School of Nursing and Midwifery

Comparison of two semi-quantitative wound swabbing techniques to establish the clinical efficacy in identifying the causative organism(s) in infected cutaneous wounds

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Declaration

"This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made".

Signature: ----------

Date: ----------

Abstract

All wounds are contaminated with bacteria; the decision to perform a wound swab is based on the presence of clinical signs and symptoms of infection. In acute wounds these include: pain, erythema, localised oedema, heat and purulence. Patients with chronic wounds may display covert signs of infection such as: an increase in serous exudate, delayed healing, friable granulation tissue, pocketing at the wound base and malodour. A wound swab should only be performed when the wound has been clinical diagnosed as infected. The problem that arises for the clinician is which method of collecting the wound swab should be employed.

The aim of this research was to compare two semi-quantitative wound-swabbing techniques (Levine versus Z technique) to establish which method was more effective in determining the presence of bacteria in cutaneous wounds. The Levine technique involves rotating the wound swab over a 1 cm^2 area of the wound with sufficient pressure to express fluid from within the wound tissue. The Z technique involves rotating the swab between the fingers as the swab is manipulated in a 10-point zigzag fashion across the wound without touching the wound edge or peri wound skin. There is a scarcity of evidence in the literature to support the use of one method over the other, hence the need to undertake the study.

A prospective randomised study of two paired wound swabbing techniques (Levine versus Z technique) was conducted. Two semi-quantitative wound swabs were collected no more than five minutes apart, on each patient. The order of wound swab technique was randomised. All wounds were cleansed with normal saline using an aseptic technique prior to specimen collection. Differences between the detected microbiological bioburden values were analysed with t-test for paired sample.

There were 28 males and 22 females. Acute wounds represented 42% (n=21) of the study population and the remaining 58% (n=29) were chronic wounds. Clinical signs of infection were present in 42% of patients with acute wounds. All patients with chronic wounds had one or more overt clinical sign of infection. A statistical difference in the number of organisms was detected between the two swabbing techniques. The Levine method detected more organisms *(t = 15.46, p = <0.001)*, than the Z technique. There was also a statistically significant difference in the number of organisms detected in acute and chronic wounds. In acute wounds the Levine technique detected more organisms *(t = 9.55, p = <0.001)*. In chronic wounds the Levine technique detected more organisms *(t = 12.04, p <0.001)*.

The Levine method proved to be more sensitive in detecting organisms present in cutaneous wounds in both acute and chronic wounds. Based on the results of this study, the Levine technique is the recommended method for collecting a wound swab.

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Chapter One

Introduction

The aim of this study was to compare two wound-swabbing techniques, to determine which method is more effective in determining the causative organism(s) in infected cutaneous wounds. Initially a wound is identified as infected by the presence of recognised signs and symptoms of infection such as: heat, pain, erythema, oedema and purulent discharge. It is necessary to culture a wound to identify the causative organism(s) and the organisms' sensitivities to antibiotics. The two most commonly used methods of culturing a wound are wound swab and tissue biopsy. Tissue biopsy is not used routinely because it is painful, invasive, expensive, labour intensive, and requires trained personnel to perform the procedure.

A wound swab is a universally accepted method of obtaining a wound culture. However, there is no consensus amongst healthcare practitioners as to which is the best method for obtaining wound swab cultures. Regardless of this lack of consensus, wound swabbing remains the most frequent method of sampling wounds for microbiological analysis. Some practitioners clean the wound prior to taking the sample and some do not, others take the sample from one place on the wound bed, and some wipe the swab all over the wound in an adhoc fashion. Wound swabs that are collected incorrectly can identify bacteria on the wound surface alone and not those that penetrate the soft tissue, giving a false positive result (Kelly, 2003).

Background

Acute wound infection is initially identified by the presence of clinical signs and symptoms of infection such as: pain, erythema, localised

oedema, heat and purulence (Stotts & Hunt, 1997). In chronic wounds clinical signs of covert infection include: serous exudate, delayed healing, discolouration of granulation tissue, pocketing at the wound base, malodour, and friable granulation tissue (Cutting & Harding, 1994; Gardner, Frantz, Bradley, & Dobbeling, 2001; Schultz et al., 2003). Since all wounds are contaminated with bacteria a wound should only be cultured after it has been clinically diagnosed as infected (Dow, 2003; Fleck, 2006; Kingsley, 2003a). The indiscriminate use of antibiotics for all open wounds would raise healthcare costs and potentially add to the development of multi-resistant stains of microorganisms (Kingsley, 2003b). Therefore the accurate identification of the causative organism is essential to guide clinical practise.

Wound infection is defined as "The deposition and multiplication of bacterial in tissue with an associated host reaction" (Ayton, 1985). It is necessary to culture a wound to determine the clinically significant isolates, carry out antimicrobial susceptibility testing, and to guide antimicrobial therapy (Bowler, Duerden, & Armstrong, 2001; Washinton, 1999). Two methods of collecting a wound sample are tissue biopsy and wound swab (Bowler et al., 2001). Tissue biopsy is expensive, invasive, painful, and disrupts the wound from healing (Fleck, 2006). It is also not standard practice in health care settings (Stotts, 1995). In clinical practice, wound swabbing is the most frequently used method of collecting a wound sample, it is simple and inexpensive (Kelly, 2003). However, controversy exists in the literature as to how to carry out this procedure (Bowler et al., 2001; Kelly, 2003). To date there is not a universally accepted method of collecting a wound swab (Bowler et al., 2001; Cooper & Lawrence, 1996; Donovan, 1998; Kingsley, 2003a), despite this, wound swabbing remains the most common method of collecting a wound sample (Kelly, 2003).

Purpose of the Research

The purpose of this research was to compare two semi-quantitative wound-swabbing techniques (Levine versus Z technique) to define which method is more effective in determining the causative organism(s) in infected cutaneous wounds. The 'Levine technique' involves rotating the wound swab over a 1-cm^2 area of the wound with sufficient pressure to express fluid from within the wound tissue (Levine et al., 1976). The 'Z technique' involves rotating the swab between the fingers as the swab is manipulated in 10-point zigzag fashion across the wound without touching the wound edges or peri wound skin (Cooper & Lawrence, 1996; Cuzzell, 1993; Donovan, 1998; Gilchrist, 1996). There is insufficient evidence in the literature to indicate which method is more accurate, consequently the need to undertake the study.

Statement of the Problem

Research Aim.

To compare two wound swabbing techniques (Levine versus Z technique) to establish which method is more effective in determining the causative organism(s) in infected cutaneous wounds.

Significance of the Study.

Untreated or poorly treated wound infections can lead to delayed wound healing, systemic infection, septicaemia and death (Lawrence, 1993; Stotts & Whitney, 1999), as well as being a burden on health care financial resources (Bowler et al., 2001). There is a lack of consensus in the literature as to which is the optimal method of obtaining a wound swab (Ayello, 2004; Bowler et al., 2001; Cooper & Lawrence, 1996; Cuzzell, 1993; Donovan, 1998; Dow, 2003; Kingsley, 2003a). Incorrect specimen

collection can potentially lead to either over diagnosis or under diagnosis of wound infection (Dow, 2003). To date there is not a standardised method of collecting a wound swab (Bowler et al., 2001; Cooper & Lawrence, 1996; Donovan, 1998; Kelly, 2003; Kingsley, 2003a). The significance of this study is that it will add to the body of knowledge and potentially guide clinical practice in relation to the most clinically efficacious technique of wound swabbing, for the collection of microbiological specimens in infected cutaneous wounds. This will therefore improve wound care by measuring the effectiveness of microbiological wound specimen collection.

Key Definitions

Acute wounds: is any surgical wound that heals by primary intention, or any traumatic or surgical wound that heals by secondary intention, and which proceeds through an orderly and timely reparative process that results in sustained restoration of anatomical integrity (Carville, 2005).

Biofilm: Bacteria attach to the wound surface and may enchase themselves in a gelatinous matrix, which provides protection against antimicrobial agents and the immune system. Biofilms may contain several species of bacteria (Carville et al., 2008).

Chronic wounds: occurs when the reparative process does not proceed through an orderly and timely process as anticipated and healing is complicated and delayed by intrinsic and extrinsic factors that impact on the person, the wound or the environment (Lazarus et al., 1994).

Colonisation: the presence of replicating microorganisms, there is no host reaction and healing is not delayed (Schultz et al., 2003).

Contamination: the presence of bacteria on the wound bed, that do not replicate or cause clinical problems (Dow, Browne, & Sibbald, 1999).

Covert infection: wounds particularly chronic wounds, bacteria may cause a problems, eg delayed or (stalled) healing, in the absence of such obvious indicators of inflammation (Carville et al., 2008).

Critical colonisation: increased bacterial burden, or local infection on the wound bed, occurs when there is replication of bacteria, without overt clinical signs of infection such as; pain, heat swelling, erythema and purulence, causing a delay in healing but not invading the soft tissue

(Cutting & White, 2005; Dow, 2003; Sibbald, Orsted, Schultz, Coutts, & Keast, 2003).

Overt infection: wounds displaying the classical signs and symptoms of inflammation - pain, heat, swelling, redness and loss of function (Carville et al., 2008).

Quantitative bacteriology: analysis of wound specimens that identifies the species of bacteria present and virulence, expressed in number of organisms per gram of tissue (Bowler et al., 2001; Robson & Heggers, 1969; Sibbald et al., 2003).

Semi-quantitative bacteriology: analysis of wound specimens identifies the species of organisms present and virulence. The number of bacteria present is estimated, and expressed as scant, small, moderate or abundant. This method does not provide quantitative information regarding bacteria per gram of tissue (Bowler et al., 2001; Miller, 1998; Stotts & Whitney, 1999).

Chapter Two

Literature review

Wound types

Wounds are broadly classified as being either acute or chronic. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts, abrasions, lacerations and those caused by crush or gun shot injuries (Flanagan, 1998). In contrast, chronic wounds are usually caused by endogenous mechanisms associated with predisposing conditions that compromise the integrity of the dermis and epidermis. Pathophysiological conditions such as arterial disease, venous disease, malignancy, and diabetes mellitus may predispose an individual to the development of chronic wounds such as leg ulcers, foot ulcers, malignant wounds, and pressure ulcers.

Advancing age, obesity, smoking, poor nutrition, and immunosuppression, or certain medications such as chemotherapy and non-steroidal antiinflammatory agents, may also exacerbate chronicity of a wound (Bowler et al., 2001; Flanagan, 1998).

Assuming a relatively healthy host, acute wounds heals within a predictable time frame, progressing through a reparative process of coagulation, inflammation, proliferation or regeneration, maturation, and re-modelling (Enoch & Harding, 2003; Schultz et al., 2003; Sibbald et al., 2003). The variance in the time frame occurs according to the type, anatomical location and the depth of the wound (Bowler et al., 2001). Chronic wounds do not heal within a predictable time frame. The normal reparative process of wound healing is disrupted at one or more points in the stages of wound healing (Enoch & Harding, 2003). Usually as a result of

endogenous factors associated with the person's underlying condition (Sibbald et al., 2003).

Wound microbiology

As early as 2000 BC medical practitioners incorporated wound management principles such as wound cleansing with agents such as; hypertonic substances (honey), antiseptic agents (malachite and vinegar), and topical oils, which facilitated reducing the bacterial burden in a wound. Bacteria were not described until 1692, by Antony van Leeuwenhoek. Ignatz Semmelweis utilised principles of antisepsis to reduce puerperal fever mortality, prior to the founding of modern bacteriology by Louis Pasteur and Robert Koch. Clinical practise was changed when Joseph Lister introduced the use of antiseptics during surgery. The next major advancement was in the 1940s with the discovery of sulfonamides and penicillin (Dow et al., 1999).

In recent times our knowledge of bacteriology and wound healing and microbial virulence has increased. Never the less, there is inconsistency in wound specimen collection and culture techniques. This has led to haphazard topical and systemic antibiotic prescribing (Mollering, 1998). The antibiotic era led to prolific antimicrobial resistance of organisms such as methicillin-resistant *staphylococcus aures* (MRSA) and vancomycin resistant *enterococcus* (VRE) (Mollering, 1998). There are new fears of infectious disease mortality with the recent discovery of glycopeptide intermediate sensitivity *Staphylococcus aureus* (GISA) (Smith, Pearson, Wilcox, & et, 1999). It is important issues such as these that have increased the demand on health care providers for consistent methods of diagnosis of wound infection.

Optimal diagnosis and management of wound infection is crucial if healing is to be promoted and associated morbidity and mortality reduced (Edwards & Harding, 2004). All open wounds are colonised with bacteria,

and the progression of wound healing can still occur in their presence (Edwards & Harding, 2004; Enoch & Harding, 2003; Schultz et al., 2003). According to Bowler et al. (2001) wound infection occurs when:

> Virulence factors expressed by one or more micro-organisms in a wound out compete the host natural immune system and subsequent invasion and dissemination of micro-organisms in viable tissue provokes a series of local and systemic host responses. (p. 247)

Thus it is the interaction between the host and the bacteria that will determine the organisms' influence on wound healing, not the presence of bacterial alone.

Bacterial involvement in a wound can be defined in four ways; contamination, colonisation, critical colonisation and wound infection (Edwards & Harding, 2004; Enoch & Harding, 2003; Schultz et al., 2003; Sibbald et al., 2003).

Contamination.

Contamination is the presence of non-replicating organisms within a wound (Bowler et al., 2001; Dow et al., 1999; Sibbald et al., 2003). Virtually from the time of wounding all wounds are contaminated with bacteria. The majority of organisms entering the wound fall into this category and are incapable of replicating in soft human tissue. These microorganisms originate from the external environment (exogenous contamination) such as airborne transmission introduced following a traumatic injury, or contamination of an open wound with soil. Many of the organisms within soil do not replicate in soft tissue and are promptly cleared by the hosts immune system (Dow et al., 1999). Likewise contamination from endogenous sources such as the gastrointestinal, oropharyngeal and genitourinary mucosae are incapable of replicating in soft tissue (Bowler et al., 2001; Durden, 1994; Sibbald et al., 2003).

Usually the host defences, if not compromised will swiftly act to phagocytose bacteria (Enoch & Harding, 2003). Within the chronic wound environment organisms may establish large colonies. Chronic wounds become contaminated from endogenous secretions and exogenous sources such as poor hand hygiene practised by health care providers, or via environment exposure (Sibbald et al., 2003). In the majority of instances wound contaminants far out number pathogenic bacteria. However, most of these organisms are not readily cultured with routine culture techniques, hence the under reporting of their presence in the literature or pathology reports (Barer, 1997).

