcutaneous tissue (superficial incisional SSI) and deeper soft tissues of the incision (deep incisional SSI). Organ/Space SSI involve all other anatomy, except incised wall layers, that was open or manipulated during the surgical procedure (Figure 1.3)

1.2.2.2 Microbiology of Surgical Site Infection

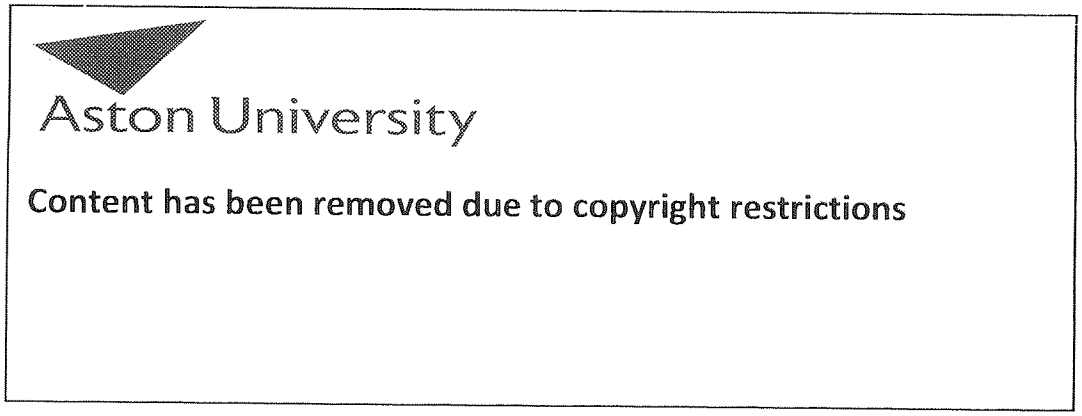
Microbial contamination of the surgical site must occur to result in a surgical site infection. The risk of SSI is specifically related to the number of microorganisms, the virulence of the microorganisms, and the patient's susceptibility. The source of microorganisms, or pathogens, for most SSI is the endogenous flora of the patient's skin (Table 1.4), mucous membranes or hollow viscera (Mangram, *et al.*, 1999). Operative wounds have been categorized according to the likely inoculum of microorganism and the possibility of causing a subsequent infection (Table 1.3).

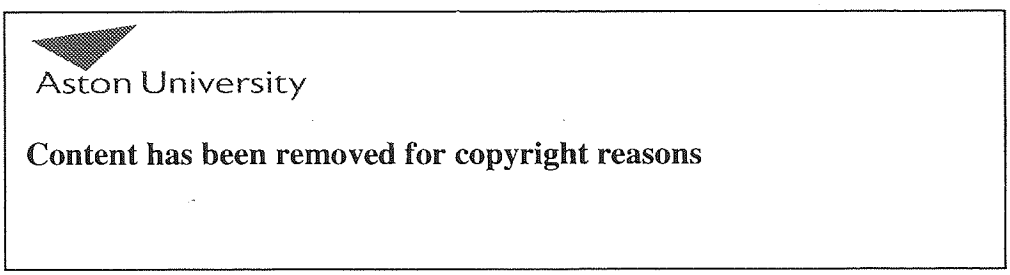
The microbiology of surgical site infections has changed in the past two decades, with the emergence of multi-drug resistant microorganisms, such as meticillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase producing coliforms. The most common causes of SSI are outlined in Tables 1.4 and 1.5 below.

1.3 Prevention of Healthcare-Associated Infection

Prevention of HAI is now a major goal for all health care providers. Many strategies have been derived ranging from improved surveillance, the formulation of specific evidence based guidelines to the introduction of bundles of care. Examples of some of these initiatives are outlined below.

Table 1.2: Criteria for defining a surgical site infection (SSI)





Note: Adapted from Horan, *et al*, 1992.

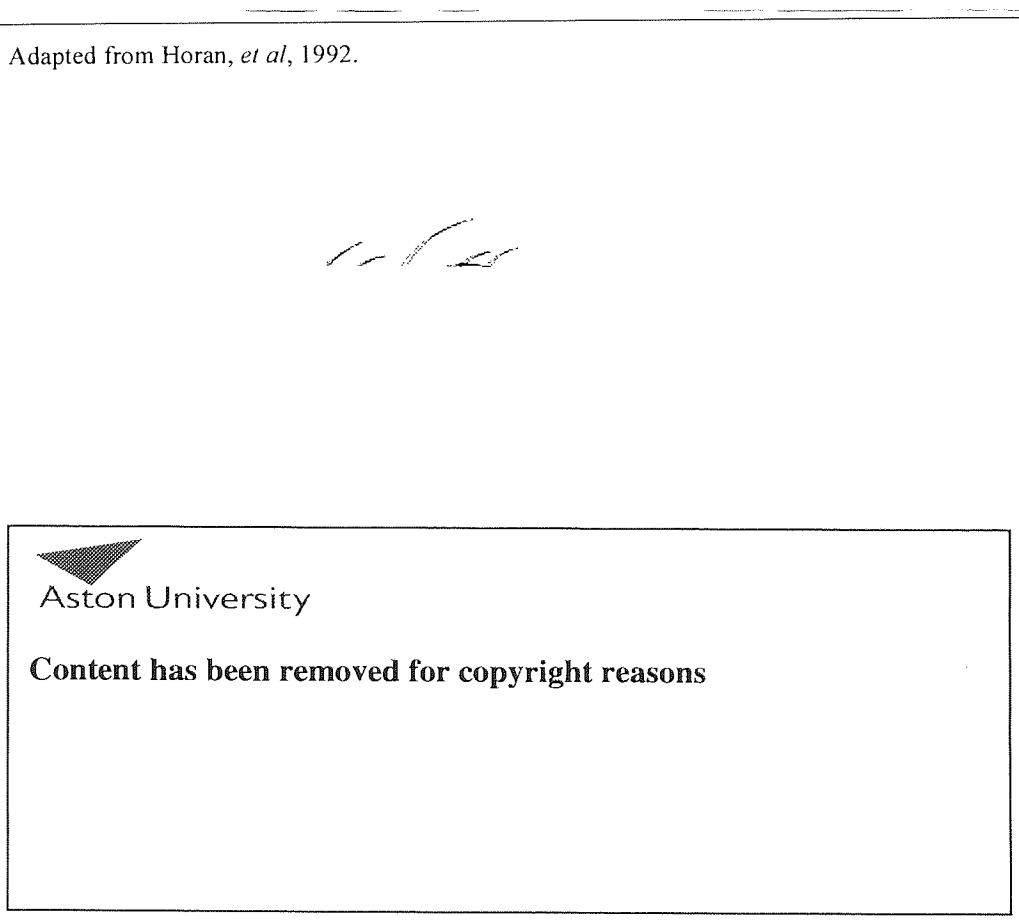


Figure 1.3: Diagrammatic representation of a cross-section of abdominal wall depicting CDC classifications of surgical site infection.

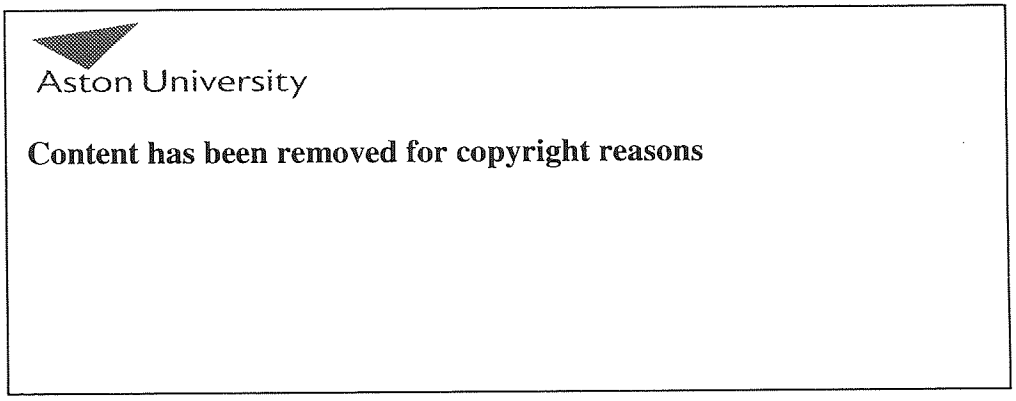
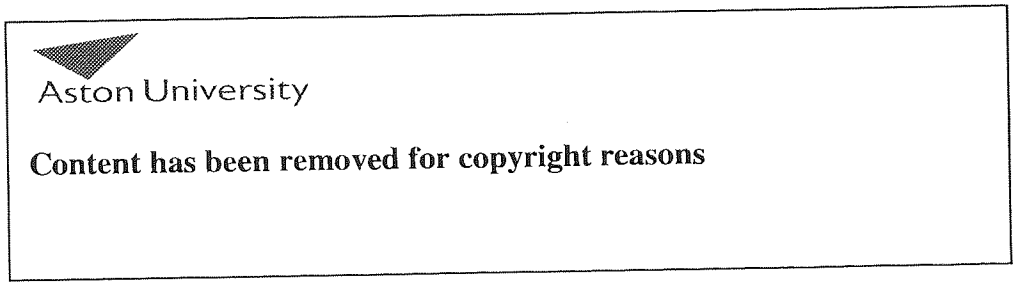


Figure 1.4: Diagrammatic representation of a cross-section of the integumentary system. From Todar 2006.

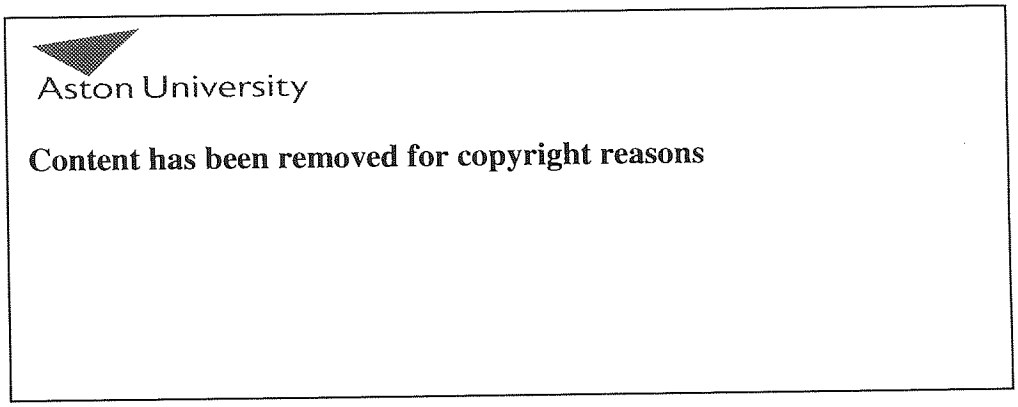
Table 1.3: Classification of operative wounds based on degree of microbial contamination

Classification	Criteria
Clean	Elective, not emergency, non-traumatic



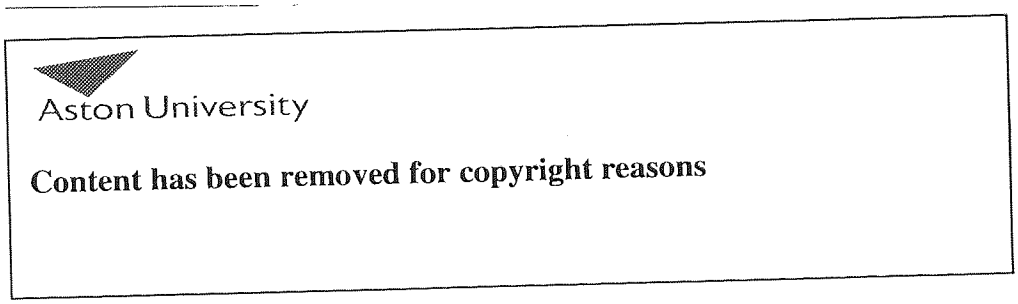
Note: Adapted from Garner 1986 and Simmons 1983.

Table 1.4: Pathogens isolated from SSI in the United States



Note: Adapted from Weiss, *et al.*, 1999.

Table 1.5: Pathogens isolated from patients with SSI reported in Europe



Note: Adapted from Leaper 2004.

1. Surveillance initiative includes the National Healthcare Safety Network (NHSN) which is a CDC derived programme from the NNIS, the Dialysis Surveillance Network (DSN), and the National Surveillance System for Health Care Workers (NaSH). This new NHSN is designed to gather and exchange broad information about infectious and noninfectious adverse events associated with healthcare delivery and to allow organizations to compare their infection rates to national performance measures. The NHSN system is intended to improve infection surveillance to increase patient safety.
2. The CDC's Healthcare Infection Control Practices Advisory Committee (HICPAC) has established a guidance document for reporting healthcare-associated infections (McKibben, *et al.*, 2005). This document was designed by SSI policymakers who created mandatory public reporting systems to track health-associated infections. Four states in the US—Illinois, Pennsylvania, Missouri, and Florida—now require hospitals to report health-associated infections, and 30 more states have legislation pending. The HICPAC recommendations suggest using outcome measures including SSI following selected procedures.
3. 5M lives Campaign: Since achieving the goal of the 100k lives Campaign in 2006, the Institute for Healthcare Improvement initiated the 5M lives campaign aimed at hospitals to improve patient outcomes and reduce mortality rates. The initial interventions include:
    a. Deploying rapid response teams
    b. Delivering reliable, evidence-based care for acute myocardial infarction
    c. Preventing adverse drug events
    d. Preventing central line infections
    e. Preventing SSI
    f. Preventing ventilator-associated pneumonia
4. In the UK the Saving Lives programme has been developed. This outlines basic requirements for prevention of HAI. It has for example focused on intravascular catheter related sepsis and gives clear guidelines for key preventative measures. In the UK there is now also mandatory reporting of infections such as MRSA bacteraemia. As in the US this has enabled comparative tables of different health care

organizations to be produced. This in turn has encouraged providers to examine their infection prevention strategies and to implement the saving lives programme.

The next sections deal with specific approaches to the prevention of defined infection including catheter related blood stream infections and surgical site infections.

1.3.1 Prevention of Catheter-Related Bloodstream Infections

To improve patient outcome and reduce health-care costs, strategies should be implemented to reduce the incidence of both local and systemic catheter-related infections. This effort should be multidisciplinary, involving health-care professionals who insert and maintain intravascular catheters, health-care managers who allocate resources and patients who are capable of assisting in the care of their own catheters. The Guidelines for the Prevention of Intravascular Catheter-Related Infections were published based on NNIS outcomes data demonstrated that catheter-related bloodstream infections pose a substantial risk to patient health within the hospital (O'Grady, *et al.*, 2002). Recommendations for catheter selection, placement and care, as well as a substantial emphasis on education, have influenced the manner in which vascular catheterization is implemented in hospital settings.

1. The CDC intravascular catheter-related guidelines include several recommendations for the placement and care of intravascular catheters. Major areas of emphasis include:
    a. educating and training health-care providers in the insertion and maintenance of catheters
    b. use maximal sterile barriers during central venous catheter insertion
    c. using a 2% chlorhexidine-based solution for skin antisepsis
    d. avoid routine replacement of CVC
    e. use of antiseptic/antibiotic coated short-term CVC if the rate of infection is high, despite adherence to the above mentioned strategies
2. More recently the National UK guidelines on the prevention of CVC related sepsis (Pratt, *et al.*, 2007) have also made recommendations with emphasis in the following areas:
    a. Asepsis; an aseptic non-touch technique (ANTT) must be used and strict adherence to hand hygiene
    b. Skin decontamination; decontaminate the skin with a single patient use application of 2% chlorhexidine gluconate in 70% isopropyl alcohol

c. Dressing and insertion site care; sterile, transparent semi-permeable dressing should cover the catheter insertion site and be changed every 7 days. A single patient use application of 2% chlorhexidine gluconate in 70% isopropyl alcohol should be used to clean the catheter insertion site during dressing changes.
d. Filters, locks and needle-free devices; inline filters should not be used routinely. New intravascular devices that include needle-free devices should be monitored for an increase in infection. Decontamination of the access port of the needle-free device should be carried out before and after use.
e. Administration Sets and IV fluids; replace administration sets every 72 hours unless disconnected earlier. Sets for blood and blood components should be changed after transfusion or every 12 hours (whichever is the sooner). Parenteral nutrition sets should be replaced every 24 hours.

The first strategy identified in the above catheter-related infection control guidelines includes quality assurance and continuing education critical for decreasing infection rates. Emphasis is placed on education programmes that enable healthcare workers to provide, monitor and evaluate care while also incorporating advances in infection control and prevention. The need for awareness of new technology and the evolution of care based on documented improvements in clinical outcomes is critical as new products and evidence-based information become available.

1.3.2 Prevention of Surgical Site Infections

To effectively address SSI, healthcare practitioners must first understand the contributors to infection. All surgical wounds are contaminated by microorganisms, but the risk for development into infection depends on various factors, including the size of the inoculum of microorganisms, the virulence of the microbial contaminant, the microenvironment of the wound, and the integrity of host defenses (Fry 2006). Microorganisms may enter the wound from the skin, the air, the operating room environment, or from surgical instruments. Most SSI develops from microorganisms that are colonising the skin, mucous membranes, or hollow viscera (Mangram, *et al.*, 1999, Fry 2006). The risk of SSI varies depending on the type of surgical wound and other factors, such as the underlying health status of the patient and the duration of the operation (Fry 2006). Effective risk management to reduce SSI involves a multifaceted approach that includes understanding not only the pathogenesis of SSI, but also

assessing individual patient risk, and keeping apprised of new evidence-based recommendations and current initiatives to reduce the incidence of SSI.

Initiatives to reduce SSI focus on aspects of risk ranging from improving control of the physical care of the patient to information-gathering efforts such as broader reporting of infection rates. Major initiatives to prevent SSI are described in the following section.

1. Guideline for Prevention of Surgical Site Infection, 1999: This CDC guideline provides comprehensive and specific details about risk and prevention of SSI (Mangram, *et al.*, 1999). Information includes patient characteristics that contribute to increased SSI risk, such as diabetes, smoking, or prolonged hospital stay. Evidence-based information is provided about preoperative, operative, and postoperative procedures that have been clinically demonstrated to reduce infection risk. Surveillance methods with appropriate feedback to surgeons and infection control professionals are described to assist institutions in monitoring the incidence of SSI. Each recommendation in the CDC guideline is ranked by the level of scientific information available to support it. Preoperative recommendations from this guideline are described in more detail in the section below.
2. Surgical Infection Prevention Project (SIPP): The Centers for Medicare & Medicaid Services (CMS) and the CDC developed an initiative to reduce postoperative SSI by improving the selection and timing of preventive antibiotic therapy. Specific recommendations include the administration of antibiotics within 1 hour before surgical incision, prophylactic antibiotic therapy consistent with current recommendations, and discontinuation of prophylactic antibiotics within 24 hours after surgery. The need for this initiative is supported by clinical data that demonstrated that only 44% of US Medicare patients who undergo surgery receive antimicrobial prophylaxis within 1 hour of surgical incision (Bratzler, *et al.*, 2005).
3. The National Institute for Health and Clinical Excellence (NICE, 2008) recently released Guidelines for the Prevention of Surgical Site Infection that has specific recommendations for the preoperative, intraoperative, and postoperative phases of the surgical procedure. These are summarised in Figure 1.5.

Figure 1.5. Guidelines for the prevention of surgical site infection. From National Institute for Health and Clinical Excellence, 2008.

4. The Association of Perioperative Registered Nurses Recommended Practices for Skin Preparation of Patients (AORN 2008): These guidelines recommend skin preparation procedures designed to reduce damage to skin tissue and decrease the risk of infection. Specific guidelines include:
    a. Assessing and documenting the condition of the surgical site before skin preparation
    b. Leaving hair intact at the surgical site whenever possible to avoid skin damage during removal
    c. Thoroughly cleaning the surgical site
    d. Applying an antiseptic agent to the surgical site and surrounding areas
    e. Preparing the skin in a manner that preserves skin integrity and prevents injury

1.3.3 Cutaneous Antiseptics for the prevention of HAI

The skin, which consists of the epidermis, dermis, and subcutaneous tissue, is crucial for human survival. A mature and intact epidermis is an effective barrier in preventing skin infection (Figures 1.6).

However, invasive procedures, such as vascular access, hemodynamic monitoring, blood cultures, drainage tubes and surgical intervention procedures break the skin's barrier. Cutaneous antisepsis is vital in infection control.

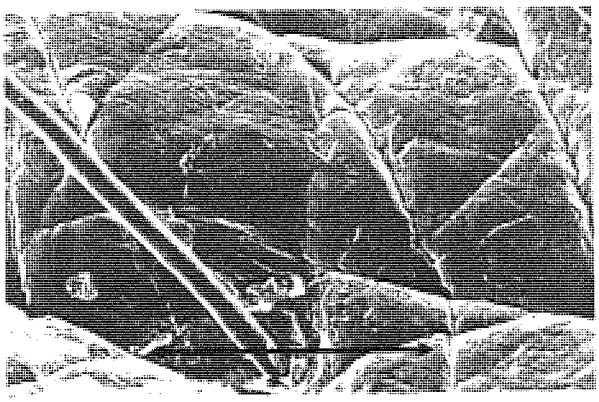


Figure 1.6: Magnified photograph of the normal human skin surface showing epidermal cells and hair entering through the skin. Bar = 500 μm^2.

Cutaneous or topical antiseptics are antimicrobial agents that kill, inhibit, or reduce the number of microorganisms on the skin. Antiseptics must be active against both resident and transient microorganisms on intact skin and ideally reduce the number on the skin by mechanical removal, chemical action, or both. Healthcare antiseptic formulations have a variety of modes and mechanisms, speed of antimicrobial activity, and persistent or residual properties. They also demonstrate varying levels of toxicity. There are specific characteristics to consider when selecting appropriate antiseptics for healthcare personnel hand washing, surgeons and operating room surgical scrub, and patient preoperative skin preparation. The selection should consider the safety and efficacy, quality of the evidence, ease of implementing, availability of product, affordability, and health economic value. The ideal cutaneous antiseptic should have the following properties:

1. broad spectrum of activity against Gram positive bacteria, Gram negative bacteria and fungi;
2. rapid bactericidal and fungicidal activity;
3. persistence or residual antimicrobial properties on the skin;
4. maintain its activity in the presence of organic matter, including blood;

5. be non-irritating or have low allergic and/or toxic responses; and
6. no or minimal systemic absorption.

The need to educate and update procedures is addressed in the recommendation to use 2% chlorhexidine gluconate as the preferred method of skin antisepsis (O'Grady, *et al.*, 2002; Pratt *et al.*, 2 2007). In the case of cutaneous antisepsis, the recommendation to use 2% chlorhexidine represents a change in procedure based on data demonstrating the superiority of this antiseptic in lowering intravascular-device related bloodstream infections (Maki, *et al.*, 1991; Sheehan, *et al.*, 1993; Meffre, *et al.*, 1995; Mimoz, *et al.*, 1996, Humar, *et al.*, 2000; Knasinski and Maki 2000; Kelly, *et al.*, 2005).

1.4 Summary

Published infection control guidelines include evidence-based recommendations that have been demonstrated to decrease infection rates, subsequently improving patient outcomes. Reduction of infection rates is known to decrease the total cost of patient care. Education of personnel who are responsible for infection control is critical to success and requires ongoing assessment of new data and materials to provide continual renewal of procedures and constant re-evaluation of results. A commitment to education, evaluation, and re-assessment creates an environment that promotes enhancement of patient care.

CRBSI and SSI produce significant morbidity and mortality, as well as increasing the costs of care. Several major initiatives have been undertaken to identify contributors to both of these healthcare-associated infections and to reduce risks to patients. The most comprehensive information is found in the national and international guidelines for prevention of CRBSI and SSI. According to these guidelines, preoperative procedures can substantially reduce risks for developing SSI. Several preoperative procedures are based on the use of chlorhexidine gluconate for preoperative showering, general skin antisepsis, preoperative hand hygiene, and patient preoperative skin preparation. The current international guidelines for the prevention of intravascular device infection have very specific recommendation for 2% chlorhexidine patient skin preparation for skin preparation prior to insertion and maintenance of care of central venous catheters. Currently there are no published prospective, randomized, controlled studies in the prevention of surgical site infection in respect to the surgeon's choice of patient preoperative skin preparation.

1.5 Outline of Current Project

In this project various characteristics of chlorhexidine were compared to povidone iodine to facilitate the choice of appropriate skin antisepsis. This included *in-vitro* and *in-vivo* testing that evaluated rates of kill of various microorganisms and the effect of the presence of proteins on the antiseptics antimicrobial activity. The methodology of the actual application of antiseptics was also evaluated in an in-vivo simulation of preparation of the patient's skin prior to the surgical procedure. In addition, the application of 2% chlorhexidine for the prevention of subsequent SSI in patients undergoing varying types of surgery was also studied.

CHAPTER 2: CHLORHEXIDINE CUTANEOUS ANTISEPSIS

2.1 Antisepsis in the Healthcare Setting

The term "antisepsis" (Greek: anti=against, sepsis=putrefaction) was first utilized by Sir John Pringle in 1772 and not until the introduction of carbolic spray and dressings by Joseph Lister was antiseptic actions were instituted in healthcare and are vital today in modern medicine.

Antiseptics are agents that destroy or inhibit the growth and development of microorganisms in or on living tissue. Unlike antibiotics that act selectively on a specific target, antiseptics have multiple targets and a broader spectrum of activity, which include bacteria, fungi, viruses, protozoa, and even prions (McDonnell and Russell 1999, Taylor 1999). There are several antiseptic categories, including alcohols (ethanol), anilides (triclocarban), biguanides (chlorhexidine), bisphenols (triclosan), chlorine compounds, iodine compounds, silver compounds, peroxygens, and quaternary ammonium compounds (McDonnell and Russell 1999). The most commonly used products in clinical practice today include povidone iodine, chlorhexidine, and alcohol.

Many antiseptic agents mainly focus on cleansing intact skin and are used for prepping patients preoperatively and prior to intramuscular injections or venous punctures, pre- and postoperative scrubbing in the operating room, and hand washing by medical personnel.

The usefulness of antiseptics on intact skin is well established and broadly accepted. However, the use of antiseptics as prophylactic anti-infective agents for the prevention of healthcare associated infections has been an area of intense controversy for several years among clinicians and government regulatory bodies.

Prophylactic antisepsis is indicated:

1. Before any division of skin and mucosa
2. Prior to diagnostic or therapeutic procedures in body cavities without division of the integument, such as transurethral catheterisation and transvaginal endoscopic uteroscopy)
3. To treat unwanted colonization, such as multiresistant pathogens such as MRSA
4. After accidental contamination of the skin, mucosa, eye or wounds where there is a risk of infection
5. As protection against unwanted colonization with subsequent infection

The requirements for classifying a substance as an antiseptic are defined in the basic test of phase 1 of the European Standard for disinfectants and antiseptics (EN 1040 and 1275).

EN 1040: Basic bactericidal activity – Test method and requirement (phase 1)

This European Standard describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have a bactericidal activity under the laboratory conditions defined by this European Standard. If a product complies with the test requirements, it can be considered as possessing a bactericidal activity. The product, when tested shall demonstrate at least a 10^5 log reduction in viable counts when the test organism is *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Suggested strains are *Pseudomonas aeruginosa* ATCC 15442** and *Staphylococcus aureus* ATCC 6538**.

EN 1275 Basic fungicidal activity – Test method and requirement (phase 1)

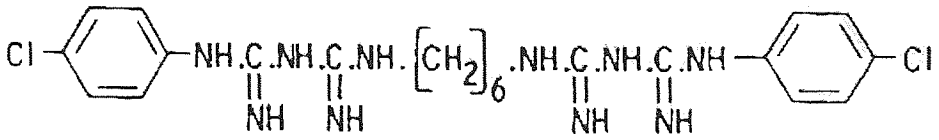
This European Standard describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have a fungicidal activity under the laboratory conditions defined by this European Standard. If a product complies with the test requirements, it can be considered as possessing a fungicidal activity. The product, when tested shall demonstrate at least a 10^5 log reduction in viable counts when the test organism is *Candida albicans* and *Aspergillus niger*. Suggested strains are *Candida albicans* ATCC 10231** and *Aspergillus niger* ATCC 16404.

2.2 Chlorhexidine

Chlorhexidine was first synthesized in 1950 by Imperial Chemical Industries (ICI) while researching synthetic compounds related to the antimalarial, proguanil. Compounds containing the biguanide structure have demonstrated potent antibacterial effect and chlorhexidine was quickly recognized for its antimicrobial properties. Chlorhexidine, a bisbiguanide, is a symmetrical molecule containing a hexamethylene chain that links two biguanide groups with *p*-chlorophenyl substituents. (Hugo and Russell 1998, Russell 2002) It is identified as 1,1'-hexamethylenebis(5-[p-chlorophenyl]biguanide, CAS # 55-56-1, with a molecular weight of 505.44, and molecular formula $C_{22}H_{30}C_{12}N_{10}$ (Figure 2.1).

Chlorhexidine is available as the acetate (diacetate), hydrochloride and gluconate salts.

1. Chlorhexidine acetate identified as 1,1'-hexamethylenebis(5-[p-chlorophenyl]biguanide diacetate, CAS # 56-95-1, with a molecular weight (MW) of 625.56, and molecular formula $C_{22}H_{30}C_{12}N_{10}\bullet 2C_2H_4O_2$.



Chlorhexidine

Figure 2.1: Chlorhexidine molecule.

2. Chlorhexidine hydrochloride identified as 1,1'-hexamethylenebis(5-[p-chlorophenyl]biguanide dihydrochloride , CAS # 3697-42-5, with a molecular weight of 578.37 and a molecular formula $C_{22}H_{30}C_{12}N_{10}$•2HCl.
3. Chlorhexidine Gluconate identified as 1,1'-hexamethylenebis(5-(p-chlorophenyl)biguanide) digluconate, CAS# 18472-51-0, with a molecular weight (MW) of 897.88, and molecular formula $C_{22}H_{30}C_{12}N_{10}$•$2C_6H_{12}O_7$

Chlorhexidine is an important medical, dental and pharmaceutical antiseptic, disinfectant and preservative (Hugo *et al.,* 1992; Scott *et al.*, 1992; Russell *et al.*, 1986 and Russell *et al.,* 1990). It has an extensive history of use in cosmetics, antiseptics, ophthalmic solutions, pharmaceuticals, rinses and toothpastes. It is reported under several trade names, for example, Hibiclens, Hibiscrub, Hibitane, 2% (w/v) Chlorhexidine gluconate in 70% (v/v) isopropyl alcohol, Chlorascrub, Orahexal, Peridex, PLAC out, Unisept, *etc.*

2.2.1 Microbiology

Chlorhexidine is active against many Gram-positive, Gram-negative bacteria and yeasts (Figure 2.2). Viruses are rapidly inactivated by chlorhexidine. Chlorhexidine inhibits vegetative cell of spore forming bacteria and spore germination. Chlorhexidine does not inhibit bacterial spores except at elevated temperatures. At a temperature of 70°C, chlorhexidine reduced the number of *Bacillus subtilis* spores by 5 logarithms (Shaker 1986). In addition, it has residual antimicrobial activity that is due to the affinity of the compound for binding to skin while remaining in an active form (Denton, 2001).

Figure 2.2: Activity of chlorhexidine towards different microorganisms. From McDonnell and Russell, 1999.

2.2.2 Mechanism of Activity

Chlorhexidine is bacteriostatic at low concentrations and rapidly bactericidal at higher concentrations. The mechanisms surrounding cell inhibition and death are related to cytologic and physiologic changes. Chlorhexidine's antimicrobial effects are associated with the attractions between chlorhexidine (cationic) and the negatively charged surface and membranes of bacterial cells.

Hugo and Longworth in the 1960s (Hugo and Longworth, 1964) demonstrated that chlorhexidine was a membrane-active agent, causing extensive damage to the bacterial cytoplasmic (inner) membrane, followed by the precipitation or coagulation of protein and nucleic acids. Chawner and Gilbert (1989) showed that the positively-charged biguanide regions of the chlorhexidine molecule bind strongly with anionic sites on the cell membrane and cell wall, particularly the acidic phospholipids and proteins. This binding causes displacement of divalent cations (Mg^{2+}, Ca^{2+}) and disrupts the cell wall and membrane (Davies 1973). The hexamethylene region, being 6 carbons in length, allows the biguanides to bind to adjacent phospholipid headgroups of the cell membrane and displaces the associated divalent cations (Davies 1973).

Studies in which bacteria were exposed to varying concentrations of chorhexidine and examined for cytoplasmic leakage showed that leakage increases with increasing chlorhexidine concentrations, maximizes, and then declines at higher concentrations (Russell 1996 and Russell *et al.*, 1999). Jones showed through electron microscopy that the bacteria cells exposed to higher concentrations of chlorhexidine are altered substantially (Jones, 1997). The reduced

leakage at higher concentrations is thought to be due to precipitation and coagulation of the cytoplasmic contents of the cell.

Harold (1969) stated that chlorhexidine inhibited the uptake of membrane-bound and soluble ATPase and K^+ in *Enterococcus faecalis*. However, only low biguanide concentrations inhibit membrane-bound ATPase (Chopra *et al.*, 1987). Chlorhexidine collapses the membrane potential; it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects (Barett *et al.*, 1991, McDonnell *et al.*, 1999).

2.2.3 Antimicrobial Spectrum

In review of the published literature, the antimicrobial activity of chlorhexidine is directed mainly toward vegetative Gram-positive and Gram-negative bacteria; it is inactive against bacterial spores except at elevated temperatures, and acid-fast bacilli are inhibited but not killed by aqueous solutions. Yeasts (including *Candida albicans*) and dermatophytes are usually sensitive; although chlorhexidine's fungicidal action in general is subject to species variation, as are other agents (Denton 2001).

Although numerous publications refer to the bacteriostatic and bactericidal properties of chlorhexidine against particular organisms, the methods used vary, and it is often difficult to compare results. A series of studies provide a comprehensive spectrum of activity for chlorhexidine using both minimum inhibitory concentrations and time-kill methods. Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism (Andrews, 2002), and time-kill kinetics is a study that determines how fast an antimicrobial can kill certain bacteria and prevent their regrowth. The strains of organisms tested included clinical isolates, laboratory strains, and standard culture collection types. Each strain was tested to determine the minimum inhibitory concentration (MIC) of chlorhexidine and its susceptibility to the bactericidal action of 0.05% aqueous chlorhexidine gluconate using a rate of time-kill method. The bacteriostatic activity and fungistatic activity of chlorhexidine is shown in Table 2.1 and 2.2, respectively. The in vitro bactericidal and fungicidal rate of activity for chlorhexidine (0.05%) is shown in Tables 2.3 and 2.4, respectively (Denton 2001).

Table 2.1: Bacteriostatic activity of chlorhexidine gluconate

Test organism	MIC (mg/L)[a]		
	No. of strains	Mean	Range
Gram-positive cocci			
Micrococcus flavus	1	0.5	
Micrococcus lutea	1	0.5	
Staphylococcus aureus	16	1.6	1-4
Staphylococcus epidermidis	41	1.8	0.25-8
Streptococcus faecalis	5	38	32-64
Streptococcus mutans	2	2.5	
Streptococcus pneumoniae	5	11	8-16
Streptococcus pyogenes	9	3	1-8
Streptococcus sanguis	3	9	4-16
Streptococcus viridans	5	25	2-32
Gram-positive *bacilli*			
Bacillus cereus	1	8	
Bacillus subtilis	2	1	
Clostridium difficile	7	16	8-32
Clostridium welchii	5	14	4-32
Corynebacterium spp	8	1.6	0.5-8
Lactobacillus casei	1	128	
Listeria monocytogenes	1	4	
Propionibacterium acne	2	8	
Gram-negative *bacilli*			
Acinetobacter anitratus	3	32	16-64
Acinetobacter lwoffi	2	0.5	
Alkaligenes faecalis	1	64	
Bacteroides distastonis	4	16	
Bacteroides fragilis	11	34	8-64
Campylobacter pyloridis	5	17	8-32
Citrobacter freundii	1	18	4-32
Enterobacter cloacae	12	45	16-64
Escherichia coli	14	4	2-32
Gardnerella vaginalis	1	8	
Haemophilus influenzae	10	5	2-8
Klebsiella aerogenes	5	25	16-64
Klebsiella oxytoca		32	

Table 2.1 (*continued*)

Test organism	MIC (mg/L)[a]		
	No. of strains	Mean	Range
Klebsiella pneumoniae	5	64	82-128
Proteus mirabilis	5	115	64->128
Proteus morganii	5	73	16-128
Proteus vulgaris	5	57	32-128
Providencia stuartii	5	102	64-128
Pseudomonas aeruginosa	15	20	16-32
Pseudomonas cepacia	1	16	
Pseudomonas fluorescens	1	4	
Salmonella bredeney	1	16	
Salmonella dublin	1	4	
Salmonella gallinarum	1	8	
Salmonella montivideo	1	8	
Salmonella typhimurium	4	13	8-16
Salmonella virchow	1	8	
Serratia marcescens	10	30	16-64

[a]minimal inhibitory concentration.

Table 2.2: Fungistatic activity chlorhexidine gluconate

Organism	No. of strains	Mean MIC[a] (mg/L)
Mold fungi		
Aspergillus flavus	1	64
Aspergillus fumigatus	1	32
Aspergillus niger	1	16
Penicillium notatum	1	16
Rhizopus sp.	1	8
Scopulariopsis spp.	1	8
Yeasts		
Candida albicans	2	9
Candida guillermondii	1	4
Candida parapsilosis	2	4
Candida pseudotropicalis	1	3
Cryptococcus neoformans	1	1
Prototheca zopfii	1	6
Saccharomyces cerevissia	1	1
Torulopsis glabrata	1	6
Dermatophytes		
Epidermophyton floccosum	1	4
Microsporum canis	2	4
Microsporum fulvum	1	6
Microsporum gypseum	1	6
Trichophyton equinum	1	4
Trichophyton interdigitale	2	3
Trichophyton mentagrophytes	1	3
Trichophyton quinkeanum	1	3
Trichophyton rubrum	2	3
Trichophyton tonsurans	1	3

[a]minimal inhibitory concentration.

Table 2.3: Bactericidal activity of 0.05% chlorhexidine gluconate

Test organism	No. of Strains	Mean log_{10} reduction after 1/3 min	1 min	10 min
Gram-positive cocci				
Micrococcus flavus	(1)	0.1	0.4	2.1
Micrococcus lutea	(1)	0.2	0.7	2.9
Staphylococcus aureus	(16)	0.4	0.7	2.5
Staphylococcus epidermidis	(41)	2.2	3.4	>5.1
Streptococcus faecalis	(5)	0.4	0.4	1.1
Streptococcus mutans	(2)	0.8	>4.6	5.8
Streptococcus pneumoniae	(5)	0.8	1.5	>3.5
Streptococcus pyogenes	(9)	1.2	1.8	>3.7
Streptococcus sanguis	(3)	1.1	2.2	>3.9
Streptococcus viridans	(5)	0.4	0.8	2.3
Gram-positive *bacilli*				
Bacillus cereus	(1)	2.0	2.0	4.7
Bacillus subtilis	(2)	0.5	0.5	0.3
Clostridium difficile	(7)	0.2	0.3	0.3
Clostridium welchii	(5)	2.1	3.1	>4.8
Corynebacterium spp	(8)	1.1	1.4	3.7
Lactobacillus casei	(1)	0.2	0.2	4.1
Listeria monocytogenes	(1)	0.6	2.2	4.8
Propionibacterium acne	(2)	0.7	1.8	3.6
Gram-negative *bacilli*				
Acinetobacter anitratus	(3)	1.4	2.6	>5.3
Acinetobacter lwoffi	(2)	>4.0	>4.3	>4.8
Alkaligenes faecalis	(1)	1.5	2.7	4.1
Bacteroides distastonis	(4)	0.9	2.7	>4.9
Bacteroides fragilis	(11)	3.0	4.2	5.2
Campylobacter pyloridis	(5)	N.T.	2.8	>4.0
Citrobacter freundii	(10)	3.4	4.9	>6.0
Enterobacter cloacae	(12)	3.5	4.5	>6.3
Escherichia coli	(14)	3.2	5.0	>6.4
Gardnerella vaginalis	(1)	2.3	3.3	>5.8
Haemophilus influenzae	(10)	>4.1	>4.1	>4.1
Klebsiella aerogenes	(5)	2.	3.9	>5.9
Klebsiella oxytoca	(2)	3.	5.2	>6.4

Table 2.3 (*continued*)

Test organism	No. of Strains	Mean $\log_{10}$ reduction after		
		1/3 min	1 min	10 min
Klebsiella pneumoniae	(5)	3.0	4.8	>6.2
Proteus mirabilis	(5)	0.8	0.9	2.9
Proteus morganii	(5)	1.0	1.5	4.2
Proteus vulgaris	(5)	0.8	1.0	4.1
Providencia stuartii	(5)	0.6	0.9	1.8
Pseudomonas aeruginosa	(15)	1.7	2.7	4.9
Pseudomonas cepacia	(1)	1.1	1.3	>4.6
Pseudomonas fluorescens	(1)	3.8	5.0	>6.7
Salmonella bredeney	(1)	1.6	3.4	>6.4
Salmonella dublin	(1)	1.5	2.9	3.2
Salmonella gallinarum	(1)	2.5	4.0	>6.2
Salmonella montivideo	(1)	2.4	3.8	>6.3
Salmonella typhimurium	(4)	2.0	3.7	>6.0
Salmonella virchow	(1)	1.9	3.9	>6.2
Serratia marcescens	(10)	1.5	3.7	>5.9

Table 2.4: Fungicidal activity of 0.05% chlorhexidine gluconate

Test organism	No. of Strains	Mean $\log_{10}$ reduction after		
		1/3 min	1 min	10 min
Mold fungi				
Aspergillus flavus	1	0.4	0.8	1.7
Aspergillus fumigatus	1	0.7	1.2	2.4
Aspergillus niger	1	0.7	1.2	3.0
Penicillium notatum	1	0.6	2.0	3.5
Rhizopus sp.	1	0.4	0.4	0.5
Scopulariopsis spp.	1	0.6	1.1	2.3
Yeasts				
Candida albicans	2	2.8	>4.1	>4.2
Candida guillermondii	1	3.5	>4.3	>4.3
Candida parapsilosis	2	2.1	3.4	>4.2
Candida pseudotropicalis	1	3.6	>4.4	>4.4
Cryptococcus neoformans	1	4.0	>4.2	>4.2
Prototheca zopfii	1	3.3	>3.6	>3.6
Saccharomyces cerevissia	1	3.7	>3.7	>3.7
Torulopsis glabrata	1	1.3	2.2	>4.4
Dermatophytes				
Epidermophyton floccosum	1	0.7	0.5	>1.8
Microsporum canis	2	0.4	1.0	>2.0
Microsporum fulvum	1	0.2	0.6	>2.4
Microsporum gypseum	1	0.1	0.3	2.0
Trichophyton equinum	1	0.5	1.1	>2.1
Trichophyton interdigitale	2	0.4	0.9	>2.4
Trichophyton mentagrophytes	1	1.3	>2.1	>2.1
Trichophyton quinkeanum	1	0.2	0.9	>2.8
Trichophyton rubrum	2	0.3	0.6	>2.4
Trichophyton tonsurans	1	0.4	0.3	1.6

Adams and colleagues assessed the efficacy of 6 skin disinfectants against *S. epidermidis* RP26A in the presence or absence of protein, using quantitative time-kill suspension and carrier tests. The skin disinfectants were 2% (w/v) chlorhexidine gluconate in 70% (v/v) IPA, 70% (v/v) IPA, 0.5% (w/v) aqueous chlorhexidine gluconate, 2% (w/v) aqueous chlorhexidine gluconate, 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) IPA, and 10% (w/v) aqueous povidone iodine (PI) (Adams, *et al.*, 2005).

In the suspension test 10μL broth containing 3 x 10^6 cfu *S. epidermidis* RP62A was added to 990μL disinfectant and mixed. After 30 seconds contact time at room temperature, 100μL suspension was removed and added to 990μL neutralising agent, mixed and left to dwell for 5 minutes. Serial dilutions were inoculated on to brain heart infusion (BHI) agar plates which were incubated at 37°C for up to 48 hours. Further suspension tests were done by adding 10% (v/v) human serum to the suspension prior to adding the disinfectant. The evaluations were carried out in triplicate.

In the carrier biofilm test, a suspension of *S. epidermidis* RP26A was diluted in BHI to approximately 1 X 10^4. Aliquots of 200μL suspension were inoculated into 16 wells of a microtitre tray. This was covered and incubated 37°C for up to 48 hours. Biofilm production was confirmed by O'Toole and Kolter's technique (O'Toole and Kolter, 1998). To determine the efficacy of the disinfectants against the biofilm in the presence of protein, the carrier test was repeated; a suspension of *S. epidermidis* RP26A was diluted in BHI to approximately 1 x 10^4 cfu/mL, and 10% human serum was added.

In all tests, the controls containing no disinfectant resulted in the complete recovery of the initial inocula. The results as represented by the $\log_{10}$ reduction factor of the initial cfu/mL are shown in Table 2.5.

When evaluating the effectiveness of the 6 disinfectants against *S. epidermidis* RP26A in a biofilm enriched with 10% (v/v) human serum, 4 of the disinfectants achieved a $\log_{10}$ reduction factor between 2 and 4 at 30 seconds. The 2% (w/v) CHG in 70% (v/v) IPA and 10% (w/v) aqueous povidone-iodine achieved a $\log_{10}$ reduction factor between 4 and 5. There was no statistical difference between these 2 disinfectants (P=0.28).

However, when challenged with biofilm, the antimicrobial effectiveness was reduced. The study demonstrated that the 2% (w/v) chlorhexidine gluconate in 70% (v/v) IPA may offer an advantage over the other chlorhexidine products tested.

Table 2.5: Comparing the efficacy of 2% (9 w/v) chlorhexidine gluconate (CHG) in 70% (v/v) IPA against 5 standard skin disinfectants on *Staphylococcus epidermidis* RP62A after 30 seconds contact time utilizing suspension and carrier tests.

	Log_{10} reduction factor in CFU[a]/mL of *S. epidermidis* RP62A			
Antiseptic	Suspension test	Suspension test with 10% human serum	Carrier test: Biofilm	Carrier test: Biofilm enriched with 10% human serum
2% (w/v) CHG in 70% (v/v) IPA	6.5	6.3	5.3	4.7
70% (v/v) IPA	6.5	6.3	5.4	2.8
0.5% (w/v) aqueous CHG	6.5	6.3	4.1	2.3
2% (w/v) aqueous CHG	6.5	6.3	4.8	2.8
0.5% (w/v) CHG in 70% (v/v) IPA	6.5	6.3	5.8	3.6
10% (w/v) aqueous povidone iodine	6.5	6.3	5.9	4.4

[a]colony-forming units.

2.2.4 Resistance

The emergence of bacteria that are resistant to multiple antibiotics has caused the same concern to be raised regarding antiseptics and disinfectants. Several investigators have looked for resistance to CHG or have tested strains of bacteria that are resistant to antibiotics. It is important to understand the mechanisms of microbial responses to biocides so that susceptibility and the possibility of development of resistance to these compounds can be predicted (Russell, 1997). The resistance mechanisms to antibiotics must be differentiated from the resistance to biocides such as CHG. Antibiotic resistance mechanisms are in some instances intrinsic, but most are acquired through mutation or through gene transfer. Examples of antibiotic resistance mechanisms include: impaired uptake, modification of the target site, bypass of a sensitive step, overproduction of the target, absence of the enzyme of a metabolic pathway, or efflux of the antimicrobial drug. Biocide resistance is usually intrinsic in nature and is often unspecific.

Impaired uptake and proteins that pump the antimicrobial out of the cell are examples of this nonspecific resistance (Russell, 1997).

There has been minimal evidence for the development of resistance to CHG, or for cross-resistance arising from antibiotic resistance mechanisms antibiotic resistance common in Gram-negative microorganisms with the use of CHG. Several studies specifically evaluating sensitivity or increased resistance to CHG in antibiotic-resistant strains of *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens,* or *Proteus mirabilis* demonstrated no significant increase in sensitivity (Denton, 1991). Although, a study in patients undergoing intermittent bladder catheterization demonstrated that the application of chlorhexidine to the perineal skin prior to catheter insertion was effective against Gram-positive skin flora but not against Gram-negative orgainisms, such as *Providencia stuartii*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, which ultimately lead to the cause of urinary infections (Stickler 2002).

Strains of MRSA and MSSA tested against 10% povidone-iodine, 0.5% aqueous CHG and 0.5% CHG in 80% ethanol showed no evidence of resistance to CHG (Sakuragi *et al.*, 1995).

The testing of the drug product of antibiotic-resistant bacteria showed no more resistance than shown by the antibiotic sensitive strains of the same organisms. The MIC for the 2% (w/v) Chlorhexidine gluconate in 70% (v/v) isopropyl alcohol drug product and aqueous 2% CHG solution did not exceed 6.25 ppm for the following organisms: MRSA, vancomycin-resistant *Staphylococcus epidermidis* (VRSE), vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Enterococcus faecalis* (VRE), and vancomycin-resistant *Enterococcus faecium* (VRE).

Another major difference between antibiotics and topical antimicrobial agents is the achievable factor over the MIC for each. Antibiotics typically have maximum serum concentrations that become toxic for the patient at 2 to 3 times the minimum effective serum concentration.

2.3 Modified *In-Vitro* Time-Kill Evaluation

Purpose: The Modified *In-Vitro* Time-Kill Method evaluates the antimicrobial properties of topical antimicrobial products in the presence of 0.9% Normal Saline or Blood.

2.3.1 Test Product(s)

1. 2% (w/v) Chlorhexidine Gluconate in 70% (v/v) Isopropyl Alcohol
2. 2% (w/v) Chlorhexidine Gluconate

3. 10% Povidone-Iodine Solution (1% available iodine)

2.3.2 Neutralisation Study

1. A neutralisation study was performed using a representative microorganisms strain ensuring that the neutralising solution (BBP++) was effective in neutralising the antimicrobial properties of the test product(s).
2. A 1.0 mL aliquot of the appropriate product concentration (i.e., undiluted, 50% [v/v] or 10% [v/v], was added to a sterile test tube containing 8.9 mL of BPB++ solution, and mixed thoroughly 1 x 10^9 CFU/mL was added to the tube containing product/neutraliser and mixed thoroughly (10^{-3} dilution).
3. Appropriate ten-fold dilutions (e.g., 10^{-4}, 10^{-5}, 10^{-6}) were prepared in (BPB++) solution, mixing thoroughly using a vortex mixer between dilutions.
4. From the final of the product/neutraliser challenge suspension, 0.1 mL or 1.0 mL aliquots were pour-plated or spread-plated, in duplicate, using the appropriate agar. The plates were incubated at the temperature and under the conditions appropriate for each species for forty-eight (48) to seventy-two (72) hours, or sufficient growth was observed.
5. Following incubation, the colonies on the plates were counted. Counts in the thirty (30) to three-hundred (300) CFU range were used preferentially in the data calculations.
6. If the colony counts (CFU/mL) recovered from the neutralisation control were no more than 0.25 Log_{10} lower than those observed for the initial population successful neutralisation was assumed to have been demonstrated.

2.3.3 Methodology

2.3.3.1 Inoculum Preparation

1. Approximately forty-eight hours prior to testing, separate sterile tubes containing the appropriate broth media were inoculated from lyophilized vials or cryogenic stock cultures containing the challenge microorganisms. The broth cultures were incubated at the temperatures and under the conditions appropriate for each species for approximately twenty-four hours, or until sufficient growth was observed.

2. Approximately twenty-four hours prior to testing, the broth cultures prepared as described in section 2.2.3.1.1 were used to inoculate the surface of Petri plates containing the appropriate agar media (Table 2.6). These plates were incubated appropriately until sufficient growth is observed. This produced lawns of the bacteria or yeast on the surface of the agar plates, and these were used to prepare the challenge suspensions.

2.3.3.2 Challenge Suspensions

Immediately prior to initiating the test procedure, a suspension of each challenge microorganism were prepared in sterile 0.9% Sodium Chloride Irrigation (SCI) by suspending the microorganisms from the solid media previously prepared to achieve challenge suspensions concentrations of approximately 1 x 10^9 CFU/mL. The suspensions will be homogenized, as necessary, using sterile glass tissue grinders. The prepared suspensions may be stored at 2° - 8°C for up to two weeks prior to use in testing.

2.3.3.3 Initial Population Determinations

The initial population of each challenge suspension was determined by preparing ten-fold dilutions of each microorganism in (BBP++). Using the appropriate agar, pour- or spread-plates were prepared, in duplicate from the inoculum dilutions for each microorganism by plating 0.1 mL to achieve plated dilutions of 10^{-6}, 10^{-7}, and 10^{-8}. The plates will be incubated at the temperature and under the conditions appropriate for each species for forty-eight to seventy-two hours, or until sufficient growth is observed.

2.3.4 Testing Procedures

1. For Products to be evaluated at a 99% (v/v) concentration, a 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^9 CFU/mL were transferred to a sterile test tube containing 9.9 mL of test product and mixed thoroughly using a vortex mixer and/or positive displacement pipetter to achieve the 99% (v/v) concentration of the product.
2. For Products to be evaluated at a 50% (v/v) concentration, a 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^9 CFU/mLwere transferred to a sterile test tube containing 5.0 mL of test product and 4.9 mL of sterile Water-for-Irrigation (WFI), mixed thoroughly using a vortex mixer and/or positive displacement pipetter to achieve the 50% (v/v) concentration of the product.

3. For Product to be evaluated at a 10% (v/v) concentration, a 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^9 CFU/mL will be transferred

Table 2.6: General challenge microorganisms

Microorganisms species	Incubation time	Incubation temperature	Media[a]
Acinetobacter species	24 - 72 hours	35° ± 2°C	BHIB/BHIA/BHIA+
Aspergillus species	2 – 10 days	25° ± 2°C	PDA/SDA/SDA+
Bacteroides species	24 - 72 hours	35° ± 2°C (anaerobic)	SB/SA-B
Bacillus species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Camplobacter species	24 - 72 hours	35° ± 2°C (microaerophilic)	FTM/SBA
Candida species	24 - 72 hours	30° ± 2°C	TSB/SDA/SDA+
Citrobacter species	24 – 72 hours	35° ± 2°C	TSB/TSA/TSA+
Clostridium species	24 - 72 hours	35° ± 2°C (anaerobic)	RCM/RCA
Corynebacterium species	24 - 72 hours	35° ± 2°C	SBA
Enterobacter species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Enterococcus species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Escherichia coli	24 – 72 hours	35° ± 2°C	TSB/TSA/TSA+
Haemophilus species	24 - 72 hours	35° ± 2°C	CAE
Klebsiella species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Lactobacillus species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Listeria species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Micrococcus species	24 – 72 hours	35° ± 2°C	TSB/TSA/TSA+
Penicillium species	2 – 10 days	25° ± 2°C	PDA/SDA/SDA+
Proteus species	24 – 72 hours	35° ± 2°C	TSB/TSA/TSA+
Pseudomonas species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Rhodotorula species	24 - 72 hours	25° ± 2°C	TSB/TSA/TSA+
Salmonella species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Serratia species	24 - 72 hours	30° ± 2°C	TSB/TSA/TSA+
Shigella species	24 – 72 hours	35° ± 2°C	TSB/TSA/TSA+
Staphylococcus species	24 - 72 hours	35° ± 2°C	TSB/TSA/TSA+
Streptococcus pneumoniae	24 - 72 hours	35° ± 2°C	SBA
Streptococcus pyogenes	24 – 72 hours	35° ± 2°C	BHIB/BHIA/BHIA+

[a]Butterfield's Phosphate Buffer (BBP), Butterfield's Phosphate Buffer solution with product neutralisers (BBP++), Phosphate Buffered Saline Solution (PBS), Brain-Heart Infusion Broth (BHIB), Schaedler's Broth (SB), Tryptic Soy Broth (TSB), Fluid Thioglycollate Medium (FTM), Brain-Heart Infusion Agar (BHIA), Brain-Heart Infusion Agar with product neutralisers (BHIA+), Chocolate Agar with Enrichment (CAE),

to a sterile test tube containing 1.0 mL of test product and 8.9 mL of sterile WFI, mixed thoroughly to achieve the 10% (v/v) concentration of the product.

4. Product for All Product Evaluations: Each of the challenge microorganisms were exposed to the test product(s) for the designated exposure time(s).
5. After each exposure time has elapsed, 1.0 mL were removed from each tube containing product/challenge suspension, placed in separate sterile test tubes containing 9.0 mL of BPB++ solution (10^{-3} dilution), and mixed thoroughly. Appropriate ten-fold dilutions were prepared for each challenge microorganism in BPB++, mixing thoroughly.
6. For the final dilutions of the product/neutraliser/challenge suspension. 0.1 or 1.0 mL aliquots were pour- or spread- plated, in duplicate, using the appropriate solid medium, producing final plated dilutions of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}. The plates were incubated for forty-eight (48) to seventy-two (72) hours, or until sufficient growth was observed.

2.3.5 Data Collection

Following incubation, the colonies on the plates were counted. Counts of thirty (30) to three-hundred (300) CFU range were used in the data calculations.

The Log_{10} Average and the CFU/mL of the average of the duplicate plate counts for the initial population after each timed exposure to the product(s) will be calculated for each challenge microorganism as follows:

$$Log_{10}\ Average = Log_{10}\ (C_i \times 10^{-D})$$

$$CFU/mL = (C_i \times 10^{-D})$$

where: Log IP = Log_{10} of the Initial Population of Challenge Microorganism

Log P_{EX} = Log_{10} of the Average Population after Exposure to the Product

The Percent Reductions will be calculated for each microorganism for each timed exposure to the product as follows:

$$\text{Percent Reduction} = \frac{IP - P_{EX}}{IP} \times 100$$

where: IP = Initial Population of Challenge Microorganism (CFU/mL)

P_{FX} = Average Population after Exposure to the Product (CFU/mL)

2.3.6 Neutralisation Study Results

The neutralisation of *Staphylococcus epidermidis* (ATCC #12228) and the neutralising solution is outlined in Table 2.7. The neutralisation of the 3 antiseptic agents, 2% CHG in 70% isopropyl alcohol, 2% CHG, and 10% povidone-iodine against *Staphylococcus epidermidis* (ATCC #12228) are outlined in Table 2.8, Table 2.9, and Table 2.10.

Table 2.7: Neutralisation evaluation – initial population (inoculum) and neutraliser toxicity *Staphylococcus epidermidis* (ATCC #12228). The data demonstrates that the neutraliser has no antimicrobial activity.

Test description	Dilution 10^{-D}	Plate counts A	Plate counts B	Average colony count (C_i)	Log_{10} average[a]
Initial population (inoculum)					
Replicate #1	-2	TNTC	TNTC		
	-3	185	175	180.0	5.3
	-4	25	18		
Replicate #2	-2	TNTC	TNTC		
	-3	194	196	195.0	5.3
	-4	17	20		
Replicate #3	-2	TNTC	TNTC		
	-3	187	175	181.0	5.3
	-4	17	19		
Neutraliser toxicity with inoculum					
Replicate #1	-2	TNTC	TNTC		
	-3	165	169	165.0	5.2
	-4	30	12		
Replicate #2	-2	TNTC	TNTC		
	-3	192	187	189.5	5.2
	-4	19	16		
Replicate #3	-2	TNTC	TNTC		
	-3	163	156	159.5	5.2
	-4	22	22		

[a] Log_{10} Average = Log_{10} (C_i x 10^{-D}).

Table 2.8: Neutralisation evaluation – 2% chlorhexidine gluconate in 70% isopropyl alcohol *Staphylococcus epidermidis* (ATCC #12228)

Test description	Dilution 10^{-D}	Plate counts A	Plate counts B	Average colony count (C_i)	Log_{10} average[a]
Product – 2% Chlorhexidine gluconate in 70% isopropyl alcohol					
Replicate #1	-2	TNTC	TNTC		
	-3	189	157	173.0	5.3
	-4	16	8		
Replicate #2	-2	TNTC	TNTC		
	-3	163	167	165.0	5.2
	-4	11	12		
Replicate #3	-2	TNTC	TNTC		
	-3	173	154	163.5	5.2
	-4	16	19		

[a]Log_{10} Average = Log_{10} (C_i x 10^{-D})

Table 2.9: Neutralisation evaluation – 2% chlorhexidine gluconate *Staphylococcus epidermidis* (ATCC #12228)

Test description	Dilution 10^{-D}	Plate counts A	Plate counts B	Average colony count (C_i)	Log_{10} average[a]
Product – 2% Chlorhexidine gluconate					
Replicate #1	-2	TNTC	TNTC		
	-3	165	169	167.0	5.2
	-4	30	12		
Replicate #2	-2	TNTC	TNTC		
	-3	192	187	189.5	5.2
	-4	19	16		
Replicate #3	-2	TNTC	TNTC		
	-3	163	156	159.5	5.2
	-4	22	22		

[a]Log_{10} Average = Log_{10} (C_i x 10^{-D})

Table 2.10: Neutralisation evaluation – 10% povidone-iodine (1% available iodine) *Staphylococcus epidermidis* (ATCC #12228)

Test description	Dilution 10^{-D}	Plate counts A	Plate counts B	Average colony count (C_i)	Log_{10} average[a]
Product – 10% povidone-iodine (1% available iodine)					
Replicate #1	-2	TNTC	TNTC		
	-3	198	179	188.5	5.3
	-4	16	13		
Replicate #2	-2	TNTC	TNTC		
	-3	148	170	159.0	5.2
	-4	16	19		
Replicate #3	-2	TNTC	TNTC		
	-3	186	156	171.0	5.2
	-4	12	16		

[a]Log_{10} Average = Log_{10} (C_i x 10^{-D})

2.3.7 Modified Time-Kill Kinetics Results

The time kill kinetics results for the efficacy of 3 antiseptic agents, 2% CHG in 70% isopropyl alcohol, 2% CHG, and 10% povidone-iodine against *Staphylococcus epidermidis* (ATCC #12228) in the presence of 0.9% normal saline or blood can be found in Tables 2.11, Table 2.12, and Table 2.13.

Table 2.11: Time kill kinetics - Challenge microorganism *Staphylococcus epidermidis* (ATCC #12228)

Product -2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol (lot number: 7474)	Exposure time	Diluent: 0.9% NaCl irrigation, USP[a] Initial inoculum (CFU/mL): 1.5×10^9 Post exposure (CFU/mL)	Log_{10} reduction	Percent reduction	Diluent: Bovine whole blood (in Na Heparin)[b] Initial inoculum (CFU/mL): 1.3×10^9 Post Exposure (CFU/mL)	Log_{10} reduction	Percent reduction
50% (v/v) product concentration 10.0 mL of product diluted with 10.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
75% (v/v) product concentration 15.0 mL product diluted with 5.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
90% (v/v) product concentration 18.0 mL product diluted with 2.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
95% (v/v) product concentration 19.0 mL Product diluted with 1.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
99% (v/v) product concentration 19.8 mL of roduct diluted with 0.2 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%

[a]Baxter Healthcare Corp; Lot #G062836

[b]PML Microbiologicals; Lot #1219-H001

Table 2.12: Time kill kinetics - challenge microorganism *Staphylococcus epidermidis* (ATCC #12228)

Product- aqueous 2% (w/v) chlorhexidine gluconate solution (lot number:2007-ET054)	Exposure time	Diluent: 0.9% NaCl irrigation, USP[a] Initial inoculum (CFU/mL): 1.5×10^9 Post exposure (CFU/mL)	Log_{10} reduction	Percent reduction	Diluent: Bovine whole blood (in Na Heparin)[b] Initial inoculum (CFU/mL): 1.3×10^9 Post exposure (CFU/mL)	Log_{10} reduction	Percent reduction
50% (v/v) product concentration 10.0 mL of product diluted with 10.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
75% (v/v) product concentration 15.0 mL product diluted with 5.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
90% (v/v) product concentration 18.0 mL product diluted with 2.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
95% (v/v) product concentration 19.0 mL product diluted with 1.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
99% (v/v) product concentration 19.8 mL of product diluted with 0.2 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	99.9%	$< 1.0 \times 10^3$	6.1	99.9%

[a]Baxter Healthcare Corp; Lot #G062836

[b]PML Microbiologicals; Lot #1219-H001

Table 2.13: Time kill kinetics - challenge microorganism *Staphylococcus epidermidis* (ATCC #12228)

Product -10% povidone - iodine surgical solution (1% available iodine; lot number:057-0208)	Exposure time	Diluent: 0.9% NaCl irrigation, USP[a] Initial inoculum (CFU/mL): 1.5×10^9			Diluent: Bovine whole blood (in Na Heparin)[b] Initial inoculum (CFU/mL): 1.3×10^9		
		Post exposure (CFU/mL)	Log_{10} reduction	Percent reduction	Post exposure (CFU/mL)	Log_{10} reduction	Percent reduction
50% (v/v) product concentration 10.0 mL of product diluted with 10.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	<99.9%	6.4×10^8	0.3	51.2%
	5 min	$< 1.0 \times 10^3$	6.2	<99.9%	1.1×10^8	1.1	91.8%
75% (v/v) product concentration 15.0 mL product diluted with 5.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	<99.9%	3.8×10^7	1.5	97.0%
	5 min	$< 1.0 \times 10^3$	6.2	<99.9%	9.8×10^6	2.1	99.2%
90% (v/v) product concentration 18.0 mL product diluted with 2.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	<99.9%	1.3×10^6	3.0	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	<99.9%	4.5×10^4	4.5	99.9%
95% (v/v) product concentration 19.0 mL product diluted with 1.0 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	<99.9%	1.5×10^4	4.9	99.9%
	5 min	$< 1.0 \times 10^3$	6.2	<99.9%	$< 1.0 \times 10^3$	6.1	<99.9%
99% (v/v) product concentration 19.8 mL of Product diluted with 0.2 mL of blood or saline	3 min	$< 1.0 \times 10^3$	6.2	<99.9%	$< 1.0 \times 10^3$	6.1	<99.9%
	5 min	$< 1.0 \times 10^3$	6.2	<99.9%	$< 1.0 \times 10^3$	6.1	<99.9%

[a]Baxter Healthcare Corp; Lot #G062836

[b]PML Microbiologicals; Lot #1219-H001

2.3.8 Conclusion

This study compared the efficacy of 3 antiseptic agents, 2% CHG in 70% isopropyl alcohol, 2% CHG, and 10% povidone-iodine against *Staphylococcus epidermidis* (ATCC #12228) in the presence of 0.9% normal saline or blood. The 2% (w/v) chlorhexidine gluconate in 70% isopropyl alcohol and 2% (w/v) chlorhexidine solutions antimicrobial activity was not diminished in the presence of 0.9% normal saline or blood. The 10% povidone-iodine solution antimicrobial activity was not diminished in the presence of 0.9% normal saline, but was diminished in the presence of blood when the concentration of povidone-iodine solution was diluted to 75%.

2.4 Pharmacokinetics

The pharmacology information for chlorhexidine gluconate is based on the published literature since the active ingredients have a recognized efficacy and level of safety for numerous years in the medical community. In reviewing the literature, chlorhexidine presents minimal pharmacokinetic properties and no internal bioavailability to human infants, children or adults.

For animal and/or human pharmacokinetics to begin, at least a moiety of the drug product must enter the body. The drug product containing chlorhexidine gluconate (CHG) does not present an absorbable drug to normal intact skin. Chlorhexidine is traditionally applied topically and no clinical pharmacological effects are observed since the compound is poorly absorbed through intact skin and mucous membranes (Martindale, 2004).

Reported toxicity parameters include acute oral effects (LD_{50} 2 g/kg (rat), 1.26 g/kg (mouse)), and toxicity by subcutaneous administration (LD_{50} 3.32 g/kg (rat), 1.14 g/kg (mouse)). Testing for mutagenic potential in mammalian systems is reported as negative, as are standard carcinogenicity assay determinations. Reported clinical uses include mouthrinses (0.2% concentration, 40 mg/day CHG) and gum bases (20 mg/day CHG). A solution of 2% CHG has been employed as an endodontic irrigant.

Limited reports of contact irritant dermatitis in health care workers (non-allergenic) were reviewed, and a single citation of allergic reaction in six patients following dermal application was noted. The Cosmetic Ingredient Review (CIR) Expert Panel has established a level of safe use as 0.14% in cosmetics, with a manufacturer recommendation of <0.05% CHG in wound creams (cited in anecdotal allergic response reference as acceptable).

Based upon the animal data for subcutaneous toxicity, application of uncertainty and safety factors yields a Health-based Exposure Limits (HBEL) of 0.4 mg/kg including consideration of chronic extrapolation. Assuming a default body weight for adults as specified in the cited ISO guidelines (70 kg), yields a total dose HBEL of 28.0 mg.

The declared contaminants in the nominal CHG product include a maximum of 500 ppm p-chloroaniline and a presumably trace amount of chlorhexidine. The former, p-chloroaniline (PCA), CAS# 106-47-8, has been reported as a breakdown product of surgical scrub products, with an analytically determined average concentration of 5 ppm. The PCA compound is reported to be a mild skin irritant, with both acute and chronic potential toxicological effects. The oral toxicity is represented by an LD_{50} of 0.1 to 0.3 g/kg in the mouse or rat, respectively. Similar information for exposure via skin is given by an $LD_{50\ of}$ 3.2 g/kg (rat) and 0.36 g/kg (rabbit). The material is also reported to be tumorigenic and produce methemoglobinemia with possible liver and kidney lesions. The International Agency for Research on Cancer (IARC) has classified this compound as 2B (animal sufficient evidence) and Environmental Protection Agency (EPA) has established an oral reference dose of 0.004 mg/kg/day based upon neoplastic effects in the spleen. An HBEL derived from the dermal results in the rabbit model would be somewhat larger, *i.e.* 0.12 mg/kg. It is noted that an allowable limit has been established as 500 ppm PCA for surgical scrubs and preoperative skin preparations. For comparison, assume use of 50 ml of surgical scrub/patient preoperative skin preparation per exposure, resulting in a dose of 25 mg PCA from this source.

With respect to chlorhexidine, toxicity via subcutaneous exposure has been reported as LD_{50} of 0.63 to >1.0 g/kg (mouse, rat). Applying parallel safety and uncertainty factors would yield an HBEL of 0.21 mg/kg. It is suggested that both PCA and chlorhexidine levels be determined in the current raw material and/or product.

2.4.1 Absorption

Gongwer and colleagues showed that daily washing of up to 600 cm^2 of body surface area of newborn Rhesus monkeys for 90-92 days with a skin cleanser consisting of 8% (w/v) chlorhexidine resulted in no measurable or recognizable effect on the health of the animal (Gongwer *et al.*, 1980). Moreover, there were no appreciable levels of chlorhexidine in the blood, bile, bladder, urine, brain, cerebrospinal fluid, or lungs of these animals despite the finding of significant levels in the skin, demonstrating the absence of percutaneous absorption of chlorhexidine even after such extended use. Finally, necropsy histologic examination of liver,

adipose tissue, kidney, central nervous system, lungs, and skin, as well as hematologic assessment, failed to reveal any changes (Gongwer, *et al.*, 1980). This animal model of bathing neonatal Rhesus monkeys has been validated as the most appropriate model with relevance to human neonates (Gongwer *et al.*, 1980; Wester & Maibach, 1975).

CHG has been studied extensively for its safety as an antimicrobial agent in neonatal and pediatric subjects. These studies have generally focused on the lack of percutaneous absorption of CHG into the systemic circulation, especially through immature neonatal skin, and the favorable tolerance of young or immature skin to the local effects of CHG in a detergent formulation or in alcohol.

Absorption of chlorhexidine, through the skin barrier, has been demonstrated to be very low with the exception being the pre-term infant under 26 week gestational age. Human studies demonstrating the absence of percutaneous absorption of CHG in term and preterm infants have also been reported. Johnsson reported finding no appreciable CHG in venous samples taken from 25 full term infants whose umbilical cord stumps had been treated for five consecutive postpartum days with 0.2 ml of a commercial solution of 4% CHG (Hibiscrub®) (Johnsson *et al.*, 1987). Moreover, no skin irritation or overgrowth of Gram-negative bacteria or yeast was observed in these infants.

Even more interesting is a report by Cowen and colleagues, in which 34 preterm infants first seen in a neonatal intensive care unit (NICU) were bathed in 4% CHG (Hibiscrub®), including exposure of the head and the body, starting as early as two days postpartum for up to 32 days (Cowen *et al.*, 1979). Gestational age at birth for these infants averaged 33 weeks (range from 28 weeks to 39 weeks), and birth weight averaged 1900 gm (range from 700 gm to 3020 gm). Venous blood was taken from these neonates as soon as 1 hr after bathing. The results showed that chlorhexidine may be detectable at minute levels (ng/ml) in the blood of some preterm and term infants after whole body bathing, but that the presence or amount of CHG that may be absorbed percutaneously is unrelated to gestational age. Indeed, the authors speculate that the small amounts detected could even be due to contamination of the blood sample by the presence of CHG in the skin through which the blood was drawn (Cowen *et al.*, 1979).

Further investigation of percutaneous absorption of chlorhexidine in 23 preterm infants and 25 term infants was carried out by Aggett and colleagues (Aggett *et al.*, 1981). In these studies, 1% chlorhexidine in ethanol was used for umbilical cord care every 4 hr, followed by dusting of the umbilical stump with 1% chlorhexidine in a 3% zinc oxide dusting powder. In

addition, the alcoholic chlorhexidine solution was also used as a site prep for all invasive procedures the infants required (e.g., lumbar puncture, umbilical catheterization). Gestational age for the preterm infants ranged from 31 to 36.5 weeks. Venous or cord blood was collected from these neonates five and nine days after birth. As in other similar studies, chlorhexidine levels were detected in the ng/ml range in some infants, more often in the preterm babies than in the term infants (Aggett *et al.*, 1981). Further, the authors speculate that the humidity within the NICU incubator and exposure to phototherapy required by the preterm infants may have increased cutaneous vascular perfusion, thereby enhancing skin permeability and causing an apparent but not real increase in percutaneous absorption of chlorhexidine. Despite these confounding variables, at no time did the chlorhexidine levels in the preterm infants rise above 32 ng/ml.

Reduced thickness of the stratum corneum and diminished cohesion between dermal and epidermal layers in preterm infants not only increase their susceptibility to percutaneous absorption of drugs, but also may increase the likelihood of local skin damage or irritation in response to compounds applied cutaneously.

In this regard, even with the increasingly widespread use of CHG in NICUs and pediatric units, few local allergic reactions have been reported following application with the antiseptic, and no neonate has developed contact dermatitis after CHG cleansing (Garland *et al.*, 1995). Furthermore, Garland reported on the results of a randomized, multicenter clinical trial in preterm neonates admitted to Level III nurseries, that was designed to test the safety and efficacy of Biopatch®, an occlusive CHG-impregnated opaque foam patch used as a site prep prior to placement of a vascular percutaneous device. Nine of the 300 (3%) preterm infants enrolled in the trial developed local reactions; all nine were approximately 24 weeks gestational age and weighed <880 gm (Garland 1996). Subsequently, a second leg of the study was performed on infants >26 weeks gestational age (>800 gm); local skin reactions were observed in 3 of the 75 infants treated (4%). All local reactions resolved spontaneously in both groups of infants who survived their primary morbid conditions. Because no local skin reactions were seen by the authors when they used a 0.5% CHG scrub, they postulate that many, if not all, of the local reactions they observed in their study were due to the occlusive nature of the Biopatch® or improper use of the dressing (Garland 1996). Based on these results, the authors recommend use of CHG for infants who are gestationally >26 weeks and chronologically >7 days post-partum.

A recent study of 50 maternity units in France in which the most appropriate neonatal umbilical cord treatment modality was surveyed, revealed that chlorhexidine was the consensus choice of neonatologists in terms of safe use, excellent tolerance by neonatal skin, and effectiveness as an antimicrobial agent (Lacour *et al.*, 1999).

Full term infants bathed in a 4% chlorhexidine handwash, showed no detectable skin absorption of chlorhexidine on each day following the bathing for three consecutive days (Denton, 1991). A previous study of 10 infants had indicated detectable levels of chlorhexidine in blood samples, when heel pricks were completed in obtaining the samples. This was probably due to contamination of the blood sample. Follow-up of 7 infants, with venous blood samples, to avoid contamination, showed very low levels of chlorhexidine in some infants, primarily babies of 36 week gestational age or younger. Venous samples of 17 infants drawn 12 hours after bathing and then weekly intervals to study accumulation of the chlorhexidine found 2 infants positive for detectable levels (Cowen *et al.*, 1979).

Routine neonatal cord care with a 4% chlorhexidine gluconate solution was examined with 32 full-term infants born vaginally and 36 full term infants delivered by Caesarian section. This study concluded that rountine cord care during the first five days does not result in percutaneous absorption of chlorhexidine gluconate (Johnnson *et al.*, 1987).

In the full term infant, child, and adult, the stratum corneum is developed and provides a protective barrier to toxic compounds. A series of studies evaluating percutaneous absorption of radiolabeled chlorhexidine in a hand scrub regimen demonstrated no radioactivity in blood or urine. A small amount of radioactivity was seen in one volunteer's fecal sample (Case 1977) and a later study, two volunteers' fecal sample (Case *et al.*, 1980). A study that included 25 hospital staff members that had used a 4% chlorhexidine preoperative antiseptic for hand disinfection for a minimum of 6 month showed no detectable levels of chlorhexidine in blood samples. Level of detection for the analytical method was 0.01μg/m. Percutaneous absorption of chlorhexidine is extraordinarily low. It appears that CHG eventually leaves the skin by sloughing, not absorption (Case *et al.*, 1980).

The intent of the chlorhexidine gluconate 2% (w/v) and 70% isopropyl alcohol (v/v) is a topical solution and contains a warning label for oral consumption. Orally administered chlorhexidine is poorly absorbed in the gastrointestinal tract. Human oral studies have shown extremely low levels in urine samples of adult volunteers (Case 1977).

2.4.2 Wound

The definition for a wound is the injury to the person, by which the skin is divided, or its continuity broken and sensation intact. An uncomfortable stinging sensation would be present. In the course of treating 16,000 minor injuries a case history of a 53-year old male, with a hypersensitivity to neomycin, with a wound to the shin was cleaned with 1% Savlon developed localized dermatitis with subcutaneous edema. Patch testing with a 1% solution of savlon and a control dressing resulted in an area of erthema with vesiculature formation. The strength of the solution suggests that the response was of a primary irritation and not hypersensitivity (Nickol, 1965). A case report study of 3 patients with chronic *Staphylococcus aureus* empyema, after postpneumonectomy, displayed symptoms of cerebral intoxication after irrigation of cavities with a 0.02% chlorhexidine solution. Symptoms resolved after irrigation was discontinued. Use of a continuous chlorhexidine irrigation treatment of large wounds and cavities is discouraged (Friis-Møller *et al.*, 1984).

2.4.3 Burns

The definition of a burn depends on the depth, area and location of the burn. Burn depth is categorized as first, second or third degree. A first-degree burn is typical of sunburn, the skin is red and sensation is intact, somewhat painful. Second-degree burns look similar to first degree burns with blistering of the skin and the pain is intense. Third degree burns is at the point of skin death, the skin is white without sensation. The treatment is dependent of the degree classification of the burn. In a case study, where a 19-year old man with a burn to his left arm, suffered acute anaphylaxis due to a topical application of chlorhexidine actetate. The man exhibited a positive patch test for a 0.05% chlorhexidine actetate (Evans, 1992).

2.4.4 Leg Ulcers

A leg ulcer is the local defect or excavation of the surface of the leg tissue, which is produced by the sloughing of inflammatory necrotic tissue. A study with 297 patients with leg ulcers tested with 1% chlorhexidine gluconate, 39 had positive reactions. A 1% chlorhexidine acetate demonstrated to be a very strong irritant when compared to 1% CHG. Greater than 13% of the leg ulcer patients in this study may be sensitized to chlorhexidine, with a recommendation that indication for the chlorhexidine in leg ulcer patients should be reconsidered (Knudsen *et al.*, 1991). Another study with 551 patients were patch tested with 1% chlorhexidine gluconate, 14 patients exhibited a strong reaction. Severe dermatitis

developed in 10 patients with leg ulcers. Sensitizing potential of chlorhexidine has been underestimated (Osmundsen 1982). Additionally, 1063 eczema patients were patch tested with a 1% chlorhexidine gluconate solution, 52 showed a positive reaction. At retesting 21/29 was still positive and indicated allergic sensitization. Leg ulcers were present in 10 of the 21 patients, which provide optimal sensitization conditions. Patients with leg ulcers seem to be particularly at risk (Lasthein-Andersen and Branderup 1985).

2.4.5 Eczema

Eczema is a pruritic papulovesicular dermatitis occuring as a reaction to many endogenous and exogenous agents. It is characterized by erythema, edema with exudate between the cells of the epidermis and the dermis. In a patch test with 1% aqueous chlorhexidine solution 48 of 2061 patients, with eczematous lesions exhibited a positive reaction (Bechgaard *et al.*, 1985). Another study with 551 patients were patch tested with 1% chlorhexidine gluconate, 14 patients exhibited a strong reaction. Severe dermatitis developed in 4 patients with skin infection on the face and/or scalp (Osmundsen 1982). Additionally, 1063 eczema patients were patch tested with a 1% chlorhexidine gluconate solution, 52 showed a positive reaction. At retesting 21/29 was still positive and indicated allergic sensitization. Patients with eczema seem to be particularly at risk (Lasthein-Anderson and Banderup 1985).

The most relevant information for the pharmacokinetic assessment chlorhexidine is that it is not systemically available after cutaneous application and only trace amounts are absorbed following oral administration.

2.5 Discussion

Skin antisepsis prior to surgery is a standard of practice. The criteria for the antimicrobial agent and their effects on surgical site infection and wound healing are unclear (Garibaldi *et al.*, 1991; Edwards *et al.*, 2004; Ellenhorn *et al.*, 2005; Viljanto 2006;). Iodophors and chlorhexidine gluconate are currently the most commonly used antimicrobial agents in preoperative skin preparation (McDonnell and Russell 1999). There have been questions as to what effect wound irrigation fluids and/or blood have on the antimicrobial activity of an antiseptic agent to remain on the skin and to remain active throughout the surgical procedure. Iodophors and chlorhexidine antimicrobial activity demonstrate variable susceptibility in the presence of organic matter (blood and proteins), either by inactivation or diminished antimicrobial activity. In addition, can

the antimicrobial agent be affected by saline and/or irrigation fluids (Bloomfield 1996; Russell and Day 1996)?

CHAPTER 3: EVALUATION OF THE EFFECT OF BLOOD AND SALINE ON RESIDUAL ANTIMICROBIAL ACTIVITIES OF PREOPERATIVE SKIN PREPARATIONS

3.1 Introduction

Skin antisepsis prior to surgery or any invasive procedure is an expected and recognised standard of practice. However the selection and use of the various available skin antiseptic preparations and their subsequent effects on the rates heathcare-associated infection, especially surgical site infection and wound healing are still not fully established (Garibaldi *et al.*, 1991; Edwards *et al.*, 2004; Ellenhorn *et al.*, 2005; Viljanto 2006). Iodophors and chlorhexidine gluconate are currently the most commonly used antimicrobial agents for preoperative skin preparation (McDonnell and Russell 1999). There have been many queries as to what the effect of wound irrigation fluids and/or blood have on the residual antimicrobial activity of these antiseptic agents particularly on the skin and there ability to remain active throughout the surgical procedures. Indeed the antimicrobial activity of both iodophors and chlorhexidine demonstrate variable susceptibility in the presence of organic matter including blood and proteins, either by inactivation or diminished antimicrobial activity. In addition, it is still unclear whether or not either or both of these antiseptic agents can remain *in situ* and active on the skin following lavage or irrigation with saline or other fluids (Bloomfield 1996; Russell and Day 1996). This clinical study investigated the effect of blood and saline irrigation on the efficacy of antiseptic applied to the skin of volunteers.