Colonisation.

Colonisation is the presence of replicating microorganisms that adhere to the wound surface without a host reaction and healing is not delayed (Dow et al., 1999; Enoch & Harding, 2003). Larger or chronic wounds, because of the prolonged exposure of wound tissue increase the risk colonisation by a wide variety of microorganisms (Bowler et al., 2001). Colonising organisms are usually commensal skin flora such as *Staphylococcus epidermis, Corynebacterium* species (diptheroids), and low levels of *Staphylococcus aureus* species (DeHaan, Ellis, & Wilkes, 1974; Marks, Harding, & Hughes, 1987; Pollack, 1984).

Commensal organisms rather than cause harm to the host, have been shown to enhance wound healing (DeHaan et al., 1974; Pollack, 1984; Rodeheaver, Smith, Thacker, Edgerton, & Edlich, 1975). These organisms stimulate an inflammatory response, increasing, monocytes, macrophages, collagen hydroxpryproline, granulation tissue and blood flow (DeHaan et al., 1974; Schultz et al., 2003; Stone, 1980).

A reasonable large number of studies have analysed the microbiology of clinically non-infected acute and chronic wounds (Bowler, 1998; Bowler & Davies, 1999a, 1999b; Brook, 1987, 1989a, 1989b, 1989c, 1991, 1995;

Brook & Finegold, 1981; Brook & Frazier, 1990, 1997, 1998a, 1998b; Di Rosa, Di Rosa, & Panichi, 1994; Gilchrist & Reed, 1989; Hansson, Hoborn, Moller, & Swanbeck, 1995; Louie, Bartlett, Tally, & Gorbach, 1976; Mousa, 1997; Pathare, Bal, Talvalkar, & Antani, 1998; Sapico et al., 1986; Summanen et al., 1995). None of these studies investigated the effect of bacteria on wound healing. However of significance they did report anaerobes, which are often under reported in the literature. Table 1 provides an overview of the studies that provided detailed microbiological analysis of microorganisms in clinically non-infected wounds (colonised wounds), anaerobes accounted for 38% of microbial isolates.

The opinion of many wound care practitioners is that aerobic or facultative pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Beta-hemolytic streptococci* are principally responsible for delayed healing and infection in acute and chronic wounds (Bowler et al., 2001; Sibbald et al., 2003). This opinion is formulated on studies undertaken in the last two decades in which the identification of anaerobic bacteria was either negligible or absent (Daltrey, Rhodes, & Chattwood, 1981; Danielsen et al., 1998; Gilland, Nathwani, Dore, & Lewis, 1988; Halbert, Stacey, Rohr, & Jopp-McKay, 1992; MacFarlane, Baum, & Serjeant, 1986; Pal'tsyn et al., 1996; Schraibman, 1990; Sehgal & Arunkumar, 1992; Twum-Danso et al., 1992). Culture, isolation, and identification of anaerobic bacteria are considered to be time-consuming, labour intensive, and expensive, and this may in part explain the under reporting of anaerobes. Another reason for the under reporting of anaerobes is that some clinicians do not consider anaerobes detrimental to wound healing (Eriksson, Eklund, & Kallings, 1984; Gilchrist & Reed, 1989; Majewski et al., 1995; Sehgal & Arunkumar, 1992).

A wide diversity of aerobic and anaerobic organisms contaminate and colonise acute and chronic wounds. Bowler & Davies (1999a) their work cultured 367 isolates from 61 acute wounds and 45 chronic wounds, as illustrated by Table 2 .

Table 1 Studies involving detailed analysis of the aerobic and anaerobic microbiology of non-infected wounds without specifically investigating the role of microorganisms in wound healing.

Adapted from Bowler, Duerden, & Armstrong (2001).

Author	Study description	No. of microbial isolates (% that were anaerobes)	Predominant isolates
Louie, Bartlett, Tally, Gorbach (1976)	Microbiology of 20 diabetic ulcers	116 (45)	*Bacteriodies* spp., *Peptostreptococcus* spp., *Proteus* spp.
Brook & Finegold (1981)	392 specimens taken from burn sites in 180 children	580 (23)	S. *epidermidis*, S. *aureus*, alpha-hemolytic streptococci, *Propionibacterium acnes*, *Peptostreptocuccus* spp., *Bacteroides* spp.
Sapico et al (1986)	49 specimens from 25 pressure sores	130 (24)	*Bacteroides* spp., coliforms, *P.aeruginosa*
Brook (1987)	Aspirates from human and animal bite wounds in 39 children	59 from animal bites (37); 97 from human bites (55)	*Peptostreptococcus* spp., *Peptostreptococcus* spp., *Bacteroides* spp. (in both wound groups)
Brook & Frazer (1990)	Analysis of decubitus ulcers in 58 children	1,470 (62)	*Bacteroides* Spp., *Peptostreptococcus* spp., S. *Aureus, Clostridium* Spp., *Fusobacterium* Spp.
Brook (1991)	Analysis of decubitus ulcers in 58 children	132 (40)	*Peptostreptococcus* spp., S. *Aureus, B. Fragilis* group, *P. aeruginosa*
Hansson, Hoborn, Moller, Swanbeck. (1995)	Analysis of leg ulcers without clinical signs of infection in 58 patients	325 (22)	S. *aureus, Enterococcus faecalis, Enterobacter cloacae, Peptostreptococcus magnus*
Brook & Frazier (1998a)	43 swab specimens from chronic leg ulcers in 41 patients	97 (34)	S. *aureus, Peptostreptococcus* spp., *B. fragilis* group
Bowler & Davies (1999a)	Swab specimens from 30 noninfected leg ulcers without clinical signs of infection	110 (36)	S. *aureus*, coliforms, coagulase-negative staphylcocci, fecal streptococci, *Pepto-streptococcus* spp.

Table 2 Aerobic and anaerobic isolates from acute and chronic wounds of varied aetiology

Acute wounds (a) comprise of cutaneous abscess, post surgical wounds; chronic wounds (C) included primarily leg ulcers, foot ulcers and pressure ulcers

Adapted from Bowler & Davies (1999a).

Aerobic and facultative microorganisms	**Type of wound**	**Anaerobic bacteria**	**Type of wound**
Coagulase-negative staphylococci	A, C	*Peptrostreptococcus asaccharolyticus*	A, C
Mirococcus sp.	C	*Peptrostreptococcus anaerobius*	A, C
Staphylococcus aureus	A, C	*Peptrostreptococcus magnus*	A, C
Beta-hemolytic streptococcus (group C)	A	*Peptrostreptococcus micros*	A, C
Beta-hemolytic streptococcus (group G)	C	*Peptrostreptococcus prevotii*	A, C
Streptococcus spp. (fecal)	A, C	*Peptrostreptococcus indolicus*	C
Streptococcus spp. (viridans)	A, C	*Peptrostreptococcus* sp.	A, C
Corynebacterium xerosis	C	*Streptococcus intermedius*	C
Corynebacterium spp.	A, C	*Clostridium perfingens*	A, C
Bacillus spp.	A	*Clostridium clostridioforme*	A, C
Escherichia hermanii	A, C	*Clostridium cadaveris*	A, C
Serratia liquefaciens	A	*Clostridium baratii*	C
Klebsiella pneumoniae	C	*Clostridium septicum*	A
Klebsiella oxytoca	A, C	*Clostridium histolyticum*	A, C
Enterobacter cloacae	A, C	*Clostridium tertium*	A
Enterobacter aerogenes	A, C	*Clostridium ramosum*	C
Citobacter freundii	C	*Clostridium sporogenes*	A, C
Proteus mirabilis	C	*Clostridium difficile*	C
Proteus vulgaris	A, C	*Clostridium bifermentans*	A
Providencia stuartii	C	*Clostridium limosum*	A
Morganella morganii	A	*Eubacterium limosum*	C
Acineobacter calcoaceticus	C	*Propionibacterium acnes*	A, C
Pseudomonas aeruginosa	A, C	*Bacteroides fragilis*	A, C
Stenotrophomonas maltophilia	A, C	*Bacteroides ureolyticus*	A, C

Aerobic and facultative microorganisms	Type of wound	Anaerobic bacteria	Type of wound
Sphingobacterium multivorum	A	*Bacteroides ovatus*	A
Candida parapsilosis	C	*Bacteroides uniformis*	A, C
Candida krusei	A	*Bacteroides stercoris*	C
	A	*Bacteroides capillosus*	C
		Bacteroides thetaiotamicron	C
		Bacteroides caccae	C
		Prevotella oralis	A, C
		Prevotella oris	A, C
		Prevotella disiens	A
		Prevotella bivia	C
		Prevotella buccae	C
		Prevotella Prevotella sp.	A
		Prevotella corporis	A, C
		Prevotella intermedia	A
		Prevotella melaninogenica	C
		Porphyromonas asaccarolytica	A, C
		Gram-negative pigmented bacillus	A, C
		Fusobacterium necrophprum	C
		Veillonella	A

Critical Colonisation.

Critical colonisation is postulated to be an intermediate phase between colonisation and infection. There is an increase in the bacterial burden within the wound without overt signs of infection, causing a delay in wound healing, but not invading the soft tissue (Enoch & Harding, 2003). The concept first came to light when Davis (1998) treated two patients with non-infected, non-healing venous leg ulcers with a topical anti-microbial agent, both responded to treatment. The presumption from this report was that there is a transitional stage between colonisation and infection (European Wound Management Association, 2005).

The term ‘critical colonisation’ was introduced into the wound management literature in 2001, following two studies conducted by

Browne, Vearncombe & Sibbald, and Sibbald, Browne, Coutts & Queen, in 2001 (Browne, Vearncombe, & Sibbald, 2001; Sibbald, Browne, Coutts, & Queen, 2001), and the terminology and concept remains a contentious issue. In the first study eight patients with chronic non-infected diabetic foot ulcers (mean duration 11 months), had a 3 mm tissue biopsy. Patients with necrotic tissue, osteomyelitis, or had evidence of cellulitis were excluded from the study. Despite the absence of clinical signs of infection, 75% had greater or equal to 10^5 colony forming units/gram organisms present in the wound. The authors concluded that increased bacterial burden or critical colonisation hinders wound healing, despite the lack of overt signs of infection (Browne et al., 2001).

In the second study 29 patents with a variety of chronic non-healing wounds were treated with ionised nanocrystalline silver. Prior to application, bacterial activity was measured by semi-quantitative wound swab analysis and quantitative biopsy. Patients with systemic infections including osteomyelitis required treatment with appropriate systemic antimicrobial therapy. Patients with increased bacterial burden or critical colonisation localised to the dermal compartment, treated with the application of the topical antibacterial agent (nanocrystalline silver) demonstrated an improvement in wounds healing, as well as reduction in pain, in these otherwise non-healing wounds (Sibbald et al., 2001).

In the clinical setting, diagnosing critical colonisation rather than infection can prove challenging. Sibbald et al. (2003) elaborate “the host is harmed enough to impede healing but not enough to cause typical inflammatory symptoms”. Delayed healing, pain/tenderness, increased serous exudate, change in the colour of the wound bed, friable granulation tissue, absent or abnormal granulation tissue and malodour are potential signs of critical colonisation (Enoch & Harding, 2003; Gardner et al., 2001; Sibbald et al., 2003). There is a lack of large-scale longitudinal studies to validate that these signs are an intermediary phase between colonisation

and infection, or related to constant chronic inflammation (European Wound Management Association, 2005).

The host-pathogen interaction, associated with critical colonisation in studies published to date, is conflicting (Browne et al., 2001; Davis, 1998; Fumal, Braham, Paquet, & al., 2002; Jørgensen, Price, Anderson, & al., 2005; Sibbald et al., 2001). Resolution of a wound with subsequent antimicrobial intervention indicates that microbial involvement and host involvement was present, and suggests a possible transition between colonisation (host is unaffected) towards overt infection (host injury occurs) (Ayton, 1985; Bowler et al., 2001; Davis, 1998; Kingsley, 2001; Sibbald, Williamson, Orsted, & al., 2000).

A definite diagnosis of critical colonisation is further compromised by the presence of biofilms. It is well documented that biofilms form on catheters, medical implant devices, endoscopic equipment (Kolter & Losick, 1998) and are difficult to remove. It has been proposed that biofilms also form on the wound bed (Wysocki, 2002). The wound bed may be healthy in appearance while playing host to colonies of bacteria (Wysocki, 2002). Attached to the wound bed are replicating bacteria which secrete a extracellular polymeric substance (a polysaccaride that holds the community of organisms together) and which provides protection against antimicrobial agents such as antibiotics and antiseptics (Association for the Advancement of Wound Care., 2008; Enoch & Harding, 2003; Percival & Cutting, 2009; Sibbald et al., 2003) and host defence mechanisms (Costerton & Stewart, 2001; Costerton et al., 2003; Marion et al., 2006). Biofilm colonies undergo genetic mutation to alter their sensitivity to antibacterial agents. Single viable bacterial cells are released intermittently (planktonic or free floating bacteria) from the biofilms onto the wound bed forming new colonies, leading to local infection or weakening of the collagen matrix in healed wounds, and can also cause breakdown of healed tissue. (Costerton, Stewart, & Greenberg, 1999; Kolter & Losick, 1998; Potera, 1999).

Biofilms are difficult to detect, suppress and remove. In addition they contribute to delayed wound healing (Davey & O'Toole, 2000; Rhoads, Wolcott, & Percical, 2008). Management strategies to reduces biofilms include sharp debridement of the wound bed; antimicrobial dressings that have 'antibiofilm' properties (although not currently available in Australia), and systemic antibiotics when there is a significant wound infection (Percival & Cutting, 2009; Rhoads et al., 2008).

Certain non-infectious conditions such as pyoderma gangrenosum and vasculitis can further complicate the diagnosis of critical colonisation. These disorders produce an inflammatory response in the absence of infection (Sibbald et al., 2003). These inflammatory conditions produce persistent inflammation with associated swelling, redness and elevated temperature, unless there is systemic signs of infection that recruits cellular infiltrates and inflammatory mediators, then critical colonisation should be excluded (Sibbald et al., 2003).