3.2 Scope

This clinical volunteer study was designed to mimic the conditions of a surgical procedure in which the skin antiseptic preparations are exposed, post-incision, to blood and the saline irrigation at the surgical incision site. This test model allowed an evaluation of the effect of both the presence of blood and saline irrigation on the subsequent antimicrobial efficacy of two test skin antiseptics.

The Study Protocol was approved by the Gallatin Institutional Review Board (GIRB) on February 21, 2008. All volunteer's signed an informed consent. Good Laboratory Practices requirements were met during the course of this evaluation.

3.3 Test Materials

1. Test product #1: ChloraPrep® One-Step 26 mL

   Active Ingredient: chlorhexidine gluconate 2% (w/v) and isopropyl alcohol 70% (v/v) isopropyl alcohol in a 26 ml Applicator

   Lot number: 7474

   Expiration date: February 2009

2. Test Product #2: Scrub Care® Preoperative Skin Prep Tray

   Active ingredient: 109 mL Bottle 7.5% povidone-iodine Cleansing Solution [Scrub]
   88.5 mL Bottle 10% Povidone-iodine Topical Solution [Paint]

   Lot number: 077-0428 and 077-0420

   Expiration date: July 2010

3.4 Test Methods

1. Seven days prior to the test day, designated as the "pre-test" period, volunteers avoided the use of medicated soaps, lotions, shampoos, deordorants, etc., as well as skin contact with solvents, acids, and bases.
2. Forty-eight hours prior to study initiation the inoculum was prepared in sterile tubes of tryptic soy broth (TSB) was inoculated from cryogenic stock cultures of *Staphylococcus aureus* (ATCC #6538). The cultures were incubated at 30°±2C°for twenty-four hours. After twenty-four hours the cultures were then inoculated onto the surface of tryptic soy agar (TSA) and incubated at 30°±2C°for twenty-four hours.
3. The day of the study, the challenge suspension of *Staphylococcus aureus* (ATCC #6538) was prepared by transferring colonies from the TSA plates into test tubes containing phosphate buffer solution (PBS). Suspension concentrations of 1.0×10^9 cfu/mL was prepared and evaluated by turbidity. Serial dilution of this suspension was made in PBS to achieve a final challenge inoculum of 1×10^6 cfu/mL. The final challenge inoculum was assayed for number of microorganisms at the beginning and end of the study period.
4. Each forearm of the each volunteer was randomly assigned a test product.
5. Prior to the assigned test product application, the skin of the forearms was rinsed with 70% isopropyl alcohol (IPA) and allowed to air-dry.
6. The volunteer's forearms were marked with four test sites with a permanent ink marker on the anterior skin of each forearm. The sites were spaced uniformly and sequentially (one through three) from the elbow moving distally toward the wrist, and the ink was allowed to

dry thoroughly before continuing.

7. Test sample one: Prior to a test product application a baseline sample was contaminated with 10 µl (0.01 mL) of the challenge suspension of *Staphylococcus aureus* (ATCC #6538). Following a five minute ± thirty second exposure, the site was sampled using the Cylinder Sampling Technique (Appendix G) and then decontaminated with 70% IPA.
8. The randomly-assigned test product was then applied to the remaining test sites on each forearm, described in detail below. Any product that dripped to the underside of the arm was blotted away with a sterile towel.

a. Application of Chlorhexidine Gluconate 2% (w/v) and Isopropyl Alcohol 70% (v/v) isopropyl alcohol in a 26 ml Applicator. Pinching the wing on the applicator to break the ampoule and release the antiseptic, not touching the sponge. Wetting the sponge by repeatedly pressing and releasing the sponge against the treatment area until liquid is visible on the skin. With repeated repeated back-and-forth strokes of the sponge apply solution to skin for approximately thirty seconds. Completely wetting the treatment area with antiseptic. Allowing the area to air-dry for approximately three minutes. Solution was not blotted or wiped away.

b. Application of 7.5% povidone-iodine Cleansing Solution [Scrub] followed by 10% Povidone-iodine Topical Solution [Paint]. First: 7.5% povidone-iodine Cleaning Solution [Scrub] – The skin was wetted with water, the one mL applied and lather was lather was developed with gently scrubbing the skin thoroughly for approximately five minutes. Second: 10% povidone-iodine Topical Solution [Paint] – Application of one mL with sterile gauze to the skin and allowed to dry.

9. Second test sample: Ten minutes ± one minute following application of assigned test product, the test site was exposed to 10 µl of the challenge suspension of *Staphylococcus aureus* (ATCC #6538). Following a five minute ± thirty second exposure, the site was sampled using the Cylinder Sampling Technique (Appendix G).
10. Using a sterile sixty mL syringe, the entire forearm was then rinsed with thirty mL of bovine blood, and after one minute ± ten seconds, the forearm was rinsed clean with sixty mL of sterile 0.9% Sodium Chloride Irrigation and blotted dry using sterile gauze. This procedure was repeated immediately one additional time.

11. Third test sample: Ten minutes ± one minute following the second blood and saline challenge, the test site was exposed to 10 µl of the challenge suspension of *Staphylococcus aureus* (ATCC #6538). Following a five minute ± thirty second exposure, the site was sampled using the Cylinder Sampling Technique (Appendix G).
12. Using a sterile sixty mL syringe, the entire forearm was then rinsed with thirty mL of bovine blood, and after one minute ± ten seconds, the forearm was rinsed clean with sixty mL of sterile 0.9% Sodium Chloride Irrigation and blotted dry using sterile gauze. This procedure was repeated immediately one additional time.
13. Fourth test sample: Four hours ± fifteen minutes following application of assigned test product the test site was exposed to 10 µl of the challenge suspension of *Staphylococcus aureus* (ATCC #6538). Following a five minute ± thirty second exposure, the site was sampled using the Cylinder Sampling Technique (Appendix G).
14. All test site samples were serially diluted in Butterfield's Phosphate Buffer Solution with neutralisers (BPP++). Spread plates, in duplicate, were prepared from each of the dilutions on tryptic soy agar with neutrializers (TSA+) and incubated at 30°±2°C for seventy-two hours.

3.5 Neutralisation Studies

The results of a neutralisation study indicated that the neutraliser(s) used in the recovery medium successfully repressed the antimicrobial activity of the test products (Appendix I). Study procedures followed guidelines set forth in *ASTM E 1054-02, Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* (Appendix H), except that the microorganism was added to the neutraliser prior to the addition of the test products. *Staphylococcus aureus* (ATCC #6538) was used as the challenge species in the neutraliser validation study.

3.6 Human Volunteer Demographics

Fifty-seven healthy human volunteers at least eighteen years of age were admitted into the study. The group of volunteers selected was of mixed gender, age, and race, and the skin of the test sites was free from dermatoses, injuries, and/or any other disorders that may have compromised the volunteer and the study. Thirty-six volunteers completed testing. All subjects signed the Study Description and Informed Consent Form, Subject Confidential Information and Acceptance Criteria prior to participating in the study. The demographics for the volunteers who participated in the study are presented in Table 3.1.

3.7 Adverse Events

No volunteers experienced an adverse event during or following completion of this study.

Table 3.1: Volunteer demographics

Demographic summary	All subjects	
	Recruited	Received product
Age		
Minimum age	18	18
Median age	25	25
Maximum age	75	75
Sex		
Males (M)	19	10
Females (F)	38	26
Total	57	36
Race		
White/Caucasian (C)	56	35
Black/African-American (AA)	0	0
Latino (L)	1	1
Asian (A)	0	0
Native American/Alaskan Native (NA)	0	0
Native Hawaiian/Pacific (P)	0	0
Other (O)	0	0
Total	57	36
Did not participate in testing		
SC = schedule conflict	1	
QC = qualification (inclusion/exclusion) criteria failure	7	
NS = no show	8	
SF = study requirements fulfilled	4	
VW = voluntary withdrawal	1	
SD = dismissed by study director	0	

3.7 Results

The 2% CHG in 70% IPA produced mean log10 reductions of microorganisms from baseline populations (Table 3.2) of 6.55 ten minutes following application of product, 1.67 ten minutes following the first series of blood and saline challenges, and 0.96 four hours post-application and following the second series of blood and saline challenges (Table 3.3). The mean

reduction produced by 2% CHG in 70% IPA ten minutes following application of product was significantly greater than those produced ten minutes following the first series of blood and

Table 3.2: Log_{10} values and log_{10} reduction from baseline values by subject, post-prep with 2% CHG in 70% IPA

Subject	Baseline log_{10} values	10 minutes post-prep log_{10} reductions from baseline	10 minutes post- prep and 1st blood & saline exposure log_{10} reductions from baseline	4 hours post-prep and 2nd blood & saline exposure log_{10} reductions from baseline
1	6.91	6.75	1.17	0.78
2	6.97	6.97	1.96	1.22
3	7.01	7.01	1.03	0.97
4	6.98	6.52	2.10	0.95
5	7.03	5.41	1.74	0.96
6	6.65	6.65	1.79	0.68
7	7.02	7.02	1.09	0.66
8	6.94	6.78	0.89	0.84
9	6.82	3.89	0.86	0.76
10	7.05	6.72	1.88	0.50
11	7.05	7.05	1.49	0.68
12	7.00	6.84	2.15	1.22
13	7.04	6.88	2.27	1.33
14	7.01	6.45	1.99	1.01
15	6.89	6.89	2.35	1.72
16	7.02	7.02	1.97	1.06

saline challenges ($p = 0.00$) and four hours post-application, following the second series of blood and saline challenges ($p = 0.00$) are outlined in Table 3.3.

The PVP-I, produced mean log_{10} reductions of microorganisms from baseline populations (Table 3.4) of 6.70 ten minutes following application of product, 0.41 ten minutes following the first series of blood and saline challenges, and 0.38 at four hours post-application and following the second series of blood and saline challenges (Table 3.5). The mean reduction produced by PVP-I ten minutes following application of product was significantly greater than those produced ten minutes following the first series of blood and saline challenges ($p = 0.00$) and four hours

post-application, following the second series of blood and saline challenges (p = 0.00) are outlined in Table 3.5.

Table 3.3: Statistical summary of the mean $\log_{10}$ values post-prep with 2% CHG in 70% IPA

Mean $\log_{10}$ value	Sample size	Mean $\log_{10}$	Standard deviation	95% confidence interval	Comparison to 10 minutes post-prep $\log_{10}$ reductions from baseline
At baseline	16	6.96	0.11	6.91 to 7.02	N/A
10 minutes post-prep reduction from baseline	16	6.55	0.81	6.12 to 6.99	N/A
10 minutes post- prep and 1st blood & saline exposure $\log_{10}$ reductions from baseline	16	1.67	0.53	1.37 to 1.96	p Value = 0.000[a]
4 hours post-prep and 2nd blood & saline exposure $\log_{10}$ reductions from baseline	16	0.96	0.32	0.78 to 1.14	p Value = 0.000[a]

[a] p value from Two-Sample Student's t Test, α = 0.05

Table 3.4: Log_{10} values and log_{10} reduction from baseline values, by subject, post prep with PVP-I

Subject	Baseline log_{10} values	10 minutes post-prep log_{10} reductions from baseline	10 minutes post- prep and 1st blood & saline exposure log_{10} reductions from baseline	4 hours post-prep and 2nd blood & saline exposure log_{10} reductions from baseline
1	6.83	6.83	1.67	0.34
2	6.92	6.92	0.47	0.40
3	6.95	6.95	0.59	0.41
4	6.80	6.80	0.35	0.29
5	6.79	6.79	0.00	0.07
6	6.88	6.88	0.63	0.61
7	6.72	6.72	0.31	0.43
8	7.12	5.43	0.30	0.21
9	7.09	7.09	0.15	0.33
10	7.04	7.04	0.05	0.20
11	7.01	7.01	0.08	0.12
12	7.05	6.89	0.17	0.10
13	7.01	6.85	0.60	0.64
14	6.88	5.30	0.55	0.47
15	6.97	6.97	0.30	0.38

Table 3.5: Statistical summary of the log_{10} values post-prep with PVP-I

Mean log_{10} value	Sample size	Mean log_{10}	Standard deviation	95% confidence interval	Comparison to 10 minutes post-prep log_{10} reductions from baseline
At baseline	15	6.94	0.12	6.87 to 7.00	N/A
10 minutes post-prep reduction from baseline	15	6.70	0.55	6.39 to 7.00	N/A
10 minutes post-prep and 1st blood & saline exposure log_{10} reductions from baseline	15	0.41	0.40	0.19 to 0.64	p Value = 0.000[a]
4 hours post-prep and 2nd blood & saline exposure log_{10} reductions from baseline	15	0.34	0.18	0.23 to 0.44	p Value = 0.000[a]

[a] p value from Two-Sample Student's t Test, $\alpha = 0.05$

The Negative Control (untreated skin) produced mean log_{10} reductions of microorganisms from baseline populations (Table 3.6) of -0.02 ten minutes following, -0.01 ten minutes following the first series of blood and saline challenges, and 0.08 at four following the second series of blood and saline challenges (Table 3.7). The mean reduction produced by the Negative Control ten minutes following application of product was not statistically different from that produced ten minutes following the first series of blood and saline challenges (p = 0.660) but was significantly greater than that produced four hours post-application, following the second series of blood and saline challenges (p = 0.006) are outlined in Table 3.7.

Table 3.7 presents a statistical summary of the mean log_{10} microbial populations and reduction from baseline values from the Negative Control (No product applied). Figure 3.1 presents graphically the log_{10} reductions from baseline produced 2% CHG in 70% IPA, PVP-I, and the Negative Control.

Table 3.6: Log_{10} values and log_{10} reduction from baseline values, by subject, from negative control

Subject	Baseline log_{10} values	10 minutes post-prep log_{10} reductions from baseline	10 minutes post- prep and 1st blood & saline exposure log_{10} reductions from baseline	4 hours post-prep and 2nd blood & saline exposure log_{10} reductions from baseline
1	6.68	-0.20	-0.15	-0.06
2	6.98	0.02	0.09	0.24
3	6.82	-0.05	0.12	0.19
4	7.12	0.09	0.13	0.25
5	6.98	-0.05	-0.10	-0.12
6	7.04	-0.05	-0.06	0.08
7	7.09	0.03	-0.01	0.00
8	7.10	-0.01	-0.01	0.17
9	7.12	0.06	0.05	-0.04
10	6.95	-0.02	-0.03	0.08
11	7.00	0.04	0.03	0.06
12	6.95	0.01	-0.05	-0.01
13	7.04	0.00	0.09	0.22
14	6.92	-0.13	-0.08	0.11
15	6.99	-0.01	0.02	0.12
16	6.99	0.00	-0.02	0.10

Table 3.7: Statistical summary of the log_{10} values from negative control - no product applied

Mean log_{10} value	Sample size	Mean log_{10}	Standard deviation	95% confidence interval	Comparison to 10 minutes post-prep log_{10} reductions from baseline
At baseline	16	6.99	0.11	6.93 to 7.05	N/A
10 minutes post-prep reduction from baseline	16	-0.02	0.07	-0.06 to 0.02	N/A
10 minutes post-prep and 1st blood & saline exposure log_{10} reductions from baseline	16	-0.01	0.08	-0.05 to 0.04	p Value = 0.661[a]
4 hours post-prep and 2nd blood & saline exposure log_{10} reductions from baseline	16	0.08	0.11	0.02 to 0.14	p Value = 0.008[a]

[a] p value from Two-Sample Student's t Test, $\alpha = 0.05$

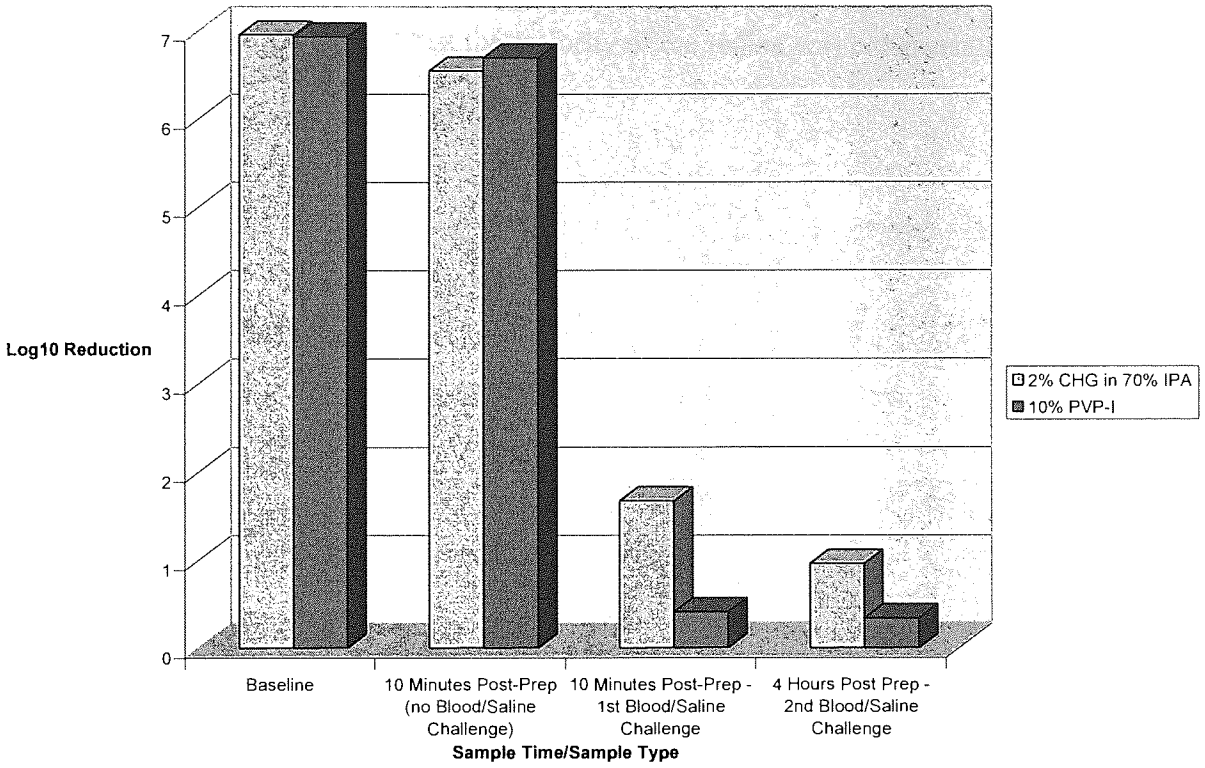


Figure 3.1: Graphical presentation of the $\log_{10}$ reductions from baseline produced by the two (2) test products and the negative control.

3.8 Discussion

This study was designed to mimic the conditions of a surgery in which skin preparations are exposed to blood and the saline irrigation of the surgical site. Specifically, this study examined whether the ionic components of blood combined with the irrigation (mechanical removal) of the saline rinse would affect the populations of *Staphylococcus aureus* (ATCC# 6538) recovered from the skin of the forearms.

The antimicrobial activities of 2% CHG in 70% IPA and PVP-I were reduced following each of the series of blood and saline rinses. Following the first series of blood and saline rinses, the reduction provided by the 2% CHG in 70% IPA was significant, while that produced by the PVP-I was not significant.

The ability of 2% CHG in 70% IPA was significant, while that produced by the PVP-I to significantly reduce microorganisms post-application is important in preventing healthcare associated infections. That 2% CHG in 70% IPA continues to reduce the microorganisms after

exposure to blood and saline, while that produced by the PVP-I does not, suggests that the level of chlorhexidine gluconate remaining bound to the skin following the first series of blood and saline rinses was significant, and that the majority of the iodine was either neutralised or removed.

CHAPTER 4: EVALUATION OF THE EFFECT OF APPLICATION PROCEDURE ON THE ANTIMICROBIAL EFFECTS OF AN AQUEOUS CHG AND A TRADITIONAL IODOPHOR SCRUB PAINT

4.1 Introduction

Patient preoperative skin preparation is mandatory prior to surgical or invasive medical procedures. Currently the predominant skin patient preoperative skin preparation is an aqueous povidone iodine scrub followed by aqueous povidone iodine paint (2-step application methodology). Manufacturer's directions for these and many other patient preoperative skin preparations state that once the solution(s) is applied to the procedural site, do not blot or wipe away, allow product to dry completely. There is a wide variety of policy and procedures for the actual application of skin antiseptic solutions in the medical field, especially in allowing these solutions to naturally dry. Therefore, many clinicians will "blot" or "wipe off" these solutions to prepare the patient prior to the medical procedure. Research of current literature addressing the application or "lack of dry time" on the efficacy of topical antiseptics solutions is not available. Therefore, the following evaluation was designed to replicate current patient preoperative skin preparation with aqueous based antiseptic solutions.

4.2 Scope

This study was designed to evaluate the effect of application procedure on the antimicrobial efficacy of two test products, an aqueous 2% CHG solution and a 7.5% iodophor scrub followed by a 10% iodophor paint, used for patient preoperative skin preparation. Specifically, this study evaluated the blot of wet product from the sites of application immediately post-application when compared to the same application procedure applied without blot to determine if blot affects antimicrobial efficacy.

This design required twenty subjects in order to provide ten sites per product and product configuration at both the inguinal and abdominal sites. One-hundred twenty-two human test subjects were baseline-screened following a seven day restriction period, and forty-seven completed testing. Each of the products was applied to the skin of the inguinal and the abdomen, and microbial reductions were assessed at fifteen seconds, ten minutes, and six hours post-product application. The Study Protocol was approved by the Gallatin Institutional Review

Board (GIRB) on 09 Oct 2007. All volunteer's signed an informed consent. Good Laboratory Practices requirements were met during the course of this evaluation.

4.3 Test Materials

1. Test Product #1: ChloraPrep™2% w/v chlorhexidine gluconate in a 26 mL Applicator
2. Test Product #2: Scrub Care® Preoperative Skin Prep Tray
   109 mL Bottle of 7.5% Povidone Iodine Cleansing Solution (scrub)
   88.5 mL Bottle of 10% Povidone Iodine Topical Solution (paint)

4.4 Test Methods:

1. Seven days prior to the test day, designated as the "pre-test" period, volunteers avoided the use of medicated soaps, lotions, shampoos, deordorants, etc., as well as skin contact with solvents, acids, and bases.
2. Baseline-screening was conducted after the "pre-test" period. Volunteers did not shower seventy-two hours prior to their sampling times. Samples were attained using the Cup Scrub Technique (Appendix G) at the center of the sampling areas of the inguinal (Figure 4.2) and abdominal (Figure 4.12) sites.
3. The test products were randomly assigned to the volunteers per a computer-generated randomization schedule. The two configurations of application methodology (described below) for each of the test products was assigned to the left and right sides of the inguinal and abdominal sites.
4. Test site preparation – on the abdomen, a sterile surgical marker was used to demarcate two (2) 4 inches by 4 inch areas of skin, to the right and left side of the naval. In the inguinal region, a ; a sterile surgical marker was used to demarcate two (2) 2 inch by 5 inch areas of skin, placed in the crease of the groin below the gracilis muscle ridge proceeding up toward the hip.
5. Test Products were applied and configurations of application performed to the inguinal and abdominal sites per directions below.
   - Test Product #1 - Application Instructions for 2% (w/v) chlorhexidine gluconate solution in 26 mL Applicator (2% CHG) - The wing(s) on the applicator was pinched to break the ampoule and release the antiseptic. The sponge was not touched. The sponge was wetted by being repeatedly pressed and released at the treatment site until liquid was visible on the skin. The treatment area was

completely wetted prior to beginning the scrub.

- Abdominal test site (location illustrated in Figure 4.12) was prepped by alternating from horizontal to vertical and using repeated back-and-forth strokes of the sponge, product was applied to the site for thirty seconds, completely wetting the treatment area. Inguinal test site (location illustrated in Figure 4.2) was prepped by alternating from horizontal to vertical and using repeated back-and-forth strokes of the sponge, product was applied to the site for two minutes, completely wetting the treatment area. (For completion of application, see Configurations #1 and #2, below.)

- Application Instructions for 7.5% Povidone Iodine Cleansing Solution (scrub) and 10% Povidone Iodine Topical Solution (paint) (PVP-I)–
  - Abdominal test site and inguinal test site application area was prepped using five iodine scrub saturated sponges to sites, one per minute. Saturated sponge was scrubbed in circular motion for one minute starting at center and moving outward. This was performed an additional four times for a total of five minutes of scrubbing. Site was blotted with a sterile towel. The iodine scrub solution was followed with application of iodine paint, using a circular motion. Application was started at the center and moved outward. (For completion of application, see Configurations #1 and #2, below.)

6. After test product was applied the following application configurations were employed.
   - Configuration #1 - No Blot Technique: The 2% CHG test site area was allowed to air-dry until completely dry, but for no less than three minutes. Product was not blotted or wiped away. The PVP-I scrub and paint test sites were allowed to air-dry.
   - Configuration #2 (Blot Technique, Both Products) the sites were blotted by lightly pressing a sterile towel over the site repeatedly until the site appeared dry.
7. After the test products where applied and configuration of application performed, the inguinal and abdominal test sites were sampled at fifteen ± fifteen seconds, ten minutes ± fifteen seconds, and 6 hours ± 30 minutes using the Cup Scrub Technique (Appendix G). The six hour sites were covered with sterile gauze and semi-occlusive dressings.

8. Test site samples were serially diluted in Butterfield's Phosphate Buffer Solution with product neutralisers (BBP++). Spread plates, in duplicate, were prepared from each of the dilutions on tryptic soy agar with neutralisers (TSA+) and incubated at 30°±2°C for seventy-two hours.

4.5 Neutralisation

A neutralisation validation study showed that the neutraliser(s) used in the recovery medium suppressed the antimicrobial activity of the test products (Appendix J). The procedure followed guidelines set forth in ASTM E 1054-02, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* (Appendix H)., except that the microorganism was added to the neutraliser prior to the addition of the test products. *Staphylococcus epidermidis* (ATCC #51625) was used as the challenge species in the neutraliser validation study

4.6 Subject Demographics

One-hundred and twenty-two healthy subjects at least eighteen years of age were recruited into the study. Insofar as possible, the group of subjects selected was of mixed sex, age, and race, and the skin of the test sites was free from clinically evident dermatoses, injuries, and/or any other disorders that may have compromised the subject and the study. Forty-seven subjects completed testing. All subjects signed the Study Description and Informed Consent Form, Subject Confidential Information and Acceptance Criteria, and Authorization to Use and Disclose Protected Health Information Form prior to participating in the study. The demographics of the test portion of the study are presented in Table 4.1.

4.7 Adverse Events

No subject experienced an adverse event during or following completion of this study.

Table 4.1 Subject demographics

Demographic summary	All subjects	
	Recruited	Tested
Age		
Minimum age	18	18
Median age	26	25
Maximum age	69	69
Sex		
Males (M)	67	31
Females (F)	55	16
Total	122	47
Race		
White/Caucasian (C)	118	46
Native American/Alaskan Native (NA)	1	0
Native Hawaiian/Pacific (P)	1	1
Other (O)	2	0
Total	122	47
Did not participate in testing		
SC = schedule conflict	10	
QC = qualification (inclusion/exclusion) criteria failure	3	
NS = no show	5	
SF = study requirements fulfilled	4	
SD= dismissed by study director	7	
LBL= low Baseline	33	
NU = numbers not used in the test system	13	
Total	75	

4.8 Statistical Analysis and Interpretation

The $\log_{10}$ microbial reduction data produced by the two test products on each of the two application regions, inguinal and abdomen were analyzed using a Two Factor Analysis of Variance (ANOVA). The two factors are A: Sample Time and B: Configuration. Significance level of the statistical test was set at $\alpha = 0.05$. The test hypotheses were:

1. Hypothesis A

   H_0: Configuration #1 (no blot) $\log_{10}$ microbial reduction = Configuration #2 (blot) $\log_{10}$ microbial reduction

   H_A: Configuration #1 $\log_{10}$ microbial reduction $\neq$ Configuration #2 $\log_{10}$ microbial reduction

2. Hypothesis B

   H_0: Configuration #1 $\log_{10}$ microbial reduction at fifteen seconds, ten minutes, and six hours = Configuration #2 $\log_{10}$ microbial reduction at fifteen seconds, ten minutes, and six hours

   H_A: Configuration #1 $\log_{10}$ microbial reduction at fifteen seconds, ten minutes, and six hours $\neq$ Configuration #2 $\log_{10}$ microbial reduction at fifteen seconds, ten minutes, and six hours

3. Hypothesis A x B

   H_0: Interaction does not exist between the two factors

   H_A: Interaction exists between the two factors

4.9 Results

The 2% CHG when applied to the inguinal sites per the "no blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 2.04 at fifteen seconds post-product application, 2.14 at ten minutes post-product application and 2.88 at six hours post-product application. Test Product #1, when applied to the inguinal region per the "blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 1.91 at fifteen seconds post-product application, 2.10 at ten minutes post-product application, and 2.73 at six hours post-product application.

The PVP-I scrub and paint, when applied to the inguinal sites per the "no blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 3.18 at fifteen seconds post-product application, 3.62 at ten minutes post-product application, and 4.28 at six hours post-

product application. This test product, when applied to the inguinal region per the "blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 3.11 at fifteen seconds post-product application, 4.23 at ten minutes post-product application, and 3.85 at six hours post-product application.

The 2% CHG when applied to the abdominal sites per the "no blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 1.36 at fifteen seconds post-product application, 1.79 at ten minutes post-product application, and 1.62 at six hours post-product application. This test product, when applied to the abdominal region per the "blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 1.35 at fifteen seconds post-product application, 1.80 at ten minutes post-product application, and 1.82 at six hours post-product application

The PVP-I scrub and paint when applied to the abdominal sites per the "no blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 2.48 at fifteen seconds post-product application, 2.65 at ten minutes post-product application, and 2.79 at six hours post-product application. This test product, when applied to the abdominal sites per the "blot" technique, produced mean $\log_{10}$ reductions from baseline populations of 2.36 at fifteen seconds post-product application, 2.74 at ten minutes post-product application, and 2.37 at six hours post-product application.

Table 4.2 shows the statistical summary of the mean $\log_{10}$ microbial reductions from baseline values produced by 2%CHG when applied to the inguinal site per the "no blot" technique, followed by a three minute air-dry.

Table 4.2: 2% CHG inguinal site with "no blot" technique

Inguinal site 2% CHG with "no blot"	Sample size	Log_{10} reduction	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	18	2.04	1.00	1.55 to 2.54
10 minutes post-prep reduction from baseline	18	2.14	0.80	1.74 to 2.54
6 hours post-prep reduction from baseline	18	2.88	1.01	2.38 to 3.39

Table 4.3 shows the statistical summary of the mean log_{10} microbial reductions from baseline values produced by 2% CHG when applied to the inguinal site per the "blot" technique.

Table 4.3: 2% CHG - Inguinal Site with the "blot" technique

Inguinal site 2% CHG IPA "with blot"	Sample size	Log_{10} reduction	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	18	1.91	0.66	1.58 to 2.24
10 minutes post-prep reduction from baseline	18	2.10	0.94	1.64 to 2.57
6 hours post-prep reduction from baseline	18	2.73	1.56	1.95 to 3.50

Figure 4.1 presents a statistical comparison of the mean $\log_{10}$ reduction values produced by 2% CHG when applied to the inguinal site "no blot" versus "blot" using a Two-Way ANOVA with individual 95% confidence intervals based on a pooled standard deviation.

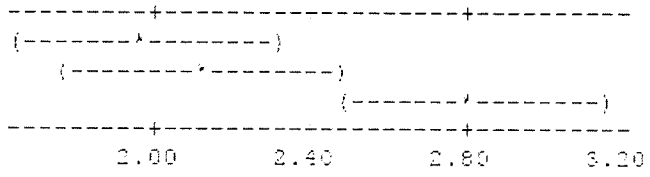
Two-way ANOVA: log(CFU/mL) versus sample, configuration – Inguinal Site

Source	DF	SS	MS	F	P
Sample	2	14.105	7.05268	6.62	0.002
Configuration	1	0.315	0.31543	0.30	0.588
Interaction	2	0.068	0.03376	0.03	0.969
Error	102	108.650	1.06519		
Total	107	123.138			

S = 1.032 R-Sq = 11. 77% R-Sq (adj) = 7.44%

Sample	Mean
1 – 15 second	1.97675
2 – 10 minute	2.12079
3 – 6 hour	2.80518

Individual 95% CIs for Mean Based on Pooled StDev



Configuration	Mean
1 - No Blot	2.35495
2 - Blot	2.24687

Individual 95% CIs for Mean Based on Pooled StDev

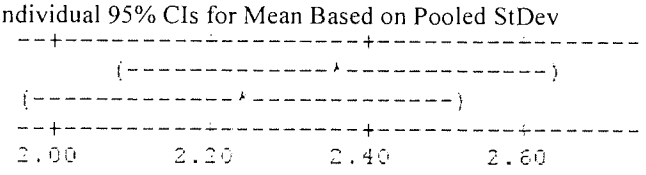


Figure 4.1: 2% CHG mean $\log_{10}$ reduction in the inguinal site with "no blot" versus "blot."

(----------) Standard Deviation

* Mean

Figure 4.2 presents a graphical comparison of the mean $\log_{10}$ reductions from baseline values produced by 2% CHG when applied to the inguinal site "no blot" vs "blot technique.

Figure 4.3 presents the statistical comparison of mean $\log_{10}$ reduction values produced by 2% CHG when applied to the inguinal site "no blot" versus "blot"; fifteen second post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 0.8458.

Figure 4.4 presents the statistical comparison of mean $\log_{10}$ reduction values produced by 2% (w/v) CHG when applied to the inguinal site "no blot" versus "blot"; ten minute post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 0.8702.

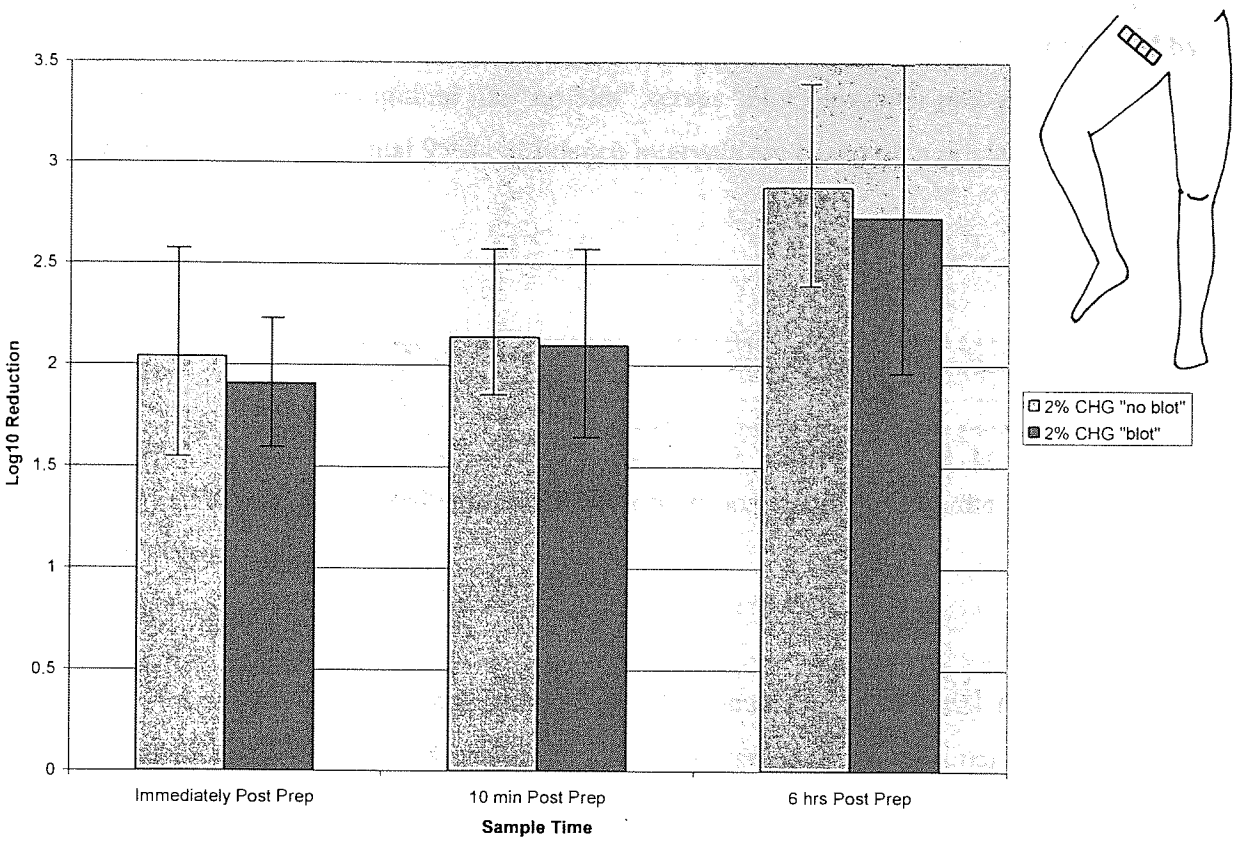


Figure 4.2: 2% CHG mean $\log_{10}$ reductions in the inguinal site with "no blot" and "blot."

Config.	N	Mean	StDev
No Blot	18	2.0420	0.9964
Blot	18	1.9115	0.6618

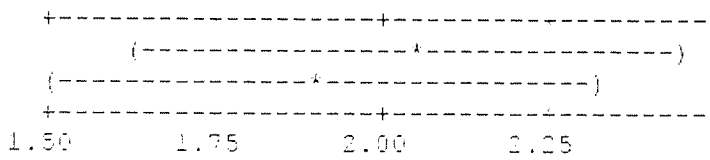


Figure 4.3: 2% CHG mean $\log_{10}$ reduction values fifteen second post application in the inguinal site with "no blot" versus "blot."

Config.	N	Mean	StDev
No Blot	18	2.1402	.7968
Blot	18	2.1014	.9379

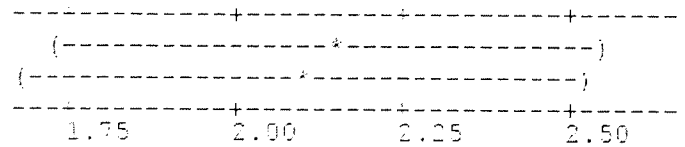


Figure 4.4: 2% CHG mean $\log_{10}$ reduction values ten minute post application in the inguinal site with "no blot" versus "blot."

Figure 4.5 presents the statistical comparison of mean log_{10} reduction values produced by 2% CHG when applied to the inguinal site "no blot" versus "blot"; six hour post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.313.

Config.	N	Mean	StDev
No Blot	18	2.883	1.010
Blot	18	2.728	1.557

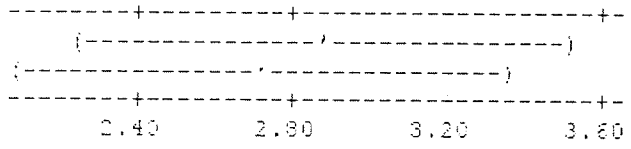


Figure 4.5: 2% CHG mean log_{10} reduction values six hour post application in the inguinal site with "no blot" versus "blot"

Table 4.4 shows the statistical summary of the mean log_{10} microbial reductions from baseline values produced by PVP-I scrub and paint when applied to the inguinal site per the "no blot" technique, followed by an air-dry.

Table 4.4: PVP-I scrub and paint mean log_{10} microbial reductions in the inguinal site with "no blot"

Inguinal Site PVP-I scrub/paint with "no blot"	Sample size	Log_{10} reduction	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	9	3.18	1.36	(2.14 to 4.23)
10 minutes post-prep reduction from baseline	9	3.62	1.32	(2.60 to 4.63)
6 hours post-prep reduction from baseline	9	4.28	1.79	(2.91 to 5.66)

Table 4.5 shows the statistical summary of the mean log_{10} microbial reductions from baseline values produced by PVP-I scrub and paint when applied to the inguinal site per the "blot" technique.

Table 4.5: PVP-I scrub and paint mean log_{10} microbial reductions in the inguinal site with" blot" technique

Inguinal Site PVP-I scrub/paint with "no blot"	Sample size	Log_{10} reduction	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	9	3.11	1.46	(1.99 to 4.24)
10 minutes post-prep reduction from baseline	9	4.23	1.52	(3.06 to 5.39)
6 hours post-prep reduction from baseline	9	3.85	1.96	(2.34 to 5.36)

Figure 4.6 presents a statistical comparison of mean log_{10} reduction values produced by PVP-I scrub and paint when applied to the inguinal site "no blot" versus "blot" using a Two-Way ANOVA with individual 95% confidence intervals based on a pooled standard deviation.

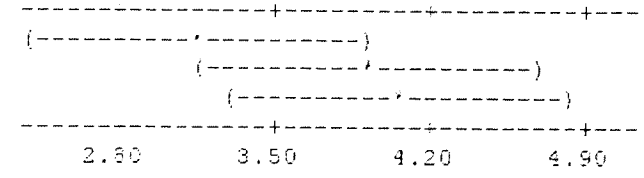
Two-way ANOVA: log(CFU/mL) versus sample, configuration – Inguinal Site

Source	DF	SS	MS	F	P
Sample	2	8.792	4.39609	1.75	0.185
Configuration	1	0.017	0.01657	0.01	0.936
Interaction	2	2.521	1.26044	0.50	0.609
Error	48	120.858	2.51788		
Total	53	132.188			

S = 1.587 R-Sq = 8.57% R-Sq (adj) = 0.00%

Sample	Mean
1 – 15 second	3.14693
2 – 10 minute	3.92133
3 – 6 hour	4.06603

Individual 95% CIs for Mean Based on Pooled St Dev



Configuration	Mean
1 - No Blot	3.69391
2 - Blot	3.72895

Individual 95% CIs for Mean Based on Pooled StDev

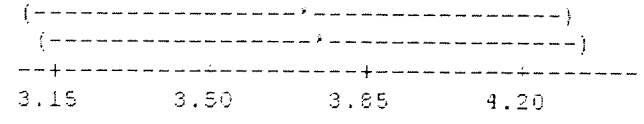


Figure 4.6: PVP-I scrub and paint at the inguinal site mean log_{10} reduction values with "no blot" versus "blot."