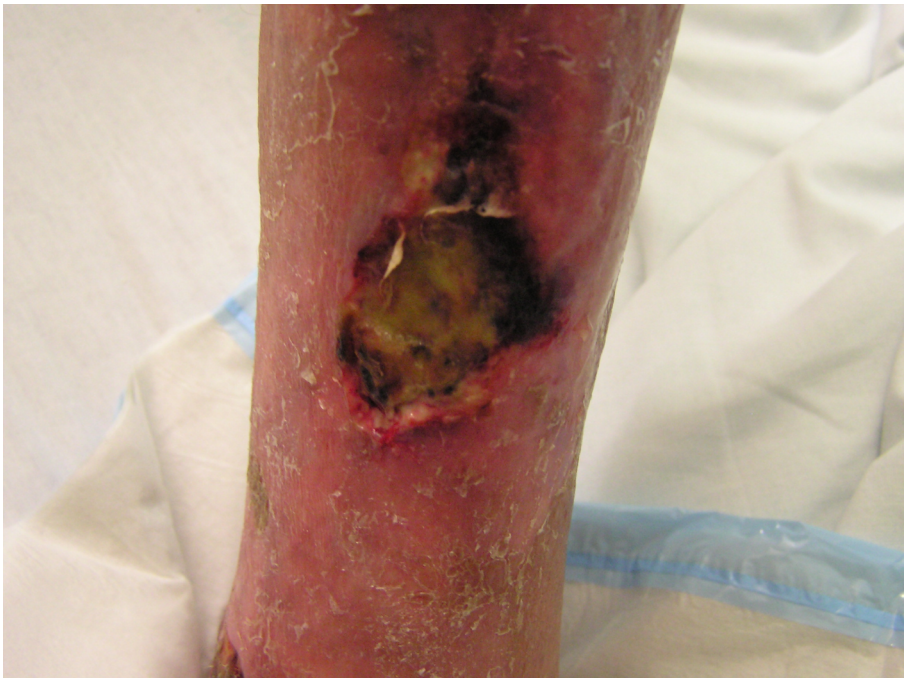

Figure 1. Pyoderma gangrenousum, producing an inflammatory response in the absence of infection

Wound Infection.

Wound infection occurs when there is replication of one or more microorganisms in a wound, which provokes a series of local and systemic host responses that leads to a delay in wound healing (Bowler et al., 2001; Heggers, 1998; Sibbald et al., 2003). Typical features are cellulitis (spreading erythema) and purulent exudate (Peel, 1992). The time it takes for wound to convert to colonised to an infected wound is difficult even for the expert clinician to determine at the bedside. A wound can be initially colonised with pathogenic bacteria without inducing host injury, as the bacteria replicate covert infection results (Thompson & Smith, 1994). A covert wound infection may not entail significant tissue invasion, yet will include sufficient injury at the wound-host interface to impair wound healing. Ultimately the opportunistic pathogenic bacteria replicate further with local invasion of the soft tissue (overt wound infection), or systemic dissemination (sepsis) (Dow et al., 1999).

Wound infection is directly related to the host's ability to resist infection, the number and virulence of the organisms. Elek (1956) described infection as:

$$\text{Infection} = \frac{\text{number x virulence}}{\text{Host resistance}}$$

Of prevailing importance in this equation is the host's resistance to the bacteria present in a wound, and the virulence of the organisms' present (Sibbald et al., 2000).

Pathogenesis of wound infection

Physiological responses to microbial pathogens varies greatly in acute and chronic wounds. In the acute wound environment, the invasion

of pathogenic pathogens stimulates the host's immune system and provokes an inflammatory response. This is initiated by the release of cytokines and growth factors. This initial inflammatory reaction produces vasodilatation and an increase in blood flow to the area of injury (Dow et al., 1999; Stotts, 2000). Increased vascular permeability permits the entrance of neutrophils and macrophages to phagocytose the invading organisms. Lymphocytes release lymphokines that recruit neutrophils and monocytes to the area of injury. There is an increase in exudate due to the increase in permeability and number of cells recruited to the area. Antibodies are activated if the individual has had previous exposure to the same organism. Collectively they remove microorganisms, foreign debris, bacterial toxins, and enzymes. The initiation of the coagulation cascade activates and contains the inflammatory response, by isolating the site of infection within a gel-like matrix, protecting the host (Dow et al., 1999; Stotts, 2000). The molecular and cellular actions are expressed by the classical signs of inflammation: rubor (erthema due to vasodilatation), callor (due to increased temperature releated to enchanced metabolic activity and blood flow), tumor (swelling due to increased vessel permeability), and dolor (pain as a result of cytokine mediated stimulation of nociceptive nerve fibres) (Dow et al., 1999).

In chronic wounds the continuous presence of virulent microorganisms can lead to host injury by prolonging the inflammatory response. There is a persistent production of inflammatory mediators such as prostaglandin E2 and thromboxane together with a steady influx of neutrophils, which release cytolytic enzymes and free oxygen radicals (Laato, Niinikoski, Lundberg, & et, 1988). Vasoconstricting metabolites and localised thrombosis can lead to further replication of pathogens and tissue destruction. The increase in bacterial numbers can cause depletion of complement and platelets (Fearon, Ruddy, Schur, & al., 1975).

There can also be an adaptive down regulation of the immune response in chronic wounds that are infected. This prevents microbial

clearance and reduces auto injury. Chronically infected wounds can also give the false impression that host destruction is not occurring because of the relative immune tolerance (Dow et al., 1999). Experimental surveillance of infected wounds has demonstrated that granulation tissue is inclined to be: fragile, bleed easily, and oedematous as compared to non-infected wounds (Bucknall, 1980). These features appear to be related to over exuberant small vessel angiogenesis, whilst larger vessels have shown occlusion with thrombus formation and reduced wound perfusion. There is a reduction in the number of fibroblasts and collagen is produced in disorganized patterns (Robson, 1988). There is an increased production of collagen and hydroxyproline suggesting increased collagenolytic activity in infected wounds (Dow et al., 1999).

In summary chronic wound infection is related to wound hypoxeamia resulting from large vessel occlusion, fragile granulation tissue linked to proliferation of small vessels, and reduced fibroblast numbers with disorganised collagen production. This results in impaired granulation tissue and reduced tensile strength (Robson, Stenberg, & Heggers, 1990).

Diagnosis of wound infection

In acute wounds it is recognised that the invasion of bacteria into the surrounding tissue provokes a sequence of systemic and local host responses. Left untreated sepsis, multi-organ failure and death can occur (Dow et al., 1999; Enoch & Harding, 2003). Overt signs of infection include cellulitis (erythema and local heat), pain, swelling, and purulent discharge (see Figure 2) (Bowler, 2003; Carville et al., 2008; Schultz et al., 2003; Stotts & Whitney, 1999). Cutting and Harding (1994), identified additional criteria for identifying infection in open wounds including; delayed healing (compared with normal rate for site/type of wound), discolouration, friable granulation tissue which bleeds easily, unexpected pain/tenderness, pocketing at base of wound, bridging at base of wound, abnormal smell,

and wound breakdown. Systemic signs include; fever, chills, rigours, hypotension, and multiple organ failure (Sibbald et al., 2003).

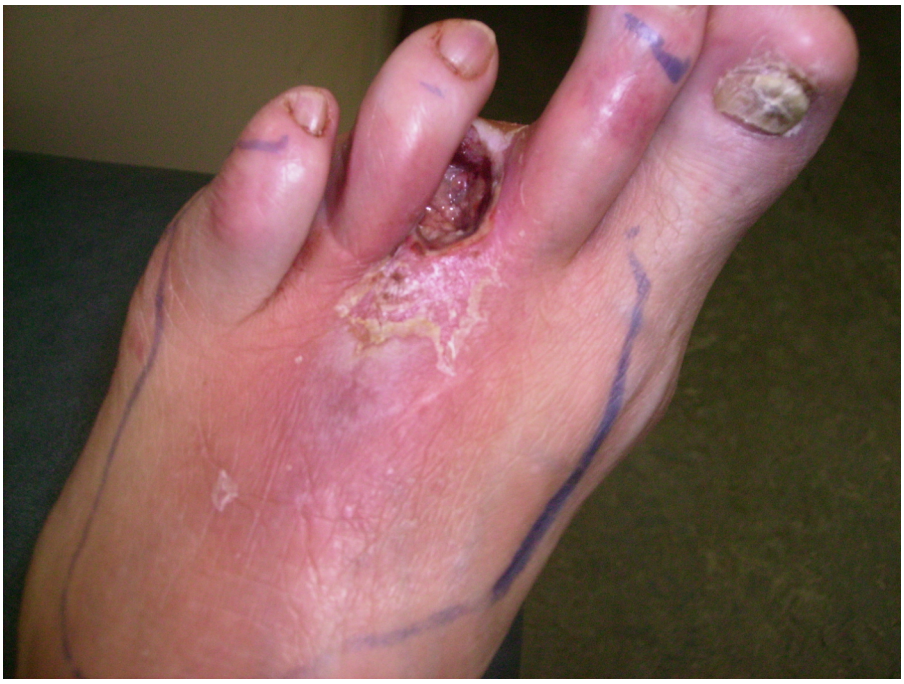

Figure 2. The presence of erythema indicating the presence of deep tissue infection such as cellulitis that may be limb threatening

Frequently failure of the wound to heal, with progressive deterioration are features common to chronic wound infections (see Figure 3) (Carville et al., 2008; Dow et al., 1999; Schultz et al., 2003).

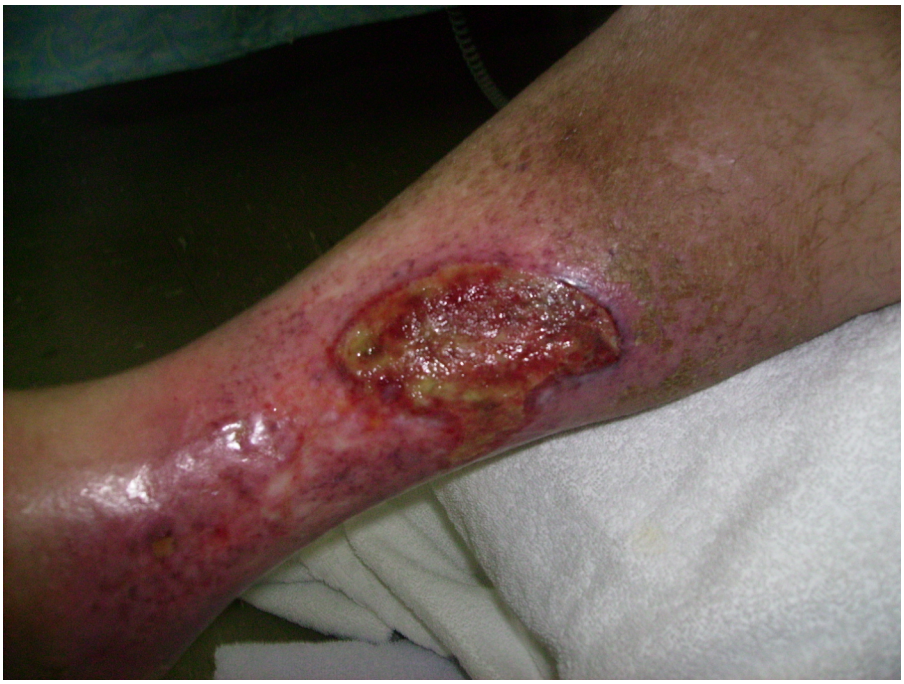

Figure 3. In chronic wounds with delayed healing, failure of wound healing and progressive deterioration is a common feature of wound infection

Gardner, Frantz, Bradely, & Doebbeling (2001) validated; pain, increased wound size, odour, and new areas of wound breakdown as indicators of infection in chronic wounds. In their study five registered nurses subjectively assessed 36 chronic wounds. The wounds were then quantitatively cultured, by tissue biopsy. Results demonstrated a high correlation with $>10^5$ colony-forming organisms of bacteria per gram of tissue, with the subjectively identified signs of infection. Nonetheless, the difficulty in assessing all the contributing factors in chronic wounds hampers the clinical diagnosis of infection (Schultz et al., 2003).

Significance of microorganisms in wounds

The consequence of bacterial quantity and ensuing wound infection was first described by Elek (1956). Even with large inocula of *Staph aureus* (from nasopharyngeal or abscesses), injected into young volunteers, Elek

demonstrated that unless inoculates were greater that 7.5 x 10^6 overt infection did not occur. The presence of a foreign body was the only identified factor that reduced the infective dose. In the clinical setting Kass (1956) was the first to prove quantitation of bacterial burden to diagnose wound infection, in his work with urinary tract infections. Bendy et al. (1964) reported that healing was delayed in pressure ulcers if the bacterial burden was greater that 10^6 colony forming units (CFU)/mL of wound fluid. Their work demonstrated that significant numbers of bacteria were required to produce enough toxins and proteolytic enzymes to cause damage to living tissue. When analysing skin graft survival Krizek, Robson, and Kho (1967) established that the procedure would fail if there were greater than 10^5 bacteria on the wound bed. Successful delayed wound closures were related to wound beds with fewer that 10^5 or less bacteria per gram of tissue in Robson, Lea, Dalton and Heggers (1968) study.

Pathogen characteristics

Authors have suggested that in both acute and chronic wounds it is the bacterial species, not the number of organisms present that is significant (Dow et al., 1999). Almroth Wright, Alexander Fleming, and Leonard Colebrook (1918) were the first to report that regardless of the quantity of organisms, surgical wounds could not be successfully closed if a haemolytic *Streptococcus pyogenes* strain was present. Robson and Heggers (1970) identified *Beta-hemolytic streptococcus* as the only pathogen at levels less than 10^5 CFU/g tissue capable of causing infection.

Since then other pathogens have also been identified as impairing wound healing and causing infection regardless of quantity such as; Mycobacteria (*M tuberculosis, M leprae,* and atypical mycobacteria), *bacillus anthracis, Yersina pestis, Corynebacterium diphtheriae* (toxic producing), *Erysipelothrix sp., Leptospira sp., Treponema sp., Brucella sp.*, highly adapted cutaneous viruses (chronic infections with Herpes Zoster or Herpes Simplex), invasive dimorphic fungi (*Histoplasma sp.,*

Blastomyces sp., Coccidioides immitis), and some parasitic organisms (leishmaniasis) (Dow et al., 1999).

Gram-positive organisms are usually present in higher numbers in infected wounds that have been present for less than a month. For example in acute wounds such as traumatic, surgical or burn wounds *S. aureus* is considered to be the main culprit in causing wound infection (Bowler, 1998; Haneke, 1997; Klimek, 1985; Mayhall, 1993; Nichols & Smith, 1994; Page & Beattie, 1992).

Chronic wounds or those that have been present for longer that one-month are likely to be polymicrobial in nature, including, Gram-negatives and anaerobes in addition to Gram-positive bacteria (Dow et al., 1999). *S. aureus, P. aerginosa,* and beta haemolytic streptococci are the most commonly cited pathogens causing delayed healing and wound infection in chronic wounds (Brook, 1996; Daltrey et al., 1981; Danielsen et al., 1998; Gilland et al., 1988; Halbert et al., 1992; MacFarlane et al., 1986; Madsen, Westh, Danielsen, & Roshahl, 1996; Schraibman, 1990; Sehgal & Arunkumar, 1992; Twum-Danso et al., 1992).

Despite polymicrobial wounds being colonised with organisms such as *S. aureus, P. aeruginosa,* and beta-hemolytic streptococci it still needs to be substantiated that a correlation exists between these organisms and wound infection (Bowler & Davies, 1999a, 1999b; Hansson et al., 1995). This could only be confirmed if they existed within the wound environment as monomicrobial flora. *S. aureus* was identified as the only organism present 24 to 29% in two studies concerning cutaneous abscesses (Brook & Finegold, 1981; Meislin et al., 1977). In the case of necrotising fasciitis *S. pyogenes* and *S. aureus* have been reported as the sole isolate (Regev, Weinberger, Fishman, Samra, & Pitlik, 1998). Single isolates have also been reported from animal bite wound infections (Dutta, 1998).

Specific pathogens were identified in studies conducted by Danielsen et al (1998) and Orenstien et al (1997) as the cause of wound infection in pressure ulcers and burn wound. However, it is not clear if they are present as mono, or polymicrobial organisms. Selective culture media was used in other studies which can bias the results (Rastegar Lari, Bahrami Honar, & Alaghehbandan, 1998; Schraibman, 1990). Other authors have demonstrated that resident microflora does not affect wound healing making the role of specific organisms causing wound infections difficult to determine (Annoni, Rosina, Chiurazzi, & Ceva, 1989; Eriksson et al., 1984; Handfield-Jones, Grattan, Simpson, & Kennedy, 1988; Hansson et al., 1995; Sapico et al., 1986).

Diagnosing infection should be based on overt clinical signs of infection, such as heat, pain, swelling, suppuration, erythema, and fever. Reliance on microbiological results alone can be misleading, particularly for polymicrobical infections. It is not possible to differentiate between the pathogenic and non-pathogenic species. However, diabetic foot infections do not display classic clinical signs of infection, this is related to microvascular changes and peripheral neuropathy. In this instance importance should be given to microbiology results (Bowler et al., 2001).

Host resistance

Host resistance must be considered in every individual with a wound. A wound infection occurs when the host's immune system is compromised by the virulence of organisms present within a wound (Bowler et al., 2001; Dow et al., 1999). As discussed previously all open wounds are either contaminated or colonised with bacteria, but this does not always result in wound infection. The symbiotic relationship between the host and the colonising microorganisms becomes pathogenic when the hosts immune system is compromised (Bowler, 2003). This can occur through a number of mechanisms, such as bacteria interacting with each other to take advantage of the host, different species of bacteria communicating with

each other and dictate the virulence of the organisms, or the bacteria's ability to increase the production of toxins, producing cell adhesion and cell protecting components (biofilms) (Bowler, 2003).

In chronic wounds local and systemic factors are taken into account when assessing host resistance and the likelihood of wound infection (Dow et al., 1999) and are summarised in Table 3. Local factors include the size, depth, and duration of the wound. There is greater host impairment associated with larger wounds, increasing the risk of infection. There is a high correlation of infection between wounds that are over bony prominences or those that probe to bone with osteomyelitis. The exposure of or probing to bone in individuals with diabetes as a clinical sign of infection was substantiated as a useful bedside tool in research undertaken by Grayson, Gibbons, Balough, & Karchmer (1995). The authors concluded that there was a sensitivity of 66%; specificity 85%; positive predictive value 89% and negative predictive value 56% for osteomyelitis when bone is exposed or can be probed. Wounds over the lower extremities or with less tissue perfusion are more prone to infection than proximal wounds or those with good blood flow. For example scalp wounds are less likely to become infected than those in the lower extremity (Dow et al., 1999).

A crucial component of assessing host resistance is vascular assessment. Inadequate arterial pressure alters the hosts' response to infection (Dow et al., 1999). A wound with reduced arterial perfusion will not display the 'classic' feature of inflammation, even in the presence of an aggressive infection. In this instance the threshold to assess for infection using semi-quantitative bacteriology should be lowered.

The presence of necrotic tissue and foreign materials such as dressing fragments, also have the potential to decrease the host's resistance and reduce the number or virulence of the bacteria required to potentiate infection (Sibbald et al., 2003)

Systemic host factors that determine the presence of infection are multifactorial and encompass many variables. Malnutrition, depressive illness, poorly controlled blood sugar levels, smoking and alcohol abuse all reduce the host resistance and increase the probability of infection (Dow et al., 1999; Schultz et al., 2003). Individuals with right sided heart failure and chronic leg ulcers have an increased risk of infection. Odeama reduces lymphatic flow and subsequently increases the risk of gram-positive infection. The threshold to use semi-quantitatve bacteriology must be lowered with the use of cytotoxic agents and corticosteroids, as these agents can completely mask all local and systemic signs of infection (Dow et al., 1999; Schultz et al., 2003).

Table 3 Local and systemic factors that increase infection risk in chronic wounds

Adapted from Dow et al. (1999).

Local factors	Systemic factors
Large wound area	Vascular disease
Increased wound depth (subdermal)	Oedema
Degree of chronicity	Malnutrition
Anatomic location (distal extremity, perineal)	Diabetes mellitus
Foreign body present	Alcoholism
Necrotic tissue	Prior surgery or radiation
Mechanism of injury (bites, perforated viscous)	Corticosteroids
Degree of wound contamination, post wounding	Inherited neutrophil defects
Reduced perfusion	

Microbiological analysis of wounds

It is necessary to culture a wound for a number of reasons, first to identify the causative organism(s) and for the provision of an antibiogram for a particular pathogen. Second, to ensure empirical antibiotic therapy is correct and to provide information to guide antimicrobial therapy when healing is delayed without overt clinical signs of infection (Bowler et al., 2001; Washinton, 1999). Since all wounds are contaminated with bacteria a wound should only be cultured after a wound has been clinically diagnosed as infected (Dow, 2003; Fleck, 2006; Kingsley, 2003a). Accurate identification of the organism(s) responsible for causing the wound infection is essential in clinical practice to maximise antibiotic use. Suboptimal antibiotic prescribing would as a consequence, raise health care costs and add to the development of multi-resistant strains of micro-organisms (Kingsley, 2003b). Accurate reporting assists in the prescribing of appropriate antibiotics, curtails the spread of antibiotic-resistant strains of bacteria, and facilitates successful wound healing (Bowler et al., 2001). Untreated wound infection leads to delayed healing, systemic infection, septicaemia and in some instances death (Lawrence, 1993; Stotts & Whitney, 1999), as well as burden on financial resources (Bowler et al., 2001).

The three methods for collecting a wound sample are: tissue biopsy, wound fluid aspirate and wound swab. Biopsy is not standard practice in the vast majority of health care settings (Stotts, 1995). Across settings wound swabbing is the moist frequently used method of collecting a wound sample, it is simple, non-invasive and inexpensive (Kelly, 2003). However, controversy exists in the literature as to how best to carry out this procedure (Bowler et al., 2001; Kelly, 2003). To date there is no single universally accepted method of collecting a wound swab (Bowler et al., 2001; Cooper & Lawrence, 1996; Donovan, 1998; Kingsley, 2003a), despite this, swabbing remains the most frequent method of collecting a wound sample (Kelly, 2003).

Tissue biopsy.

Tissue biopsy is considered the 'gold standard' for determining the species and the number of organisms, which penetrate soft tissue (Dow, 2003; Gardner et al., 2001; Kingsley, 2003b; Robson, 1997; Robson & Heggers, 1969; Rudensky, Lipschits, Isaacsohn, & Sonnenblick, 1992; Stotts, 1995; Thompson & Smith, 1994). Tissue biopsy provides both quantitative and qualitative information, that is it identifies the species of bacteria and virulence of the strain respectively, expressed in number of organisms per gram of tissue (Bowler et al., 2001; Robson & Heggers, 1969; Sibbald et al., 2003). Samples are obtained aseptically by removal of a piece of tissue with either a scalpel or a punch biopsy. The tissue samples are then weighed, homogenized, serially diluted, and cultured on selective and non-selective agar media under aerobic and anaerobic conditions (Bowler et al., 2001; Kingsley, 2003b; Stotts, 1995). Quantitative biopsy provides information from only a small area of the wound bed and can therefore have poor sensitivity and reliability (Sibbald et al., 2003). Woolfery, Fox, & Quall (1981) demonstrated that due to the unequal distribution of organisms within the wound there is a 25% chance of missing organisms.

Tissue biopsy effectively causes tissue damage; potentially delaying wound healing and can lead to infection. In a prospective study of 100 patients conducted by Wahie & Lawrence (2007), a complication rate of 29% occurred in patients following diagnostic skin biopsy. Of those individuals who developed complications, 93% resulted in wound infection.

Staff also need to be specifically trained in this method of specimen collection (Stotts, 1995). Dow (2003) and Bowler (2001) postulate that this method is more suitable for clinical research rather than routine situations. Sibbald et al. (2003) state that invasive tissue biopsy to quantify the bacterial burden is not always required and that less invasive methods should be utilized such as wound swab, which provides comparable

information. It is for these reasons that tissue biopsy has been excluded from this study.

Clinical studies comparing punch biopsy with wound swab.

In order to understand the relationship between punch biopsy and wound swabbing, the author has conducted an extensive review of both human and animal studies. The results from these studies are summarised in Table 4 and although a lengthy summation it is presented within the thesis to aid reader clarity.

Levine et al. (1976) demonstrated that the bacteria counts from wound swabs is linearly related to biopsy quantification in open burn wounds (that is burn wounds not covered by necrotic tissue). They recruited 24 patients and collected wounds swabs and tissue biopsy from 41 granulating wounds. They concurred from the results that wound swabbing is a reliable method for identifying bacteria colonising open wounds, and that there is a direct relationship between wound swab and tissue biopsy counts of viable bacteria.

On the other hand, infection was monitored in 50 patients with burns via tissue biopsy, wound swab and blood cultures by Bharadwaj, Joshi and Phadke (1983). In their study only 62.5% of patients with positive wound swabs showed clinical signs of sepsis compared with 87.5% of tissue biopsy. Blood cultures were only useful for prognostic value. Although wound swab is non-invasive the authors believe that they are not of any value in determining organisms invading the soft tissue, and which cause wound infection or sepsis.

Sepsis was also better correlated to tissue biopsy than wound swab in a study of 50 patients with burns, conducted by Sjöberg, Mzezewa,

Jönsson, Robertson and Salemark (2003). This study demonstrated a high mortality rate of 58% and there was a correlation of identical organisms in only 29% of cases between each of the methods. Different bacterial species was identified in 41% of the samples. In the remaining 30%, the results were almost identical. Although there was a better relationship between infection and biopsy, the authors propose that the time required to process this method, limited its predictive and therapeutic value in terms of initiating treatment.

A larger study of 100 patients with burns was undertaken by Uppal, Ram, Kwatra, Garg, and Gupt (2007). In 95% of cases both wound swab and biopsy identified the same organisms. Basak, Dutta, Gupta, Ganguly, and Ranjan, (1992), found concordance of 72% between wound swab and biopsy in a larger study of 171 patients with wounds of various aetiology. They also found that wound swab was reliable in 95% for both assessment of wounds as well as monitoring response to treatment.

Wound swab and biopsy culture methods was compared by Lookingbill, Miller and Knowles (1978), in a small study of 13 chronic leg ulcers. Their main objective was to predict wound healing rates with bacteriological measurement. The authors found that there was a correlation in 12 out of 17 cases between both methods. When bacterial levels were less than 10^5 the wounds healed (3 out of 5 wounds). However when levels of bacteria were greater than 10^5 none of the wounds healed (n=8).

Neil and Munro (1997) also examined the chronic wound environment in their small study comprising of 10 patients. A tissue sample and wound swab was collected from the same site in each of the patients. The Wilcoxon test indicated that tissue cultures had higher levels of colony forming units of bacteria (z score = -2.1915; p = 0.0284). The authors concluded that although bacteria counts were underestimated in the wound swab, eight of the 10 swab cultures did indicate infection,

compared with nine out of 10 in the tissue sample group. Both methods also identified the same species of bacteria, thus fulfilling the purpose for collecting the sample. There was a correlation of 79% between biopsy and wound swab in a larger study of 38 patients with chronic wounds, in a study conducted by Bill et al. (2001). The chronic wound environment was also the topic of a study conducted by Lim, Mwipatayi, Sieunarine, Abbas and Angel (2006), in their prospective observational cohort study of 39 patients, a wound swab and punch biopsy was taken from each ulcer. They found that concordance was poor between each of the methods. The results demonstrated a difference in the number and type of organisms present. In this study 46% of the cases had one organism in common, 26% had no concordance and there was non-concordance in 28% of cases. The correlation between biopsy and wound swab was poor.

A prospective study to evaluate wound-healing outcomes was undertaken by Davis et al. (2007). Punch biopsy versus wound swab was compared in 70 patients with chronic venous leg ulcers. None of the wounds were infected. Logistic regression demonstrated that biopsy offered no predictive information in terms of wound healing outcomes when compared with a wound swab ($p=0.27$). The authors recommend that biopsy should be discouraged in clinically non-infected wounds.

A study of 25 pressure ulcers in 25 patients was undertaken by Sapico et al. (1986). This group found a concordance of 75% between biopsy and wound swab culture results. Rudensky, Lipschits, Isaachohn, and Sonnenblick (1992) studied three methods of specimen collection in pressure ulcers. The researchers compared needle aspiration with wound swab and biopsy. Seventy-two samples were collected from 51 patients. Polymicrobial infection was diagnosed from 68% of the swabs, 50% of needle aspirations and 41% of tissue biopsies. Based on the results the authors propose that swab specimens reflected surface colonisation as compared with needle aspirate and tissue biopsy results, which suggests

underestimated bacterial isolates. In patients with sepsis the authors recommend biopsy as accurate measure of soft tissue infection.