Figure 4.7 presents a graphical comparison of the mean log_{10} reductions from baseline values produced by PVP-I scrub and paint when applied to the inguinal site per "no blot" and "blot".

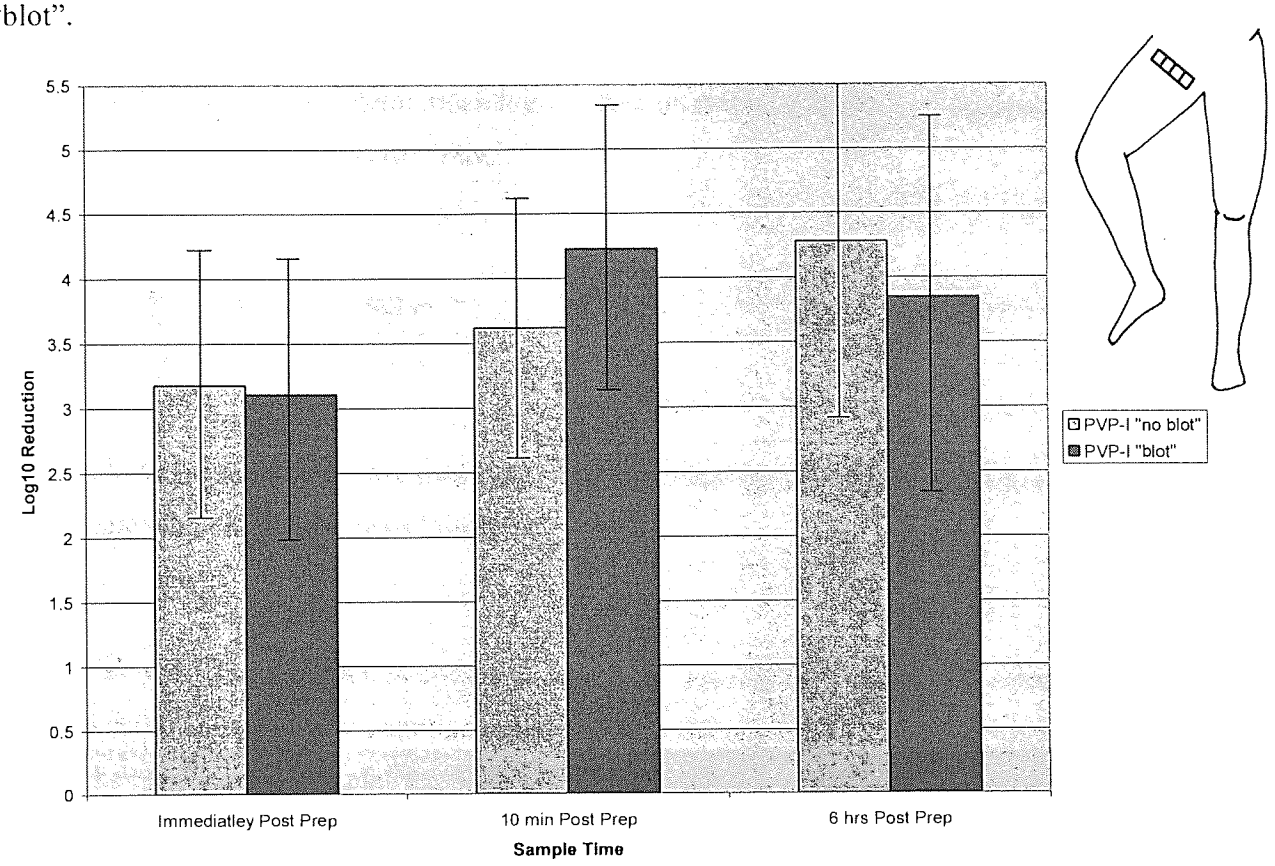


Figure 4.7: PVP-I scrub and paint in the inguinal site with "not blot" and "blot"

Figure 4.8 presents the statistical comparison of mean log_{10} reduction values produced by PVP-I scrub and paint when applied to the inguinal site "no blot" versus "blot"; fifteen second post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.414.

Figure 4.9 presents the statistical comparison of mean log10 reduction values produced by PVP-I scrub and paint when applied to the inguinal site "no blot" versus "blot"; ten minutes post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.424.

Config.	N	Mean	StDev
No Blot	9	3.183	1.363
Blot	9	3.111	1.463

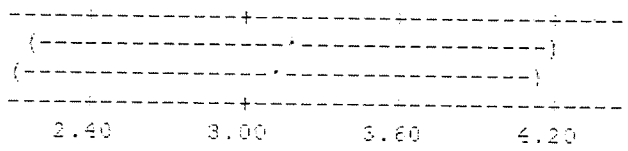


Figure 4.8: PVP-I scrub and paint mean $\log_{10}$ reduction fifteen seconds post application in the inguinal site with "no blot" versus "blot."

Config.	N	Mean	StDev
No Blot	9	3.617	1.321
Blot	9	4.226	1.519

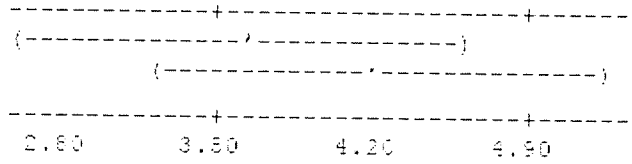


Figure 4.9: PVP-I scrub and paint mean $\log_{10}$ reduction ten minutes post application in the inguinal site with "no blot" versus "blot"

Figure 4.10 presents the statistical comparison of mean log10 reduction values produced by PVP-I scrub and paint when applied to the inguinal site per "no blot" versus "blot"; six hours post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.878.

Config.	N	Mean	StDev
No Blot	9	4.283	1.789
Blot	9	3.849	1.963

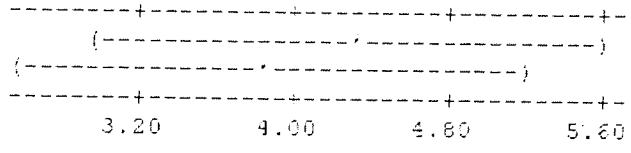


Figure 4.10: PVP-I scrub and paint mean $\log_{10}$ reduction six hours post application in the inguinal site with "no blot" versus "blot"

Table 4.6 shows the statistical summary of the mean log10 microbial reductions from baseline values produced by 2% CHG when applied to the abdominal site per the "no blot" technique, followed by a three minute air-dry.

Table 4.6: 2% CHG mean log_{10} microbial reductions at the abdominal site with "no blot"

Sample	Sample size	Mean	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	10	1.36	0.50	0.22 to 2.50
10 minutes post-prep reduction from baseline	10	1.79	0.36	0.98 to 2.59
6 hours post-prep reduction from baseline	10	1.62	0.44	0.62 to 2.62

Table 4.7 shows the statistical summary of the mean log10 microbial reductions from baseline values produced by 2% CHG when applied to the abdominal site per the "blot" technique.

Table 4.7: 2% CHG mean log_{10} microbial reductions at the abdominal site with "blot"

Sample	Sample size	Mean	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	10	1.35	0.41	0.42 to 2.27
10 minutes post-prep reduction from baseline	10	1.80	0.39	0.92 to 2.68
6 hours post-prep reduction from baseline	10	1.82	0.48	0.73 to 2.91

Figure 4.11 presents a statistical comparison of mean log10 reduction values produced by 2% CHG when applied to the abdominal site "no blot" versus "blot" using a Two-Way ANOVA with individual 95% confidence intervals based on a pooled standard deviation.

Figure 4.12 presents a graphical comparison of the mean log10 reductions from baseline values produced by 2% CHG when applied to the abdominal site per "no blot" and "blot".

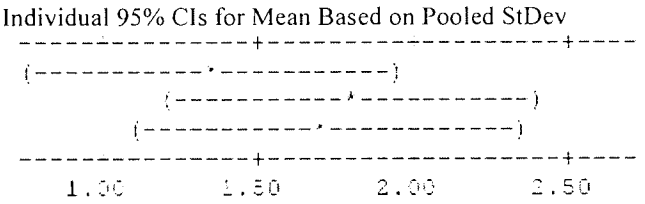
Figure 4.13 presents the statistical comparison of mean log10 reduction values produced by 2% CHG when applied to the abdominal site "no blot" versus "blot"; fifteen second post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.450.

Two-way ANOVA: log(CFU/mL) versus sample, configuration

Source	DF	SS	MS	F	P
Sample	2	2.201	1.10059	0.59	0.560
Configuration	1	0.065	0.06501	0.03	0.853
Interaction	2	0.139	0.06941	0.04	0.964
Error	54	101.270	1.87537		
Total	59	103.675			

S = 1.369 R-Sq = 2.32% R-Sq (adj) = 0.00%

Sample	Mean
1 – 15 second	1.35441
2 – 10 minute	1.79277
3 – 6 hour	1.71840



Configuration	Mean
1 – No Blot	1.58895
2 - Blot	1.65478

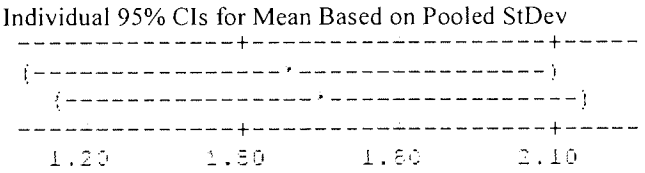


Figure 4.11: 2% (w/v) chorhexidine gluconate in 70% (v/v) isopropyl alcohol in a 26 ml applicator mean $\log_{10}$ reduction at the abdominal site "no blot" versus "blot."

Config.	N	Mean	StDev
No Blot	10	1.360	1.591
Blot	10	1.348	1.294

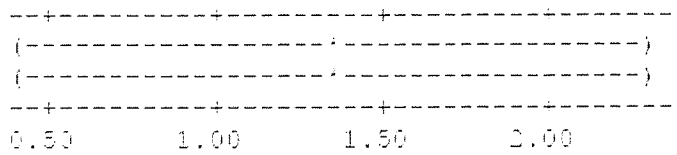


Figure 4.13: 2% CHG mean $\log_{10}$ reduction fifteen second post with "no blot" versus "blot.

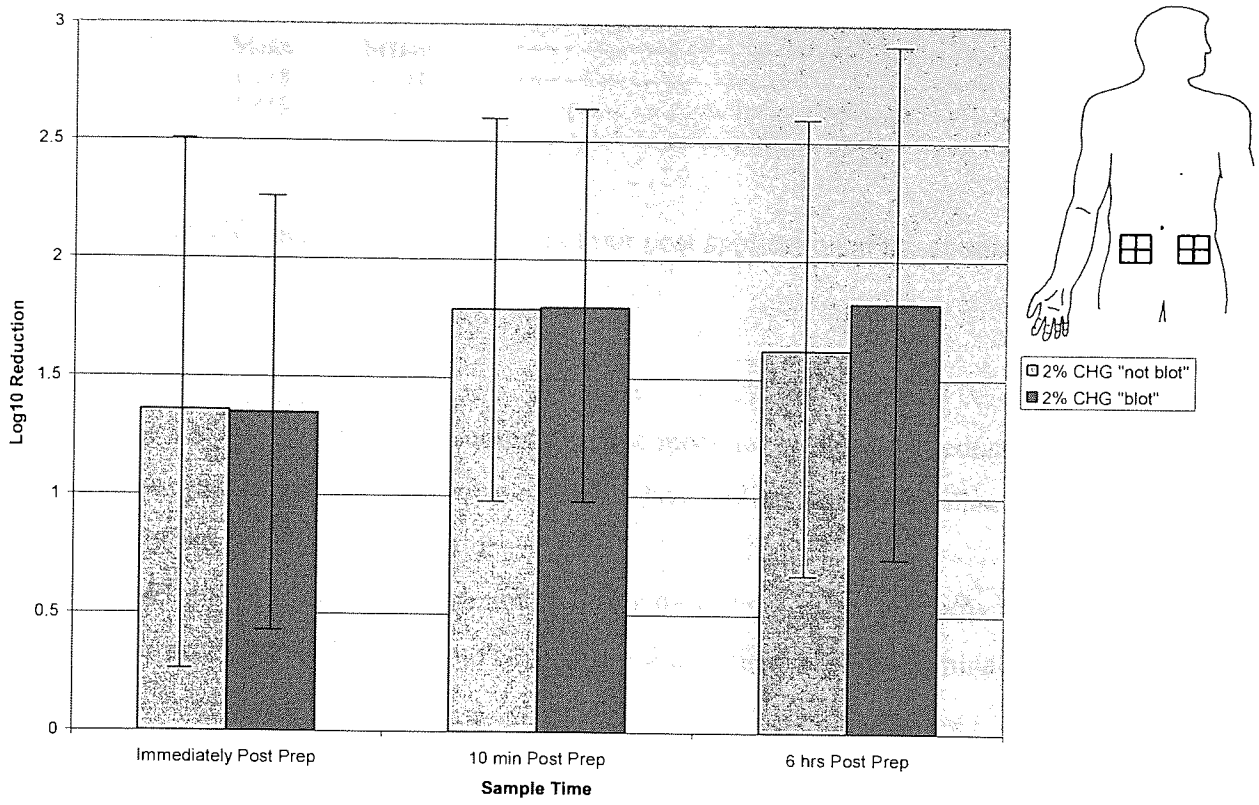


Figure 4.12: 2% CHG mean $\log_{10}$ reductions at the abdominal site "no blot" versus "blot."

Figure 4.14 presents the statistical comparison of mean $\log_{10}$ reduction values produced by 2% CHG when applied to the abdominal site "no blot" versus "blot"; ten minute post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.178.

Figure 4.15 presents the statistical comparison of mean $\log_{10}$ reduction values produced by 2% CHG when applied to the abdominal site "no blot" versus "blot"; six hour post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.461.

Config.	N	Mean	StDev
No Blot	10	1.789	1.125
Blot	10	1.797	1.230

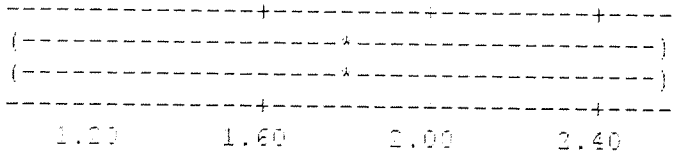


Figure 4.14: 2% CHG mean $\log_{10}$ reduction ten minute post application at the abdominal site "no blot" versus "blot"

Config.	N	Mean	StDev
No Blot	10	1.618	1.394
Blot	10	1.819	1.525

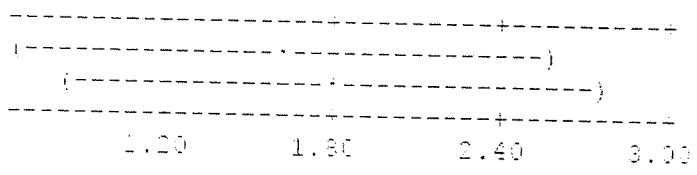


Figure 4.15: 2% CHG mean $\log_{10}$ reduction six hour post application at the abdominal site "no blot" versus "blot"

Table 4.8 shows the statistical summary of the mean $\log_{10}$ microbial reductions from baseline values produced by PVP-I scrub and paint when applied to the abdominal site per the "no blot" technique, followed by an air-dry.

Table 4.9 shows the statistical summary of the mean $\log_{10}$ microbial reductions from baseline values produced by PVP-I scrub and paint when applied to the abdominal site the "blot" technique.

Table 4.8: PVP-I scrub and paint mean $\log_{10}$ microbial reductions at the abdominal site "no blot" technique

Sample	Sample size	Mean	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	12	2.48	1.55	(1.49 to 3.46)
10 minutes post-prep reduction from baseline	12	2.65	1.33	(1.81 to 3.49)
6 hours post-prep reduction from baseline	12	2.79	0.81	(2.27 to 3.30)

Table 4.9: PVP-I scrub and paint mean $\log_{10}$ microbial reductions at the abdominal site "blot" technique

Sample	Sample size	Mean	Standard deviation	95% confidence interval
Immediately post-prep reduction from baseline	12	2.36	1.29	(1.54 to 3.17)
10 minutes post-prep reduction from baseline	12	2.74	1.06	(2.06 to 3.41)
6 hours post-prep reduction from baseline	12	2.37	1.03	(1.72 to 3.03)

Figure 4.16 presents a statistical comparison of mean $\log_{10}$ reduction values produced by PVP-I scrub and paint when applied to the abdominal site "no blot" versus "blot" using a One-Way ANOVA with individual 95% confidence intervals based on a pooled standard deviation.

Figure 4.17 presents a graphical comparison of the mean $\log_{10}$ reductions from baseline values produced by PVP-I scrub and paint when applied to the abdominal site "no blot" and "blot".

Figure 4.18 presents the statistical comparison of mean $\log_{10}$ reduction values produced by PVP-I scrub and paint when applied to the abdominal site "no blot" versus "blot"; fifteen second post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.423.

Figure 4.19 presents the statistical comparison of mean $\log_{10}$ reduction values produced by PVP-I scrub and paint when applied to the abdominal site "no blot" versus "blot"; ten minute post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 1.423.

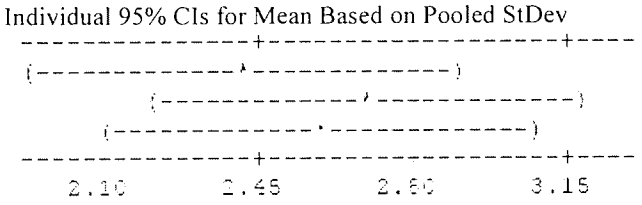
Figure 4.20 presents the statistical comparison of mean $\log_{10}$ reduction values produced by PVP-I scrub and paint when applied to the abdominal site "no blot" versus "blot"; six hour post-product application sample. The individual 95% confidence intervals are based on a pooled standard deviation of 0.9252.

Two-way ANOVA: log(CFU/mL) versus sample, configuration

Source	DF	SS	MS	F	P
Sample	2	0.9126	0.45632	0.32	0.730
Configuration	1	0.3927	0.39266	0.27	0.603
Interaction	2	0.7670	0.38349	0.27	0.767
Error	66	95.0514	1.44017		
Total	71	97.1237			

S = 1.200 R-Sq = 2.13% R-Sq (adj) = 0.00%

Sample	Mean
1 – 15 second	2.41824
2 – 10 minute	2.69264
3 – 6 hours	2.57930



Configuration	Mean
1 – No Blot	2.63724
2 – Blot	2.48955

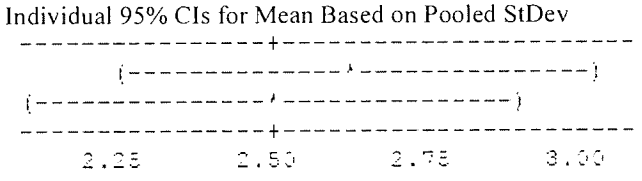


Figure 4.16: PVP-I scrub and paint mean $\log_{10}$ reduction at the abdominal site per "no blot" versus "blot"

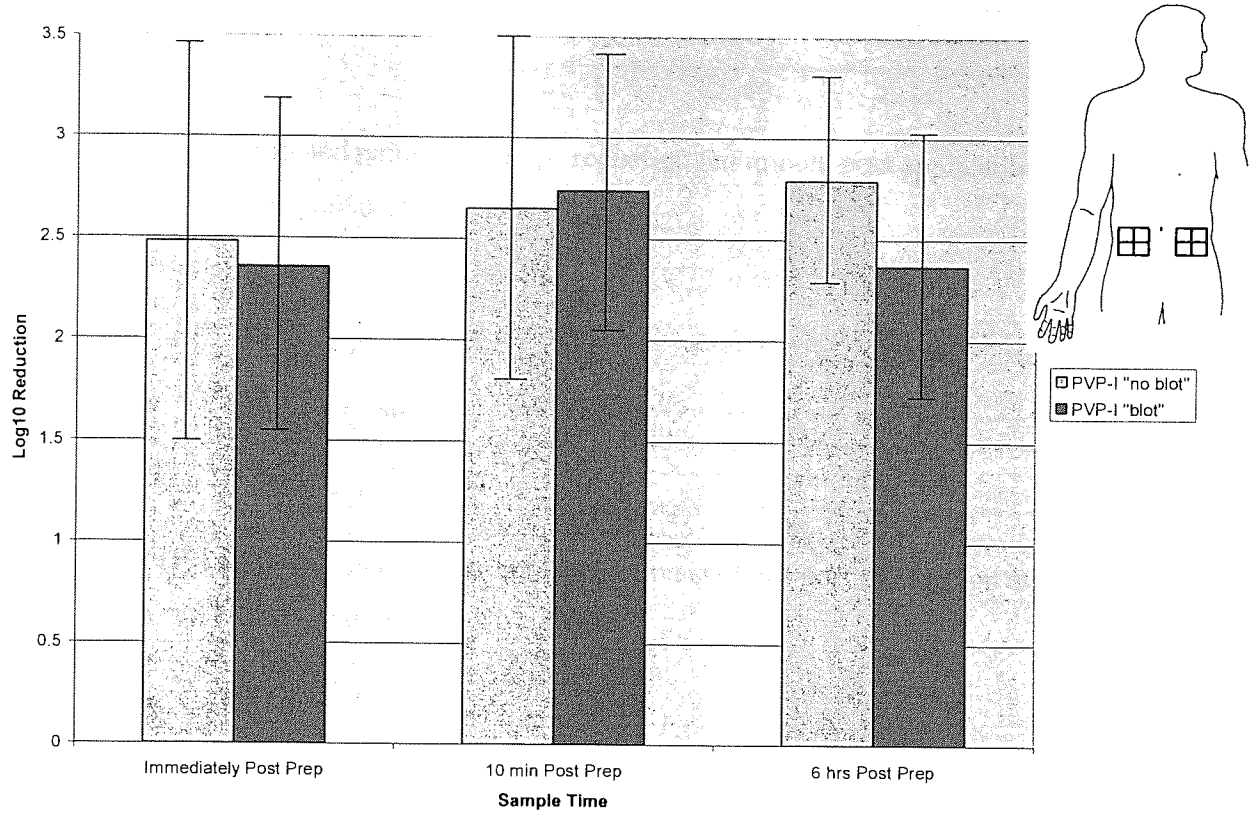


Figure 4.17: PVP-I scrub and paint Mean Log_{10} Reductions at the Abdominal Site "no blot" versus "blot."

Config.	N	Mean	StDev
No Blot	12	2.477	1.547
Blot	12	2.360	1.288

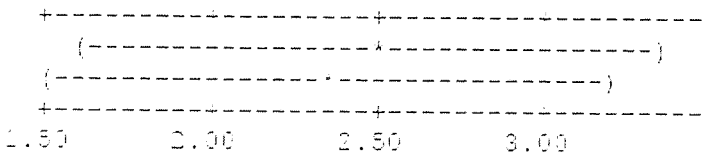


Figure 4.18: PVP-I scrub and paint mean log_{10} reduction fifteen second post application at the abdominal site "no blot" versus "blot."

Config.	N	Mean	StDev
No Blot	12	2.648	1.325
Blot	12	2.737	1.059

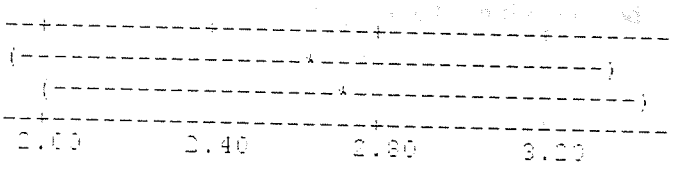


Figure 4.19: PVP-I scrub and paint mean $\log_{10}$ reduction ten minute post application at the abdominal sites "no blot" versus "blot."

Config.	N	Mean	StDev
No Blot	12	2.7865	0.8082
Blot	12	2.3721	1.0291

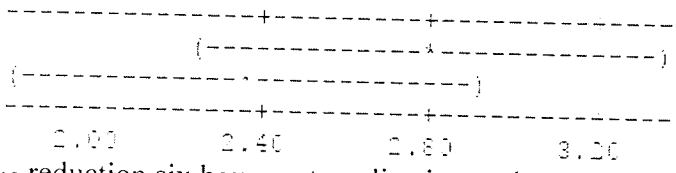


Figure 4.20: PVP-I scrub and paint mean $\log_{10}$ reduction six hour post application at the abdominal site "no blot" versus "blot."

4.10 Summary

The hypothesis of this evaluation was that "blotting" the PVP-I scrub and paint solution from the test sites would significantly reduce the mean $\log_{10}$ reduction in comparison to the 2% CHG. This hypothesis was based on the fact that CHG has a huge affinity to bind to the skin due to its cationic positive charge and the skin being negatively charged. Where as the assumption that PV P-I scrub and paint would be removed prior to the I^2 being released from the iodophor solutions. The data suggests that this is not a correct hypothesis, there was no significance between 2% CHG and PVP-I scrub and paint OR the configuration of "blot" versus "no-blot".

4.11 Discussion

The potential for reduction of the antimicrobial efficacy of patient preoperative skin preparations due to mechanical removal of wet product from the application site (blot) has been a topic of debate for many years. Products that require the evaporation of the antiseptic as a contribution to the reduction in resident flora, such as alcohol-containing products, or for the antiseptic to dry completely, such as iodophors, could potentially be compromised by prior removal of the product. The practice of blot reduces the amount of antiseptic on the application site, as well as hinders the cidal effects of the evaporation of the antiseptic.

In this evaluation, the blot of the wet antiseptic from the application site did not produce a significant difference in product efficacy. This was as expected for the aqueous chlorhexidine

product, but was not for the iodophor. However, a significant factor may have been the method of and volume provided by the iodophor product application procedure. The two-step application procedure for the Scrub Care® Preoperative Skin Prep Trays (7.5% Povidone Iodine Cleansing Solution USP [Scrub] and 10% Povidone Iodine Topical Solution USP [Paint]) required that the entire contents of the scrub and paint be poured onto sponges and applied at each of the sites of application. Therefore, the entire contents of the 109 mL bottle of Povidone Iodine Cleansing Solution USP (Scrub) and the 88.5 mL bottle of Povidone Iodine Topical Solution USP (Paint) were applied at each site of application. Furthermore, the scrub was performed for five minutes, a new saturated sponge used each minute prior to the performance of the paint application. Given that the regulatory agencies has questioned whether a 26 mL application of product was appropriate or excessive for prepping of the inguinal region, a total combined scrub and paint volume of 197.5 mL applied to the inguinal region would appear to be excessive. Additionally, data Betadine™, a competitor product with a identical composition and activity, states that one mL of the scrub is sufficient to cover an area of 20 to 30 square inches, which then is followed by application of the paint. It appears that the robust nature of the iodophor application may have reduced the resident flora prior to any effect afforded by air-drying or blot of the product. Therefore, in order to evaluate the effect of a mechanical removal (blot) of wet antiseptic, different application configurations should be considered that vary the volume, and perhaps, the method for the application of the test products.

CHAPTER 5: A CLINICAL TRIAL TO COMPARE CHLORHEXIDINE AND POVIDONE IODINE SKIN PREPARATIONS FOR THE PREVENTION OF SURGICAL SITE INFECTIONS - METHODOLOGY

5.1 Summary

The patient's skin is a major source of microorganisms that can cause surgical site infections. Optimization of preoperative skin antisepsis may therefore decrease postoperative infections. We hypothesized that preoperative skin cleansing with chlorhexidine-alcohol reduces the number of microorganisms on the skin surface more effectively than the traditional povidone-iodine antiseptics, therefore offering a greater protective potential against subsequent infection.

Patients undergoing clean-contaminated surgery were randomly assigned in 6 hospitals to preoperative skin preparation with either 2% chlorhexidine gluconate in 70% isopropyl alcohol scrub or 10% povidone-iodine scrub and paint. The primary outcome of any surgical site infection was assessed at thirty days post surgery. Secondary outcomes included individual type of surgical site infection. The methodology used for this trial is described in this chapter. In chapter 6 the findings are presented.

5.2 Introduction

More than 23 million surgical procedures are performed each year in the United States (Kluytmans 1997). In general, surgical site infection is the most common post-operative infection and accounts for over 500,000 surgical site infections per year in the US (Wenzel 1991, Kluytmans 1997). Surgical site infections significantly increase the postoperative length of stay and hospital costs (Green and Wenzel 1997). The medical sequelae and economical burden associated with surgical site infections have highlighted the importance of preventing these infections. Perioperative administration of systemic antibiotic prophylaxis, local application of antiseptic agents, and adherence to aseptic guidelines has helped reduce but has not eliminated the occurrence of surgical site infections. For instance, between 2.4 to 7.7% of patients undergoing "clean-contaminated" surgery still develop post-operative wound infection (Cruse and Foord 1980, Haley, *et al.*, 1985, Olson and Lee 1990, Culver, *et al.*, 1991). Because clean-contaminated wounds generally exclude the possibility of gross spillage of microorganisms from the gastrointestinal tract (as in "contaminated" wounds) and preoperative infection (as in "dirty-infected" wounds), most cases of infection of clean-contaminated wounds are thought to

originate from the patients skin. Therefore, it is conceivable that application of an optimal antiseptic agent can reduce the rate of such surgical site infections. Betadine (an iodophor) continues to be used to disinfect the skin prior to most surgeries in the United States. Although both Betadine and chlorhexidine possess broad-spectrum antimicrobial activity, the latter antiseptic agent provides extra advantages such as rapid bactericidal activity, persistent activity on the skin that is not altered by presence of organic matter, and no-to-minimal systemic absorption (Crosby, *et al.*, 2009) Moreover, chlorhexidine-based preparations have been demonstrated to be more effective than Betadine in preventing infections associated with indwelling vascular catheters (Maki, *et al.* 2001). These factors help explain why the most recent HICPAC guidelines for the prevention of intravascular catheter-related infection (O'Grady, *et al.* 2002) have preferentially recommended disinfecting the skin with a 2% chlorhexidine-based preparation (category IA). Recently, the FDA approved the use of ChloraPrep applicator (2% chlorhexidine and 70% isopropyl alcohol) for disinfecting the skin prior to procedures.

The primary objective of this prospective, randomized, multicenter clinical trial was to compare the impact of disinfecting the skin with ChloraPrep vs. Betadine on the rates of infection of clean-contaminated surgical wounds.

5.3 Background

Despite implementation of potentially preventive measures that include preoperative skin cleansing with povidone-iodine, approximately 500,000 patients suffer from surgical site infections annually in the US (Wolf, *et al.*, 2008, Hawn, *et al.*,2008, Belda, *et al.*, 2005, Kurz, *et al.*, 1996, Yasunaga, *et al.*, 2007, Kirkland, *et al.*, 1999). In view of the fact that the patient's skin is a major source of pathogens, it is plausible that improving skin antisepsis would decrease surgical site infections (Napolitano 2006). The CDC recommended the use of 2% chlorhexidine-based preparations for cleansing the insertion site of intravascular catheters (O'Grady *et al.*, 2002). However, the CDC has not issued a preference as to which antiseptic should be used preoperatively in the prevention of surgical site infections. This is mainly because there are no published randomized studies that compare the impact of using one antiseptic preparation versus another on the incidence of surgical site infection. The main objective of this current study was to compare the efficacy of disinfecting, preoperatively, the patient's skin with chlorhexidine-alcohol versus povidone-iodine in preventing surgical site infections.

5.4 Scope

This prospective, randomized, multicenter clinical trial included patients who had clean-contaminated surgical wound. The two antiseptics used were 10% povidone iodine scrub and paint and a 2% chlorhexidine in 70% isopropyl alcohol in a 26 mL applicator.

5.5 Study Design

The prospective, randomized clinical trial was conducted between April 2004 and May 2008 at six US university-affiliated hospitals. The institution review board at each hospital approved the study protocol, and a written informed consent was obtained from each patient before enrollment.

5.5.1 Patients

Patients 18 years of age or older undergoing clean-contaminated surgery including colorectal, small intestinal, gastroesophageal, biliary, thoracic, gynaecological or urological operations carried out under controlled conditions without significant spillage or unusual contamination or major break in sterile techniques were eligible for enrollment.

Patients exclusion criteria included: (1) they are unable or unwilling to give informed consent; (2) the patient was less than 18 years of age; (3) evidence of pre-existing infection at or adjacent to the operative sites; (4) a break in sterile technique occurs; (5) history of allergy to chlorhexidine, alcohol or iodophor; or, (6) a perceived inability to complete a patient follow-up 30 day post surgery.

5.5.2 Patient Location – Multicenter Study

The following center's entered patients into the study:

1. Michael E Debakey Medical Center, Houston TX, USA
2. Ben Taub General Hospital, Houston TX, USA
3. Milwaukee VA Medical Center, Milwaukee, WI, USA
4. Medical College of Wisconsin, Milwaukee, WI, USA
5. VA Medical Affairs New England Medical, MA, USA
6. VA Medical Affairs Duke, NC, USA

5.5.3 Patient Consent Procedures

With the verbal permission of each patient's care-givers, both physicians and nurses, and written consent from the patient or his/her family or guardian, each patient enrolled were randomized using a preset randomization schedule to one of two treatment groups:

1. 10% povidone-iodine an aqueous solution of polyvinylpyrrolidone polymer complexed with 1% titratable iodine (Iodophor, PVP –I, Betadine™, etc.)
2. 2% chlorhexidine and 70% isopropyl alcohol (ChloraPrep w/Tint 26 mL applicator, Cardinal Health, USA).

5.5.4 Protection of Patient

The costs of the study were borne by independent research funds. There was no additional cost to subjects.

All information pertaining to subjects and their medical condition were kept in strictest confidence.

Patients were under no obligation to participate in the study and, if they did participate, they were free to withdraw at any time. Their care was not affected in any way by their decision to participate or not to participate.

5.5.5 Interventions

Enrolled patients were randomized in a 1:1 ratio to have the skin at the surgical site either preoperatively prepped with an applicator that contained two-26mL applicators of 2%(w/v) chorhexidine gluconate and 70% (v/v) isopropyl alcohol or prepped with 90 mL of 10% iodophor scrub and followed by 90 mL of 10% iodophor paint solutions. A stratified randomization by hospital was implemented with the use of computer-generated randomization numbers without blocking.

Patients randomized to the 2% chlorhexidine gluconate in 70% isopropyl alcohol in a 26 mL applicator had their antiseptic applied as follows: The activate the 2% CHG in 70% IPA 26 mL applicator(s) the wing on the applicator was pinched to break the ampoule and release the solution into the sponge head of the applicator. The sponge head was gently pressed and released against the operative site until the solution was visible on the skin. The solution was then applied to the operative site by using repeated back and forth or up and down strokes of the applicator sponge at the incision site for approximately 30 seconds. The entire operative site was prepped for approximately 2 minutes which resulted in completely wetting the site with

antiseptic solution. Without any blotting or wiping, the operative site was allowed to air dry for approximately 3 minutes. All solution soaked drapes and towels around and under the patient were removed. The solution was not allowed to pool under the patient.

Patient's randomized to the 10% povidone-iodine scrub and paint solution had their antiseptic applied as follows: The povidone-iodine scrub cleansing solution was poured into the sectioned prep tray and 6 split sponges were saturated with the solution. The scrub solution was applied to the operative site with the saturated sponge(s) in a circular motion for 5 minutes starting at the incision site and moving outward to the periphery. The scrub solution that had not dried was then blotted away with a sterile towel. Next the iodophor paint solution was poured into the sectioned prep tray and 3 sponge sticks were saturated with solution. The paint solution was applied to the operative site using cicular motion, starting at the incision site and moving outward to the periphery. All solution soaked drapes and towels around and under the patient were removed. The solution was not allowed to pool under the patient.

Institutional routine care including bowel preparation and perioperative systemic antibiotic prophylaxis was practiced at all centers and followed a commen protocol. The only difference in the operative procedure was the antiseptic used to disinfect the skin. Povidone-iodine scrub and paint was pre trial the standard antiseptic. Those enrolled in the povidone-iodine group had their surgical site scrubbed and then painted with 10% povidone-iodine as stated above.

5.5.6 Clinical Assessment

Preoperative patient evaluation included a medical history, physical examination, and routine haematology and chemistry laboratory tests. The surgical site and vital signs were assessed at least once a day during hospitalisation, upon discharge, at the time of follow-up evaluation, and whenever surgical site infection occurred. Patients that were discharged during the 30-day follow-up period were contacted by telephone and arranged for prompt clinical evaluation if infection was suspected. Whenever surgical site infection was suspected or diagnosed, clinically relevant microbiological samples were cultured. Healthcare physicians, masked to the study group, assessed the seriousness of all adverse events and determined whether they were related to the study. The criteria used for diagnosing a SSI is outlined in Table 1.2.

5.5.7 Efficacy Outcomes

The primary endpoint of the study was the occurrence of any surgical site infection within 30 days post surgery. The operating surgeon became aware of the assigned type of intervention only after the randomized patient was brought to the operating room. Both the patients and sites' investigators who diagnosed surgical site infection using criteria developed by the CDC remained blinded as to the assigned group. The secondary endpoints included (1) the occurrence of different types of surgical site infection (as outlined in Table 1.2); (2) adverse effects on the skin; and, (3) cost-savings associated with the use of 2% chorhexidine gluconate and 70% isopropyl alcohol versus 10% iodophor scrub and paint solutions.

5.6 Statistical Analysis

Taking into consideration that the average baseline rate of surgical site infection after clean-contaminated surgery at the six participating hospitals while using povidone-iodine was 14%, it was estimated that application of chlorhexidine-alcohol would decrease the rate of surgical site infection to 7%. This 50% reduction estimate was based on the effect of CHG versus PVP-I in the reduction of CRBSI (Chaiyakunapruk, *et al.*, 2003). Therefore, it was planned in advance to obtain a sample size of approximately 430 evaluable patients in each study group to have 90% power to detect a significant difference in the rates of surgical site infection between the two groups at a two-tailed significance level of $p \leq 0.05$.

The criteria for including patients in the intention-to-treat analysis included randomization and application of study antiseptic preparation. Inclusion in the per-protocol analysis required application of study medication before clean-contaminated surgery and completion of 30-day follow-up. An independent monitoring board composed of an infectious disease physician, a surgeon, and a statistician met annually to review the conduct of the study. There were no formal criteria to stop the study.

In working with a statistician, the significance of the differences in patients' characteristics between the two study groups was determined with the use of the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. For efficacy outcomes, the proportions of evaluable patients in the two study groups who developed any type or individual types of surgical site infection with use of Fisher's exact test and computed the relative risks of infection and the 95% confidence intervals. The consistency of the study intervention on infections across different types of surgery was examined using an interaction test. To determine whether the results were consistent across the six participating

hospitals, a Breslow-Day test for homogeneity was performed. The proportions of patients who were free of surgical site infection as a function of the length of time were compared between the study groups with the use of log-rank test of Kaplein-Meier estimates in which patients not experiencing infection were censored at 30 days post surgery. The frequency of isolating certain microorganisms and categories of microorganisms and the incidence of adverse and serious adverse events were compared between the study groups with use of Fisher's exact test. All reported p values were based on two-tailed tests of significance and not adjusted for multiple testing.

Univariate and multivariate analyses to assess whether risk factor contributed to the occurrence of surgical site infection were performed. The univariate analysis for categorical factors was performed with use of Fisher's exact test. For continuous factors, a single variable logistic-regression model using generalized estimating equation (GEE) that accounted for hospital site as a random effect was utilized. A multivariate logistic-regression analysis which also adjusted for the hospital site as a random effect (via GEE) was performed to assess factors deemed significant ($p \leq 0.10$) by univariate analysis or considered clinically important. The assessed risk factors were pre-specified in the protocol and the statistical methods were pre-planned, with the exception of including hospital site as a random effect. Since some types of surgery did not result in infection in both study groups, a dichotomous variable of "abdominal" surgery (including colorectal, biliary, small intestinal, and gastroesophageal versus "non-abdominal" surgery (including thoracic, gynecologic, and urologic) was created for the GEE logistic regression model.

5.7 Case Report Forms

The case report forms that were collected for each individual patient are listed in Appendixes A–F.

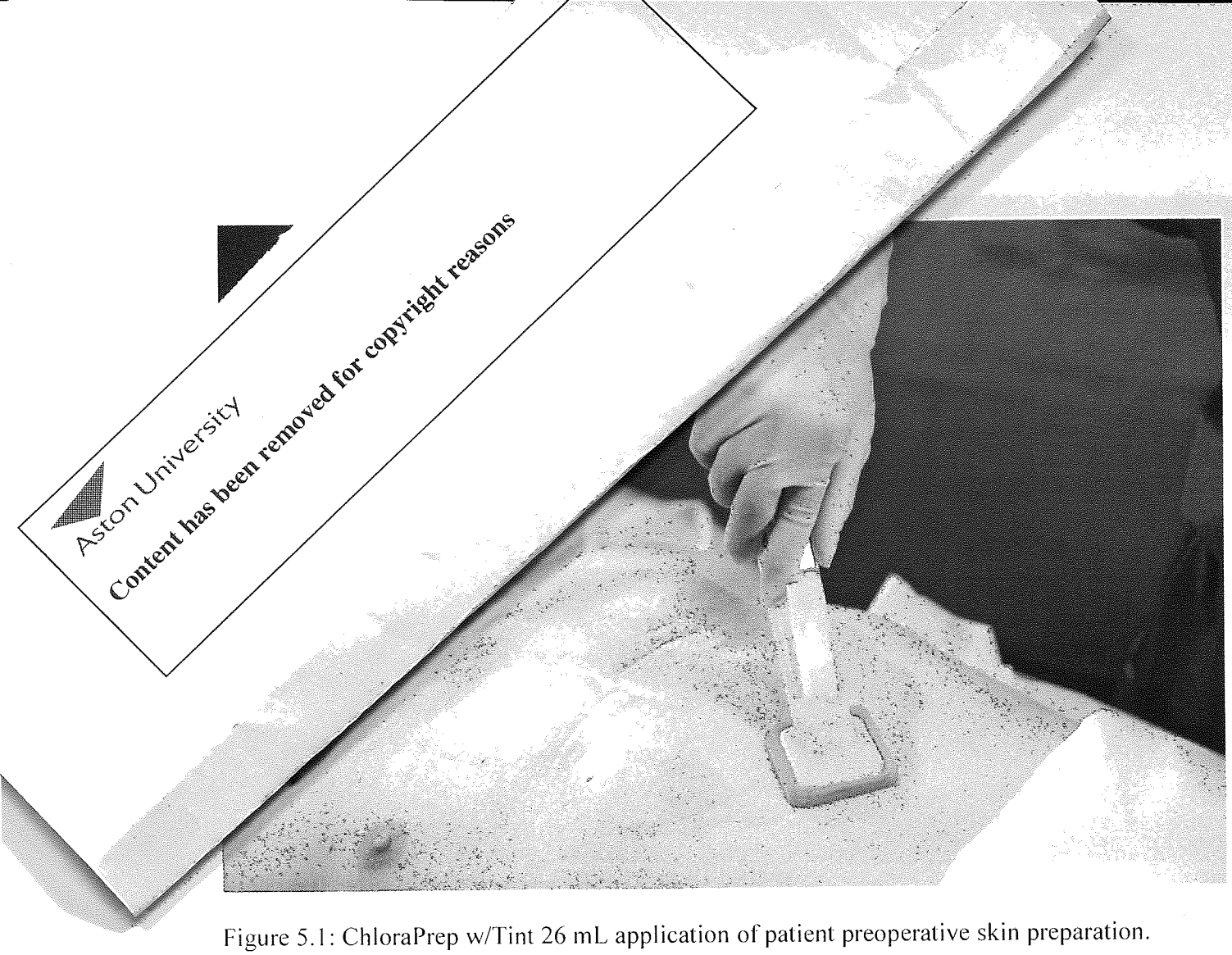


Figure 5.1: ChloraPrep w/Tint 26 mL application of patient preoperative skin preparation.

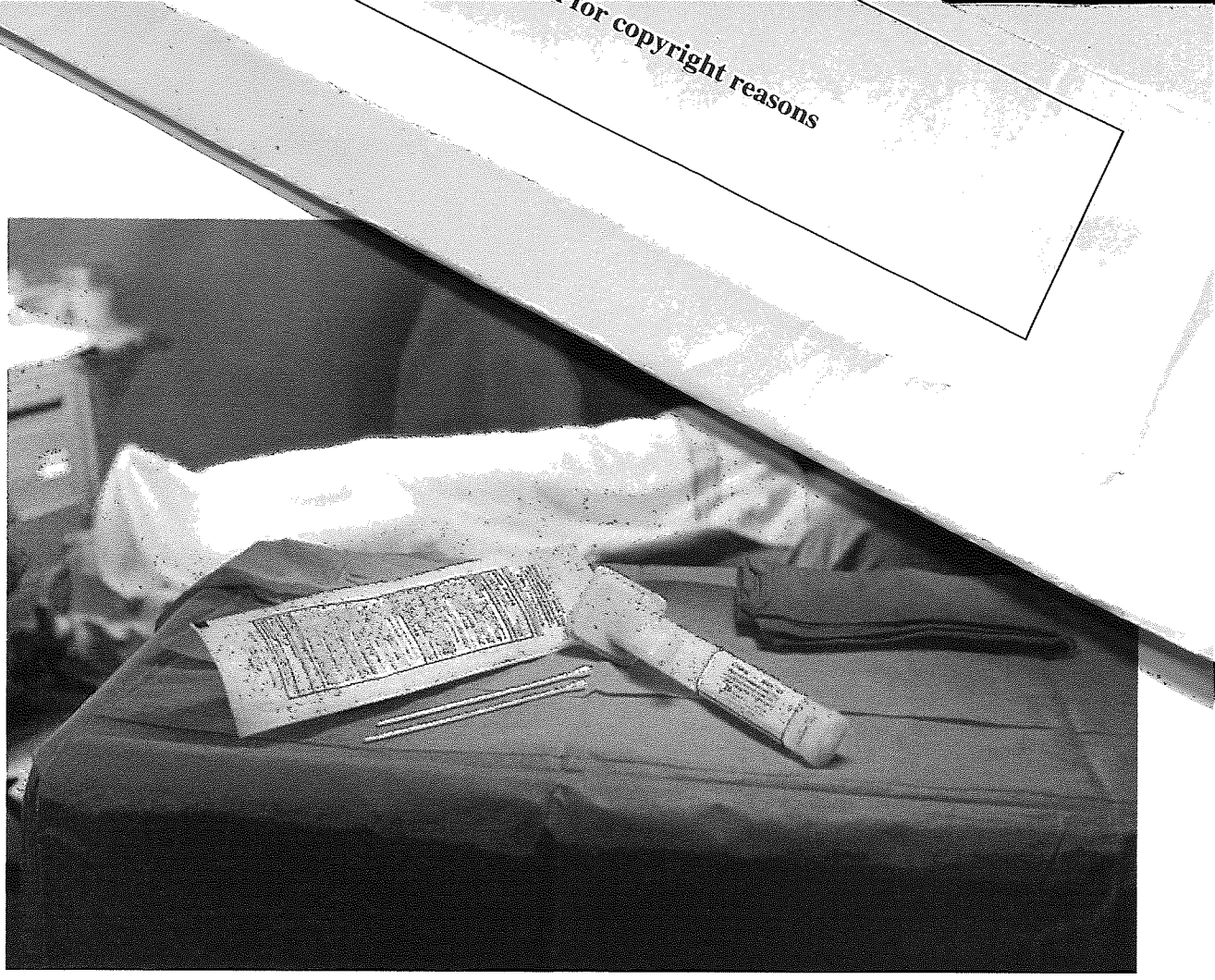


Figure 5.2: ChloraPrep w/Tint 26 mL applicator with 2% chlorhexidine and 70% isopropyl alcohol.

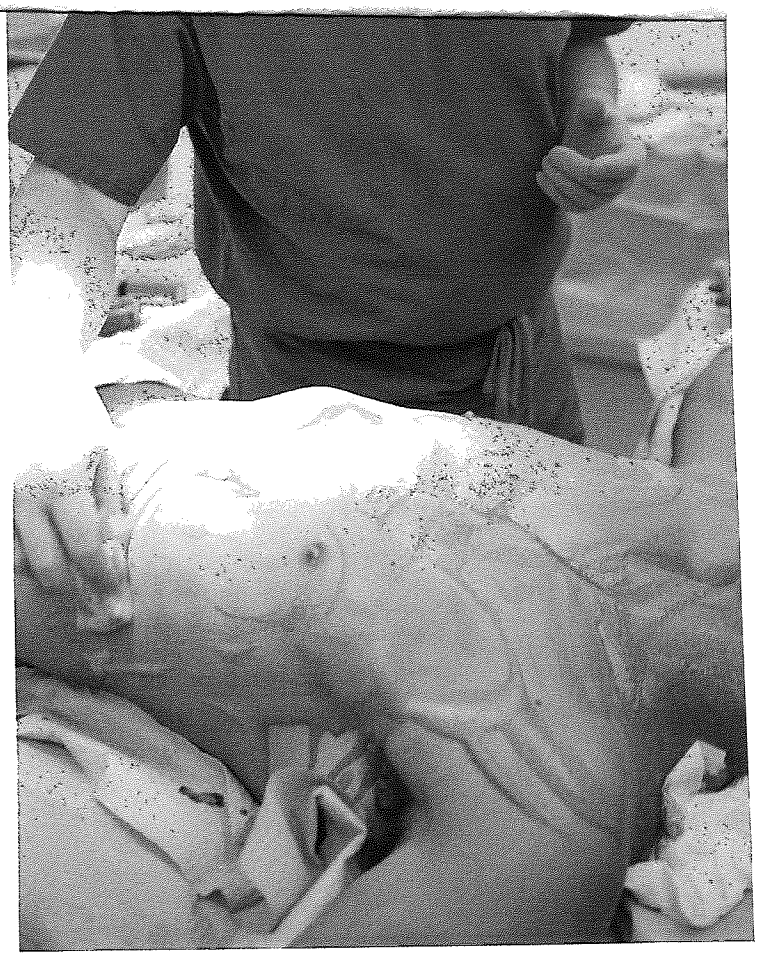


Figure 5.3: Scrub and Paint povidone-iodine Tray applicat n preparation.

Figure 5.4: Scrub and Paint povidone-iodine Tray with an aqueous solution of polyvinylpyrrolidone polymer complexed with 1% titratable iodine.

CHAPTER 6: A CLINICAL TRIAL TO COMPARE CHLORHEXIDINE AND POVIDONE IODINE SKIN PREPARATIONS FOR THE PREVENTION OF SURGICAL SITE INFECTIONS - RESULTS

6.1 Summary

There were 849 patients randomly assigned to the study groups (409 in the chlorhexidine-alcohol and 440 in the povidone-iodine group) who qualified for the intention-to-treat analysis. The overall surgical site infection was significantly lower in the chlorhexidine-alcohol (2% chlorhexidine in 70% isopropyl alcohol) group than in the povidone-iodine (10% povidone-iodine scrub and paint) group (9.5% versus 16.1%, p=0.004; relative risk, 0.59 with 95% confidence interval of 0.41 to 0.85). When comparing specific types of infection, chlorhexidine-alcohol was significantly more protective than povidone-iodine against both superficial (4.2% versus 8.6%, p=0.008) and deep (1% versus 3%, p=0.05) incisional infections but not organ-space infections (4.4% versus 4.6%). Similar results were observed in the per-protocol analysis of 813 patients who remained alive during the 30-day post follow-up period. Adverse events were comparable in the two study groups.

Preoperative cleansing of the patient's skin with chlorhexidine-alcohol is superior to povidone-iodine in preventing surgical site infection after clean-contaminated surgery.

6.2 Results

6.2.1 Patients

A total of 897 patients were randomized, 431 to the chlorhexidine-alcohol group and 466 to the povidone-iodine group (Figure 6.1). Of the 849 patients who qualified for the intention to treat analysis, 409 received chlorhexidine-alcohol and 440 received povidone-iodine. Thirty six patients, who underwent clean rather than clean contaminated surgery (25), or dropped out of the study 1 to 2 days after surgery (4) or died before completing the 30-day follow-up (7) were excluded from the per-protocol analysis of 813 patients (391 in the chlorhexidine-alcohol group and 422 in the povidone-iodine group). When assessed in both the intention-to-treat and per-protocol analyses, patients in the two study groups had similar demographics, co-morbidities, risk factors for infection, antimicrobial exposure, and duration and types of surgery (Table 6.1).

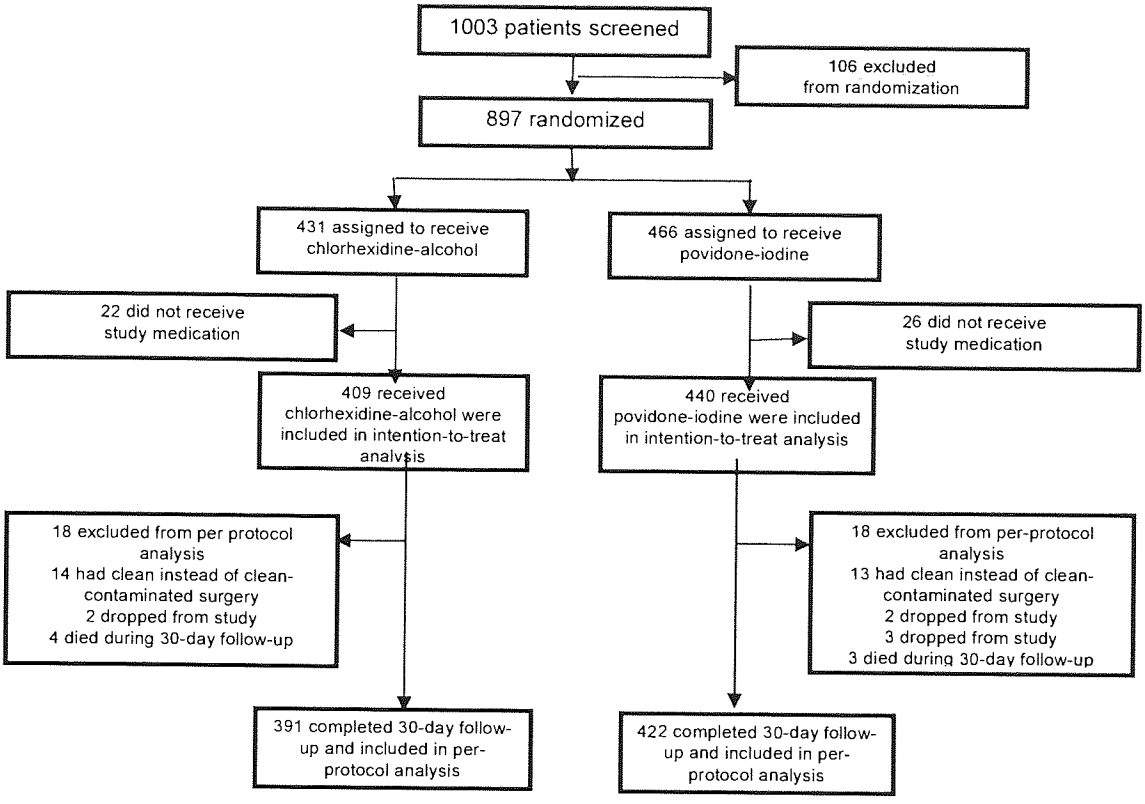


Figure 6.1 Outlines of screening, randomization, and follow-up of study patients.

Table 6.1: Demographics of the patients in each antiseptic group

Demographics	Chlorhexidine-alcohol (n = 409)	Povidone-iodine (n = 440)	p-value[a]
Male sex - %	58.9	55.9	0.40
Age			0.87
Mean	53.3±14.6	52.9±14.2	
Range	18 to 100	19 to 85	
Gastrointestinal disease - %	67.0	65.0	0.56
Cardiopulmonary disease - %	33.5	37.1	0.28
Gynecological disease - %	13.0	12.1	0.76
Neurologic disease - %	12.5	10.0	0.28
Renal disease - %	7.3	8.4	0.61
Immunologic disease - %	4.7	4.8	>0.99
Cancer - %	58.0	61.4	0.33
Diabetes mellitus- %	15.4	13.9	0.56
Malnutrition - %[b]	6.1	5.2	0.66
Liver cirrhosis - %	2.2	3.0	0.52
History of Alcohol abuse - %	17.9	19.3	0.60
History of smoking - %	37.2	34.6	0.43
Mechanical ventilation - %	0.2	0.5	>0.99
Documented colonization with MRSA - %	2.0	2.3	0.81
Systemic antibiotics			
Received preoperatively - %	100.0	100.0	>0.99
Duration of administration - days			>0.99
Mean	1.1±1.2	1.1±0.8	
Range	1 to 20	1 to 11	
Received postoperatively - %	51.7	48.9	0.41
Duration of surgery - hours			>0.99
Mean	3.0±1.5	3.0±1.5	
Range	0.5 to 10.0	0.3 to 12.0	
Preoperative shower (overall) - %	26.5	27.3	0.82
With 4% chlorhexidine gluconate - %	16.1	18.9	0.32
With 10% povidone-iodine - %	7.3	5.2	0.26
With soap bar (0.6% triclocarban)-%	3.2	3.0	>0.99

[a]P-values based on Fisher's exact test for categorial variables and Wilcoxon Rank Sum test for continuous variables. Plus-minus values are means ± SD.

[b]Malnutrition defined as >10% decrease in weight over 2 months.

6.2.2 Infection Rates

In the intention-to-treat patient population (Table 6.2), the overall rate of surgical site infection was significantly lower (p=0.004) in the chlorhexidine-alcohol group (9.5%) than in the povidone-iodine group (16.1%). The relative risk of any surgical site infection among patients whose skin was preoperatively cleansed with chlorhexidine-alcohol versus povidone-iodine was 0.59 with a 95% CI of 0.41 to 0.85. Similarly, significantly lower proportions of patients who received chlorhexidine-alcohol versus povidone-iodine developed superficial (relative risk, 0.48 with a 95% CI of 0.28 to 0.84) or deep (relative risk, 0.33 with a 95% CI of 0.11 to 1.01) incisional infection. However, there were no significant differences between the two study groups in the incidence of organ-space infection (relative risk, 0.97 with a 95% CI of 0.52 to 1.80) or sepsis from surgical site infection (relative risk, 0.62 with a 95% CI of 0.30 to 1.29). The per-protocol analysis yielded similar efficacy results.

Table 6.2: The number and type of infections associated with each antiseptic group

Type of infection	Chlorhexidine-alcohol (N = 409) no. (%)	Povidone-iodine (N = 440) no. (%)	Relative risk (95% CI)	p-value[a]
Any surgical site infection	39 (9.5)	71 (16.1)	0.59 (0.41-0.85)	0.004
Superficial incisional infection	17 (4.2)	38 (8.6)	0.48(0.28-0.84)	0.008
Deep incisional infection	4 (1.0)	13 (3.0)	0.33 (0.11-1.01)	0.05
Organ-space infection	18 (4.4)	20 (4.6)	0.97 (0.52-1.80)	>0.99
Sepsis from surgical site infection	11 (2.7)	19 (4.3)	0.62 (0.30-1.29)	0.26

[a] p-values were based on Fisher's exact test and 95% confidence intervals for relative risks were computed with use of the asymptotic standard error estimates.

The interaction of treatment group with type of surgery (abdominal versus non-abdominal) was included in a logistic regression model with the main effects of group and surgery type, and found to be non-significant (p=0.41). When considered separately as a subgroup analysis (Table 6.3), the rates of infection after abdominal surgery were 12.5% in the chlorhexidine-alcohol group versus 20.5% in the povidone-iodine group, with a 95% CI for the difference (chlorhexidine-alcohol minus povidone-iodine) of -13.9% to -2.1%. For patients undergoing non-abdominal surgery the rates of infection were 1.8% in the chlorhexidine-

Table 6.3: The rates of infection with type of surgery

Type of surgery	Chlorhexidine-alcohol			Povidone-iodine		
	N	# infected	(%) infected	*N*	# infected	(%) infected
Abdominal	297	37	(12.5)	308	63	(20.5)
Colorectal	186	28	(15.1)	191	42	(22.0)
Biliary	44	2	(4.6)	54	5	(9.3)
Small Intestinal	41	4	(9.8)	34	10	(29.4)
Gastroesophageal	26	3	(11.5)	29	6	(20.7)
Non-Abdominal	112	2	(1.8)	132	8	(6.1)
Thoracic	44	2	(4.6)	57	4	(7.0)
Gynecologic	42	0	(0.0)	40	1	(2.5)
Urologic	14	0	(0.0)	22	3	(13.6)

alcohol group versus 6.1% in the povidone-iodine group, with a 95% CI for the difference of -7.9% to 2.6%.

Both the intention-to-treat and per-protocol analyses demonstrated lower rates of surgical site infection in the chlorhexidine-alcohol group than in the povidone-iodine group for each of the seven types of studied operations. Although the trial was not powered to compare the rates of infection for subcategories of patients, infection occurred significantly less in the chlorhexidine-alcohol group than in the povidone-iodine group when performing intention-to-treat analysis of patients who underwent small intestinal surgery ($p = 0.04$), had abdominal surgery ($p = 0.009$) or did not shower preoperatively ($p = 0.02$).

The Breslow-Day tests indicated homogeneity by showing no significant differences between hospitals in the incidence of any type ($p = 0.35$) or individual types ($p \geq 0.19$) of surgical site infections. Even so, all logistic regression models accounted for hospital site by including this term as a random effect through the use of GEE.

Figure 6.2 show Kaplan-Meier estimates of the risk of surgical site infection and demonstrates a significantly longer time to infection after surgery in the chlorhexidine-alcohol versus povidone-iodine group ($p = 0.004$ by long-rank test).

6.2.3 Risk Factors

Univariate and multivariate logistic regression analysis identified the following risk factors for surgical site infection: use of chlorhexidine-alcohol, abdominal surgery, alcohol

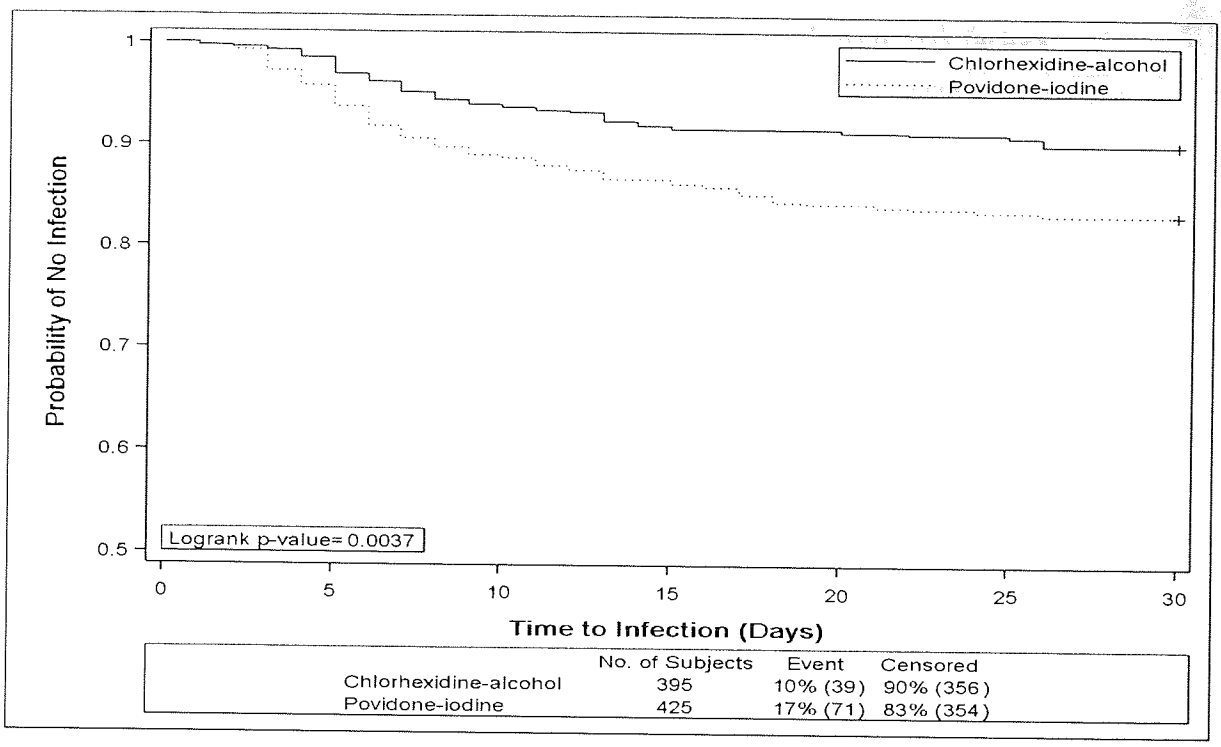


Figure 6.2: Kaplein Meyer for time to infection.

abuse, liver cirrhosis, cancer, diabetes mellitus, malnutrition, gastrointestinal disease, duration of surgery, duration of placement of surgical drain, and preoperative shower with povidone-iodine (Table 6.4). Since analysis of risk factors other than the assigned intervention is an exploratory analysis with multiple simultaneous statistical tests, it could inflate the probability of a false-positive finding (type I error).

6.2.4 Microbiology

Sixty of 61 infected cases that had surgical site cultures yielded growth of microorganisms (total of 107 isolates) and comparable proportions of infection cases in the two study groups (23/39 = 59% in the chlorhexidine-alcohol group and 37/71=52% in the povidone-iodine group) had an identifiable microbiological cause (Table 6.5). Gram-positive aerobic bacteria (63 isolates) outnumbered Gram-negative aerobic bacteria 925 isolates) by 2.5 fold and 38% of cultures were polymicrobial. There were no significant differences in the frequency of isolating certain microorganisms in the chlorhexidine-alcohol group (total of 44 isolates) versus the povidone-iodine group (total of 63 isolates), except for streptococci which were less common in the former group (1/44=3% versus 10/63=16%, $p = 0.03$).

Table 6.4: Univariate and multivariate analysis regression analysis of risk factors

	Univariate analyses			Multivariable analysis		
Factor	Odds ratio	95% CI	*p*-value[a]	Odds ratio	95% CI	Post exposure (CFU/mL)
Use of chlorhexidine-alcohol (vs. povidone iodine)	0.55	(0.36- 0.83)	0.004	0.383	(0.26-0.56)	<.0001
Abdominal surgery (vs. non-abdominal)	4.14	(2.12- 8.10)	<0.001	2.72	(1.20- 6.16)	0.02
Anastomotic leakage	14.37	(5.27- 39.15)	<0.001	17.64	(4.91- 63.29)	<0.001
ASA score of 4 (vs. 1)	14.14	(3.25- 61.53)	<0.001	5.79	(0.48- 69.96)	0.17
ASA score of 3 (vs.1)	5.26	(1.26- 22.03)	0.009	2.09	(0.24- 18.00)	0.50
ASA score of 2 (vs. 1)	2.77	(0.62- 12.42)	0.25	2.36	(0.35- 15.82)	0.38
Age (per year)	1.02	(1.01-1.0)	0.005	1.00	(0.98-1.01)	0.44
Female sex	0.59	(0.39-0.91)	0.02	0.90	(0.74-1.11)	0.33
Alcohol abuse	1.07	(0.64-1.76)	0.79	1.11	(1.04-1.18)	0.001
Liver cirrhosis	3.15	(1.25-7.91)	0.020	2.54	(1.69-3.82)	<0.001
Immunologic disease	2.61	(1.26-5.39)	0.02	1.53	(0.87-2.69)	0.14
Cancer	1.93	(1.23-3.02)	0.004	1.76	(1.03-3.00)	0.04
Diabetes mellitus	1.88	(1.15-3.09)	0.01	1.77	(1.03-3.03)	0.04
Malnutrition	2.90	(1.50-5.60)	0.003	2.89	(1.56-5.35)	<0.001
Gastrointestinal disease	2.83	(1.67-4.80)	<0.001	1. 31	(1.03-1.66)	0.03
Infection at another bodily site (vs. no infection)	0.89	(0.30-2.57)	1.000	0.84	(0.60-1.19)	0.33
Duration of surgery (per hour)	1.31	(1.16-1.47)	<0.001	1.09	(1.00-1.20)	0.05
Days that surgical drain was in place (per day)	1.03	(1.00-1.05)	0.03	1.04	(1.02-1.06)	<0.001
Preop shower with Chlorhexidine (vs. no shower)	1.50	(0.55-4.10)	0.39	1.07	(0.80 -1.44)	0.64
Preop shower with povidone-iodine (vs. no shower)	0.13	(0.02-0.95)	0.01	0.34	(0.30-0.40)	<0.001
Preop shower with soap (vs. no shower)	1.11	(0.66-1.86)	0.69	0.86	(0.68-1.09)	0.22

[a] *p*-values for univariate analyses were computed using Fisher's Exact tests for categorical variables and a logistic regression model adjusting for hospital site as a random effect (GEE method).

[b] *p*-values for the multivariable analysis were computed using a logistic regression model with all terms included, and adjusting for hospital site as a random effect (GEE method).

Table 6.5 lists only microorganisms that were isolated from >5% of cases of infection with documented microorganisms. Less frequently isolated organisms included, *Pseudomonas aeruginosa*, (3 cases), *Citrobacter* species (2 cases), *Morganella morganii* (1 case), *Proteus mirabilis* (1 case), *Aeromonas hydrophila* (1 case), *Peptostreptococcus* species (1 case), *Propionibacterium acne* (1 case), *Provetella melaninogenica* (1 case), and 5 unidentified Gram-negative aerobic bacilli.

The total of 107 isolates included 44 strains from the chlorhexidine-alcohol group vs. 63 strains from the povidone-iodine group and comprised 63 Gram-positive aerobic bacterial isolates (22 vs. 41), 25 Gram-negative aerobic bacterial isolates (13 vs. 12), 15 anaerobic bacterial isolates (9 vs. 6), and 4 fungal isolates (0 vs. 4). The 14 excess cases of infection in the povidone-iodine group were primarily accounted for by *S. aureus* and streptococci which caused 22 cases of superficial and deep incisional infection in this group vs. 7 in the chlorhexidine-alcohol group. The 60 cases of infection with identified microbiology occurred after 53 abdominal, 5 thoracic, 1 gynecologic, and 1 urologic surgeries. Ten of the 24 strains of *S. aureus* were meticillin-resistant and were proportionately distributed between the two study group (3 in the chlorhexidine-alcohol group and 7 in the povidone-iodine group. All isolates of *S. epidermidis* and Corynebacteria grew from polymicrobial cultures except in 1 case of infection each

6.2.5 Adverse Events

Equal proportions of patients in the chlorhexidine-alcohol and povidone iodine groups developed adverse events (228/409 = 57% versus 256/440 = 58.2%, respectively) or serious adverse events (72/409 = 17.6% versus 70/440 = 15.9%, respectively) in the intention-to-treat analysis (Table 6.6). Three patients (0.7%) in each study group developed an adverse event (pruritis and/or erythema around surgical wound) that was judged to be related to the study drugs. No serious adverse events were related to the study drugs. There were no cases of heat or chemical skin burn in the operating room. A total of 7 patients expired, including 4 (1%) without surgical site infection in the chlorhexidine-alcohol group and 3 (0.7%) who died from sepsis due to organ-space infection in the povidone-iodine group.

Table 6.5: Microbiology of infections with documented causative pathogens according to study group and type of surgical site infection

	No. of Isolates (n = 107)								
	Chlorhexidine-alcohol				Povidone-iodine				Both groups
Microorganism	Superficial incisional (12 cases)	Deep incisional (3 cases)	Organ-space (8 cases)	Any (23 cases)	Superficial incisional (19 cases)	Deep incisional (9 cases)	Organ-space (9 cases)	Any (37 cases)	Any (60 cases)
Gram-positive aerobic bacteria									
Staphylococcus aureus[a]	7	0	1	8	12	2	2	16	24
Staphylococcus epidermidis	4	1	0	5	4	2	1	7	12
Enterococci	3	1	2	6	2	2	2	6	12
Streptococci	0	0	1	1	5	3	2	10	11
Corynebacteria[b]	2	0	0	2	1	0	1	2	4
Gram-negative bacteria									
Escherichia coli	1	1	1	3	1	0	0	1	4
Klebsiella pneumoniae	1	0	1	2	1	1	0	2	4
Enterobacter species	3	2	2	7	1	2	2	5	12
Anaerobic bacteria									
Bacteroides species	3	2	2	7	1	2	2	5	12
Fungi									
Candida species	0	0	0	0	0	3	1	4	4

[a]Ten of the 24 strains of *S. aureus* were meticillin-resistant and were proportionately distributed between the two study group (3 in the chlorhexidine-alcohol group and 7 in the povidone-iodine group

Table 6.6: Adverse events recorded for each antiseptic patient study group

Clinical adverse event	Chlorhexidine-alcohol (N = 409) no.(%)	Povidone-iodine (N = 440) no.(%)	Absolute difference %	(95% CI)	p-value
Adverse events (>5% of patients in either study group)					
Patients with adverse events	228 (55.7)	256 (958.2)	-2.4	(-9.1 to 4.2)	0.49
Patients with drug-related adverse events	3 (0.7)	3 (0.7)	0.1	(-1.1 to 1.2)	>0.99
Serious adverse events (≥1% of patients in either study groups)					
Patients with serious adverse events	71 (17.6)	70 (15.9)	1.7	(-3.3 to 6.7)	0.52
Patients with serious drug-related adverse events	0	0	–	–	–
Death	4 (1)	3 (0.7)	0.3	(-0.9 to 1.5)	0.72

6.3 Discussion

Randomized studies have compared the efficacy of different types (Arnaud, *et al.*, 1992, Milsom, *et al.*, 1998, Itani, *et al.*, 2006, Ishizaka, *et al.*, 2007) or dosing (Fujita, *et al.*, 2007, Mohri, *et al.*, 2007) of systemic antibiotics in preventing surgical site infection, but not the impact of preoperative skin antisepsis. In this large randomized study, application of chlorhexidine-alcohol reduced the risk of surgical site infection by 41% as compared with the most common practice in the US of using aqueous povidone-iodine (Napolitano, 2006). This degree of protection is comparable to the 49% reduction in the risk of vascular catheter-related bloodstream infection in a meta-analysis that demonstrated the superiority of skin disinfection with chlorhexidine-based solutions versus 10% povidone-iodine (Chaiyakunapruk, *et al.*, 2002). Although the overall rates of surgical site infection of 10-16% in this study are higher than in some reports (Uchiyama, *et al.*, 2007, Grief, *et al.*, 2000), they are comparable to the pre-study rates at the participating hospitals and those reported in other studies (Arnaud *et al.*,

1992) and are lower than reported rates in trials that instituted the CDC definition of infection and adequate follow-up (Milsom, *et al.*, 1998, Smith, *et al.*, 2004, Itani, *et al.*, 2006) as in this trial. Based on the findings, the estimated number of patients needed to receive chlorhexidine-alcohol instead of povidone-iodine skin preparation to prevent one case of surgical site infection is approximately seventeen.

Although both studied antiseptic preparations possess broad-spectrum antimicrobial activity (Mangram, *et al.*, 1999) the superior clinical protection provided by chlorhexidine-alcohol is likely to be attributed to its more rapid action, persistent activity despite exposure to bodily fluids, and residual effect (Denton, 2001). The findings of superior clinical efficacy of chlorhexidine-alcohol correlate well with previous microbiological studies which demonstrated that chlorhexidine-based antiseptic preparations are more effective than iodine-containing solutions in reducing bacterial concentration in the operative field for vaginal hysterectomy (Culligan, *et al.*, 2005), foot and ankle (Bibbo, *et al.*, 2005, Ostrander, *et al.*, 2005) and shoulder surgery (Saltzman, *et al.*, 2009). Although the use of flammable alcohol-based products in the operating room raises the potential, though infrequent, risk for fire or chemical skin burn, no such adverse events occurred in this and other studies (Ostrander, *et al.*, 2005, Saltzman, *et al.*, 2009).

During this trial, standard of care preventive measures were enforced, such as administering systemic prophylactic antibiotics within one hour prior to incision, and if needed, clipping hair immediately prior to surgery (Mangram, *et al.*, 1999; Tanner, *et al.*, 2006). The hospitals were allowed to continue their preexisting practices that have a potential but non-established protective efficacy, such as patient preoperative showering (Webster and Osbourne, 2007). However, the impact was controlled by utilizing hospital-stratified randomization that ensured close matching of the two study groups while serving to apply the trial results to a wide sector of the population.

Topical antiseptics act only against microorganisms residing on the patient's integument, the overall superior protection afforded by chlorhexidine-alcohol was attributed primarily to reduction in the rates of superficial and deep incision infections that were mostly caused by Gram-positive skin microflora. Approximately two-thirds of surgical site infections are confined to the incision (Mangram, *et al.*, 1999, Itani, *et al.*, 2006) therefore optimizing skin antisepsis prior to the surgery could result in a significant clinical benefit. The findings of this study prompt evaluation of the economic impact of this approach.

CHAPTER 7: FINAL DISCUSSION

7.1 Introduction

Major initiatives worldwide have been undertaken by many healthcare organizations to identify contributors to healthcare-associated infections and to reduce risks to patients. Catheter-related bloodstream infection and SSI produce significant morbidity and mortality, as well as increasing the costs of healthcare. The most comprehensive information is found in the national and international guidelines for prevention of CRBSI and SSI. According to these guidelines, preoperative procedures can substantially reduce risks for developing CRBSI and SSI. The current international guidelines for the prevention of intravascular device infection have very specific recommendation for 2% chlorhexidine patient skin preparation for skin preparation prior to insertion and maintenance of care of central venous catheters. Currently there are no published prospective, randomised, controlled studies in the prevention of surgical site infection in respect to the surgeon's choice of patient preoperative skin preparation. Therefore the guidelines for SSI lack the recommendation for patient preoperative skin preparation.

7.2 *In-Vitro* and *In-Vivo* Efficacy of Chlorhexidine

Cutaneous antiseptics are also defined as substances that prevent or arrest the growth or action of microorganisms either by inhibiting their activity or by destroying them by means of a fast and, when compared with antibiotics, a more unspecific mode of action. The extent of eradicating a microorganism is governed by three principle factors: the concentration of the antiseptic, the bacterial (or fungal/viral) cell density (cell load), and the time of contact.

Many medical procedures are invasive to the natural protection of the integumentary system; when the integrity of this system is compromised the risk of infection increases. Therefore the reduction of resident and transient microorganisms with a topical cutaneous antiseptic is critical in infection prevention. The ideal antiseptic agent provides an immediate kill of transient and resident microorganisms that inhabit the superficial cell layers of the stratum corneum of the epidermis and provides a residual, or persistent, property that prevents bacterial regrowth. In addition, an antiseptic agent has to remain activity in the presence of organic matter.

Organic material in the healthcare environment is always present, such as, blood, body fluids, pus, or colloidal proteins and can challenge the efficacy of the antiseptics antimicrobial activity, either by absorption or chemical inactivation. Organic matter can reduce the initial

concentration of the antiseptic agent or it can act as a barrier to penetration of the antiseptic agent into the microorganism.

In Chapter 2 the research study compared the efficacy of 3 antiseptic agents, 2% CHG in 70% isopropyl alcohol, 2% CHG, and 10% povidone-iodine against *Staphylococcus epidermidis* (ATCC #12228) in the presence of 0.9% normal saline or blood. The 2% CHG in 70% IPA and 2% aqueous CHG solutions antimicrobial activity was not diminished in the presence of 0.9% normal saline or blood. The 10% povidone-iodine solution antimicrobial activity was not diminished in the presence of 0.9% normal saline, but was diminished in the presence of blood. Adams, *et. al.* (2006) compared the efficacy of 2% w/v chlorhexidine gluconate in 70% v/v isopropyl alcohol against five standard skin disinfectants. This *in-vitro* study concluded that compared to all three standards of preparation of chlorhexidine (eg. 2% aqueous chlorhexidine, 0.5% aqueous chlorhexidine, 0.5% chlorhexidine tincture) currently available in the UK market, the combination of 2% w/v chlorhexidine gluconate and 70% v/v isopropyl alcohol, demonstrated improved antimicrobial effect when challenged with *S. epidermidis* RP62A in a biofilm in the presence of 10% human serum. Future work should evaluate the antiseptic agents' efficacy in the presence of biofilm

In a recently published *in-vitro* evaluation, iodine povacrylex in alcohol and chlorhexidine and alcohol residual efficacy was evaluated following exposure to saline (Stahl and Parks 2007). The antiseptics were applied to the forearms of healthy human volunteers and allowed to dry. The prepped sites where then exposed to saline rinse or to a saline-soaked gauze. The test sites were then seeded with an indicator microorganisms, *Staphylococcus aureus* (ATCC 27217) at 10^8 (CFU)/mL. After 30 minutes, samples were collected from the test sites and surviving colonies were enumerated and log reductions were evaluated. Both antiseptic agents significantly reduced the density of the indicator microorganisms on the skin surface. A second analysis was performed with the saline-soaked gauze, where it was evaluated chemically for the presence of iodine or chlorhexidine. In this analysis, chlorhexidine was detected in the saline-soaked gauze, yet no saline-soaked gauze had detectable iodine. The authors concluded that the chlohrexideine was removed by saline-soaked gauze, while the iodine povacrylex water insoluble film remained intact (Stahl and Parks 2007).

Therefore, the research conducted in Chapter 3 was designed to mimic the conditions of a surgery in which skin preparations exposed to blood and the saline irrigation of the surgical site. Specifically, this study examined whether the ionic components of blood combined with the

irrigation (mechanical removal) of the saline rinse would affect the efficacy of 2% CHG in 70% IPA and PVP-I. The 2% CHG in 70% IPA continued to reduce the microorganisms after exposure to blood and saline, while that produced by the PVP-I did not. This study demonstrated that the level of chlorhexidine gluconate remaining bound to the skin following the first series of blood and saline rinses was significant, and that the majority of the iodine was either neutralised or removed.

The discrepancy between the two studies could be explained by the water-insoluble iodine povacrylex film. The saline-soaked gauze would not have been able to absorb or adsorb the iodine contained in the water-insoluble barrier. Both studies demonstrated that a saline rinse does not affect the antiseptic agents' efficacy, yet in the research studies conducted in Chapter 2 and 3 of this research project, both *in-vitro* and *in-vivo* exposure to blood serum has shown that chlorhexidine efficacy was not compromised. Future studies need to assess the water-insoluble iodine povacrylex product with blood serum. In addition, neutralisation protocols need to be addressed for each antiseptic agent that is compared. Neutralising ingredient(s) should be used in the sampling method, either on the swab, in the transport media, or the culture media. A neutraliser inactivates an antimicrobial at sampling so that there is no further kill in the test tube while the sample is waiting to be diluted and plated for quantification. If a neutraliser is not present, the antimicrobial agent will continue to kill bacteria in vitro resulting in an erroneous higher kill rate. Water-insoluble films are difficult to sample and neutralise, therefore validation studies need to be conducted with sampling technique and culture media.

7.3 Prevention of Surgical Site Infections with Chlorhexidine

Sub-optimal skin antisepsis is considered to be one of the primary sources of health-care-associated infections. Adequate skin cleansing and protection is essential to pre- and post-care of patients undergoing invasive medical procedures. The skin, which consists of the epidermis, dermis, and subcutaneous tissue, is vital for human survival.