Two studies have been performed in patients with diabetic foot ulcers (Kelkar & Kagal, 2004; Slater et al., 2004). Keller and Kagal (2004) recruited 50 patients with deep ulcers into their study and compared wound swabs with tissue biopsies. Patients with superficial wounds were excluded. Biopsies identified higher numbers of bacteria ($p=<0.01$), than wound swabs. The authors concluded that although wound swabs may provide useful information they argue that certain organisms might be missed on wound swabbing. In a larger study of 60 patients with diabetic foot wounds, Slater et al. (2004) collected a wound swab prior to debridement of each wound and a tissue specimen at the end of surgical debridement. In 62% of cases the results were the same, in 20% of cases the swab identified more organisms. Further analysis demonstrated that wounds not extending to bone 36 out of 40 of cases (90%) the swab identified all organism isolated from the tissue sample. In wounds with bone exposed the correlation was poor at 13 out of 20 wounds (65%). Of importance from this study is the correlation when bone is exposed.

Two studies were performed in the experimental rat model. Borneside and Bornside (1979) performed 200 experimental incision wounds in female white rats under sterile conditions. The wounds were then inoculated with *Escherichia coli* prior to suturing. In 50% of the subjects the wounds were also traumatised by crushing the paraspinous muscle with a sterile hemostat, 10mg of garden soil was introduced into the wound prior to adding bacteria. At day 10 a wound swab and tissue sample was collected from all wounds. There results demonstrated that 10^3 bacteria/gm or ml of specimen obtained from the wound swab was equivalent to 10^5 bacteria/gm of the tissue sample. Although there is a less than perfect correlation between the two methods the authors concluded that the wound swab method reaches equivalency to that of tissue sample. They postulate that the wound swab method removes the need for the

invasive biopsy in detecting wound infection in patients. A smaller animal study was conducted by Sullivan, Conner-Kerr, Hamilton, Smith, Tefertiller and Webb (2004). This group performed surgical wounds on 14 female rats and collected wound swab and tissue samples at day 2, 4, and 12. On analysis of the samples there was an 18% difference between bacteria colony counts. Since both methods detected the same microorganisms, their findings indicate that swab culture provides useful guidance for initial treatment of wound infection.

In summary, there is conflicting evidence as to which is the best method for collecting a wound sample. Based on the evidence from larger human studies (Basak et al., 1992; Bill et al., 2001; Levine et al., 1976; Sapico et al., 1986; Slater et al., 2004; Uppal et al., 2007) and the results from animal studies (Bornside & Bornside, 1979; Sullivan et al., 2004), there is mounting evidence to indicate that wound swab cultures are a useful alternative to invasive tissue biopsy

Table 4 Studies comparing punch biopsy with wound swab

Author	Year	Study	Results
Levine, Robert, Linberg, Mason, Basil, Pruitt & Colonel	(1976)	41 granulating wounds in 24 patients	Correlation between punch biopsy and wound swab
Lookingbill, Miller, & Knowles	(1978)	13 chronic leg ulcers	Correlation between punch biopsy and wound swab in 12/17 wounds
Bornside & Bornside	(1979)	Experimental incision wounds in 200 rats	Colony counts of 10^5 bacteria/gm tissue were equivalent to colony counts of 10^3 bacteria/ml of specimen on a moist wound swab

Author	Year	Study	Results
Bharadwaj, Joshi, & Phadke	(1983)	50 patients with burns. Biopsy compared with wound swab to monitor for sepsis	Wound swab gave poor indication of organisms invading the soft tissue
Basak, Dutta, Ganguly, Ranjan	(1992)	171 wounds of various aetiology	72% concordance between wound swab and tissue biopsy
Rudensky, Lipschits, Isaacsohn, Sonnenblick	(1992)	72 pressure ulcers. Compares wound swab with needle aspiration and biopsy.	Swab specimen reflected surface colonisation, needle aspiration underestimated bacterial isolates. Biopsy the accurate method
Steer, Papini, Wilson, McGrouther, Parkhouse	(1996)	69 biopsy/wound swab pairs collected from 47 patients	Biopsy or wound swab does not aid in the prediction of sepsis or graft loss
Neil & Munro	(1997)	10 chronic wounds. Biopsy verus wound swab	Wound swab did not accurately predict the tissue bacteria
Bill, Ratcliff, Donovan, Knox, Morgan, Rodeheaver	(2001)	38 chronic wounds	79% correlation between biopsy and wound swab
Sjöberg, Mzezewa, Jönsson, Robertson, & Salemark	(2003)	50 patients with burns	Sepsis was better correlated to biopsy than wound swab
Kelkar, Kagal	(2004)	50 diabetic foot wounds. Compares wound swab with deep tissue	Swabs yielded 150 organisms compared with deep tissue samples which yielded 180 organisms

Author	Year	Study	Results
Slater, Lazarovitch, Boldur, Ramot, Buchs, Weiss, Hindi, Rapoport	(2004)	60 infected diabetic foot wounds. Compares wound swab with deep tissue specimen	90% correlation when bone not involved. 65% correlation when the wound extended to bone
Sullivan, Conner-Kerr, Hamilton, Smith, Tefertiller, & Webb	(2004)	Compares biopsy versus wound swab in a rat model	Both methods detected the same type of bacteria
Lim, Mwipatayi, Murry, Sieunarine, Abbas, & Angel	(2006)	39 chronic leg wounds. Compares biopsy with wound swab	Concordance in 46% of cases
Davies, Hill, Newcombe, Stephens, Wilson, Path, Harding, Thomas	(2007)	70 patients with non-infected chronic venous leg ulcers.	Biopsy did not contribute to patient management
Uppal, Ram, Kwatra, Garg, & Gupta	(2007)	Biopsy versus wound swab in 100 burn wounds	Wound swab correlates with biopsy in identifying causative organisms

Wound Swabbing

Although wound swabbing is commonly referred to in the literature there is only one study that directly compares the 'Z' technique with the Levine technique (Gardner et al., 2006). This group recruited 83 patients with chronic wounds excluding arterial ulcers. Amongst these, 30 participants had clinically infected wounds and 53 had non-infected wounds. From each wound concurrent swab specimens were obtained using wound exudate (the authors of the study do not elaborate as to how the wound exudate was obtained), the 'Z' swabbing technique, the Levine technique and a sample of viable tissue (the authors of the study do not elaborate as to how the sample of viable tissue was obtained). Laboratory procedures used to culture, isolate and identify organisms were explained in detail. The mean concordance between Levine's technique and tissue specimens was 78%. Their findings also indicated that wound swabs using

Levine's technique provided more accuracy than wound exudate or the 'Z' swabbing technique. Their findings suggest that Levine's technique provided acceptable accuracy of wound bioburden.

Chapter Three

Methodology

Introduction

This section will outline the: design of the research; sample size, setting, sample size justification, inclusion criteria and exclusion criteria. The method of data collection will be discussed in detail, as will the protocols that outline the specimen collection.

Research Design

A prospective randomised study of two paired wound swabbing techniques (Levine versus Z technique) was conducted in patients with wounds of any aetiology where wound infection was clinically suspected.

Sample size and setting

The following points outline the sample size and setting of the study.

- A total of 50 patients with wounds of any aetiology with clinically infected wounds were invited to participate in the study;
- Patients were recruited by the chief investigator, from a large 855 bed, adult metropolitan public teaching hospital in Perth, Western Australia
- Patients were verbally informed about the study, and invited to participate by the chief investigator;

- All patients were provided with an information sheet about the study (Appendix I);
- Written consent was obtained from participants by the chief investigator (Appendix II).

Sample size justification

The sample size of this study was calculated using methods defined by Cohen (1987). The sample size of 50 paired swabs (100 swabs in total) is based on detecting an effect size difference of 25% in detected microbiological bioburden between the two swabbing techniques with a standard deviation of 6.25% when alpha is set at 0.05 and power at 80%.

Inclusion criteria

The following points describe in detail the inclusion criteria for the study.

- Adults who were able to provide informed consent;
- Presence of a wound greater than 1cm^2 and which demonstrated clinical signs of infection;
- In acute wounds the clinical signs of infection are: inflammation present for longer than 5 days, purulent drainage, elevated temperature >38^0 C, spontaneous dehiscence or presence of an abscess;
- In chronic wounds the clinical signs of infection are: increased exudate, presence of odour, erythema >1-2cm, warmth around the wound, poor quality granualtion tissue, pain or tenderness at the site, or no improvement in healing in 2 weeks in a clean wound.

Exclusion criteria

The following points describe in detail the exclusion criteria for the study.

- Children under the age of 18;
- Cognitive impairment;
- Non-English speaking patients who were unable to understand or read English;
- Wounds that were less than 1cm^2;
- Wounds that did not present with clinical signs of infection.

Data collection

Nursing staff working at the organisation were notified via global e-mail about the study. The e-mail also provided contact information for the investigator should they identify an eligible patient that could be recruited into the study.

1. Patients (either inpatients or outpatients) with acute or chronic wounds of any aetiology demonstrating clinical signs of infection were invited to participate in the study.
2. A semi-quantitative Levine and Z method wound swabs were collected, from the same wound area, no more than 5 minutes apart on each patient.
3. The order of the swab collection was randomised by the flip of a coin. This was to ensure that there was no specimen collection bias and to ensure that any possible effect of the first swabbing method did not have an effect on the second method. This design was believed to minimise alterations in culture findings related to preceding manipulations of the wound bed or the progression of time. This method of randomisation has been outlined by Polt and Hunger, (p. 120-121) “the random assignment of subjects to one

group or another is designed to perform and equalization function. Subjects who are randomly assigned to groups are expected to be comparable". They further explain that "Random assignment can be accomplished by flipping a coin or pulling names from a hat" (Polt & Hungler, 1989).

The following protocols outline the specimen collection, transportation to the laboratory, and analysis of the specimens in the study:

a) Two sterile cotton tipped swabs were used for each method. The cotton tip on the swab was pre-moistened with sterile 0.9% normal saline. One swab was used to obtain a Gram stain and the other was placed in Stuart's medium to identify the specific organisms present.
b) Using a sterile dressing pack, the wound was cleansed with normal saline once prior to the collection of both the 'Z' and the Levine technique. The wound was not cleansed again between each method. Different swabs were used for each method.
c) The 'Z' technique involves rotating the swab between the fingers as the swab is manipulated in a 10-point zigzag fashion (side to side across the wound without touching the wound edges or peri wound skin from one edge to the other).
d) With the 'Levine technique', the specimen is obtained from a limited area within the wound and excluding the wound edge or peri wound skin. The swab is rotated over a $1cm^2$ area with sufficient pressure to express fluid within the wound.
e) In order to minimise interrater error there were only two wound swab data collectors responsible for collecting all wound swabs. Both data collectors followed the same protocol for collecting the wounds swabs as outlined above in points a to d. Prior to commencement of the study both of the data collectors were assessed for technique reliability for both methods of specimen

collection by an independent observer. The findings of this reliability testing indicated that the wound swab collectors met the parameters of the study methodology.

f) All specimens were labelled, with the patient's name, ward, unique medical record number (URMN), date, time of collection, and anatomical location of the wound. Two separate labelled request forms was used stating clearly the method of wound swab collection for each sample. This allowed the reporting of the results to identify which method of wound swabbing was used.

g) The specimens were placed in a biohazard-sealed bag and transported to the microbiology laboratory via the hospital courier. All samples were collected within laboratory working hours therefore there was no delay in the processing of the specimens.

h) All specimens were analysed by the same scientist, on the day of specimen collection.

i) Gram stain procedure was performed by heat fixing the smear. The smear was then flooded with methanol fixative. The specimen was examined under low power (x10 objective lens) to quantify leucocytes and oil immersion lens (x100 objective) for microorganisms). Gram-positive organisms appeared as dark blue or purple. Gram-negative organisms appeared red. Inflammatory cells appeared as Gram negative. Epithelial cells could appear Gram positive and/or Gram negative depending on the thickness of the smear.

j) Aerobic culture was performed by inoculating the swab (that is the one that was transported in the Stuarts medium) onto horse blood agar (BA) medium and cysteine lactose electrolyte deficient agar (CLED). Both plates were then stored at 35^{o} C in a carbon dioxide holding chamber. The plates were initially examined after 24 hours and then reincubated for a further 24 hours if there was no growth. Microscopy was reported as

leucocytes not seen, few, moderate or many. Microorganisms were reported as no growth, scant, moderate, or abundant. Culture – all potential pathogens were reported and growth quantified as + (scant growth), ++ (small growth), +++ (moderate growth), ++++ (abundant growth). As was the practise of the laboratory performing the analysis of the samples, only *Staphylococcus aureus* and Groups A, B, C or G *Streptococci* were reported with susceptibilities. For the purpose of this study analysis was conducted on organism species identified by either the Levine or Z method. Analysis was not conducted on the bacterial load for each specimen.

k) Anaerobic culture was not performed routinely.

Data entry

Data entry was conducted by the chief investigator. The chief and co-investigator, Professor Nick Santamaria, conducted all data analysis.

Data analysis

Data analysis procedures are explained in detail in the following points.

1. All statistical procedures were carried out with Statistical Package for the Social Sciences (SPSS) windows version 16. For all continuous variables, descriptive statistics including means and standard deviations were calculated. Frequencies and proportions were determined for all categorical variables. Differences between the detected microbiological bioburden species were analysed with t-test for paired sample.

2. All wound swabs were semi-quantitatively analysed by the same scientist from the Department of Microbiology and Infectious Disease at Royal Perth Hospital.

Ethical issues

Ethical considerations are outlined in detail the following points below.

- Human Research Ethics Committee approval was granted by Royal Perth Hospital - certificate No EC 2006/085;
- Approval was also granted from Curtin University of Technology's Human Research and Ethics Committee;
- The National Statement on Ethical Conduct in Human Research (2007) was adhered to;
- A data collection form was developed (Appendix III);
- A patient consent form was used (Appendix II);
- A patient information sheet was given to each patient (Appendix I).

Facilities and resources

Curtin University School of Nursing and Midwifery provided access to statistical advice, on campus office/study accommodation, computer access and telephone access as required.

Data storage

The study required medical chart review (which incorporates pathology reports). It was necessary for the data collection tool to be name identified.