Many studies have evaluated the difference in efficacy of the various cutaneous antiseptic solutions. Chlorhexidine gluconate has repeatedly been shown to be more effective than povidone-iodine or alcohol in the prevention of intravascular bloodstream infections. There are, however, few studies focusing specifically on preoperative antisepsis of patients before surgery. In foot and ankle surgery, patients' skin that was prepared with 2% chlorhexidine gluconate in 70% isopropyl alcohol demonstrated a significant reduction in microorganisms compared with that prepared with 0.7% iodine in 74% isopropyl alcohol or 3.0% chloroxylenol (Ostrander, *et*

al., 2005). In a similar study for elective foot and ankle surgery, chlorhexidine gluconate and alcohol paint preparations were found to be better than povidone-iodine (Bibbo, *et al*, 2005). In a coronary artery bypass graft surgery study, the skin for the harvest of the saphenous vein was prepared with either 2% chlorhexidine gluconate in 70% isopropyl alcohol or 0.5% chlorhexidine gluconate in 70% isopropyl alcohol. There was a trend for a greater reduction in the total number of organisms with 2% chlorhexidine gluconate in 70% isopropyl alcohol compared with 0.5% chlorhexidine gluconate in 70% isopropyl alcohol ($p = 0.07$). In the 2% chlorhexidine gluconate in 70% isopropyl alcohol group both the absorbent and the adhesive components of the dressings removed 24 hours post-surgery contained a significantly lower number of microorganisms than those of the 0.5% chlorhexidine gluconate in 70% isopropyl alcohol group ($p = 0.02$ and $p = 0.007$ respectively) (Casey, *et al*, 2008). There are no currently published randomized controlled studies that compare the impact of using one antiseptic preparation versus another on the incidence of surgical site infection. The main objective of this research study outlined in Chapter 5 was to compare the efficacy of disinfecting, preoperatively, the patient's skin with chlorhexidine-alcohol versus povidone-iodine in preventing surgical site infections. The results of the study, reported in Chapter 6, demonstrated the overall surgical site infection was significantly lower in the 2% chlorhexidine in 70% isopropyl alcohol group than in the povidone-iodine scrub and paint group (9.5% versus 16.1%, $p = 0.004$; relative risk, 0.59 with 95% confidence interval of 0.41 to 0.85). When comparing specific types of infection, 2% chlorhexidine in 70% isopropyl alcohol was significantly more protective than povidone-iodine scrub and paint against both superficial (4.2% versus 8.6%, $p = 0.008$) and deep (1% versus 3%, $p = 0.05$) incisional infections but not organ-space infections (4.4% versus 4.6%).

Topical antiseptics act only against microorganisms residing on the patient's integument, the overall superior protection afforded by 2% chlorhexidine in 70% isopropyl alcohol was attributed primarily to reduction in the rates of superficial and deep incision infections that were mostly caused by Gram-positive skin microflora. Approximately two-thirds of surgical site infections are confined to the incision (Mangram, *et al.*, 1999; Itani, *et al.*, 2006) therefore optimizing skin antisepsis prior to the surgery could result in a significant clinical benefit.

7.4 Conclusion

Patient preoperative skin preparation with 2% chlorhexidine in 70% isopropyl alcohol is superior to povidone-iodine scrub and paint in preventing surgical site infection after clean-contaminated surgery. The findings reported in Chapter 6 of this research study prompt evaluation of the economic impact of this approach. Additional studies should evaluate iodophor in alcoholic solution in comparison to chlorhexidine in alcohol solutions. Studies are warranted in clean surgery classifications, especially in orthopedic device-implant surgeries where SSI can be devastating to the patient and an economic burden on the healthcare system.

References

Adams D, Quayum M, Worthington T, Lambert P, Elliott T. Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant. *J Hosp Infect.* 2005;61:287-90.

Aggett, PJ, Cooper, LV, Ellis SH, McAinsh J. Percutaneous absorption of chlorhexidine in neonatal cord care. *Arch Dis Child.* 1981;56(11):878-80.

Andrews JM. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy.* 2001;48:5-16

Arnaud JP, Bellissant E, Boissel P, *et al.* Single-dose amoxycillin-clavulinic acid vs. Cefotetan for prophylaxis in elective colorectal surgery: a multicentr, prospective, randomized study. The PRODIGE Group. *J Hosp Infect.*1992;22(Suppl A):23-32.

Barrette, W. C., Jr., D. M. Hannum, W. D. Wheeler, and J. K. Hurst. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. *Biochemistry* 1989;28:9172–9178.

Bates DW, Goldman L, Lee TH. Contaminant blood cultures and resource utilization. The true consequences of false-positive results. *JAMA* 1991 Jan 16;265(3):365-9.

Bechgaard E, Ploug E, Hjorth N. Contact sensitivity to chlorhexidine? *Contact Dermatitis.* 1985;13(2):53-55.

Belda Fj, Aguilera L, Garcia AJ, *et al.* Supplemental perioperative oxygen and the risk of surgical wound infection: a randomized controlled trial. *JAMA.* 2005;294:2035-42.

Bibbo C, Patel DV, Gehrmann RM, Lin SS. Chlorhexidine provides superior skin decontamination in foot and ankle surgery: a prospective randomized study. *Clin Orthop Relat Res.* 2005;438:204-8.

Bloomfield S.F. Chlorine and iodine formulations. In: Ascenzi J.M., ed. *Handbook of disinfectants and antiseptics*, Marcel Dekker, Inc.New York, NY.1996: 133-158.

Blot F, Nitenberg G, Chachaty E, Raynard B, Germann N, Antoun S, *et al.* Diagnosis of catheter-related bacteraemia: a prospective comparison of the time to positivity of hub-blood versus peripheral-blood cultures. *Lancet.* 1999;354:1071-7.

Blot S.I., Depuydt P., Annemans L., *et al*: Clinical and economic outcomes in critically ill patients with nosocomial catheter-related bloodstream infections. *Clin Infect Dis.* 2005;41:1591-1598.

Boyce JM, Pittet D, Healthcare Infection Control Practices Advisory Committee; HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Guidelines for hand hygiene in health-care settings. *MMWR Recomm Rep.* 2002;51(RR-16):1-45.

Bratzler DW, Houck PM, for the Surgical Infection Prevention Guideline Writers Workgroup. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Am J Surgery* 2005;189:395-404.

Brun Buisson C, Rauss A, Legrand P. Semiquantitative culture of catheter tips. *J Clin Microbiol.* 1987 Jul;25(7):1343-4.

Calfee DP, Farr BM. Comparison of four antiseptic preparations for skin in the prevention of contamination of percutaneously drawn blood cultures: a randomized trial. *J Clin Microbiol.* 2002 May;40(5):1660-5.

Case, DE. 1977. "Percutaneous Absorption of Chlorhexidine Gluconate". Report on paper read at symposium entitled: Skin Antisepsis: Chlorhexidine Gluconate. University of California School of Medicine, San Francisco.

Case, DE, McAinsh J, Rushton A,Winrow MJ. 1980. Chlorhexidine: attempts to detect percutaneous absorption in man, *In*: Williams JD, Geddes AM, (eds.). *Chemotherapy*, New York:Plenum, Vol. 3, 367-74.

Chatzinikolaou I, Hanna H, Darouiche R, Samonis G, Tarrand J, Raad I. Prospective study of the value of quantitative culture of organisms from blood collected through central venous catheters in differentiating between contamination and bloodstream infection. *J Clin Microbiol.* 2006;44(5):1834-5.

Chaiyakunapruk N, Veenstra DL, Lipsky BA, Sullivan SD, Saint S. Vascular catheter site care: the clinical and economic benefits of chlorhexidine gluconate compared with povidone iodine. *Clin Infect Dis.* 2003;37(6):764-71.

Chawner, J. A., and P. Gilbert. A comparative study of the bactericidal and growth inhibitory activities of the bisbiguanides alexidine andchlorhexidine. *J. Appl. Bacteriol.* 1989;66:243–252.

Chawner, J. A., and P. Gilbert. Interaction of the bisbiguanides chlorhexidine and alexidine with phospholipid vesicles: evidence for separate modes of action. *J. Appl. Bacteriol.* 1989;66:253-8.

Chen YY, Chou YC, Chou P. Impact of Nosocomial infection on cost of illness and length of stay in intensive care units. *Infect Control Hosp Epidemiol.*2005;26:281-287.

Chopra, I., Johnson SC, Bennett PM. Inhibition of *Providencia stuartii* cell envelope enzymes by chlorhexidine. *J. Antimicrob. Chemother.* 1987;19:743–751.

Cleri DJ, Corrado ML, Seligman SJ. Quantitative culture of intravenous catheters and other intravascular inserts. *J Infect Dis.* 1980 Jun;141(6):781-6.

Collignon PJ. Intravascular catheter associated sepsis: a common problem. The Australian Study on Intravascular Catheter Associated Sepsis. *Med J Aust.* 1994 Sep 19;161(6):374-8.

Cowen J, Ellis S, McAinsh J. Absorption of chlorhexidine from intact skin of newborn infants. *Arch Dis Child.* 1979;54:379-383.

Crinch CJ, Maki DG. The promise of novel technology for the prevention of intrvascular device-related bloodstream infection 1. Pathogenesis and short- term devices. *Healthcare Epidemiology* 2002:34:1232-42.

Crosby CT, Elliott TSJ, Lambert PA, Adams D. Preoperative skin preparation: a historical perspective. *British J Hosp Med.* 2009;70(9):634-37.

Cruse PJE, Foord R. The epidemiology of wound infection: a 10-year prospective study of 62,939 wounds. *Surg Clin North Amer* 1980;60:27-40.

Culligan PJ, Kubik K, Murphy M, Blackwell L, Snyder J. A randomized trial that compared povidone iodine and chlorhexidine as antiseptics for vaginal hysterectomy. *Am J Obstet Gynecol.* 2005;192:422-5.

Culver DH, Horan TC, Gaynes RP, *et al.* Surgical wound infection rates by wound class, operative procedure, and patient risk index. *Am J Med* 1991; 91(suppl. 3B):152S-157S.

Darouiche RO, Raad II, Heard SO, Thornby JI, Wenker OC, Gabrielli A, Berg J, Khardori N, Hanna H, Hachem R, Harris RL, Mayhall G. A comparison of two antimicrobial-impregnated central venous catheters. Catheter Study Group. *N Engl J Med* 1999 Jan 7;340(1):1-8.

Davies A. The mode of action of chlorhexidine. *J Periodont Res* 1973: 8(suppl 12): 68-75.

Denton, G. Chlorhexidine: *In S. S. Block (ed.), Disinfection, sterilization and preservation, 5th ed.* 2001;321-336. Lea & Febiger, Philadelphia, Pa

Digiovine B, Chenoweth C, Watts C, Higgins M. The attributable mortality and costs of primary nosocomial bloodstreami infections in the intensive care unit. *Am J Respir Crit Care Med.* 1999 Sep;160(3):976-81.

Dimick JB, Pelz RK, Consunji R, Swoboda SM, Hendrix CW, Lipsett PA. Increased resource use associated with catheter-related bloodstream infection in the surgical intensive care unit. *Arch Surg.* 2001 Feb;136(2):229-34.

DoH/PHLS. *Hospital infection control. Guidance on the control of infection in hospitals.* Prepared by the Hospital Infection Working Group of the Department of Health and Public Health Laboratory Service, London, 1995.

DoH/PHLS. *Surveillance of hospital-acquired bacteraemia.* Prepared by the Nosocomial Infection National Surveillance Service and Public Health Laboratory Service, London, 2002.

Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: A three year analysis. *Clinical Infectious Disease.* 1999;29:239-44.

Edwards PS, Lipp A, Holmes A. Preoperative skin antiseptics for preventing surgical wound infections after clean surgery. *Cochrane Database Syst Rev 3.*2004;CD003949.

Elliott TSJ. Line-associated bacteramia. *Communicable Disease Report.* 1993;33(7):R91-R95.

Ellenhourn JDI, Smit DD, Schwarz RE, Kawachi MH, Wilson TG, McGonigle KF. Paint only is equivalent to scrub and paint in preoperative preparation of abdominal surgery sites. *J Am Coll Surg. 2005;201:737-741.*

Elliott TSJ, Moss HA, Tebbs SE, Wilson IC, Bonser RS, Graham TR, Burke LP, Faroqui MH. Novel approach to investigate a source of microbial contamination of central venous catheters. *Eur J Clin Microbiol Infect Dis.*1997;16(3):210-213.

Emori TG, Gaynes RP. An overview of Nosocomial infections, including the role of the microbiology laboratory. *Clinical Microbiology Review.* 1993 Oct;6(4):428-442.

European Committee for Standardization (1997). *Chemical disinfectants and antiseptics. Basic Bactericidal Activity: Test Methods and Requirements (phase 1).* European Standard EN 1040. Brussels: European Committee for Standardization.

European Committee for Standardization (1997). *Chemical disinfectants and antiseptics. Basic Fungicidal Activity: Test Methods and Requirements (phase 1).* European Standard EN 1275. Brussels: European Committee for Standardization.

Evans RJ: Acute anaphylaxis due to topical chlorhexidine acetate. *Br Med J* 1992;304:686.

Fabry J. Surveillance of hospital-acquired infections. Comparison of methods. The methodology. The International Consultation on Prevention and Control of Healthcare-Associated Infections; Venice, Italy; April 26–28, 2004.

Friis-Møller A, Brynitz S, Jess P. Toxicity of chlorhexidine irrigation in chronic postpneumonectomy empyema. *Scand J thor cardiovasc Surg.* 1984;18:243-244.

Fry DE. The surgical infection prevention project: processes, outcomes, and future impact. *Surg Infec.* 2006;7(S3): S17–S26.

Fujita S, Saito N, Yamada T, *et al.* Randomized, multicenter trial of antibiotic prophylaxis in elective colorectal surgery: singe dose vs 3 doses of a second-generation cephalosporin without metronidazole and oral antibiotics. *Arch Surg.* 2007;142:657-61.

Ganter B. Regional strategy on healthcare associated infections. The International Consultation on Prevention and Control of Healthcare-Associated Infections; Venice, Italy; April 26–28, 2004.

Garibaldi RA, Cushing D, Lerer T. Risk factors for postoperative infection. *Am J Med.* 1991S158-S163.

Garland JS, Buck RK, Maloney P, *et al.* Comparison of 10% povidone-iodine and 0.5% chlorhexidine gluconate for the prevention of peripheral intravenous catheter colonization in neonates: a prospective trial. *Pediatr Infect Dis J.* 1995;14(6):510-516.

Garland JS. Local reactions to a chlorhexidine gluconate-impregnated antimicrobial dressing in very low birth weight infants. *Pediatr Infect Dis J.* 1996;15(10):912-914.

Gaynes R, Richards C, Edwards J, Emori G, Horan T, Echanove JA, Fridkin S, Lawton R, Peavy G, Tolson J, and the National Infections Surveillance (NNIS) System Hosptials. *Emerging Infectious Diseases.*2001;7(2):295-298.

Gongwer, L.E., K.Hubben, R.S.Lenkiewicz, E.R.Hart B.Y.Cockrell, 1980. The effects of daily bathing of neonatal rhesus monkeys with an antimicrobial skin cleanser containing chlorhexidine gluconate. *Toxicol Appl Pharmacol.* 52(2), pp.255-261.

Green JW, Wenzel RP. Postoperative wound infections: a controlled study of the increased duration of hospital stay and direct cost of hospitalization. *Ann Surg* 1977;185:264-268.

Grief R, Akca O, Horn EP, Kurz A, Sessler DI. Supplemental perioperative oxygen to reduce the incidence of surgical-wound infection. Outcomes Research Group. *N Eng J Med.* 2000;342:161-7.

Haley RW, Culver DG, Morgan WM, *et al.* Identifying patients at high risk of surgical wound infection; a simple multivariate index of patient susceptibility and wound contamination. *Am J Epidemiol* 1985;121:206-215.

Harold, F. M., J. R. Baarda, C. Baron, and A. Abrams. 1969. Dio 9 and chlorhexidine. Inhibition of membrane bound ATPase and of cation transport in *Streptococcus faecalis. Biochem. Biophys. Acta* 183:129–136.

Hawn MT, Itani Km, Gray SH, Vick CC, Henderson W, Houston TK. Association of timely administration of prophylactic antibiotics for major surgical procedudres and surgical site infection. *J Am Coll Surg.* 2008;206:814-9.

Horan TC, Gayness RP, Martone WJ, *et al.* CDC definitions of surgical site infections, 1992. A modification of CDCP definitions of surgical wound infections. *Infect Control Hosp Epidemiol* 1992;13:606-608

Hugo, W.B. and Longworth, A.R., Some aspects of the mode of action of chlorhexidine. *J. Pharm. Pharmacol.,* 16 (1964) 655-662.

Hugo, W. B. Disinfection mechanisms. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed.1999. Blackwell Science, Oxford, England.

Hugonnet S, Sax H, Eggimann P, Chevrolet JC, Pittet D. Nosocomial bloodstream infection and clinical sepsis. *Emerg Infect Dis.* 2004 Jan;10(1):76-81.

Humar A, Ostromecki A, Direnfeld J, Marshall JC, Lazar N, Houston PC, Boiteau P, Conly JM. Prospective Randomized Trial of 10% Povidone-Iodine versus 0.5% tincture of Chlorhexidine as Cutaneous Antisepsis for Prevention of Central Venous Catheter Infection. *Clin Infect Dis* 2000. Oct;31(4):1001-1007.

Ishizaka K, Kobayashi S, Machida T, Yoshida K. Randomized prospective comparison of fosfomycin and cefotiam for prevention of postoperative infection following urological surgery. *J Infect Chemother.* 2007;13:324-331.

Itani KMF, Wilson Se, Awad SS, *et al.* Ertapenem versus cefotetan prophylaxis in elective colorectal surgery. *N Engl J Med.* 2006;355:2640-51.

Jeske C, Raedler C, von Goedecke A, Mayr A, Hinterberger G, Aspoeck C, Lass-Floerl C, Benzer A. Early identification of bacteria leading to central venous catheter contamination. *Anesth Analg.*2003;97:940-3.

Johnsson J, Seeberg S, Kjelimer I. Short Communication. Blood concentrations of chlorhexidine in neonates undergoing routine cord care with 4% chlorhexidine gluconate solution. *Acta Pediatr Scand.* 1987;76:675-676.

Jones CG. Chlorhexidine: is it still the gold standard? *Periodontology 2000.* 1997;15:55-62.

Kelly JW, Usry G, Blackhurst D, Bushey M, Steed C. Prevention of Infections Related to Central Venous Catheters and Arterial Catheters in Intensive Care Patients: a Prospective Randomized Trial of Chlorhexidine Gluconate (CHG) versus Povidone Iodine (PI). Presented at the 15th Annual Scientific Meeting of the Society for Healthcare Epidemiology of America, April 9-12, 2005, Los Angeles, CA. Abstract No. 165.

Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: Attributable mortality, excess length of hospitalization, and extra costs. *Infect Control Hosp Epidemiol.* 1999;20:725-730

Klenerman L. The Evolution of Orthopaedic Surgery. 2002. RSM Press, London

Kluger DM, Maki DG. A meta-analysis of the risk of intravascular device-related bloodstream infection based on 223 published prospective studies. *Infect Control Hosp Epidemiol.* 2000;2:95-99.

Kluytmans J. Surgical infections including burns. *In*: Wenzel RP, ed. Prevention and Control of Nosocomial Infections. 3rd ed. Williams & Wilkins, Baltimore, MD, 1997:841-865.

Knudsen B B, Avnstorp C. Chlorhexidine gluconate and acetate in patch testing. *Contact Dermatitis* 1991: 24: 45–49.

Kohn LT, Corrigan JM, Donaldson MS. To err is human: building a safer health system. Committee on Quality of Health Care in America. Institute of Medicine. 2000; full pdf available at http://www.nap.edu/catalog/9728.html

Kurz A, Sessler DI, Lenhardt R. Perioperative normothermia to reduce the incidence of surgical wound-infection and shorten hospitalizaiton. Study of Wound Infection and Temperature Group. *N Eng J Med.* 1996;334:1209-15.

Lacour JP, Castanet J, Boutte P, Ortonne JP. Antisepsie du cordon ombilical du nouveau-ne : enquete et recommandations. *Arch Pediatr.* 1999 ;6 :631-634.

Lasthein Andersen B, Branderup F. Contact dermititis from chlorhexidine. *Contact Dermatitis.* 1985 :13(5) ;307-309.

Leape LL, Brennan TA, Laird N, Lawthers AG, Localio AR, Barnes BA, Hebert L, Newhouse JP, Weiler PC, and Hiatt H. The nature of adverse events in hospitalized patiens. *N Eng J Med.* 1991;324:377-384.

Leaper DJ, van Goor H, Reilly J, Petrosillo N, Geiss HK, Torres AJ, Berger A. Surgical site infection - a European perspective of incidence and economic burden. *Int Wound J* 2004;1:247-273

Livesley MA, Tebbs SE, Moss HA, Farouqui MH, Lambert PA, Elliott TSJ. Use of pulsed field gel electrophoresis to determine the source of microbial contamination of central venous catheters. *Eur J Clin Microbiol Infect Dis.* 1998;17:108-112.

Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med.* 1977 Jun 9;296(23):1305-9.

Maki DG. Skin as a source of nosocomial infection: directions for future research. *Infection Control.* 1986;7(2):113-16.

Maki DG, Ringer M, Alvarado CJ. Prospective randomised trial of povidone-iodine, alcohol, and chlorhexidine for prevention of infection associated with central venous and arterial catheters. *Lancet.* 1991 Aug 10;338(8763):339-43.

Maki DG. Infections caused by intravascular devices used for infusion therapy: pathogenesis, prevention, and management. *Infections associated with indwelling medical devices. 2nd ed.* Edited by Alan L. Bisno and Francis A. Waldvogel. 1994: 155-212.

Maki DG, Stolz SM, Wheeler S, Mermel LA. Prevention of Central Venous Catheter-Related Bloodstream Infection by Use of an Antiseptic-Impregnated Catheter. *Ann Intern Med.* 1997 Aug;127(4);257-266.

Maki DG, Knasinki V, Larans LL, *et al.* A randomized trial of a novel 1% chlorhexidine-75% alcohol tincture vs. 10% povidone-iodine for cutaneous disinfection with vascular catheters. Abstract #142. The 11th Annual Scientific Meeting of the Society for Healthcare Epidemiology of America, Toronto, Canada, 2001.

Maki DG, Kluger DM, Crnich CJ. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. *Mayo Clin Proc.* 2006 Sep;81(9):1159-71.

Mangram AJ, Horan TC, Pearson ML, Sliver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. *Infect Control Hosp Epidemiology.* 1999;20(4):247-78.

Martindale: The complete drug reference disinfectants and preservatives: 34th Ed. London: The Pharmaceutical Press; 2004. Chlorhexidine; pp. 1173-5.

McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clinical Microbiology Reviews.* 1999;12(1):147-179.

McKibben L, Horan T, Tokars J, Fowler G, Cardo D, Pearson M, Brennan P. Guidance on Public Reporting of Healthcare-Associated Infections: Recommendations of the Healthcare Infection Control Practices Advisory Committee. *Am J Infect Control.* 2005;33(4):217-226.

Mermel LA. Prevention of intravascular catheter-related infections. *Ann Intern Med.* 2000 Mar 7;132(5):391-402. Erratum in: *Ann Intern Med* 2000 Sep 5;133(5):5.

Meffre C, Girard R, Hajjar J, Fabry J. Povidone-Iodine vs. Alcoholic Chlorhexidine for Disinfection of the Insertion Site of Peripheral Venous Catheters: Results of a Multicenter Randomized Trial. (Abstract) In: Program and Abstracts of the Society for Healthcare Epidemiology Association. In: Program and Abstracts of the 6th Society for Healthcare Epidemiology of America, April 1996: Abstract 64.

Milsom JW, Smith DI, Corman ML, Howerton RA, Yellin AE, Luke DR. Double-blind comparison of single-dose alatrofloxacin and cefotetan as prophylaxis of infection following elective colorectal surgery. Trovalfloxacin Surgical Group. *Am J Surg.* 1998;176(6A Suppl):46S-52S.

Mimoz O, Pieroni L, Lawrence C, Edouard A, Costa Y, Samii, Brun-Buisson C. Prospective, randomized trial of two antiseptic solutions for prevention of central venous or arterial catheter colonization and infection in intensive care unit patients. *Crit Care Med.* 1996. 24(11):1818-1823.

Mimoz O, Karim A, Mercat A, Cosseron M, Falissard B, Parker F, Richard C, Samii K, Nordmann P. Chlorhexidine compared with povidone-iodine as skin preparation before blood culture. A randomized, controlled trial. *Ann Intern Med.* 1999 Dec 7;131(11):834-837.

Mohri Y, Tonouchi H, Kobayashi M, *et al.* Randomized clinical trial fo single-versus mutliple-dose antimicrobial prophylaxis in gastric cancer surgery. *Br J Surg.* 2007;94:683-8.

Napolitano LM. Decolonization of the skin of the patient and surgeon. *Surg Infect.* 2006;7(supple 3):S3-S15,

National Nosocomial Infections Surveillance System. National nosocomial infections surveillance (NNIS) report, data summary from October 1986-April 1997, issued May 1997. A report from the NNIS System. *Am J Infect Control* 1997;25:477-87.

National Collaborating Center for Women's and Children's Health. Surgical Site Infection. NICE Guideline CG74. National Institute for Health and Clinical Excellence. 2008. London

Nickol KH. Reaction to chlorhexidine and cetrimide. *Lancet.* 1965. pg. 821.

O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG, Masur H, McCormick RD, Mermel LA, Pearson ML, Raad II, Randolph A, Weinstein RA. Guidelines for the prevention of intravascular catheter-related infections. *MMWR Recomm Rep.* 2002;51(RR-10):1-29.

Olson MM, Lee JT Jr. Continuous 10-year wound infection surveillance. Results, advantages and unanswered questions. *Arch Surg* 1990;125:794-803.

Omundsen, PE. 1982. Contact dermatitis to chlorhexidine. *Contact Dermatitis.* 8:81-83.

Oppenheim BA. Optimal management of central venous catheter-related infections--what is the evidence? *J Infect.* 2000 Jan;40(1):26-30.

Orsi G.B., Raponi M., Franchi C., Rocco M., Mancini C., Venditti M.: Surveillance and infection control in an intensive care unit. *Infect Control Hosp Epidemiol* 26. 321-325.2005

Ostrander RV, Botte MJ, Brage ME. Efficacy of surgical preparation solutions in foot and ankle surgery. *J Bone Joint Surg.*2005;87:980-5.

O'Toole GA, Kolter R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol Microbiol.* 1998;28:449-461.

Pittet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients. Excess length of stay, extra costs, and attributable mortality. *JAMA.* 1994 May 25;271(20):1598-601.

Pittet D, Hulliger S, Auckenthaler R. Intravascular device-related infections in critically ill patients. *J Chemother.* 1995 Jul;7 Suppl 3:55-66.

Pittet D, Allegranzi B, Sax H, Bertinato L, Concia E, Cookson B, Fabry J, Richet H, Philip P, Spencer RC, Ganter BWK, Lazzari S. Considerations for a WHO European strategy on health-care-associated infection, surveillance, and control. *Lancet.* 2005;5:242-50.

Plowman R, Graves N, Griffin MAS, Roberts JA, Swan AV, Cookson B, Taylor L. The rate and cost of hospital-acquired infections occurring in patients admitted to selected specialties of a district general hospital in England and the natiional burden imposed. *J Hosp Infect.* 2001.41:198-209.

Raad I. Intravascular-catheter-related infections. *Lancet.* 1998 Mar;351:893-98.

Raad I. Differential time to positivity: a useful method for diagnosing catheter-related bloodstream infections. *Ann Intern Med.* 2004;140(1):18-25.

Rello J, Ochagavia A, Sabanes E, Roque M, Mariscal D, Reynaga E, Valles J. Evaluation of outcome of intravenous catheter-related infections in critically ill patients. *Am J Respir Crit Care Med.* 2000 Sep;162:1027-30.

Renaud B, Brun-Buisson C. Outcomes of primary and catheter-related bacteremia. *Am. J. Respir. Crit. Care Med.* 2001;163(7):1584-1590

Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infect Control Hosp Epidemiol.* 2000;21:510-515.

Russell AD. Chlorhexidine: antibacterial action and bacterial resistance. *Infection* 1986: 14: 212-215.

Russell, A.D. (1990) The effects of chemical and physical agents on microbes disinfection and sterilization. In *Topley and Wiison's Principles of Bacteriology, Viro/ogy and Immunity,* 8th ed, Vol. 1, ed. Linson, A.H. and Dick, H.M. pp. 72-103. London: Edward Arnold.

Russell AD, Day MJ. Antibacterial activity of chlorhexidine. *J Hosp Infect.* 1993;25:229-238.

Russell AD (1996) Activity of biocides against mycobacteria. *J Appl Microbiol* Symposium Supplement 81, 87S-107S.

Russell, AD. Plasmids and bacterial resistance to biocides. *J. Appl. Microbiol.* 1997;82:155–165.

Russell AD, Suller MTE and Maillard J-Y. Do antiseptics and disinfectants select for antibiotic resistance? *J Med Microbiol.*1999;48:613-615.

Russell AD, Hugo WB and Ayliffe GAJ (1999b) Bacterial sensitivity and resistance. In: *Principles and practice of disinfection, preservation and sterilization.* 3rd ed. Oxford: Blackwell Science.

Russell AD. Bacterial resistance to disinfectants: present knowledsge and future problems.*Journal of hospital infection.*1999;43 (*supplement*) S57-S58.

Russell AD. Do biocides select for antibiotic-resistant bacteria? *J Pharm and Pharmacol.* 2000;52: 227-233

Russell AD. Mechanisms of antimicrobial action of antiseptics and disinfectants: an increasingly important area of investigation. *J Antimicrobial Chemo.* 2002;49:597-599.

Sakuragi T, Yanagisawa K, Dan K. Bactericidal activity of skin disinfectants on methicillin-resistant *Staphylococcus aureus*. *Anesth and Analg.* 1995;81:555-558.

Shaker LA, Russell AD, Furr JR. Aspects of the action of chlorhexidine on bacterial spores. *Int. J. Pharm.* 1986;34:51–56.

Seifert H, Cornely O, Seggewiss K, Decker M, Stefanik D, Wisplinghoff H, *et al.* Bloodstream infection in neutropenic cancer patients related to shortterm nontunnelled catheters determined by quantitative blood cultures, differential time to positivity, and molecular epidemiological typing with pulsed-field gel electrophoresis. *J Clin Microbiol.* 2003;41:118-23.

Sheehan G, Leicht K, O'Brien M, *et al.* Chlorhexidine versus povidone-iodine as cutaneous antisepsis for prevention of vascular-catheter infection. In: Program and Abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, 17-20 October 1993, New Orleans. Washington, DC, American Society for Microbiology, 1993: Abstract 1616.

Smith JMB. Laboratory evaluation of antimicrobial agents. *Hugo and Russell's Pharmaceutical Microbiology, 7th Edition.* Edited by Stephen P Denyer, Norman A Hodges, Sean P Gorman. 2004 Blackwell Science Ltd.

Smith RL, Bohl JK, McElearney ST, *et al.* Wound infection after elective colorectal resection. *Ann Surg.* 2004;239:599-605.

Smith SM, Eng RHK, Lopez AB, Valena FD. Reducing Blood Culture Contamination: What Makes the Difference? *Am J Infect Control.* 2006;34:E78-E79.

Soufir L, Timsit JF, Mahe C, Carlet J, Regnier B, Chevret S. Attributable morbidity and mortality of catheter-related septicemia in critically ill patients: a matched, risk-adjusted, cohort study. *Infect Control Hosp Epidemiol.* 1999 Jun;20(6):396-401.

Stickler DJ, Morris NS, McLean RJC, Fuqua C. Biofilms on indwelling urethral catheters produce quorum-sensing signal molecules in situ and in vitro. Appl Environ Microbiol 1998;64:3486-90.

Tanner J, Woodings D, Moncaster K. Preoperative hair removal to reduce surgical site infection. Cochrane Database Syst Rev. 2006;2:CD004122.

Taylor DM. Inactivation of unconventional agents of the transmissible degenerative encephalopathies. In: Russell AD, Hugo WB, Ayliffe GAJ (eds). Principles and Practice of Disinfection, Preservation and Sterilization, Third Edition. Oxford, England: Blackwell Science, 1999.

TODAR, K. 2006. Todar's Online Textbook of Bacteriology [Accessed 18-09-2007]. 2006 Kenneth Todar University of Wisconsin-Madison Department of Bacteriology. www.textbookofbacteriology.net

Uchiyama K, Takifuji K, Tani M, *et al.* Prevention of postoperative infections by administration of antimicrobial agents immediately before surgery for patients with gastrointestinal cancers. *Hepatogastroenterology.* 2007;54:1487-93.

Viljanto J. Disinfection of surgical wounds without inhibition of normal wound healing. *Arch Surg.*2006;115:253-256.

Webster J, Osbourne S. Preoperative bathing or showering with skin antiseptics to prevent surgical site infection. *Cochrane Database Syst Rev.* 2007:2:CD004985.

Wester, RC and Maibach, HI. Percutaneous absorption in the rhesus monkey compared to man. *Toxicol. Appl. Pharmacol.* 1975;32:394-398

Weinstein RA. Nosocomial Infection Update. *Emerging Infectious Diseases.* 1998;4(3):416-20.

Wenzel RP, Edmond MB. The impact of hospital-acquired bloodstream infections. *Emerging Infectious Diseases.* 2001;7(2):174-177.

Wenzel RP, Pfaller MA. Feasible and desirable future targets for reducing the costs of hospital infections. *J Hosp Infect* 1991; 18(Suppl A):94 - 98

Wenzel RP. Preoperative antibiotic prophylaxis. N Engl J Med 1991;326:337-339

Wester RC, Maibach HI. Relationship of topical dose and percutaneous absorption on rhesus monkey and man. *J Invest Dermatol.*1976;67:518-520.

Wolf JS Jr, Bennett CJ, Dmochowski RR, Hollenbeck BK, Pearle MS, Schaeffer AJ. Urologic surgery antimicrobial prophylaxis best practice policy panel. Best practice policy statements on urologic surgery antimicrobial prophylaxix. *J Urol.* 2008;179:1379-90.

Worthington T, Elliott TSJ. Diagnosis of central venous catheter related infection in adult patients. *J Infection.* 2005;51:267-280.

Yasunaga H, Ide H, Imamura T, Ohe K. Accuracy of economic studies on surgical site infection. *J Hosp Infect.* 2007;65:102-7.

Appendix A: Surgical Site Infection Study - Enrollment Form

Patient's Initials: _ Name of Hospital: _____

Patient's Hospital Number:_____Date of Procedure:_____

Section A: To be completed by surgeon obtaining consent

Inclusion Criteria

☐ Patient is 18 years of age or older.

☐ Surgical procedure is to take place in an operative suite.

☐ Planned surgical procedure will be performed on alimentary or respiratory tract

Exclusion Criteria

☐ Patient has preexisting infection in the area of planned surgery

☐ Patient has an allergy to chlorhexidine, alcohol or iodophor

☐ Patient had a preoperative shower

Section B: To be completed by operating surgeon

Patient's Preoperative Physical Status
ASA Score

Circle one of the following:

1. Normally Healthy
2. Patient with mild systemic disease
3. Patient with severe systemic disease that is not incapacitating
4. Patient with an incapacitating systemic disease that is threat to life
5. Moribund patient who is not expected to survive for 24 hours with or without operation

Surgical Procedure Information

Surgical scrub performed by: Surgeon ☐ OR Nurse ☐
If randomized to Betadine, note name of preparation: _____

If randomized to Chloraprep, please note the number of applicators used: _____

Was Ioban placed over surgical site: Yes ☐ No ☐

Primary Surgeon: Faculty ☐ Fellow ☐ Resident ☐

Name of surgical procedure:_____ Duration of surgery:_____

Contaminated or dirty/infection status: Yes ☐ No ☐

Placement of drain or other foreign body: Yes ☐ No ☐

Signature of Surgeon:_____ Date:_____

Appendix B: Surgical Site Infection Study - Medical History

Patient's Initials: _____ Randomization Number: _____

☐ Male ☐ Female Height_____ Weight_____

Date of Birth:_____

Underlying Disease:

☐ Cardiopulmonary ☐ Cancer:_____
☐ Neurologic ☐ Other: _____

Risk Factors for Infection:

☐ Diabetes Mellitus ☐ Smoking
☐ Immunosuppressive Therapy (within 1 year) ☐ Malnutrition
☐ MRSA nasal carriage ☐ Infection at another site (within 1 year)
☐ Splenectomy ☐ Mechanical ventilation

Information on Hospitalization

Date of admission to hospital:_____
Location prior to surgery:
☐ Outpatient clinic ☐ Inpatient ward ☐ SICU ☐ MICU

Information on Surgery

Drains placed during surgery: Number:_____ Duration in days_____
Preoperative antibiotic therapy (within previous 7 days):
Name of antibiotic _____ Duration in days_____
Name of antibiotic _____ Duration in days_____
Name of antibiotic _____ Duration in days_____
Name of antibiotic _____ Duration in days_____

Surgical Wound Closed at Surgery: ☐ Yes ☐ No
Wound Care Instructions:_____

Appendix C: Surgical Site Infection Study - Investigator Evaluation Form

Patient's Initials: _____ Randomization Number: _____

Hospital Name: _____ Date: _____

This evaluation form is to be filled taking into consideration **only** episodes of infection involving the surgical site that occurred within 30-days after the first surgery and before any subsequent surgeries involving the same site.

1. Did the patient develop a superficial incisional wound infection?
   (Infection involving only the skin and subcutaneous tissue)

   Yes ☐ No ☐

2. Did the patient develop a deep incisional wound infection?
   (Infection involving fascia and muscle)

   Yes ☐ No ☐

3. Did the patient develop an organ/space infection?
   (Infection involving any part of the anatomy other than incised body wall layers)

   Yes ☐ No ☐

4. Did the patient develop sepsis from the surgical site infection?
   (Positive blood culture with clinical manifestation of sepsis)

   Yes ☐ No ☐

5. Did the patient develop sepsis with organ failure from the surgical site infection?

   Yes ☐ No ☐

Investigator's Name: _____
Investigator's Signature: _____ Date: _____

Appendix D: Surgical Site Infection Study - Medication Review

Patient's Initials: _____ Randomization Number: _____

Did the patient receive any antibiotics, immunosuppressive agents or blood products during the 30-day period post surgery?

If Yes, please list each drug below:

Drug Name	Dose	Start Date	Stop Date

Appendix E: Surgical Site Infection Study - Evidence of Infection Form

Patient's Initials: _____ Randomization Number: _____

Did the patient develop a postoperative surgical site infection? ☐Yes ☐No

If the patient did develop a surgical site infection, please complete the next two pages:

Type of Infection: (Circle one of the following)

1. Superficial Incisional
2. Deep Incisional
3. Organ/ Space
4. Surgical Site Infection with Sepsis
5. Surgical Site Infection with Sepsis and Organ Failure

Serum WBC at the time of infection:

Date	Count
_____	_____
_____	_____
_____	_____

Surgical Site Culture Results:

Date	Type of Culture	Organism	Quantity
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Was the same organism cultured from any other site? If so, please complete the following:

Date	Site	Organism	Quantity
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Did the patient have the same organism cultured from the blood? If so, please complete the following:

Date	Peripheral/ Catheter	Organism	Quantity
_____	_____	_____	_____
_____	_____	_____	_____

_____ _____ _____ _____
_____ _____ _____ _____

Did the patient have other manifestations of sepsis?(chills, hypotension, oliguria, etc)
Yes ☐ No ☐

If Yes, specify: _____

Is there radiologic evidence of a deep-seated infection?
If yes, please complete the following:

Date Procedure Findings

_____ _____ _____
_____
_____
_____ _____ _____
_____
_____

Was surgical site reopened? If yes, was there evidence of infection? If yes, please complete the following:

Date Procedure Findings

_____ _____ _____
_____
_____
_____ _____ _____
_____
_____

Appendix F: Surgical Site Infection Study - Adverse Event Form

Patient's Initials: _____ Randomization Number: _____

Did the patient experience any adverse events during the study? Yes ☐ No ☐

If Yes, please complete the following:

Adverse event	Start date	Stop date	Severity	Frequency	Outcome	Relationship	Action taken
SAE			__Mild __ Mod __ Severe	__ Single Episode __Intermittent __Continuous	__Recovered Completely __Recovered with Sequelae __Ongoing and Improved __Ongoing and Deteriorated __Death	__Unrelated __Unlikely Related __Possibly Related __Probably Related	__No Action Taken __New Non-drug Therapy Added __New Drug Therapy Added __New or Prolonged Hospitalization __Withdrawn from Study
☐ SAE			__Mild __ Mod __ Severe	__ Single Episode __Intermittent __Continuous	__Recovered Completely __Recovered with Sequelae __Ongoing and Improved __Ongoing and Deteriorated __Death	__Unrelated __Unlikely Related __Possibly Related __Probably Related	__No Action Taken __New Non-drug Therapy Added __New Drug Therapy Added __New or Prolonged Hospitalization __Withdrawn from Study
☐ SAE			__Mild __ Mod __ Severe	__ SingleEpisode __Intermittent __Continuous	__Recovered Completely __Recovered with Sequelae __Ongoing and Improved __Ongoing and Deteriorated __Death	__Unrelated __Unlikely Related __Possibly Related __Probably Related	__No Action Taken __New Non-drug Therapy Added __New Drug Therapy Added __New or Prolonged Hospitalization __Withdrawn from Study
☐ SAE			__Mild __ Mod __ Severe	__ Single Episode __Intermittent __Continuous	__Recovered Completely __Recovered with Sequelae __Ongoing and Improved __Ongoing and Deteriorated __Death	__Unrelated __Unlikely Related __Possibly Related __Probably Related	__No Action Taken __New Non-drug Therapy Added __New Drug Therapy Added __New or Prolonged Hospitalization __Withdrawn from Study

Investigator's Signature: _____

Appendix G: Recovery of Microorganisms from Skin with Cup Scrub Technique

Designation: ~~E 1874–97~~

Standard Test Method for
Recovery of Microorganisms From Skin using the Cup Scrub Technique[1]

This standard is issued under the fixed designation E 1874; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is designed to recover microorganisms from the skin of human subjects or human subject surrogates (animal skin, isolated porcine skin, human skin equivalents and other such surfaces).