All data and associated patient information was stored in a locked filling cabinet within a locked office at Royal Perth Hospital. All computer

files were stored in one password protected computer only accessible by the chief investigator. All data collected will be stored for a period of 5 years in accordance with the requirements of the Human research Ethics Committee (HREC) of Royal Perth Hospital. The National Health and Research Committee (NHMRC) Human Research Ethics Handbook (2001) guidelines approved under section 95a of the Privacy Act 1988 was adhered to.

Budget

At the participating hospital It is normal practise to collect at least one wound swab from patients with wounds that have a clinically suspected wound infection. For the purpose of this study, the pathology costs incurred from the collection of two wound swabs from each patient admitted to the study was absorbed by the hospital in accordance with an agreement made with the Microbiology department. This agreement was based on the assumption that the study would directly contribute to the development of best practise in wound swabbing techniques.

Chapter Four

Results

Introduction

This chapter will present the findings of the study including: the sample population, aetiology of the wounds included in the study, organisms identified with each clinical sign of infection in acute and chronic wounds, and the organisms identified in wounds by aetiology. Statistical analysis is also performed to determine if there is a statistical difference between the Levine and Z technique.

Sample Demographics.

A total of 50 patients were recruited to the study. There were 28 males and 22 females, with a mean age of 62.46 years (Table 5).

Table 5 Demographics

Gender	Total	%
Female	22	44
Male	28	56
Age range (mean)	(62.46) 20-88	

Wound aetiology.

The most common aetiology of wounds in this study was venous leg ulcers, 26% (n = 13), followed by surgical wounds, 16% (n = 8) neuropathic foot ulcers, 14% (n = 7), and neuro-ischaemic foot ulcers, 12% (n = 6). Five of the wounds were stage IV pressure ulcers, representing 10% of the study

participants. There were 10% (n= 5) wounds in the other category. These were comprised of four traumatic wounds and one invasive squamous cell carcinoma. Table 6 gives a detailed breakdown of wounds by aetiology.

Table 6 Wound aetiology

Wound aetiology	N	%
Arterial ulcers	5	10
Venous ulcers	13	26
Mixed arterial/venous ulcers	1	2
Neuropathic ulcers	7	14
Neuro-ischaemic ulcers	6	12
Pressure ulcer	5	10
Surgical	8	16
Other	5	10
Total	50	100%

Of these wound types 42% (n = 21) were acute wounds and 58% (n = 29) were chronic, Table 7. Acute wounds were those that had been present for less than 6 weeks. This includes some of the venous, arterial, neuropathic and neuro-ischaemic ulcers.

Table 7 Percentage of acute and chronic wounds

Wound aetiology	N	%
Acute	21	42
Chronic	29	58
Total	50	100

Clinical signs of infection in acute wounds.

Amongst patients with acute wounds, two had no clinical signs of infection present. Twenty-one patients had clinical signs of infection. Inflammation that persisted for more than 5 days (i.e. warmth, redness oedema and

localised pain) was identified in 32% (n = 16) of patients. Purulent drainage was evident in three patients with acute wounds, (see Table 8).

Table 8 Clinical signs of infection in acute wounds

Clinical Signs of infection in acute wounds	N	%
Inflammation	16	32
Purulent discharge	3	6
No symptoms	2	4
Total	21	42

Inflammation present in acute wounds and organisms identified.

Table 9 outlines the specific organisms identified when inflammation was the clinical sign of infection present, in acute wounds for both the Levine and Z technique. Several organisms were identified when inflammation was evident. Overall the Levine method detected 39 species and the Z method detected 42 species.

Both the Levine and the Z technique identified the most common organism isolated was *Staphylococcus coagulase negative*, followed by *Enterococcus species* and *Enterobacter cloacae*. The Levine technique did not detect *Cedecea species, Corynebacterium jeikeium*, and *Enterobacter species*. The Z technique did not isolate *Morganella morganii*. The laboratory reported that bacteria was not isolated in one patient with both the Levine and, Z technique.

With the Levine technique in all but three patient's wounds, two or more organisms were present when inflammation was the clinical sign of infection. Single strains of organisms were identified in three patients, six

patients had two organisms present, five patients had three organisms present and in the remaining two patients four organisms were present.

In comparison, the Z technique did not isolate *Morganella morganii* in one patient, and in one patient there was a single stain of organism present. In the remaining 14 patients there were two or more organisms present within the wound. Four patients had two organisms present, eight patients had three organisms present and in two patients four organisms were present.

Table 9 Number of organisms isolated when inflammation present using the Levine and Z techniques in acute wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Alpha-haemolytic Streptococcus	2	2
Bacteria not isolated	**1**	**1**
Bacillus species	1	1
Cedecea species	**Not isolated**	**1**
Corynebacterium jeikeium	**Not isolated**	**1**
Diptheriod bacillus	1	1
Enterobacter aerogenes	3	2
Enterobacter cloacae	4	5
Enterobacter species	**Not isolated**	**1**
Enterococcus species	5	4
Escherichia coli	1	2
Klebsiella pneumoniae	1	1
Morganella morganii	**1**	**Not isolated**
Non-epidemic Methicillin Staphylococcus Aureus (MRSA)	1	1
Proteus mirabilis	1	1
Pseudomonas aeruginosa	3	2
Staphylococcus aureus	4	4
Staphylococcus coagulase negative	6	8
Stenotrophomanas maltophila (Xanthomonas maltoph)	1	1
Streptococcus agalactiae (group B)	2	2
Streptococcus pyogenes (group A)	1	1
Total	**39**	**42**

Purulent discharge in acute wounds and organisms identified.

Table 10 provides data on the specific organisms identified when purulent discharge was the clinical sign of infection present, in acute wounds for both the Levine and Z technique.

Table 10 Number of organisms isolated when purulent discharge present using the Levine and Z techniques in acute wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Bacteria not isolated	**1**	**1**
Diptheriod bacillus	1	1
Enterobacter cloacae	1	1
Enterococcus species	2	1
Escherichia coli	1	1
Klebsiella pneumoniae	1	1
Proteus mirabilis	1	1
Total	8	7

There were similarities between both methods of wound swab collection and organisms identified when purulent discharge was the clinical sign of infection in acute wounds. The only difference was that a bacterium was not isolated with the Z technique, and that *Enterococcus species* was identified twice with the Levine technique.

The Levine technique identified different single stains of organism in two patients. In one patient, five strains of organisms were identified. In comparison, the Z technique identified the same five organisms in one patient, and the same single strain of organism was identified in another patient. It is interesting to note that the Z technique also did not detect

organisms in one patient, when inflammation was the clinical sign of infection.

Clinical signs of infection in chronic wounds.

In patients with chronic wounds the most common clinical sign of infection was increased exudate. This was identified in 20% (n = 10) of patients. No improvement in healing within 2 weeks in a clean wound was identified in 16% (n = 8). Erythema was evident in 8% (n = 4). Poor quality granulation tissue was assessed in 6% (n = 3). Warmth around the wound was identified in one patient, representing 2%, of patients. What is not represented is whether the patients had one or more clinical signs of infection present (Table 11).

Table 11 Clinical signs of infection in chronic wounds

Clinical signs of infection	Number	%
Increased exudate	10	20
Erythema > 1-2cm	4	8
Warmth	1	2
Poor quality granulation tissue	3	6
Pain at site	3	6
No improvement in wound healing	8	16
Total	29	58

Increased exudate in chronic wounds and organisms identified.

The most common isolates were *Staphylococcus aureus* and *Diphtheroid bacillus,* with both methods. *Morganella morganii* was identified twice with the Z technique and only once with the Levine technique. Otherwise

there was no difference in the number or type of isolates with either sampling method (Table 12).

A Single strain of organism was identified in three patients with the Levine technique and in one patient with the Z technique. Two organisms were detected with both techniques in one patient. Three organisms were identified in three patients with the Levine technique and three organisms were detected in four patients with the Z technique. Four organisms were detected in one patient for both methods of wound swab collection.

Table 12 Number of organisms isolated when increased exudate present using the Levine and Z techniques in chronic wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Acinetobacter haemolyticus	1	1
Bacteria not isolated	1	1
Diptheriod bacillus	4	4
Enterococcus species	2	2
Morganella morganii	1	2
Non-epidemic Methicillin Staphylococcus Aureus (MRSA)	1	1
Proteus mirabilis	1	1
Proteus species	1	1
Pseudomonas aeruginosa	1	1
Staphylococcus aureus	5	5
Staphylococcus coagulase negative	2	2
Streptococcus milleri group	1	1
Total	**21**	**22**

Erythema >1-2cm in chronic wounds and organisms identified.

Table 13 demonstrates the organisms identified when erythema >1-2cm was present. *Clostridium perfingens, Diptheriod bacillus*, and *Enterococcus species*, were not isolated with the Levine technique. *Proteus species* and *Staphylococcus aureus* were identified twice with the Z technique and only once with the Levine. Otherwise there was no difference in the number or type of organisms identified. In both instances a single strain organism was identified in one patient. With the Levine technique identified three organisms in the remaining three patients. However, with the Z technique three organisms were detected in one patient, five organisms in one patient and six organisms in one patient

Table 13 Number of organisms isolated when erythema >1-2cm is present using the Levine and the Z techniques in chronic wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Alpha-haemolytic Streptococcus	1	1
Clostridium perfingens	**Not isolated**	**1**
Diptheriod bacillus	**Not isolated**	**1**
Enterococcus species	**Not isolated**	**1**
Non-epidemic Methicillin Staphylococcus Aureus (MRSA)	1	1
Proteus species	1	2
Pseudomonas aeruginosa	2	2
Serratia marcens	1	1
Serratia odoriferans	1	1
Staphylococcus aureus	1	2
Staphylococcus coagulase negative	1	1
Total	**9**	**14**

Warmth present around the wound in chronic wounds and organisms identified.

Table 14 identifies organisms present when warmth is the clinical sign of infection for both the Levine and the Z technique. In both instances when warmth was the clinical sign of infection the same organisms were identified for both methods of wound swab collection.

Table 14 Number of organisms isolated when warmth present using the Levine and Z techniques in chronic wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Proteus species	1	1
Pseudomonas aeruginosa	1	1
Staphylococcus coagulase negative	1	1
Total	**3**	**3**

Poor quality granulation tissue in around the wound and organisms identified.

There was very little difference in the number of organisms isolated when poor quality granulation tissue was the clinical sign of infection. The only difference being that Staphylococcus aureus and Staphylococcus coagulase negative was isolated twice with the Z technique and once with the Levine technique (Table 15).

Both the Levine and the Z techniques identified the same single strain of organism in one patient. In two patients the Levine technique identified three organisms. By comparison the Z technique three organisms

were identified one patient and four organisms were present in another patient.

Table 15 Number of organisms isolated when poor quality granulation tissue is present using the Levine and Z techniques in chronic wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Acineobacter baumanii	1	1
Diptheriod bacillus	1	1
Enterococcus species	1	1
Pseudomonas aeruginosa	1	1
Staphylococcus aureus	1	2
Staphylococcus coagulase negative	1	2
Streptococcus agalactiae (group B)	1	1
Total	**7**	**9**

Pain present at the wound site in chronic wounds and organisms identified.

Table 16 demonstrates the organisms identified when pain at the wound site was present, in chronic wounds for both the Levine and the Z techniques. The only difference in this instance was that *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus agalactiae (group B)* was identified twice with the Levine technique and once with the Z technique, when pain was the clinical sign of infection present.

Four or more organisms were identified in each patient with the Levine technique. In comparison, and the Z technique identified one patient had one organism and the remaining two patients had four or more organisms identified.

Table 16 Number of organisms isolated when there is pain at the wound site using the Levine and Z techniques in chronic wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Escherichia coli	1	1
Morganella morganii	1	1
Proteus mirabilis	1	1
Pseudomonas aeruginosa	2	1
Staphylococcus aureus	2	1
Staphylococcus coagulase negative	1	1
Streptococcus agalactiae (group B)	2	1
Streptococcus pyogenes (group A)	1	1
Total	**11**	**8**

No improvement in wound healing in chronic wounds and organisms identified.

There was a similar pattern for the number of organisms isolated with both methods when there was no improvement in wound healing within the past 2 weeks (Table 17). *Corynebacterium jeikeium* was not isolated with the Levine technique in one patient. In one patient bacterium was not isolated with the Z technique. Anaerobic organisms were identified in both the Levine and the Z technique. When there was no improvement in wound healing in the past 2 weeks a similar pattern emerged for the number of organisms identified for each patient when both sampling techniques were used.

Table 17 Number of organisms isolated when there was no improvement in wound healing in the past 2 weeks using the Levine and Z techniques in chronic wounds

Organism identified	Levine Number of times organism isolated	Z Number of times organism isolated
Anaerobic organisms	1	1
Corynebacterium jeikeium	**Not isolated**	**1**
Diptheriod bacillus	2	1
Enterococcus species	3	3
Escherichia coli	1	1
Morganella morganii	1	1
Non-epidemic Methicillin Staphylococcus Aureus (MRSA)	1	1
Proteus mirabilis	1	1
Proteus vulgaris	1	2
Pseudomonas aeruginosa	2	3
Staphylococcus aureus	2	2
Staphylococcus coagulase negative	2	2
Streptococcus agalactiae (group B)	1	1
Total	**18**	**21**

Inflammatory markers.

White blood count was normal in 32% (n = 16) of cases and elevated in 18% (n = 9). This test was not performed in 48% (n = 24) of patients. In one patient it is not recorded if this investigation was or was not performed. C reactive protein was normal in 4% (n = 2) and elevated in 46% (n = 23). This test was not performed in 48% (n = 24) of patients. At the time of specimen collection 52% (n = 26), were currently taking antibiotics, 48% (n = 24), were not on antibiotics.

Detected organisms Levine versus Z techniques.

Using a one-sample *t test* there was a statistically significant difference in the number of organisms detected between the Levine and the Z technique in the study population. The Levine method detected more organisms ($t = 15.46$, $p = <0.001$), than the Z technique.

When this was further broken down into acute and chronic wounds there was also a statistically significant difference in the number of organisms detected in acute and chronic wounds. In acute wounds the Levine technique detected more organisms ($t = 9.55$, $p = <0.001$). In chronic wounds the Levine also detected more organisms ($t= 12.04$, $p <0.001$).

Identified organisms Levine verus Z techniques.

Table 18 illustrates the different species of organisms identified with each method. Although the Levine technique overall detected more organisms, there were differences in the species of organisms identified within each group.