1.2 Knowledge of microbiological techniques is required for these procedures.

1.3 In this test method, metric units are used for all applications.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.5 It is the responsibility of the investigator to determine if Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) are required.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *Scrub Cups, n*—Sterile cylinders of suitable composition (i.e., glass, ceramic, stainless steel, plastic, etc.) used to isolate a sample area of skin (or skin equivalent) and confine a aliquot of liquid which is used to facilitate the scrubbing of the skin and removal of microorganisms from the skin surface by pippetting.

3.1.2 *resident flora, n*—microorganisms that live and multiply on skin, forming a permanent population.

3.1.3 *transient organisms, n*—organisms from the environment that contaminate but do not normally colonize skin.

3.1.4 *contralateral, adj*—on or relating to the opposite side (of the body).

4. Summary of Test Method

4.1 This test method describes a technique suitable for the recovery of resident and transient microorganisms from human or animal skin; the technique may be used *in situ* within clinical protocols or *in vitro* for studies using isolated skin or skin equivalents.

4.1.1 Resident microorganisms or transient microorganisms (previously applied to a test site), are recovered from the site by pressing a rigid cylinder against the skin with sufficient pressure to form a seal and instilling recovery liquid into the cylinder. The surface of the skin is then mechanically 'scrubbed' with a glass rod, rubber policeman or some other suitable device for a prescribed period of time. The fluid is pipetted from the cylinder into a test tube, or other suitable receptacle, for further analysis.

---

[1]This tests method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved April 10, 1997. Published December 1997.

[2]For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

5. Significance and Use

5.1 The procedure can be incorporated into protocols used to evaluate test materials containing antibacterial ingredients that are intended to reduce significantly the number of organisms on intact skin. It also may be used to provide an indication of residual antibacterial activity.

5.2 Performance of this technique may require the knowledge of regulations pertaining to the protection of human subjects if the protocol involves application of the technique to the skin of human subjects.

6. Apparatus

6.1 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

7. Reagents and Materials

7.1 *Scrub Cups*—Sterile cylinders of suitable composition, preferably with rod handles to facilitate stabilization, height approximately 2.5 cm, inside diameter of convenient size. Useful sizes range from approximately 1.5 to 4.0 cm.

7.2 *Polished Glass Rod or Rubber Policeman*—Can be fashioned in the laboratory or purchased.

7.3 *Pipettor*—With disposable tips to deliver appropriate volume(s).

7.4 *Sterile Beakers, Test Tubes or other container to receive the cup scrub fluid.*

7.5 *Appropriate Bacterial Cultures*—If this method will be used within a protocol targeting transient organisms.

7.6 *Sampling and Dilution Fluid*—Sterile Butterfield's phosphate buffered water[5] or other recovery fluid of suitable composition; this should contain an antimicrobial inactivator specific for any antimicrobial that might be on the test site; inactivator efficacy should be determined by Test Method E 1054.

8. Test Control and Baseline Skin Sites

8.1 Select skin sites appropriate for target flora and the protocol objectives; where possible, contralateral sample sites are recommended for use as controls.

9. Sample Site:

9.1 *Subjects*—The number of subjects (human or animal) required (if the protocol is *in vivo*) depends on the statistical confidence needed for the expected test results, the variability encountered in the study, and the relative efficacy of any antibacterial agent that may be evaluated. There may be multiple sites available on subjects; randomization is required to suppress sample bias.

9.2 *Isolated Skin or Equivalents*—The number of replicates required to discriminate effects will depend in part on the appropriateness and design of controls within the protocol.

9.2.1 The use of this technique on isolated skin or equivalents is dependent on securing the test site in order to effectively perform the procedure.

10. Sampling Live Subjects (human or animal)

10.1 *Method*:

10.1.1 Quantitative microbial counts are obtained by the cup scrub technique.[3] This procedure is used at test and control sites.

10.1.2 Subjects are positioned for site sampling.

10.1.3 The area to be sampled is delineated by a sterile sampling cylinder. The cylinder is pressed firmly against the skin surface during sampling to ensure that the sampling fluid does not leak from the sampling site.

10.1.4 A minimum 1.5-mL aliquot of sterile sampling fluid, with or without product neutralizers, is pipetted into the cylinder. The entire area is then scrubbed with moderate pressure for 60 ± 6 s using a sterile polished glass rod or policeman. After scrubbing, the sampling fluid is transferred by pipette into a sterile sample tube. This procedure is repeated once more with a fresh aliquot of sampling fluid. The sampling fluids are pooled. This procedure is repeated for each sampling site.

10.1.5 The same pipettes, cylinders, glass rods, and policeman are used for both washes of a site, but new sterile equipment is used for each site. After samples are collected, paper toweling is used to blot the site dry.

10.1.6 Care must be taken during this process to prevent the sampling fluid from spilling into an adjacent site that has not been sampled.

---

[3]Williamson, P., and Kligman, A. M., A New Method for the Quantitative Investigation of Cutaneous Bacteria, *Journal of Investigative Dermatology*, Vol 46, 1965, pp. 498–503.

10.1.7 Following all sampling, when using marker organisms, the sampling site should be decontaminated using 70 to 90 % isopropanol or equivalent, followed by a 4 % chlorhexidine scrub.

11. Sampling Isolated Skin or Skin Equivalents

11.1 *Method*:

11.1.1 Quantitative microbial counts are obtained by the cup scrub technique.[4] This procedure is used for test and control samples.

11.1.2 Samples are positioned and secured as necessary to enable placement and effective use of the sampling cylinder.

11.1.3 The area to be sampled is delineated by a sterile sampling cylinder. The cylinder is pressed firmly against the sample surface during sampling to ensure that the sampling fluid does not leak from the sampling site.

11.1.4 A minimum 1.5-mL aliquot of sterile sampling fluid, with or without product neutralizers, is pipetted into the cylinder. The entire area is then scrubbed with moderate pressure for 60 ± 6 s using a sterile polished glass rod or policeman. After scrubbing, the sampling fluid is transferred by pipet into a sterile sample tube. This procedure is repeated once more with a fresh aliquot of sampling fluid. The sampling fluids are pooled. This procedure is repeated for each sampling site.

11.1.5 The same pipettes, cylinders, glass rods, and policeman are used for both washes of a site, but new sterile equipment is used for each site.

11.1.6 If there are multiple sample sites on the same piece of isolated tissue, care must be taken during this process to prevent the sampling fluid from spilling into an adjacent site that has not been sampled.

12. Microbial Counts

12.1 Each sample is mixed thoroughly. Tenfold serial dilutions of each sample are prepared in dilution fluid. Duplicate quantitative pour or spread plates using soybean-casein digest agar with suitable neutralizer are prepared. Incubate plated samples at suitable growth temperature, ±2°C for 24 to 72 h, or until colonies are visible on the plates.

13. Precision and Bias

15.1 A precision and bias statement cannot be made for this test method at this time.

14. Keywords

16.1 cup scrub; resident flora; transient organism; skin; skin equivalent

[4]Williamson, P., and Kligman, A. M., A New Method for the Quantitative Investigation of Cutaneous Bacteria, *Journal of Investigative Dermatology*, Vol 46, 1965, pp. 498–503.

Appendix H: Neutralisation Protocol for Antimicrobial Agents

Designation: E 1054 – 02

Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents[1]

This standard is issued under the fixed designation E 1054; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These methods are used to determine the effectiveness of procedures and agents for inactivating (neutralizing, quenching) the microbiocidal properties of antimicrobial agents and to ensure that no components of the neutralizing procedures and agents, themselves, exert an inhibitory effect on microorganisms targeted for recovery.

NOTE 1—Knowledge of microbiological and statistical techniques is required for these procedures. These methods are not applicable to testing with viruses (see Test Method E 1482).

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 645 Test Method for Effectiveness of Microbicides Used in Cooling Systems

E 1115 Test Method for Evaluation of Surgical Hand Scrub Formulations

E 1482 Test Method for Neutralization of Virucidal Agents in Virucidal Effectiveness Evaluations

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *antimicrobial agent*—a test formulation, chemical compound, or product designed to prevent the growth of microbes either by inhibiting growth or destroying the microbe.

3.1.2 *antimicrobial effectiveness evaluation*—a determination of microbiocidal properties of an antimicrobial agent by methods, such as Test Methods E 645 and E 1115.

3.1.3 *CFU/mL*—colony-forming units of a microorganism per millilitre of fluid.

3.1.4 *neutralizer*—a procedure or chemical agent used to inactivate, neutralize, or quench the microbiocidal properties of an antimicrobial agent.

3.1.5 *neutralizer effectiveness*—a neutralizer's ability to inactivate, neutralize, or quench the microbiocidal properties of an antimicrobial agent.

3.1.6 *neutralizer toxicity*—any inhibitory effects a neutralizer may have on the survival of a microbial population.

3.1.7 *test material control*—an evaluation of the activity of an test material in reducing a known population of microorganisms.

3.1.8 *test organism viability*—the population or viability of a challenge microorganism used in a neutralization assay.

4. Summary of Test Methods

NOTE 2—The neutralization test method selected must be identical to the method used in the antimicrobial effectiveness evaluation.

4.1 *Neutralization Assay with Recovery on Solid Medium*—Neutralization assay for antimicrobial effectiveness tests that recover and quantify microorganism populations on solid (agar) media. This method is appropriate for antimicrobial agents that can be chemically inactivated or diluted to sub-inhibitory levels.

4.2 *Neutralization Assay with Recovery in Liquid Medium*—Neutralization assay for antimicrobial effectiveness tests that recover surviving microorganism populations in liquid media for a growth/no growth determination. This method is appropriate for antimicrobial agents that can be chemically inactivated or diluted to sub-inhibitory levels.

4.3 *Neutralization Assay with Recovery by Membrane Filtration*—Neutralization assay for antimicrobial effectiveness tests that recover and quantify microorganism populations by using membrane filtration. This method is appropriate for antimicrobial agents that cannot be chemically inactivated or diluted to sub-inhibitory levels. This method should not be used when difficulties are incurred during the filtration process.

---

[1] These test methods are under the jurisdiction of ASTM Committee E35 on Pesticides and are the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved May 10, 2002. Published August 2002. Originally published as E 1054 – 85. Last previous edition E 1054 – 91.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

5. Significance and Use

5.1 The effectiveness of antimicrobial agents such as disinfectants, sanitizers and antiseptics are measured by their ability to kill microorganisms at or for a specified contact time. Accurate determination of antimicrobial effectiveness therefore requires efficient and effective inactivation (neutralization) of the antimicrobial agent. Inefficient or incomplete neutralization will permit killing or inactivation of microorganisms to continue beyond the experimental exposure time, resulting in an over-estimation of antimicrobial activity.

5.2 The neutralization methods commonly used in antimicrobial effectiveness evaluations are chemical inactivation, dilution and filtration. All critical parameters, for example, media, microorganism(s), equipment, and temperature of solutions, of the antimicrobial effectiveness evaluation must be mimicked when evaluating a neutralization procedure to be used in the antimicrobial effectiveness evaluation.

5.3 The evaluation must include at least three replications (five replications in Section 9) so that a statistical analysis can be performed with the recovery data. The number of replicates used in the evaluation depends on the statistical significance required for the expected results, the variability encountered in the evaluation, and the relative efficacy of the neutralization procedure.

5.4 A limitation of these evaluation procedures is that they use microorganisms that have not been exposed to an antimicrobial. Under experimental conditions, cells exposed to neutralization procedures are likely to be damaged to different degrees by the antimicrobial agent. Sublethal injury may be a factor in recovery and the role of the neutralization procedure in recovery of injured organisms should be examined.

NOTE 3—Ideally, all microorganisms used in the antimicrobial effectiveness evaluation should be tested in the neutralization assay. However, "representative" organisms may be selected for testing, as judged appropriate by the investigator. The investigator is cautioned that failure to identify neutralizer efficacy and toxicity for all microorganisms could result in exaggerated microbial reductions in an antimicrobial effectiveness evaluation. Also, for studies involving multiple antimicrobial products and a sample containing multiple species of microorganisms (for example, skin flora), a single neutralizing procedure and/or combination of agents suitable for the multiple products must be used for testing.

6. Apparatus

6.1 Standard bacteriological devices and equipment should be used for performance of the neutralization assay.

6.2 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices, or automated, computerized plater/counter systems.

6.3 *Incubator*—Any incubator capable of maintaining an appropriate temperature for growth of the microorganism may be used.

6.4 *Sterilizer*—Any steam sterilizer capable of producing the conditions of sterilization.

6.5 *Timer (stopwatch)*—One that displays hours, minutes, and seconds.

6.6 *Vortex Mixer or equivalent.*

6.7 *Membrane Filter Units*—Any sterilizable unit that permits filtration of microorganisms for enumeration. The membrane filter unit should be suitable for testing the antimicrobial agent and recovery of the microorganisms.

7. Reagents and Materials

7.1 *Phosphate Buffered Saline Dilution Water*—PBS (see Test Method E 645).

7.1.1 *Phosphate Buffer Solution, Stock*—Dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of water. Adjust pH to 7.2 ± 0.2 with 0.1 N NaOH or 0.1 N HCl and bring to 1000 mL with deionized water.

7.1.2 *Phosphate Buffer Saline Dilution Water*—Add 1.25 mL of stock phosphate buffer solution and 8.75 g of NaCl to a volumetric flask, fill with deionized water to the 1000 mL mark, and mix. Final pH should be 7.2 ± 0.2. Sterilize by filtration or autoclave.

7.2 Because the types of materials and reagents required for various antimicrobial effectiveness evaluations are so diverse, it is impractical to list them in this method. The specific materials and reagents to be used in the antimicrobial effectiveness evaluation, however, should be tested in the neutralization assay to confirm that the antimicrobial agent is being neutralized in a particular evaluation.

7.3 Table 1 provides a partial list of materials that have been employed by researchers to inactivate the microbiocidal properties of various antimicrobial agents. This list is provided as a guide for selecting neutralizers. A neutralization assay should be performed to determine a selected neutralizer's effectiveness.

8. Neutralization Assay with Recovery on Solid Medium (Fig. 1)

8.1 At least three replicates are required for these procedures. The number of replicates used in the evaluation depends on the statistical significance required for the expected results, the variability encountered in the evaluation, and the relative efficacy of the neutralization procedure.

8.2 All tests must be performed in a timely manner so that replication of the test organism does not occur.

8.3 *Test A—Neutralizer Effectiveness:*

8.3.1 Add a volume of product, or solution containing product, to neutralizer that will result in the same dilution ratio to be used in the antimicrobial effectiveness evaluation. If the antimicrobial effectiveness evaluation will employ the use of carriers, use instead a carrier containing an amount of product representative of that to be used in the test.

NOTE 4—The dilution ratio of product to neutralizer can be manipulated to determine the dilution at which adequate neutralization of the product will occur, particularly when testing products not readily neutralized by chemical means.

NOTE 5—The sequence of product-into-neutralizer, followed by the challenge microorganism, allows the neutralizing action to take place. If the microorganism is introduced into the neutralizing solution prior to adding the product, there is possibility of the product acting on the microorganism there by reducing the population and disqualifying the neutralizer.

8.3.2 Within 5 s of execution of 8.3.1, inoculate the product/neutralizer mixture with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism.

TABLE 1 Processes Applied for Neutralization of Certain Antimicrobial Agent[A]

Antimicrobial Agent	Neutralizers/Inactivators
Alcohols	
Isopropanol, Phenoxyethanol	Polysorbate 80, dilution to sub-inhibitory levels
Aldehydes	
2-Bromo-2-nitroproprane-1, 3-diol (bronopol)	Serum, cysteine, thiosulfate, thioglycolate, metabisulfite
Formaldehyde	Sodium sulfite, ammonia, histamine
Glutaraldehyde	Dilution to sub-inhibitory levels, sodium bisulfite, sodium sulfite, glycine, cystine, cysteine
Chlorallytriazaazoniaadamantane (Dowicil 200)	Dilution to sub-inhibitory levels
Dimethyloldimethyl hydantoin (Glydant)	Dilution to sub-inhibitory levels
Biguanides and Bis-biquanides	
Chlorhexidine	Lecithin/polysorbate 80, sodium oleate
Polyhexamethylene biguanide HCL (Cosmocil CQ)	Polysorbate 80/lecithin
Phenolics	
Phenylphenol, Chloroxylenol, Cresols, Chlorocresols, Phenol	Nonionic surfactants, polysorbate 80, and/or dilution to sub-inhibitory levels
Bis-Phenols	
Triclosan	>10 % polysorbate 80/lecithin, and dilution to sub-inhibitory levels
Hexachlorophene	>10 % polysorbate 80/lecithin, and dilution to sub-inhibitory levels
Quaternary Ammonium Compounds	
Cetrimide, Benzalkonium and Benzethonium Chloride	Lecithin/polysorbate, suramin sodium, organic material, 0.5 % polysorbate 80, cyclodextrins
Mercurials	Sulfhydryl compounds, thioglycolic acid, thiosulfate, bisulfite, ammonium sulfite
Organic Acids	
Benzoic, Proplonic, Sorbic	Nonionic surfactants, dilution to sub-inhibitory levels, pH 7 of above
Halogens	
Hypochlorite	Thiosulfate and/or dilution to sub-inhibitory levels
Iodine	Thiosulfate, polysorbate 80, skim milk
Bromine	Thiosulfate and/or dilution to sub-inhibitory levels
EDTA	Mg^{+2} or Ca^{+2} ions
Imidazolidinyl urea	Dilution to sub-inhibitory levels
Methyl-, and methylchloroisothiazolinone (Kathon)	Amines, sulfites, mercaptans, sodium bisulfite, heparin
Parabens	
Methyl-, ethyl-, propyl-, butyl-parahydroxybenzoate	Lecithin, filtration, dilution to sub-inhibitory levels, polysorbate surfactants, 1 % polysorbate 80 or 20
Hydrogen Peroxide	Catalase
Peroxyacetic Acid	Sodium Thiosulfate

[A] Sutton, S. V. W., "Neutralizer Evaluations as Control Experiments for Antimicrobial Effectiveness Tests," Ch. 3 in *Handbook of Disinfectants and Antiseptics*, Marcel-Dekker, NY, 1996, p. 300.

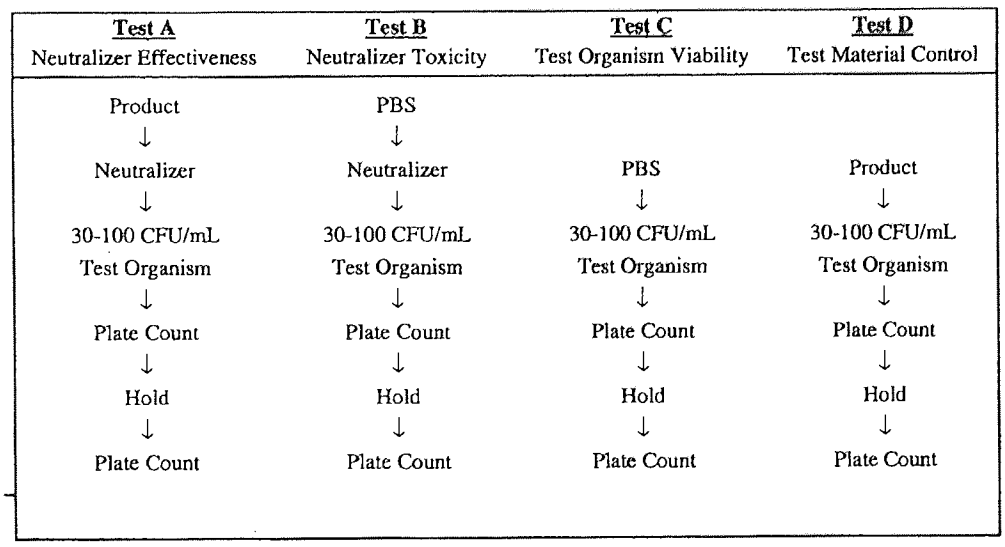


FIG. 1 Testing Schema for Neutralization Assay with Recovery on Solid Medium

NOTE 6—The challenge inoculum should be prepared in the same manner used in the antimicrobial effectiveness evaluation. The volume of

the challenge inoculum should be kept to a minimum so it does not cause significant dilution of the product/neutralizer mixture.

8.3.3 Within 1 min of execution of 8.3.2, enumerate the product/neutralizer/microorganism suspension by quantitative pour or spread plates, in duplicate, using appropriate plating medium. If neutralizers are to be incorporated in the plating medium for the antimicrobial effectiveness evaluation, use this same medium for plating the suspension.

8.3.4 Allow the product/neutralizer/microorganism suspension to stand for the longest exposure period representative of that to be used in the antimicrobial effectiveness evaluation. For example, if the product/neutralizer/microorganism from the antimicrobial effectiveness evaluation will be plated within 30 min, then the longest exposure period for the neutralization assay is 30 min.

8.3.5 After the hold-time, enumerate the product/neutralizer/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium. If neutralizers are to be incorporated in the plating medium for the antimicrobial effectiveness evaluation, use this same medium for plating the suspension.

NOTE 7—The duration of the hold time must not be such that replication of the test organism introduces a variable.

8.3.6 Repeat this procedure (8.3.1-8.3.5) an additional two times, for a total of three replicates.

8.3.7 Incubate the plates under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

8.4 *Test B—Neutralizer Toxicity*:

8.4.1 Add a volume of PBS or other appropriate buffering agent to neutralizer that will result in the same dilution ratio as that used in Test A (see 8.3.1).

8.4.2 Within 5 s of execution of 8.4.1, inoculate the PBS/neutralizer mixture with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

8.4.3 Within 1 min of execution of 8.4.2, enumerate the PBS/neutralizer/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium. If neutralizers are to be incorporated in the plating medium for the antimicrobial effectiveness evaluation, use this same medium for plating the suspension.

8.4.4 Allow the PBS/neutralizer/microorganism suspension to stand for the same period used in Test A (see 8.3.4).

8.4.5 After the hold-time, enumerate the PBS/neutralizer/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium. If neutralizers are to be incorporated in the plating medium for the antimicrobial effectiveness evaluation, use this same medium for plating the suspension.

8.4.6 Repeat this procedure (8.4.1-8.4.5) an additional two times, for a total of three replicates.

8.4.7 Incubate the plates under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

8.5 *Test C—Test Organism Viability*:

8.5.1 Inoculate a volume of PBS or other appropriate buffering agent with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

8.5.2 Within 1 min of execution of 8.5.1, enumerate the PBS/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium that does not contain neutralizers and is not a selective plating medium.

8.5.3 Allow the PBS/microorganism suspension to stand for the same exposure period used in Test A (see 8.3.4).

8.5.4 After the hold-time, enumerate the PBS/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium that does not contain neutralizers and is not a selective plating medium.

8.5.5 Repeat this procedure (8.5.1-8.5.4) an additional two times, for a total of three replicates.

8.5.6 Incubate the plates under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

8.6 *Test D—Test Material Control*:

NOTE 8—A test of a product's antimicrobial effectiveness is required to demonstrate that the neutralizer actually did neutralize the activity of an antimicrobial agent.

8.6.1 Inoculate the product with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

8.6.2 Hold the product/microorganism suspension for an exposure period necessary to allow detection of an antimicrobial effect. The hold time must not be longer than the hold time in Test A (see 8.3.4).

8.6.3 After the hold time, enumerate the product/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium that does not contain neutralizers.

8.6.4 Repeat this procedure (8.6.1 and 8.6.2) an additional two times, for a total of three replicates.

8.6.5 Incubate the plates under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

9. Neutralization Assay with Recovery in Liquid Medium (Fig. 2)

9.1 At least five replicates are required for these procedures. The number of replicates used in the evaluation depends on the statistical significance required for the expected results, the variability encountered in the evaluation, and the relative efficacy of the neutralization procedure.

9.2 All tests must be performed in a timely manner so that replication of the test organism does not occur.

9.3 *Test A—Neutralizer Effectiveness*:

9.3.1 Add a volume of product or solution containing product to neutralizer/nutrient medium that will result in the same dilution ratio to be used in the antimicrobial effectiveness evaluation (see Note 4). If the antimicrobial effectiveness evaluation will employ the use of carriers, use instead a carrier containing an amount of product representative of that to be used in the test.

9.3.2 Within 5 s of execution of 9.3.1, inoculate the product/neutralizer/nutrient medium mixture with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

Test A Neutralizer Effectiveness	Test B Neutralizer Toxicity	Test C Test Organism Viability	Test D Test Material Control
Product	PBS	PBS	Product
↓	↓	↓	↓
Neutralizer/Nutrient	Neutralizer/Nutrient	Nutrient	Nutrient
Medium	Medium	Medium	Medium
↓	↓	↓	↓
30-100 CFU/mL	30-100 CFU/mL	30-100 CFU/mL	30-100 CFU/mL
Test Organism	Test Organism	Test Organism	Test Organism
↓	↓	↓	↓
Incubate	Incubate	Incubate	Incubate
↓	↓	↓	↓
Check for Growth	Check for Growth	Check for Growth	Check for Growth

FIG. 2 Testing Schema for Neutralization Assay with Recovery in Liquid Medium

9.3.3 Repeat this procedure (9.3.1 and 9.3.2) an additional four times, for a total of five replicates.

9.3.4 Incubate the product/neutralizer/nutrient medium/microorganism suspension under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

9.3.5 After incubation, check for growth. If growth is present, record as a "1," and if no growth is present, as a zero. Confirm growth by plating the mixture.

9.4 *Test B—Neutralizer Toxicity*:

9.4.1 Add a volume of PBS to neutralizer/nutrient medium in the same volume as that used for product in Test A (see 9.3.1).

9.4.2 Within 5 s of execution of 9.4.1, inoculate the PBS/neutralizer/nutrient medium mixture with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

9.4.3 Repeat this procedure (9.4.1 and 9.4.2) an additional four times, for a total of five replicates.

9.4.4 Incubate the PBS/neutralizer/nutrient medium/microorganism suspension under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

9.4.5 After incubation, check for growth. If growth is present, record as a "1," and if no growth is present, as a zero. Confirm growth by plating the mixture.

9.5 *Test C—Test Organism Viability*:

9.5.1 Add a volume of PBS or other appropriate buffering agent to an appropriate nutrient medium in the same volume as that used for product in Test A (see 9.3.1).

9.5.2 Within 5 s of execution of 9.5.1, inoculate the PBS/nutrient medium mixture with a volume of challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

9.5.3 Repeat this procedure (9.5.1 and 9.5.2) an additional four times, for a total of five replicates.

9.5.4 Incubate the PBS/nutrient medium/microorganism suspension under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

9.5.5 After incubation, check for growth. If growth is present, record as a "1," and if no growth is present, as a zero. Confirm growth by plating the mixture.

9.6 *Test D—Test Material Control (see* Note 8):

9.6.1 Add a volume of product to an appropriate nutrient medium in the same volume as that used for product in Test A (see 9.3.1).

9.6.2 Within 5 s of execution of 9.6.1, inoculate the product/nutrient medium mixture with a volume of challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

9.6.3 Repeat this procedure (9.6.1 and 9.6.2) an additional four times, for a total of five replicates.

9.6.4 Incubate the product/nutrient medium /microorganism suspension under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

9.6.5 After incubation, check for growth. If growth is present, record as a "1," and if no growth is present, as a zero. Confirm growth by plating the mixture.

10. Neutralization Assay with Recovery by Membrane Filtration Test (Fig. 3)

10.1 At least three replicates are required for these procedures. The number of replicates used in the evaluation depends on the statistical significance required for the expected results, the variability encountered in the evaluation, and the relative efficacy of the neutralization procedure.

10.2 All tests must be performed in a timely manner so that replication of the test organism does not occur.

10.3 *Test A—Neutralizer Effectiveness*:

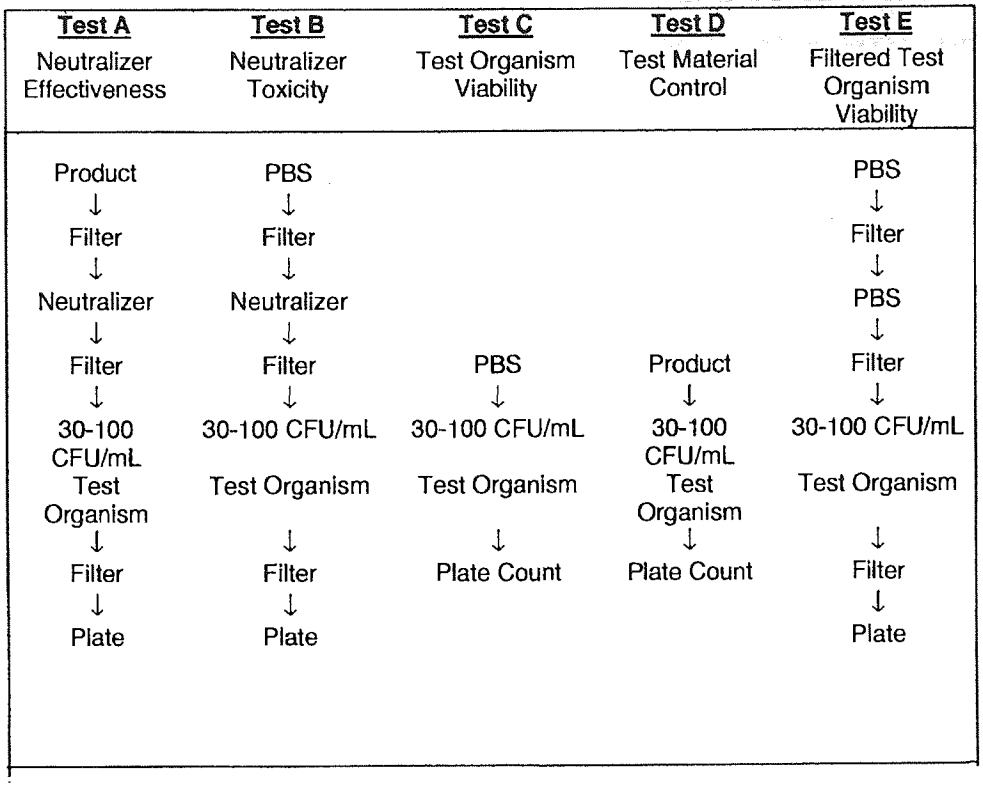


FIG. 3 Testing Schema for Neutralization Assay with Recovery by Membrane Filtration

10.3.1 Filter a volume of product or solution containing product through the membrane filter that is the same as that to be used in the antimicrobial effectiveness evaluation.

NOTE 9—The filter type and manufacturer should be the same as that to be used in the antimicrobial effectiveness evaluation.

10.3.2 Filter a volume of neutralizer through the membrane filter.

10.3.3 Inoculate a volume of PBS or other appropriate buffering agent with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU of the microorganism (see Note 6). Filter the PBS/microorganism suspension.

NOTE 10—The suspension contains a total of 30 to 100 CFU of the microorganism. This can be achieved by adding 1 mL of a microorganism suspension containing 30 to 100 CFU/mL to the PBS.

10.3.4 To enumerate the survivors, aseptically place the membrane filter on an agar plate or onto absorbent pads with appropriate nutrients. If neutralizers are to be incorporated in the plating medium for the antimicrobial effectiveness evaluation, use the same medium for plating the filter.

10.3.5 Repeat this procedure (10.3.1-10.3.4) an additional two times, for a total of three replicates.

10.3.6 Incubate the plates or pads under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

10.4 *Test B—Neutralizer Toxicity*:

10.4.1 Filter a volume of PBS through the membrane filter that is equal to the volume of product used in Test A (see 10.3.1).

10.4.2 Filter a volume of neutralizer through the membrane filter that is the same as that used in Test A (see 10.3.2).

10.4.3 Inoculate a volume of PBS with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU of the microorganism (see Note 6 and Note 10). Filter the PBS/microorganism suspension.

10.4.4 To enumerate the survivors, aseptically place the membrane filter on an agar plate or onto absorbent pads with appropriate nutrients. If neutralizers are to be incorporated in the plating medium for the antimicrobial effectiveness evaluation, use the same medium for plating the filter.

10.4.5 Repeat this procedure (10.4.1-10.4.4) an additional two times, for a total of three replicates.

10.4.6 Incubate the plates or pads under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

10.5 *Test C—Test Organism Viability*:

10.5.1 Inoculate a volume of PBS or other appropriate buffering agent with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the microorganism (see Note 6).

10.5.2 Within 1 min of execution of 10.5.1, enumerate the PBS/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium that does not contain neutralizers and is not a selective plating medium.

10.5.3 Repeat this procedure (10.5.1 and 10.5.2) an additional two times, for a total of three replicates.

10.5.4 Incubate the plates under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

10.6 *Test D—Test Material Control (see* Note 8*)*:

10.6.1 Inoculate the product with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU/mL of the test organism (see Note 6).

10.6.2 Hold the product/microorganism suspension for an exposure period necessary to allow detection of an antimicrobial effect (see Note 7).

10.6.3 After the hold-time, enumerate the product/microorganism suspension by quantitative pour or spread plates, in duplicate, using an appropriate plating medium that does not contain neutralizers.

10.6.4 Repeat this procedure (10.6.1-10.6.3) an additional two times, for a total of three replicates.

10.6.5 Incubate the plates under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

10.7 *Test E—Filtered Test Organism Viability*:

10.7.1 Filter a volume of PBS through the membrane filter that is equal to the volume of product used in Test A (see 10.3.1).

10.7.2 Filter a volume of PBS through the membrane filter that is the same as that used in Test A (see 10.3.2).

10.7.3 Inoculate a volume of PBS with a volume of the challenge microorganism suspension so that the resulting suspension contains 30 to 100 CFU of the microorganism (see Note 6 and Note 10). Filter the PBS/microorganism suspension.

10.7.4 To enumerate the survivors, aseptically place the membrane filter on an agar plate or onto absorbent pads with appropriate nutrients.

10.7.5 Repeat this procedure (10.7.1-10.7.4) an additional two times, for a total of three replicates.

10.7.6 Incubate the plates or pads under the same conditions as those to be used in the antimicrobial effectiveness evaluation.

11. Interpretation of Data

11.1 *Recovery on Solid Medium*:

11.1.1 Calculate the number of surviving challenge microorganisms for each replicate from each test using the following formula:

$$\text{number of survivors} = \frac{(\text{plate count 1} + \text{plate count 2})}{2}$$

11.1.2 Transform the number-of-survivor values to $\log_{10}$ or square root values.

11.1.3 Statistically compare the number of survivors ($\log_{10}$ or square root) from Tests A, B, and D to the test organism viability population (Test C) using a one-way ANOVA or Student's t test ($\alpha = 0.05$).[3]

NOTE 11—Prior to performing the statistical analysis, the investigator must first establish the confidence level of the test. A 95.0 % confidence level ($\alpha = 0.05$) is commonly used for biological data. The investigator must also determine the minimal difference between two samples that must be detectable by the statistical test. A $\log_{10}$ difference of 0.20 has been previously used for neutralization assays.[4] The variability of the test data can effect interpretation of the results from the statistical analysis. If a difference is determined between two samples that differ by 0.10 $\log_{10}$, then the variability of the test data is smaller than what is required to detect the minimal difference. In this case, the investigator can conclude that there was no significant difference between the two samples, because the variability was small and the difference between the samples was less than the minimal difference to be detected.

11.1.4 Neutralization is considered adequate if the Test A recovery population is not statistically different from the test organism viability population and if the Test D recovery population is statistically less than the test organism viability population (Test C).

11.1.5 The neutralizing medium is considered non-toxic if the Test B recovery population is not statistically different from the test organism viability population (Test C).

11.2 *Recovery in Liquid Medium*:

11.2.1 Compare the data from Tests A, B and C using a chi-square, one-sample goodness-of-fit test. ($\alpha = 0.05$).[5]

NOTE 12—A nonparametric test is required for analyzing data from the Liquid Medium Recovery test because the data, themselves, are ordinal and do not approximate a normal distribution.

11.2.2 Neutralization evaluation is considered adequate if Tests A, B, and C are not statistically different ($p > 0.05$) and if no growth is observed for Test D.

11.3 *Recovery by Membrane Filtration*:

11.3.1 Calculate the number of surviving challenge microorganisms for each replicate for Test C and Test E using the following formula:

$$\text{number of survivors} = \frac{(\text{plate count 1} + \text{plate count 2})}{2}$$

11.3.2 Transform the number-of-survivor values to $\log_{10}$ or square root values.

11.3.3 Statistically compare the mean number of survivors ($\log_{10}$ or square root) from Tests A, B, and D to the test organism viability population (Test C) using a one-way ANOVA or Student's t test ($\alpha = 0.05$).[3] (see Note 11)

11.3.4 Neutralization is considered adequate if the Test A recovery population is not statistically different from the test

[3] Dixon, W. J., and Massey, F. J., "Introduction to Statistical Analysis," McGraw-Hill Book Co., NY, 1983, p. 678.

[4] Reybrouk, G., "Efficacy of Inactivators Against 14 Disinfectant Substances," Zbl. Bakt. Hyg., Orig. B, Vol 168, 1979, pp. 480-492.

[5] Seigal, S., and Castellan, N. J., "Nonparametric Statistics for the Behavioral Sciences," McGraw-Hill, Boston, MA, 1988, p. 399.

organism viability population ($p > 0.05$) and if the Test D recovery population is statistically less than the test organism viability population ($p \leq 0.05$).

11.3.5 The neutralizing medium is considered non-toxic if the Test B recovery population is not statistically different from the test organism viability population ($p > 0.05$).

11.3.6 Filtration is considered adequate for enumerating viable organisms if the Test E recovery population is not statistically different from the test organism viability population ($p > 0.05$).

12. Keywords

12.1 antimicrobial agents; antimicrobial effectiveness evaluations; inactivation; neutralization; neutralizer toxicity

Appendix I: Neutralisation Data for Chapter 3, Section 5.

Staphylococcus aureus (ATCC #6538) was used as the challenge species in the neutraliser validation study.

Test Description	Sample Size	Mean	Log_{10} difference	Significantly Different
Inoculum Population 1 Minute Exposure (IP)	4	1.69	n/a	n/a
Inoculum Population 30 Minute Exposure (IP)	4	1.66	0.03	No difference
2% CHG in 70% IPA	4	0.00	1.69	[a]Significant Difference
Povidone-iodine scrub and paint tray	4	0.00	1.69	[a]Significant Difference
Neutraliser Fluid Inhibition Evaluation 1 Minute Exposure (PII)	4	1.75	0.06	No difference
Neutraliser Fluid Inhibition Evaluation 30 Minute Exposure (XPII)	4	1.71	0.02	No difference
Sampling Fluid Inhibition Evaluation 1 Minute Exposure (PIII)	4	1.72	0.03	No difference
Sampling Fluid Inhibition Evaluation 30 Minute Exposure (XPIII)	4	1.65	0.04	No difference
Neutraliser Efficacy Evaluation 2% CHG in 70% IPA 1 Minute Exposure (PIVA)	4	1.61	0.08	No difference
Neutraliser Efficacy Evaluation 2% CHG in 70% IPA 30 Minute Exposure (XPIVA)	4	1.68	0.01	No difference
Neutraliser Efficacy Evaluation Povidone-Iodine Scrub & Paint 1 Minute Exposure (PIVB)	4	1.71	0.02	No difference
Neutraliser Efficacy Evaluation Povidone-Iodine Scrub & Paint 30 Minute Exposure (XPIVB)	4	1.69	0.00	No difference

[a]Significantly Different from Inoculum Population (greater than 0.25 log_{10} difference)

Appendix J: Neutralisation Data for Chapter 4, Section 5.

Staphylococcus epidermidis (ATCC #51625) was used as the challenge species in the neutraliser validation study.

Test Description	Sample Size	Mean	Log_{10} difference	Significantly Different
Inoculum Population 1 Minute Exposure (IP)	4	2.44	0.07	n/a
Inoculum Population 30 Minute Exposure (XIP)	4	2.42	0.01	No difference
2% CHG (PIa)	4	0.00	0.00	[a]Significant Difference
Povidone-iodine scrub and paint tray (PIB)	4	0.00	0.00	[a]Significant Difference
Neutraliser Fluid Inhibition Evaluation 1 Minute Exposure (PII)	4	2.58	0.04	No difference
Neutraliser Fluid Inhibition Evaluation 30 Minute Exposure (XPII)	4	2.50	0.03	No difference
Neutraliser Efficacy Evaluation 2% CHG 1 Minute Exposure (PIVA)	4	2.43	0.03	No difference
Neutraliser Efficacy Evaluation 2% CHG 30 Minute Exposure (XPIVA)	4	2.42	0.02	No difference
Neutraliser Efficacy Evaluation Povidone-Iodine Scrub & Paint 1 Minute Exposure (PIVB)	4	2.50	0.03	No difference
Neutraliser Efficacy Evaluation Povidone-Iodine Scrub & Paint 30 Minute Exposure (XPIVB)	4	2.47	0.01	No difference

[a]Significantly Different from Inoculum Population (greater than 0.25 log_{10} difference

Letters to the Editor:

Is Chlorhexidine Prep Appropriate for Peridural Anesthesia?

To the Editor:

Hebl in 2006 stated in a review article published in *Regional Anesthesia and Pain Medicine* that chlorhexidine-based solutions should be considered the antiseptic of choice for regional anesthetic procedures and that its use be considered a Grade A recommendation.[1] In a previous *APSF Newsletter* these solutions have also been recommended, based on effectiveness, for skin preparation prior to insertion of invasive intravascular catheters to reduce the risk of catheter-related bloodstream infections.[2]

In spite of this, still in 2008 the Chloraprep "One-Step" applicator (Medi-flex, Overland, KS) has written in its contraindications in the back, that this solution should not be used when working in close proximity to meningeal structures.