Table 18 Identified organisms Levine versus Z techniques

Levine technique	Z technique
Acineobacter baumannii	*Acineobacter baumannii*
Acinetobacter haemolyticus	*Acinetobacter haemolyticus*
Alcaligenes faecalis	**Not detected**
Alpha-haemolytic Streptococcus	*Alpha-haemolytic Streptococcus*
Anaerobic organisms	*Anaerobic organisms*
Bacillus species	*Bacillus species*
Not detected	***Cedecea species***
Not detected	***Clostridium perfringens***
Not detected	***Corynebacterium jeikeium***
Diptheroid bacillus	*Diptheroid bacillus*
Enterobacter aerogenes	*Enterobacter aerogenes*
Enterobacter cloacae	*Enterobacter cloacae*
Enterococcus species	*Enterococcus species*
Escherichia coli	*Escherichia coli*
Klebsiella pneumoniae	*Klebsiella pneumoniae*
Klebsiella oxytoca	***Not detected***
Morganella morganii	*Morganella morganii*
Non-epidemic Methicillin Staphylococcus	*Non-epidemic Methicillin Staphylococcus*
Aureus (MRSA)	*Aureus (MRSA)*
Proteus mirabilis	*Proteus mirabilis*
Proteus species	*Proteus species*
Proteus vulgaris	*Proteus vulgaris*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Serratia marcescens	*Serratia marcescens*
Serratia odoriferans	*Serratia odoriferans*
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
Stenotrophomanas maltophila	*Stenotrophomanas maltophila*
(Xanthomonas maltoph)	*(Xanthomonas maltoph)*
Strep. agalactiae (group B)	*Strep. agalactiae (group B)*
Streptococcus milleri group	*Streptococcus milleri group*
Streptococcus pyogenes (group A)	*Streptococcus pyogenes (group A)*
Streptococcus pyogenes (group A)	*Streptococcus pyogenes (group A)*

Table 19 outlines the differences of organisms not detected by each method. With the Levine technique *Corynebacterium jeikeium, Cedecea species and Clostridium perfringes,* were not detected. However, these organisms were detected performing the Z technique.

Alcaligenes faecalis and *Klebsiella oxtoca*, were only detected when the Levine technique was used to collect the specimen. The majority of organisms were the same for each method of collecting the sample.

Table 19 Organisms not detected for each group Levine versus Z techniques

Levine technique organisms not identified	Z technique organisms not identified
Cedecea species	*Alcaligenes faecalis*
Clostridium perfinges	*Klebsiella oxtoca*
Corynebacterium jeikeim	

Organisms identified in acute and chronic wounds Levine versus Z technique.

A further analysis of results was performed to determine if there was a difference in the number and species of organisms identified in both acute and chronic wounds with both the Levine and Z techniques, Table 20-23 illustrates the organisms identified in acute and chronic wounds with the Levine technique.

Overall the Levine detected more organisms in both acute and chronic wounds. More organisms were detected in chronic wounds than acute wounds. This is consistent with the literature that chronic wounds are usually polymicrobial in nature.

Table 20 Organisms identified in acute and chronic wounds using the Levine technique

Acute wounds Levine technique	Chronic wounds Levine technique
Not detected	***Acineobacter baumannii***
Acinetobacter haemolyticus	*Acinetobacter haemolyticus*
Alcaligenes faecalis	**Not detected**
Alpha-haemolytic streptococcus	*Alpha-haemolytic streptococcus*
Not detected	***Anaerobic organisms***
Bacillus species	**Not detected**
Diptheroid bacillus	***Diptheroid bacillus***
Enterobacter aerogenes	**Not detected**
Enterobacter cloacae	**Not detected**
Enterococcus aerogenes	**Not detected**
Enterococcus species	*Enterococcus species*
Escherichia coli	*Escherichia coli*
Klebsiella pneumoniae	**Not detected**
Klebsiella oxytoca	*Klebsiella oxytoca*
Morganella morganii	*Morganella morganii*
Non-epidemic Methicillin Resistant	*Non-epidemic Methicillin Resistant*
Staphylococcus Aureus (MRSA)	*Staphylococcus Aureus (MRSA)*
Proteus mirabilis	*Proteus mirabilis*
Not detected	*Proteus species*
Not detected	***Proteus vulgaris***
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Serratia marcescens	*Serratia marcescens*
Serratia odorifens	*Serratia odorifens*
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
Stenotrophomanas maltophilia (Xanthomonas maltoph)	**Not detected**
Streptococcus agalactiae (group B)	*Streptococcus agalactiae (group B)*
Streptococcus milleri group	*Streptococcus milleri group*
Streptococcus pyogenes (group A)	*Streptococcus pyogenes (group A)*

There were difference in the organism detected in acute and chronic wounds with the Levine technique as illustrated in Table 21

Table 21 Organisms not detected in acute and chronic wounds with the Levine technique

Acute wounds organisms not detected Levine technique	Chronic wounds organisms not detected
Acineobacter baumannii	*Alcaligenes faecalis*
Anaerobic organisms	*Bacillus species*
Proteus species	*Enterobacter aerogenes*
Proteus vulgaris	*Enterobacter cloacae*
	Enterococcus aerogenes
	Klebsiella pneumoniae
	Stenotrophomanas maltophilia
	(Xanthomonas maltoph)

A direct comparison between acute and chronic wounds using the Levine method, demonstrated that there was a difference in the species and number of organisms present. Chronic wounds identified less species of bacteria with the Levine technique.

Table 22 demonstrates the differences in organisms detected in acute and chronic wounds with the Z techniques. The Z technique detected 22 organisms in chronic wounds compared to 18 organisms in acute wounds.

Table 22 Organisms identified in acute and chronic wounds with the Z technique

Acute wounds Z technique	Chronic wounds Z technique
Not detected	***Acineobacter baumannii***
Not detected	***Acinetobacter haemolyticus***
Alpha-haemolytic streptococcus	*Alpha-haemolytic streptococcus*
Not detected	***Anaerobic organisms***
Bacillus species	**Not detected**
Not detected	*Clostridium perfringens*
Cedecae species	**Not detected**
Corynebacteruim jeikeium	*Corynebacteruim jeikeium*
Diptheriod bacillus	*Diptheriod bacillus*
Enterobacter aerogenes	**Not detected**
Enterobacter cloacae	**Not detected**
Escherichia coli	*Escherichia coli*
Enterococcus species	*Enterococcus species*
Klebsiella pneumoniae	**Not detected**
Not detected	***Morganella morganii***
Non-epidemic Methicillin resistant	*Non-epidemic Methicillin resistant*
Staphylococcus Aureus (MRSA)	*Staphylococcus Aureus (MRSA)*
Proteus mirabilis	*Proteus mirabilis*
Not detected	***Proteus species***
Not detected	***Proteus vulgaris***
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
Stenotrophomanas maltophillia (Xanthomonas maltoph)	**Not detected**
Streptococcus agalactiae (group B)	*Streptococcus agalactiae (group B)*
Not detected	***Streptococcus milleri group***
Streptococcus pyogenes (group A)	*Streptococcus pyogenes (group A)*
Not detected	***Serratia marcescens***
Not detected	***Serratia odoriferans***

There were differences in the species of organisms detected in acute and chronic wounds with the Z technique as illustrated in Table 23.

Table 23 Organisms not detected in acute and chronic wounds with the Z technique

Acute wounds organisms not detected Z technique	Chronic wounds organisms not detected Z technique
Acineobacter baumannii	*Bacillus species*
Acinetobacter haemolyticus	*Cedecae species*
Anaerobic organisms	*Enterobacter aerogenes*
Clostridium perfringens	*Enterobacter cloacae*
Morganella morganii	*Klebsiella pneumoniae*
Proteus species	*Stenotrophomanas maltophillia (Xanthomonas maltoph)*
Proteus vulgaris	
Streptococcus milleri group	
Serratia marcescens	
Serratia odoriferans	

Organisms identified by aetiology of wounds.

A further analysis of the results was undertaken to identify the organisms detected by wound aetiology Levine verus Z technique Table 24 – 30. In all but two cases when using the Levine technique, the organisms identified were the same, between both methods.

Table 24 Organisms identified in arterial leg ulcers Levine and Z techniques

Arterial leg ulcers	Levine technique	Z technique
	Alpha haemolytic streptococcus	*Alpha haemolytic streptococcus*
	Diptheroid bacillus	*Diptheroid bacillus*
	Not detected	***Enterococcus species***
	Non-epidemic Methicillin Staphylococcus Aureus (MRSA)	*Non-epidemic Methicillin Staphylococcus Aureus (MRSA)*
	Not detected	***Proteus species***
	Staphylococcus aureus	*Staphylococcus aureus*
	Staphylococcus coagulase negative	*Staphylococcus coagulase negative*

Table 25 Organisms identified in venous leg ulcers Levine and Z techniques

Venous leg ulcers	Levine technique	Z technique
	Anaerobic organisms	Anaerobic organisms
	Bacteria not isolated	Bacteria not isolated
	Diptheroid bacillus	*Diptheroid bacillus*
	Not detected	***Clostridium perfingens***
	Enterococcus species	*Enterococcus species*
	Escherichia coli	*Escherichia coli*
	Klebsiella oxytoca	**Not detected**
	Morganella morganii	*Morganella morganii*
	Non-epidemic Methicillin	*Non-epidemic Methicillin*
	Resistant Staphylococcus	*Resistant Staphylococcus*
	Aureus (MRSA)	*Aureus (MRSA)*
	Proteus miraballis	*Proteus miraballis*
	Proteus species	*Proteus species*
	Proteus vulgaris	*Proteus vulgaris*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
	Serratia marcescens	*Serratia marcescens*
	Serratia odoriferans	*Serratia odoriferans*
	Staphylococcus aureus	*Staphylococcus aureus*
	Not detected	***Stenotrophomonas maltophilis***
	Streptococcus agalactiae (group B)	*Streptococcus agalactiae (group B)*

Apart from three cases the organisms identified between each group was the same, between both methods of wound sampling.

Bacteria were not isolated in the one patient with a mixed arterial and venous leg ulcers with both the Levine and Z technique.

Table 26 Organisms identified in neuropathic foot ulcers Levine and Z techniques

Neuropathic foot ulcers	Levine technique	Z technique
	Alpha-haemolytic streptococcus	***Not detected***
	Bacteria isolated	Bacteria not isolated
	Not detected	***Corynebacterium jeikeium***
	Diptheroid bacillus	*Diptheroid bacillus*
	Enterobacter cloacae	*Enterobacter cloacae*
	Enterococcus species	*Enterococcus species*
	Klebsiella pneumoniae	*Klebsiella pneumoniae*
	Proteus mirabilis	*Proteus mirabilis*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
	Staphylococcus aureus	*Staphylococcus aureus*
	Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
	Stenotrophomanas maltophilia (Xanthomonas species)	*Stenotrophomanas maltophilia (Xanthomonas species)*
	Streptococcus agalactiae (group B)	*Streptococcus agalactiae (group B)*
	Streptococcus pyogenes (group A)	*Streptococcus pyogenes (group A)*

Apart from *Corynebacterium jeikium*, the organisms isolated were the same for both groups.

Table 27 Organisms identified in neuro-ischaemic foot ulcers Levine and Z techniques

Neuro-ischaemic foot ulcers	Levine technique	Z technique
	Bacteria isolated	Bacteria not isolated
	Enterobacter aerogenes	*Enterobacter aerogenes*
	Enterobacter cloacae	*Enterobacter cloacae*
	Enterococcus species	*Enterococcus species*
	Escherichia coli	*Escherichia coli*
	Morganella morganii	*Morganella morganii*
	Non-epidemic Methicillin Resistant Staphylococcus Aureus (MRSA)	*Non-epidemic Methicillin Resistant Staphylococcus Aureus (MRSA)*
	Proteus vulgaris	*Proteus vulgaris*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
	Staphylococcus aureus	*Staphylococcus aureus*
	Staphylococcus coagulase negative	*Staphylococcus coagulase negative*

The organisms isolated were the same for both groups, in neuro-ischaemic foot ulcers.

Table 28 Organisms identified in pressure ulcers Levine and Z techniques

Pressure ulcers	Levine technique	Z technique
	Diptheroid bacillus	*Diptheroid bacillus*
	Enterobacter clocae	*Enterobacter clocae*
	Enterococcus species	*Enterococcus species*
	Klebsiella pneumoniae	*Klebsiella pneumoniae*
	Non-methicillin Resistant Staphylococcus Aureus (MRSA)	*Non-methicillin Resistant Staphylococcus Aureus (MRSA)*
	Proteus mirabilis	*Proteus mirabilis*
	Proteus species	*Proteus species*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
	Staphylococcus aureus	*Staphylococcus aureus*
	Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
	Streptococcus milleri group	*Streptococcus milleri group*

Identical species of organisms were identified in pressure ulcers with both the Levine and the Z techniques.

Table 29 Organisms identified in surgical wounds using Levine and Z techniques

Surgical wounds	Levine technique	Z technique
	Alcaligenes faecalis	***Not detected***
	Alpha-haemolytic streptococcus	*Alpha-haemolytic streptococcus*
	Not detected	***Cedecea species***
	Not detected	***Corynebacterium jeikeium***
	Ditheroid bacillus	*Ditheroid bacillus*
	Enterobacter aerogenes	*Enterobacter aerogenes*
	Enterobacter cloacae	*Enterobacter cloacae*
	Enterococcus species	*Enterococcus species*
	Proteus mirabilis	*Proteus mirabilis*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
	Staphylococcus coagulase negative	*Staphylococcus coagulase negative*

Apart from 2 cases the organisms identified were the same for both groups, in surgical wounds.

Table 30 Organisms identified in other wounds Levine and Z techniques

Other wounds	Levine technique	Z technique
	Acinetobacter baumannii	*Acinetobacter baumannii*
	Acinetobacter haemolyticus	*Acinetobacter haemolyticus*
	Bacillus species	*Bacillus species*
	Diptheroid bacillus	*Diptheroid bacillus*
	Enterococcus species	*Enterococcus species*
	Morganella morganii	*Morganella morganii*
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
	Staphylococcus aureus	*Staphylococcus aureus*
	Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
	Streptococcus pyogenes (group A)	*Streptococcus pyogenes (group A)*

The same species of organisms was identified between the two groups in the other wound category.

Summary

Fifty patients were recruited into the study, with a mean age of 62.46 years. There were more male (n = 28) participants than female (n = 22). The majority of patients had chronic wounds 58% (n = 29) and 42% were acute (n = 21).