In my opinion this remains a tremendous problem because we have conflicting data and the clinician remains in the middle unable to convincingly follow the published guidelines from the *American Society of Regional Anesthesia and Pain Medicine* or follow the recommendations from the manufacturer. So in the context of safety what should we do?

Felipe Urdaneta, MD
Gainesville, FL

References

1. Hebl JR. The importance and implications of aseptic techniques during regional anesthesia. *Reg Anesth Pain Med* 2006;31:311-23.

2. Wagner CL, Prielipp RC. Chlorhexidine prep decreases catheter-related infections. *APSF Newsletter* 2003;18:2

To the Editor:

I would like to thank you for the opportunity to respond to Dr. Urdaneta's question, "Should We Follow These Guidelines and Recommendations or Not?" It is a valid question that has been posed many times.

As pointed out by Dr. Urdaneta, a 2006 review article published in Regional Anesthesia and Pain Medicine, stated that chlorhexidine-based solutions should be considered the antiseptic of choice for regional anesthetic procedures and that its use be considered a Grade A recommendation.[1] As of 2008 all chlorhexidine-based topical cutaneous skin antiseptics have the warning "do not use for lumbar puncture or in contact with the meninges"[2] or "do not use in contact with the meninges"[3]

The Food and Drug Administration (FDA) approval of a drug is based on the data submitted by the manufacturer. The FDA requires that substantial evidence resulting from adequate and well-controlled investigations demonstrate that a drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling. Once the FDA determines that a drug is safe and effective the manufacturer can only advertise or promote the drug for the indication approved by the FDA, and all promotion must be based on information that was submitted for review.[4]

A physician's discretionary use of that product (the practice of medicine) is not restricted to the uses

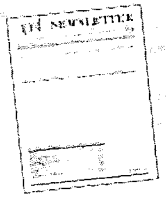
See "Chlorhexidine," Page 47

Karen Posner Named to Laura Cheney Professorship in Anesthesia Patient Safety

Dr. Karen Posner has been named to The Laura Cheney Professorship in Anesthesia Patient Safety, launched by Dr. Frederick Cheney, former chair of the department of anesthesia at the University of Washington in Seattle. This professorship is named after Dr. Cheney's mother, a nurse who encouraged him to go into the field of medicine. The Professorship provides a cornerstone for the department's patient safety research program and will provide a permanent source of research funding for anesthesia patient safety. Dr. Posner is honored as the first holder of this endowed position.

While Dr. Posner's activities continue with the ASA Closed Claims Project, some endowment funds have been committed to a junior investigator starter grant within the University of Washington's department of anesthesiology to promote patient safety research among junior faculty. Fundraising continues, with the goal of converting the professorship into an endowed chair position that would enable expansion of patient safety research activities.

APSF
NEWSLETTER
The Official Journal of the Anesthesia Patient Safety Foundation



The Anesthesia Patient Safety Foundation Newsletter is the official publication of the nonprofit Anesthesia Patient Safety Foundation and is published quarterly at Wilmington, Delaware. Annual contributor cost: Individual–$100, Corporate–$500. This and any additional contributions to the Foundation are tax deductible.

The opinions expressed in this *Newsletter* are not necessarily those of the Anesthesia Patient Safety Foundation. The APSF neither writes nor promulgates standards, and the opinions expressed herein should not be construed to constitute practice standards or practice parameters. Validity of opinions presented, drug dosages, accuracy, and completeness of content are not guaranteed by the APSF.

Address all *general, contributor, and subscription* correspondence to:
Administrator, Deanna Walker
Anesthesia Patient Safety Foundation
Building One, Suite Two
8007 South Meridian Street
Indianapolis, IN 46217-2922
e-mail address: walker@apsf.org
FAX: (317) 888-1482

Address *Newsletter* editorial comments, questions, letters, and suggestions to:
Robert C. Morell, MD
Editor, *APSF Newsletter*
c/o Addie Larimore, Editorial Assistant
Department of Anesthesiology
Wake Forest University School of Medicine
9th Floor CSB
Medical Center Boulevard
Winston-Salem, NC 27157-1009
e-mail: apsfeditor@yahoo.com

Letters to the Editor:

Chlorhexidine Question May Be For FDA

"Chlorhexidine," From Page 38

indicated on FDA-regulated labels. Off-label use is widespread in the medical community and often is essential to giving patients optimal medical care, both of which medical ethics, FDA, and most courts recognize.[3] The policy statement from the American Academy of Pediatrics for Unapproved Uses of Approved Drugs states, "An unapproved use of an approved drug refers to a use that is not included or that is disclaimed in the approved labeling. Unapproved use does not imply an improper use and certainly does not imply an illegal use. The term 'unapproved' is used merely to indicate lack of approval, not to imply disapproval or contraindication based on evidence of a lack of safety or efficacy. The distinction between contraindication and unapproved is important medically and legally". . . ."New uses, doses, or indications will not be approved by the FDA until substantial evidence of safety and efficacy for the indication or age group is submitted to the FDA. This may take years or may never occur."[4]

Since the consensus position of the American Society of Regional Anesthesia publication in the 2006 Hebl review article stating that chlorhexidine-based solutions should be considered the antiseptic of choice for regional anesthetic procedures, there has been discordance between the evidence-based practice of many clinical providers of regional anesthesia and chlorhexidine-based antiseptic product labeling. Dr. David Hepner published a correspondence in the April 2007 issue of *Anesthesiology* that stated the expert panel for *Regional Anesthesia and Pain Medicine* "felt strongly that although the US Food and Drug Administration has not approved chlorhexidine before lumbar puncture, it has a significant advantage over povidone iodine because of its onset, efficacy, and potency" and commented that "interestingly, povidone iodine is also not approved for lumbar puncture."[5] Chlorhexidine (CHG) or tinctures of CHG antiseptic solutions have been used extensively in and outside the United States for regional anesthesia procedures.[6]

The US Physician Desk Reference (PDR), as of 1984, warns that "chlorhexidine gluconate is for external use only. Keep out of eyes and ears and avoid contact with meninges." Before 1984, the PDR did not have the "avoid contact with meninges" warning. There are 2 referenced articles for neurotoxicity, both of which are animal studies. One article, published in 1955, was a study with many chemicals and detergent compounds that were injected into the cerebral spinal fluid of monkeys; neurotoxicity was seen with all compounds.[8] A second study published in 1984, based on a rat model, involved injecting a CHG solution into the anterior chamber of the eye. This study showed degeneration of adrenergic nerves and suggested that neurotoxic effects on thin myelinated fiber systems in the CNS should be investigated.[9] Since the publication of the 1984 PDR warning, a number of articles have appeared describing the use and efficacy of CHG in regional anesthesia and conclude that it is the preferred skin disinfectant.[10-12] These papers and accumulated clinical experience have been sufficient to persuade the major specialty society (ASRA) and the majority of clinicians to adopt CHG as the preferred skin disinfectant in the setting of regional anesthesia. To view this from the perspective of the FDA, their reluctance to specifically suggest a label change perhaps arises out of a philosophical reluctance, no perceived need from their perspective, and inadequately powered prospective evaluation of CHG in the setting of regional anesthesia (especially epidural and spinal) to reach their threshold for a labeling change.[13] From the perspective of a manufacturer of a CHG skin prep, the expense associated with obtaining a label change is prohibitive. Evidence based guidelines, like those in the Hebl article, make a manufacturer initiated labeling change unlikely. The question raised by Dr Urdaneta should perhaps best be posed to the FDA.

Cynthia T Crosby
VP Global Medical Affairs
Cardinal Health
Leawood, KS

References

1. Hebl JR. The importance and implications of aseptic techniques during regional anesthesia. *Reg Anesth Pain Med* 2006;31:311-23.
2. http://www.enturia.com/products/chloraPrep/pdf/Directions_of_Use_3_mL_-_9mL_Clear_label.pdf ChloraPrep™ labeling (Cardinal Health). Accessed on August 28, 2008.
3. http://www.regentmedical.com/Files/LOWE_US/Skin_Antisepsis/prodimo-hibiclens-insert.pdf Hibiclens® labeling (Mölnlycke Health Care). Accessed on August 28, 2008.
4. Committee on Drugs. Unapproved uses of approved drugs. The physician, the package insert, and the Food and Drug Administration: subject review. *Pediatrics* 1996;98:143-145.
5. Beck JM, Azan FD. FDA, off-label use, and informed consent: debunking myths and misconceptions. *Food and Drug Law Journal* 1998;53:71-104.
6. Hepner DL. Correspondence. *Anesthesiology* 2007;106:879-80.
7. Adam MN, Dimukescu I, Matheu P, et al. Comparison of the efficacy of 2 antiseptic solutions in the prevention of infection from peridural catheters. *Cah Anesthesiol* 1996;44:45-7.
8. Hurst EW. Adhesive arachnoiditis and vascular blockage caused by detergents and other chemical irritants: an experimental study. In: Stewart MI, Cameron GR, Oakley CL, Collins DH, MacDonald A, eds. The journal of pathology and bacteriology. London: Oliver and Boyd Ltd., 1955. Pp 167-178.
9. Henschen A, Olson L. Chlorhexidine-induced dengeneration of adrenergic nerves. *Acta Neuropathol* 1984;63:15-23.
10. Shapiro JM, Bond EL, Carman JK. Use of a chlorhexidine dressing to reduce microbial colonization of epidural catheters. *Anesthesiology* 1990;73:625-31.
11. Kinaroins B, Munoz O, Lafendi L, et al. Chlorhexidine versus povidone iodine in preventing colonization of continuous epidural catheters in children. *Anesthesiology* 2001;94:239-44.
12. Crowley L, Presuan R, Wong A, Brok L, Kuzejevic B. What is the best skin disinfectant solution for labour epidural analgesia? A randomized prospective trial comparing ChloraPrep™, DuraPrep™, and Chlorhexidine 0.5% in 70% alcohol. *Anesthesia & Analgesia* 2008; 106: A-3.
13. Stafford RS. Regulating off-label drug use—rethinking the role of the FDA. *N Engl J Med* 2008 Apr 3; 358 (14): 1427-1429

Errors Difficult to Completely Eliminate

To the Editor:

Dr. Workhoven is of course correct that no one should ever administer a medication based on color or shape clues without reading the label. However, it is unrealistic to predict that using words alone, written or spoken, will prevent all drug error.

Many drug names look alike (e.g., hydralazine and hydroxyzine, amrinone and amiodarone), especially if your reading glasses are smudged. The words even sound alike, when spoken in noisy operating rooms, especially those wired with the kind of hard-rock soundtracks favored by orthopedic and chest surgeons. The spoken word can also be misunderstood when expressions like "the bolus," "the antibiotic," or "start 2 of epi" are used.

Real life does not end at the operating room (OR) door, and misunderstandings will never end—despite our best efforts. The only solution I can envision is the development of a new type of OR specialist who checks every medication before it is administered and confirms the identity of every vessel before it is ligated.

Howard Schranz, MD
New York, NY

Eliminate Pattern to Prevent Drug Errors

To the Editor:

Dr. Schranz makes many good points about some of the causes for drug error in the operating room (OR). There are many distractions, and production pressure is also a major cause, I am sure. No time to "read" the label, only time to recognize the color and shape pattern.

It just seems to me that the human mind is rather adept at, and seemingly eager to use, pattern recognition for many visual tasks in and out of the OR. It is, perhaps, easier than reading the small print on the label, less time consuming, and works well, but sometimes not so very well, if your glasses are smudged or bespattered or you can't hear the surgeon well over the cacophony of sound and fury that is the modern day, boom box embattled OR.

So, eliminate the pattern, force all attention to the label and the letters printed on it. Perhaps it can serve as a small step forward in the perpetual effort to eliminate drug error.

Nick Workhoven
Coos Bay, OR

CONCISE COMMUNICATION

Efficacy of Adding 2% (w/v) Chlorhexidine Gluconate to 70% (v/v) Isopropyl Alcohol for Skin Disinfection Prior to Peripheral Venous Cannulation

Heather Small, MSc; Debra Adams, PhD;
Anna L. Casey, PhD; Cynthia T. Crosby, BS;
Peter A. Lambert, DSc;
Thomas Elliott, FRMS, PhD, DSc, MRCP, FRCPath

We undertook a clinical trial to compare the efficacy of 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol with the efficacy of 70% (v/v) isopropyl alcohol alone for skin disinfection to prevent peripheral venous catheter colonization and contamination. We found that the addition of 2% chlorhexidine gluconate reduced the number of peripheral venous catheters that were colonized or contaminated.

Infect Control Hosp Epidemiol 2008; 29:963-965

Intravenous catheters, both peripheral and central, are associated with a risk of catheter-related bloodstream infection.[1] The role of cutaneous microorganisms in the pathogenesis of these infections highlights the need to effectively decontaminate skin prior to catheterization.[2,3] Skin antiseptics frequently utilized include povidone-iodine, alcohol, and chlorhexidine. The efficacy of these antiseptics has been previously evaluated with central venous and arterial catheters.[4] The use of 2% (w/v) aqueous chlorhexidine was associated with the lowest incidence of catheter-related infection.

More recently, a solution of 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol (2% CHG in IPA) (ChloraPrep; Enturia) has been developed for skin decontamination. The 2% CHG in IPA provided more persistent antimicrobial activity on abdominal sites than either 70% IPA or 2% CHG alone at 24 hours.[5] The 2% CHG in IPA was also superior to chloroxylenol and to an alcohol-based iodine solution for eliminating skin microorganisms prior to an operation.[6] In vitro studies have further confirmed that skin antisepsis is enhanced with 2% CHG in IPA, compared with other chlorhexidine preparations.[7]

The Centers for Disease Control and Prevention guidelines recommend a 2% chlorhexidine–based preparation, tinctine of iodine, iodophor, or 70% IPA for skin antisepsis prior to insertion of intravascular catheters.[2] Evidence-based guidelines in the United Kingdom also recommend use of chlorhexidine, preferably 2% CHG in IPA, prior to the insertion of central venous catheters.[8]

We evaluated the number of peripheral venous catheter (PVC) tips that had microorganisms present on their intravascular component following skin decontamination either with 2% CHG in IPA or with 70% IPA alone, which is commonly used in the United Kingdom.

METHODS

Clinical protocol. We studied elective cardiology patients admitted for ablation or pacemaker insertion at University Hospital Birmingham, United Kingdom. We excluded patients who were less than 18 years of age, had skin dermatoses, had a chlorhexidine allergy, or were unable to give informed consent. Ethical approval was obtained from our local research ethics committee.

Patients were randomly assigned to receive skin preparation prior to PVC insertion either with the 2% CHG in IPA solution (in a Sepp 0.67 mL applicator; Enturia) or with wipes containing 0.6 mL of 70% IPA (Steret; Seton Prebble). Blinding was not achieved because of the physical differences in the antiseptic applicators.

The 2% CHG in IPA solution was applied using a standard back-and-forth stroke over the entire skin insertion site for 30 seconds. The 70% IPA wipe was also applied for 30 seconds, utilizing a circular movement as in routine clinical practice. Each antiseptic was then allowed to dry for 2 minutes before a polyurethane PVC (Optiva 2, Medex Medical) was inserted into a superficial vein of the hand. A semipermeable dressing was applied over the insertion site. Prior to PVC removal, the insertion sites were cleaned with 70% IPA. Clean, nonsterile gloves, but not masks, were worn by the operator, and the PVC tips were not handled during explantation.

Assessment of microorganisms on PVC tips. The number of microorganisms present on the PVC tips was determined by quantitative tip culture.[9] The distal 3 cm of each PVC tip was vortexed in 1 mL of saline solution for 60 seconds, then 100 μL of the liquid was inoculated onto a blood agar plate (Oxoid) that was incubated in air at 37° C for 48 hours. The number of colony-forming units was determined, and microorganisms were identified by routine methods. Data were compared using the Fisher exact test and Mann-Whitney *U* test.

RESULTS

Of the 236 patients who met the trial entry criteria, 230 gave consent and 6 declined to participate. Sixty patients were excluded, for the following reasons: the patient was discharged prior to study completion (for 1 patient), the PVC was in situ less than 24 hours (for 10 patients), the PVC was accidentally discarded (for 23 patients), a PVC different from all the others in the study was used (for 1 patient), and the explanted PVC was placed in a nonsterile dressing (for 25 patients). We analyzed PVCs from 170 patients (107 male and 63 female), with a mean age of 61.3 years (range, 21–96 years). There were 91 patients (60 male and 31 female) in the 2% CHG with IPA group and 79 patients (47 male and 32 female) in the IPA group. None of the patients exhibited

evidence of infection and no chlorhexidine hypersensitivity was observed. The mean indwell period of the PVC tips was 2.3 days (range, 1–6 days) for the 2% CHG in IPA group and 2.2 days (range, 1–4 days) for the IPA group ($P = .7$). Antibiotic prophylaxis for the cardiologic procedure (flucloxacillin) was given for 24 hours to 16 patients in the 2% CHG in IPA group and to 18 patients in the IPA group. No patients received antibiotics for treatment of infection during the study.

The use of 2% CHG in IPA was associated with a reduced number of PVC tips with microorganisms present on their surface, compared with the use of 70% IPA alone (Figure). Microorganisms were present on 39 (49.4%) of 79 PVC tips in the 70% IPA group, compared with 18 (19.8%) of 91 PVC tips in the 2% CHG in IPA group ($P < .001$; odds ratio, 4.0 [95% confidence interval, 2.0–7.8]). With the achieved sample sizes, the study had a 90% power to detect a difference between a 50% rate with 70% IPA and a 25% rate with 2% CHG in IPA; the level of significance was set at .05. Of the 16 PVCs from patients who received antibiotics in the 2% CHG in IPA group, 6 yielded microorganisms on culture; of the 18 PVCs from patients who received antibiotics in the 70% IPA group, 12 yielded microorganisms on culture.

The mean number of colony-forming units yielded from each culture-positive PVC tip was 4 in the 2% CHG in IPA group, compared with 2 in the IPA group ($P = .57$). More than one type of microorganism was present on 5 tips from the CHG in IPA group and on 8 tips from the IPA group.

DISCUSSION

Skin disinfection with 2% CHG in IPA prior to PVC insertion resulted in a significant reduction in the number of PVC tips that had microorganisms present on their surface, compared with skin disinfection with 70% IPA alone. The number of microorganisms detected could have been the result of contamination from the skin, although early colonization could not be ruled out. Both contamination and colonization are, however, likely prerequisites for the subsequent development of infection in patients with either a peripheral or a central intravenous catheter. These results concur with previous findings that demonstrated the superiority of 2% CHG in IPA in reducing skin microbial counts, compared with 70% IPA or with 2% CHG alone.[5]

IPA provides a rapid reduction in the number of skin microorganisms but does not have any residual activity. In comparison, CHG has residual activity on the skin for as long as 24 hours.[10] This offers a further explanation for the findings in this current study. CHG at a concentration of 2% (w/v) with 70% (v/v) IPA, therefore, may not only be an effective skin antiseptic but may also provide continued protection of the PVC from microbial ingress from the skin.

The results of the current study may have also been influenced by the applicators used. The different types of application may have resulted in differences in the removal of epithelial cells and bacterial commensals and may have influenced the penetration of antiseptic into the skin. A similar number of patients received antibiotic prophylaxis in each

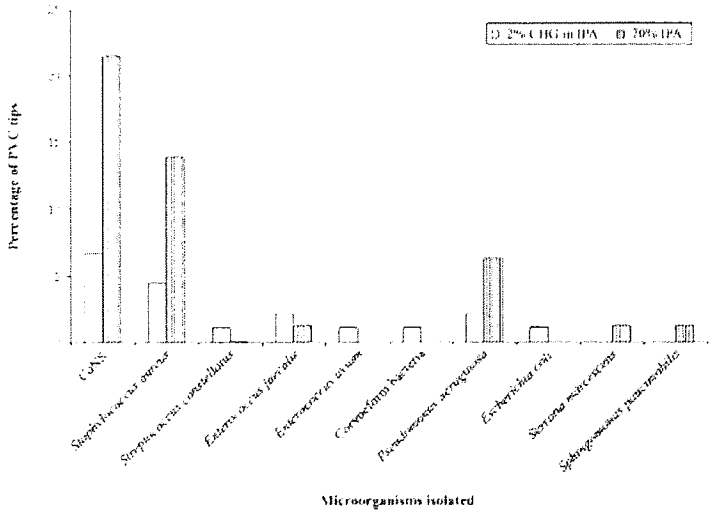


FIGURE. Comparison of the microorganisms isolated from peripheral vascular catheter (PVC) tips following removal from patients who received skin disinfection prior to catheter insertion with 2% (w/v) chlorhexidine gluconate in 70% (v/v) isopropyl alcohol (2% CHG in IPA) or with 70% (v/v) IPA alone. CoNS, coagulase-negative staphylococci.

study group, and there was no correlation between the use of these antibiotics and the presence of microorganisms on the catheter surfaces. However, the use of flucloxacillin may have decreased the overall contamination rate in both groups.

This study suggests that the use of 2% CHG in IPA for skin decontamination prior to PVC insertion may reduce the risk of subsequent PVC contamination or colonization, compared with the use of 70% IPA alone.

ACKNOWLEDGMENTS

We thank the cardiology staff for support.

Financial support. This study was supported by an educational grant from Enturia.

Potential conflicts of interest. C.T.C. is employed by Enturia and contributed to the study design. All other authors report no conflicts of interest relevant to this study.

From the Department of Clinical Microbiology and Infection Control, University Hospital Birmingham NHS Foundation Trust, The Queen Elizabeth Hospital, Edgbaston (H.S., D.A., A.L.C., T.E.), and the Department of Pharmaceutical and Biological Sciences, Aston University, Aston Triangle (P.A.L.), Birmingham, England; and Enturia, Leawood, Kansas (C.T.C.).

Address reprint requests to Thomas Elliott, PhD, Selly Oak Hospital, Raddlebarn Rd., Selly Oak, Birmingham, B29 6HD, England (tom.elliott@uhb.nhs.uk).

Presented in part: 6th International Intravenous Therapy Home and Hospital Conference; Oxford, England; March 8 and 9, 2006.

Received February 26, 2008; accepted June 5, 2008; electronically published September 4, 2008.

 0899-823X/2008/2910-0012$15.00.DOI: 10.1086/590664

REFERENCES

1. Maki DG, Crinch CJ. Line sepsis in the ICU: prevention, diagnosis, and management. *Semin Respir Crit Care Med* 2003; 24:23-36.
2. O'Grady NP, Alexander M, Dellinger EP, et al. Guidelines for the prevention of intravascular catheter- related infections. *MMWR Recomm Rep* 2002; 51:1-8.
3. Moss HA, Elliott TSJ, Tebbs SE, et al. Novel approach to investigate a source of microbial contamination of central venous catheters. *Eur J Clin Microbiol Infect Dis* 1997; 16:210-213.
4. Maki DG, Ringer M, Alvarado CJ. Prospective randomised trial of povidone-iodine, alcohol, and chlorhexidine for prevention of infection associated with central venous and arterial catheters. *Lancet* 1991; 338:339-343.
5. Hibbard JS, Mulberry GK, Brady AR. A clinical study comparing the skin antisepsis and safety of ChloraPrep, 70% isopropyl alcohol, and 2% aqueous chlorhexidine. *J Infus Nurs* 2002; 25:244-249.
6. Ostrander RV, Botte MJ, Brage ME. Efficacy of surgical preparation solutions in foot and ankle surgery. *J Bone Joint Surg Am* 2005; 87:980-985.
7. Adams D, Quayum M, Worthington T, Lambert P, Elliott T. Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant. *J Hosp Infect* 2005; 61:287-290.
8. Pratt RJ, Pellowe CM, Wilson JA, et al. Epic 2: national evidence-based guidelines for preventing healthcare associated infections in NHS hospitals in Englands. *J Hosp Infect* 2007; 65(Suppl 1):S1-S64.
9. Brun-Buisson C, Abrouk F, Legrand P, Huet Y, Larabi S, Rapin M. Diagnosis of central venous catheter-related sepsis. critical level of quantitative tip cultures. *Arch Intern Med* 1987; 147:873-877.
10. Carret L, Reverdy ME, Laforgue C, Falson F, Fleurette J, Freney J. Kinetics of chlorhexidine on intact skin following a single application. *Pathol Biol (Paris)* 1997; 45: 737-740.

Preoperative skin preparation: a historical perspective

The incidence of health-care-associated infections has reduced since skin antisepsis was introduced in the 19th century. Despite this, health-care-associated infections, including post-surgical sepsis, continue to cause significant morbidity and mortality. This article reviews the evidence for the use of preoperative skin preparations.

Before the mid-19th century, surgical sites commonly developed postoperative wound complications that included local wound infection, generalized sepsis and often death (Mangram et al, 1999). In 1847, Semmelweis was the first to identify the relationship between microorganisms and the transmission of infection and introduced chlorinated lime for hand dipping (Gaw, 1999). A few years later, Lister designed and introduced the steam-generated carbolic acid spray for surgical patients in a further attempt to keep the operative wound clean (Gaw, 1999). Indeed, when the principle of antisepsis was implemented the prevalence of postoperative wound infections decreased. However, even in the early 20th century, the prevalence of postoperative wound infection was still high and this prompted the British surgeon Sir Berkeley Moynihan to comment that 'every operation in surgery is an experiment in bacteriology and the success of the experiment, in respect of the patient, depended not only on the skill, but also on the care exercised by the surgeon in the ritual of the operation' (Klenerman, 2002).

What have we learned from these early experiences? The Health Protection Agency's *Surveillance of Surgical Site Infection in England* described the incidence of surgical site infection as ranging from 1% in knee prostheses to 13.1% in limb amputation (Health Protection Agency, 2006). Surgical site infection therefore remains, albeit reduced, a significant cause of morbidity and mortality despite the endeavours of the early workers. How can we now improve this situation?

Surgical site infection

To address prevention of surgical site infection effectively, health-care practitioners must first understand the contributors to infection. The majority of surgical wounds are contaminated by microorganisms, but the risk for development into infection depends on various factors, including the size of the inoculum, the virulence of the microbial contaminant, the microenvironment of the wound and the integrity of host defenses (Fry, 2006). Microorganisms may enter the wound endogenously from the patient's skin or by an exogenous route, including the health-care worker, the operating room environment or from surgical instruments.

Mangram and colleagues (1999) concluded that most surgical site infections develop from microorganisms already present in the skin, mucous membranes or hollow viscera. They further commented that the surgical incision of the skin or mucous membrane results in contamination of associated tissue with this endogenous flora. The risk of developing a surgical site infection varies depending on the type of surgical wound and other factors, such as the health status of the patient and the duration of the operation (Fry, 2006). Effective risk management to reduce surgical site infection involves a multifaceted approach that includes understanding the pathogenesis of surgical site infection, assessing individual patient risk, and being conversant with evidence-based recommendations and current initiatives to reduce the incidence of surgical site infection. This article reviews the role of skin antiseptics, which are a vital component of the prevention of postoperative sepsis.

Patient preoperative skin preparation

Sub-optimal skin antisepsis is considered one of the primary causes of health-care-associated infections. Adequate skin cleansing and protection is essential to pre- and post-care of patients undergoing invasive medical procedures. The skin, which consists of the epidermis, dermis, and subcutaneous tissue, is crucial for human survival. A mature and intact epidermis is an effective barrier in preventing infection. Numerous invasive procedures, such as vascular access, haemodynamic monitoring, drainage tubes and surgical intervention, break the skin's barrier allowing the ingress of microorganisms into the deeper layers. Evidence demonstrates that the most likely source of device-related infection and clean surgical site procedures is the microorganisms on the patient's skin (Maki, 1994; Elliott et al, 1997; Livesley et al, 1998;

Mrs CT Crosby *is Vice President Global Medical Affairs, Infection Prevention, CareFusion, San Diego, California, USA.* Professor TSJ Elliott *is Consultant Microbiologist, University Hospital Birmingham NHS Foundation Trust, Queen Elizabeth Hospital, Queen Elizabeth Medical Centre, Birmingham B15 2TH.* Professor PA Lambert *is Professor of Microbiology in the School of Life and Health Sciences, Aston University, Birmingham.* Dr Debra Adams *is Nurse Consultant in Infection Prevention and Control, Mid Staffordshire NHS Foundation Trust, Stafford*

Correspondence to: Professor TSJ Elliott

Jeske et al, 2003).

More recently, the Health Protection Agency suggested that 50% of surgical site infection is caused by the endogenous skin bacterium *Staphylococcus aureus* (64% of which was meticillin-resistant *S. aureus*) (Health Protection Agency, 2006). Cutaneous antisepsis is therefore critical in infection prevention and control.

Cutaneous or topical antiseptics are antimicrobial agents that kill or inhibit the microorganisms on the skin. They must be active against both resident and transient microorganisms. Health-care antiseptic formulations have a variety of mechanisms, rapidity of antimicrobial activity, persistent or residual properties, and demonstrate varying levels of toxicity.

Various antiseptics are used throughout health-care facilities including iodophors, such as povidone-iodine, chlorhexidine gluconate and isopropyl alcohol. There are specific characteristics to consider when selecting antiseptics for health-care hand washing, surgical scrub for surgeons and operating room personnel, and patients' preoperative skin preparation. The selection of an antiseptic should consider the safety and efficacy, quality of the evidence, ease of implementation, availability of product and health economic value. Ideal properties for an antiseptic agent include:

- Broad spectrum
- Rapid bactericidal (killing) activity
- Residual antimicrobial activity
- Active in the presence of organic matter, such as blood
- Non-allergic or non-toxic responses
- No systemic absorption.

Some of the important characteristics of cutaneous antiseptic solutions are outlined in *Table 1*.

Mode of action and concentration of agent

The activity of a cutaneous antiseptic solution can be affected by a number of factors, including the type of microbial species and the nature and concentration of the antiseptic solution. The higher the number of the microorganisms, referred to as the bioburden, the longer it takes to inhibit or kill the individual microorganisms. The exponential decrease in the number of pathogens will require longer exposure to the antiseptic solution.

The concentration of a cutaneous antiseptic agent can influence its efficacy in reducing skin-colonizing pathogens. For example, Adams and colleagues (2005) studied the efficacy of 2% chlorhexidine gluconate/70% isopropyl alcohol. They reported an improved antimicrobial effect compared with the other three standard preparations of chlorhexidine gluconate available in the UK, namely 0.5% aqueous chlorhexidine gluconate, 2% aqueous chlorhexidine gluconate, and 0.5% chlorhexidine gluconate/70% isopropyl alcohol when tested against *Staphylococcus epidermidis* RP62A.

Another important factor which can influence antiseptic efficacy is the time that the microorganisms are exposed to the agent – the antiseptic needs time to act. This concept is probably one of the most poorly understood and applied aspects of skin antisepsis, particularly among clinicians.

Efficacy

Many studies have evaluated the difference in efficacy of the various cutaneous antiseptic solutions. Chlorhexidine gluconate has repeatedly been shown to be more effective than povidone-iodine or alcohol in the prevention of intravascular bloodstream infections. In an evaluation of antiseptic agents using three major criteria – immediacy, persistence and cumulative action – chlorhexidine gluconate was more effective than alcohol or povidone-iodine as a preoperative skin preparation for patients (Hibbard, 2005). Similarly in a clinical trial comparing the efficacy of 2% chlorhexidine gluconate/70% isopropyl alcohol with that of 70% isopropyl alcohol for skin disinfection to prevent peripheral venous catheter colonization and contamination, the addition of 2% chlorhexidine gluconate significantly reduced (P=0.001) the number of peripheral venous catheters that were colonized or contaminated (Small et al, 2008). Similarly, a meta-analysis of the prevention of central venous catheter-related bloodstream infection demonstrated a 49% reduction in risk of bloodstream infections when chlorhexidine gluconate was the primary active agent for skin antisepsis (Chaiyakunapruk et al, 2002).

There are, however, few studies focusing specifically on preoperative antisepsis of patients before surgery. In foot and ankle surgery, patients' skin that was prepared

Table 1. Characteristics of skin antiseptic agents

	Spectrum of activity					
Antiseptic	Gram +	Fungi	Gram –	Speed of action	Residual activity	Affected by organic matter
Isopropyl alcohol (IPA)	+++	+++	++	Rapid	Minimal	Yes
Aqueous chlorhexidine	+++	++	++	Intermediate	Excellent	Minimal
Chlorhexidine in IPA	+++	+++	++	Rapid	Excellent	Minimal
Aqueous povidone-iodine	+++	+	++	Intermediate	Minimal	Yes
Povidone-iodine in IPA	+++	+++	++	Rapid	Minimal	Yes

Modified from Larson (1988)

with 2% chlorhexidine gluconate/70% isopropyl alcohol demonstrated a significant reduction in microorganisms compared with that prepared with 0.7% iodine/74% isopropyl alcohol or 3.0% chloroxylenol (Ostrander et al, 2005). In a similar study for elective foot and ankle surgery, chlorhexidine gluconate and alcohol paint preparations were found to be better than povidone-iodine (Bibbo et al, 2005). In a coronary artery bypass graft surgery study, the skin for the harvest of the saphenous vein was prepared with either 2% chlorhexidine gluconate/70% isopropyl alcohol or 0.5% chlorhexidine gluconate/70% isopropyl alcohol. There was a trend for a greater reduction in the total number of microorganisms with 2% chlorhexidine gluconate/70% isopropyl alcohol compared with 0.5% chlorhexidine gluconate/70% isopropyl alcohol (P=0.07). In addition, the 2% chlorhexidine gluconate/70% isopropyl alcohol group both the absorbent and the adhesive components of the dressings removed 24 hours post-surgery contained a significantly lower number of microorganisms than those of the 0.5% chlorhexidine gluconate/70% isopropyl alcohol group (P=0.02 and P=0.007 respectively) (Casey et al, 2008).

Guidelines

There are now a number of studies on the efficacy of antiseptic skin preparation before intravascular catheter insertion. Guidelines based on the data from these studies have subsequently been produced. For example, the epic2 guidelines clearly recommend 2% chlorhexidine gluconate/70% isopropyl alcohol solution for use before intravascular catheter insertion (Pratt et al, 2007). In comparison the American Centers for Disease Control produced guidelines for the prevention of intravascular catheter-related infection (O'Grady et al, 2002). O'Grady and colleagues recommended the use of 2% chlorhexidine gluconate but did not specify whether it should be aqueous or with alcohol. The Society of Cardiovascular Angiography and Interventions (Chambers et al, 2006) guidelines, in contrast, specify that 2% chlorhexidine gluconate/70% isopropyl alcohol is preferred for use in venous and arterial catheterization.

Despite apparent differences in the efficacy of antiseptic solutions in preoperative studies as discussed above, sub-optimal preoperative skin preparations are still commonly used in the operating theatre today.

There has generally been a lack of recommendations or preference for specific antiseptic agents in current guidelines for surgical skin preparations both nationally and internationally. However, the National Institute for Health and Clinical Excellence (NICE) issued guidelines on surgical site infection in October 2008 (National Collaborating Centre for Women's and Children's Health, 2008). These guidelines recommend either povidone-iodine or chlorhexidine but do not stipulate the concentration of the antiseptic to be used. The guidelines review a range of studies but consider many to be underpowered. Indeed, some show no statistically significant difference between the two compounds.

The Health Protection Agency Rapid Review Panel, which assesses new and novel products that add value to the NHS in reducing health-care-associated infections, issued a Recommendation 1 on 15 July 2005 for ChloraPrep (Enturia Ltd, Reigate, UK), a 2% chlorhexidine gluconate/70% isopropyl alcohol preoperative skin preparation, indicating that the product should be available to NHS bodies to include in their cleaning, hygiene or infection control protocols (Health Protection Agency, 2005). This recommendation took into account increasing evidence, some reviewed above, that suggests chlorhexidine gluconate has greater activity than iodine-based preparations and that a concentration of 2% chlorhexidine gluconate is more effective than 0.5% chlorhexidine gluconate.

Conclusions

Since the 19th century, considerable progress has been made in understanding the mechanisms of the antibacterial action of antiseptics. However, there is still an inadequate amount of data in areas such as preoperative skin care.

The formulation of specific preoperative skin preparation protocols is a key part of a wider ongoing range of measures which should be taken in response to the Department of Health (2007) Clean, Safe Care, Saving Lives initiative. It is a simple yet important way of improving procedures and reducing rates of postoperative infection. Based on the evidence related to skin antisepsis for intravascular device insertion as recommended by the epic 2 guidelines and the Health Protection Agency of using 2% chlorhexidine gluconate in 70% isopropyl alcohol formulation, the authors would suggest this approach should be taken for all preoperative skin preparations unless there is a contraindication such as allergy to chlorhexidine gluconate. In addition, care must be taken to avoid the use of excess alcohol when diathermics are used.

Conflict of interest: Mrs CT Crosby is employed by Cardinal Health, Professor TSJ Elliott, Professor PA Lambert and Dr D Adams have received educational grants from Enturia Ltd

Adams D, Quayum M, Worthington T, Lambert P, Elliott T (2005) Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant. *J Hosp Infect* **61**(4): 287–90

Bibbo C, Patel DV, Gehrmann RM, Lin SS (2005) Chlorhexidine provides superior skin decontamination in foot and ankle surgery: a prospective randomized study. *Clin Orthop Relat Res* **438**: 204–8

Casey AL, Inrakly A, Clethro A et al (2008) Prospective, randomised clinical trial to compare the efficacy of two 70% (v/v) isopropyl alcohol solutions containing either 0.5% (w/v) chlorhexidine gluconate or 2% (w/v) CHG for skin antisepsis during coronary artery bypass grafting. 18th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona April 19–22 2008. Poster #1538. *Clin Microbiol Infect* **14**(S7): S443–4

Chaiyakunapruk N, Veenstra DL, Lipsky BA, Saint S (2002) Chlorhexidine compared with povidone-iodine solution for vascular catheter site care: a meta-analysis. *Ann Intern Med* **136**: 792–801

Chambers CE, Eisenhauer MD, McNicol LB et al (2006) Infection control guidelines for the cardiac catheterization laboratory : society guidelines revisited. *Catheter Cardiovasc Interv* **67**: 78–86

Department of Health (2007) Clean, safe care. Reducing MRSA and other healthcare associated infections. www.clean-safe-care.nhs.uk/public/default.aspx?level=1&load=HomeNews (accessed 7 October 2008)

Elliott TS, Moss HA, Tebbs SE et al (1997) Novel approach to investigate a source of microbial contamination of central venous catheters. *Eur J Clin Microbiol Infect Dis* **16**: 210–13

Fry DE (2006) The surgical infection prevention project: processes, outcomes, and future impact. *Surg Infect* **7**(S3): S17–S26

Gaw JL (1999) *A Time to Heal: The Diffusion of Listerism in Victorian Britain*. Transactions of the American Philosophical Society. American Philosophical Society: Philadelphia

Health Protection Agency (2005) *Cleaning, Disinfection and Decontamination*. www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947535427 (accessed 7 October 2008)

Health Protection Agency (2006) *Surveillance of Surgical Site Infection in England, October 1997 to September 2005*. HPA: London

Hibbard JS (2005) Analyses comparing the antimicrobial activity and safety of current antiseptic agents: a review. *J Infus Nurs* **28**: 194–207

Jeske C, Raedler C, von Goedecke A et al (2003) Early identification of bacteria leading to central venous catheter contamination. *Anesth Analg* **97**: 940–5

Klenerman L (2002) *The Evolution of Orthopaedic Surgery*. RSM Press: London

Larson E (1988) Guideline for use of topical antimicrobial agents. *Am J Infect Control* **16**: 253–66

Livesley MA, Tebbs SE, Moss HA, Faroqui MH, Lambert PA, Elliott T (1998) Use of pulsed field gel ehrophoresis to determine the source of microbial contamination of central venous catheters. *Eur J Clin Microbiol Infect Dis* **17**: 108–12

Maki DG (1994) Infections caused by intravascular devices used for infusion therapy: pathogenesis, prevention and management. *Infection Assoc with Med Dev* **2**: 155–213

Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR (1999) Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* **20**: 250–78

National Collaborating Centre for Women's and Children's Health (2008) *Surgical Site Infection*. NICE Guideline CG74. NICE: London

O'Grady NP, Alexander M, Dellinger EP et al (2002) Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis* **34**(11): 1281–307

Ostrander RV, Botte MJ, Brage ME (2005) Efficacy of surgical preparation solutions in foot and ankle surgery. *J Bone Joint Surg Am* **87**(5): 980–5

Pratt RJ, Pellowe CM, Wilson JA et al (2007) epic2: national evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. *J Hosp Infect* **64S**: S1–S64

Small H, Adams D, Casey A, Crosby CT, Lambert PA, Elliott T (2008) Efficacy of adding 2% (w/v) chlorhexidine gluconate to 70% (v/v) for skin disinfection prior to peripheral venous catheterisation. *Infect Control Hosp Epidemiol* **29**(10): 963–5

KEY POINTS

- Postoperative infection remains a preventable cause of morbidity and even mortality in the UK.
- Half of all surgical site infections are caused by the endogenous skin bacteria *Staphylococcus aureus*.
- Studies have shown that 2% chlorhexidine in 70% isopropyl alcohol is the most effective skin preparation agent.
- UK surgical skin preparation guidelines have been issued by the National Institute for Health and Clinical Excellence which recommend either povidone-iodine or chlorhexidine.
- UK and US guidelines recommend 2% chlorhexidine/70% isopropyl alcohol for catheter insertion and site maintenance and for prevention of intravascular catheter-related infection respectively. The Health Protection Agency also issued a Recommendation 1 for a 2% chlorhexidine/70% isopropyl alcohol preparation in reducing health-care-associated infection.
- Using 2% chlorhexidine/70% isopropyl alcohol may be an effective way of reducing rates of surgical site infection.