The most common wound aetiology was venous leg ulcers 26% (n = 13), followed by surgical 16% (n = 8), and neuropathic foot ulcers 14% (n = 7). Neuro-ischaemic foot ulcers represented 10% (n = 6), of the study population. There was 10% (n = 5) arterial leg ulcers, pressure ulcers comprised of 10% (n = 5), and 10% (n = 5) of other wounds. The wounds in the other category comprised of four traumatic wounds and one invasive cell carcinoma.

The most common clinical sign of infection in acute wounds was erythema that persists for more than five days, which was present in 32% (n = 16). Purulent discharge was present in 6% (n = 3). In all but three patients with the Levine technique two or more organisms were present in the patients wounds when inflammation was the clinical sign of infection. By comparison with the Z technique, bacteria was not isolated in one patient, and in one patient there was a single species of organism present. In the remaining fourteen cases there was two or more organisms present with both methods. The most common organism's isolated from the patient's wounds were *Staphylococcus coagulase negative, Enterococcus species* and *Enterobacter cloacae*, with both methods of wound swabbing. When purulent discharge was the clinical sign of infection, there were similarities between both methods of wound swab collection and the species organisms (see Table 10).

In the chronic wound cohort all 58% (n = 29) had clinical signs of infection (Table 11). Increased exudate counted for 20% (n = 10) of the group, and no improvement in wound healing was present in 16% (n = 8) of the study cohort. In the remaining patients erythema was present in 8% (n = 4), poor quality granulation tissue was evident in 6% (n = 3), pain at the wound site accounted for 6% (n = 3), and warmth around the wound was present in 2% (n = 1).

With both the Levine and the Z technique when increased exudate was the clinical sign of infection in chronic wounds, no bacterium was cultured in one patient. A single strain of organism was identified in three patients with the Levine technique and in one patient with the Z technique. Two or more organisms were identified with both methods in the remainder of the patients (see Table 12).

When erythema extending greater than >1-2cm around the peri-wound area was present, in chronic wounds with both methods of wound swabbing a single strain of organism was identified in one patient, three organisms was detected in the remaining three patients with the Levine. However, with the Z technique three or more organisms were cultured in the remaining patients (see Table 13).

In both instances when warmth was the clinical sign of infection, in chronic wounds, there was no difference in the number or species of organism identified for both the Levine and the Z technique (see Table 14).

In chronic wounds when poor quality granulation tissue was the clinical sign of infection, with both the Levine and the Z technique the same single strain of organism was detected in one patient. In two patients with the Levine technique three organisms were present. By comparison with the Z technique three or more organisms were present (see Table 15).

When pain was present at the wound site in chronic wounds, the Levine technique cultured four or more organisms in each of the patients. By comparison the Z technique detected one organism in one patient's wound and in the remaining two patients four or more organisms was identified (see Table 16).

With both the Levine and the Z technique a similar pattern emerged for the number of organisms identified for each patient when there was no improvement in wound healing With both methods of wound swabbing, anaerobic organisms was cultured. Bacteria were not isolated in one patient with the Z technique. In the remaining patient's one or more organism was detected for both methods of wound swabbing (see Table 18). It is interesting to note that anaerobic organisms were not identified in acute wounds.

Inflammatory markers were tested by blood serology for elevated white cell count and C reactive protein. In 32% of cases the white cell count was normal and elevated in 18%. This test was not undertaken in nearly half of the study population (48%). C was reactive protein elevated in 46% of the group. At the time of specimen collection 52% were currently on systemic antibiotics and 48% were not on any antibiotics.

Using a one-sample *t test* there was also a statistical significant difference in the number of organisms detected between the Levine technique and the Z the technique. The Levine method detected more organisms ($t = 15.46$, $p = <0.001$), than the Z technique.

A statistically significant difference in the number of organisms detected in acute and chronic wounds was found. In acute wounds the Levine technique detected more organisms ($t = 9.55$, $p = <0.001$). In chronic wounds the Levine also detected more organisms ($t= 12.04$, p

<0.001). There was also a difference in the species of organisms detected between the Levine and the Z techniques.

When comparing the difference between the organisms identified in acute and chronic wounds with the Z technique, there were also differences in the species of organisms detected. *Acineobacter baumannii, Acinetobacter haemolyticus, Anaerobic organisms, Clostridium perfringens, Morganella morganii, Proteus species, Proteus vulgaris, Streptococcus milleri group, Serratia marcescens, Serratia odoriferans* were not detected in acute wound with the Z technique. Bacillus species, Cedecae species, *Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumoniae, Stenotrophomanas maltophillia (Xanthomonas maltoph)*, were not detected in chronic wounds with the Z technique.

Analysing by wound aetiology, demonstrated that other than pressure ulcers and wounds of "other aetiology" there was a difference in the number of organisms detected. Anaerobic organisms were only detected in the venous leg ulcers group with both the Levine and the Z technique.

Chapter five

Discussion

Comparison of wound swabbing methodology

In this study there was a statistically significant difference between the Levine and the Z technique in acute and chronic wounds (Table 20 - 23). The Levine technique detected more organisms ($t=12.04$, $p>0.001$). Gardner et al. (2006), compared wound exudate, the Z technique, the Levine technique and a sample of viable tissue, in chronic wounds. The authors did not elaborate on how they collected the sample of viable tissue. The mean concordance between Levine's technique and tissue specimens was 78%. Their findings also indicated that wound swabs using Levine's technique provided more accuracy than the Z technique. This study supports the work undertaken by Gardner et al. in chronic wounds.

In acute wounds a statistically significant difference between the two methods of wound swabbing was also demonstrated. The Levine method detected more organisms ($t=9.55$, $p=<0.001$) than the Z technique. This study also established that in acute wounds there is also a statistically significant difference between the two techniques (Tables 20 - 23).

Tissue biopsy and wound swabbing

Despite tissue biopsy being considered the 'gold standard' for collecting a wound sample for microbiological analysis, there is a growing body of evidence to support the use of wound swab cultures as a safer alternative to invasive tissue biopsy (Basak et al., 1992; Bill et al., 2001; Bornside & Bornside, 1979; Levine et al., 1976; Sapico et al., 1986; Slater et al., 2004; Sullivan et al., 2004; Uppal et al., 2007). However these

studies do not detail which method was used to collect the wound swab samples. Based on the results from this study and that of Gardner et al. (2006), one could hypothesize that the Levine technique is a safer and alternative than tissue biopsy at detecting organisms within an infected wound.

However, if the patient is not responding to systemic antibiotics and/or has sepsis, it is prudent to perform blood cultures and a tissue biopsy. Blood cultures will provide information on bacteria in the blood stream, causing sepsis. The clinician must bear in mind that tissue biopsy will only provide information from a small area of the wound bed. Therefore two or more biopsies may be required from different areas of the wound bed. If there is underlying osteomyelitis a bone sample maybe the only way of identifying the organisms involved.

Clinical signs of infection acute and chronic wounds

In the patients with acute wounds 16 out of the 21 patients had inflammation that persisted for more than 5 days (i.e. warmth redness, oedema and or localised pain). Purulent discharge was evident in six patients (Table 8). These overt signs of infection are consistent with the literature for the criteria for identifying wound infection in acute wounds (Bowler, 2003; Carville et al., 2008; Schultz et al., 2003; Stotts & Whitney, 1999).

All of the patients with chronic wounds displayed covert signs of infection. Increased exudate (n=10), and no improvement in wound healing (n=8), was the most frequent clinical sign of infection present. The remainder of the patients had warmth around the wound, poor quality granulation tissue, pain or tenderness at the wound site (Table 11). This is consistent with the literature (Carville et al., 2008; Cutting & Harding, 1994; Gardner et al., 2001; Schultz et al., 2003; Stotts & Whitney, 1999)

meeting the criteria for identifying wound infection in chronic wounds. None of this study population had documented wound odour.

Microbiology of acute and chronic wounds

Different species of organisms were detected in both acute and chronic wounds with the Levine and the Z technique in this study (Table 18 – 23). Anaerobic organisms were detected in the chronic wounds only, in particular venous leg ulcers (Table 25), by both methods of specimen collection. The laboratory did report the species of anaerobic organisms in one case *(Clostridium perfingens)*, with the Z technique. However, with the Levine technique the laboratory reported the presence of anaerobes but not the species. Identification of the species of anaerobic bacteria is generally considered to be expensive and labour intensive. Many clinicians argue that anaerobic organisms are harmful to wound healing (Eriksson et al., 1984; Gilchrist & Reed, 1989; Majewski et al., 1995; Sehgal & Arunkumar, 1992). Anaerobes are generally associated with leg ulcers, as was the case in this study (Bowler, 1998; Bowler & Davies, 1999a, 1999b). Anaerobic organisms are not commonly associated with acute wounds (Bowler & Davies, 1999a), this is thought to account for the lack of anaerobes identified in acute wounds in this study population, since the majority of wounds were chronic 58% versus 42% of acute wounds.

Gardner et al. (2006) limited their study to reporting all organisms, *Staphylococcus aureus, pseudomonas aeruginosa* and *Streptococcus* groups A and B. They did not identify which organisms were in the category 'all organisms'. This study reported all organisms identified with both techniques; consequently it is not possible to draw any conclusions from the study undertaken by Gardner et al. (2006). In comparing the organisms identified in acute and chronic wounds, with the Levine technique 25 different organisms were identified in acute wounds, compared to 20 organisms in chronic wounds. The Z technique identified 18 organisms in acute wounds compared to 23 organisms in chronic wounds. Over all, there

was a statistically significant difference between the two groups. The Levine technique identified more organisms in acute wounds ($t=9.55$, $p=<0.001$). The same statistically significant difference was found in chronic wounds with the Levine technique ($t=12.04$, $p<0.001$). There was however, a difference in the organisms detected between the two methods, as illustrated in Tables 18 - 23. Potentially this could clinically significant, as these organisms may not necessarily be covered by the appropriate antimicrobial therapy.

The findings of this is study demonstrate that that a wide variety of organisms colonise acute and chronic wounds, this is consistent with the findings in the literature (Bowler, 1998; Bowler & Davies, 1999a, 1999b; Bowler et al., 2001; Brook, 1987, 1989a, 1989b, 1989c; Brook & Frazier, 1990, 1997, 1998a, 1998b; Di Rosa et al., 1994; Gilchrist, 1996; Hansson et al., 1995; Pathare et al., 1998; Sapico et al., 1986). The majority of acute and chronic wounds in the study cohort were polymicrobial, making it difficult to determine which of the organisms were pathogenic and caused the wound infections. Gram-positive organisms, such as *Staphylococcus aureus* are usually present in acute wounds and considered the main contributor to cause wound infection (Bowler, 1998; Haneke, 1997; Klimek, 1985; Mayhall, 1993; Nichols & Smith, 1994; Page & Beattie, 1992). Based on the patients' clinical signs of infection, and the organisms identified in acute wounds (see Tables 11 – 17) similar organisms were identified in this study. This study found that the majority of chronic wounds were polymicrobial, (Tables 15-23). This is consistent with the literature, (Dow et al., 1999), *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two of the commonly cited pathogens, as was also the case in this study, (Brook, 1996; Daltrey et al., 1981; Danielsen et al., 1998; Gilland et al., 1988; Halbert et al., 1992; MacFarlane et al., 1986; Madsen et al., 1996; Schraibman, 1990; Sehgal & Arunkumar, 1992; Twum-Danso et al., 1992).

Both methods of specimen collection identified identical organisms in the pressure ulcers (Table 29) and wounds in the 'other' category (four traumatic wounds and one invasive cell carcinoma) (Table 31). Different organisms were identified with the both the Levine and the Z technique in arterial leg ulcers, venous leg ulcers, neuropathic foot ulcers, neuro-ischaemic foot ulcers and surgical wounds (Tables 24, 25, 26, 28, 30). Overall the Levine technique detected more organisms; this could be related to the method of collecting the wound sample. The Levine technique requires the specimen collector to rotate the swab over a 1 cm^2 with sufficient pressure to express fluid from within the wound. The Z involves rotating the swab in a 10-point zigziag fashion across the wound surface. It could be postulated that this lack of direct pressure in the one area has contributed to the Z technique detecting less organisms. The Z technique could perhaps be collecting organisms on the surface of the wound and none or fewer from the fluid within the soft tissue.

Biofilms and wound swabs

The presence of biofilms on the exposed extracellular matrix of the wound bed may have contributed to a difference between the organisms detected with the Levine and Z technique. Biofilms are communities of organisms encased within an extracellular polymeric substance (Association for the Advancement of Wound Care., 2008). Biofims are commonly associated with chronic wounds (Percival & Cutting, 2009). Since the majority of wounds in this study were chronic (58% versus 42% of acute wounds), it is reasonable to assume that biofilms were present in the chronic wounds swabbed. It is also therefore reasonable to suspect that biofilm formation may have had a role to play in the sensitivity of the swabbing techniques, in detecting the presence of microorganisms. However, it has been reported that antimicrobial agents and antiseptics cannot penetrate a bilofilm (Percival & Cutting, 2009), this explanation seems unlikely. More plausible is the difference between the two wounds swabbing techniques. The pressure exerted on the wound bed required to

collect the wound swab with the Levine technique may be responsible for collecting more planktonic or free-floating bacteria on the extracellular matrix, than the Z technique. With the Z technique the wound swab is rotated across the wound bed in a zigzag fashion, it is difficult for the person collecting the sample to maintain this technique and apply any pressure on the wound bed. It could be postulated that with the Levine technique, the pressure exerted may also be releasing organisms within the soft tissue that the Z technique, through the method of collecting the sample does not.

Tables 20 - 23 also illustrate that there was differences in the organisms detected with both the Levine and the Z technique in acute and chronic wounds. Acute wounds are not associated with biofilm formation (Association for the Advancement of Wound Care., 2008), and since the majority of wounds in this study were chronic, it is reasonable to postulate again that it is the technique of collecting the wound swab rather than biofilm formation that has contributed to a difference in the two techniques.

Limitations of the study

The sample size in this study was small and the study did not compare the Levine and Z techniques with tissue biopsy. Therefore the author is unable to determine the accuracy of the organisms detected between the 2 techniques employed.

Both the Levine and the Z technique are only suitable for open wounds healing be secondary intention. For the Levine technique the wound has to be greater that 1cm^2. The 'Z' technique requires a wound large enough to swab the wound in a 10-pont zigzag fashion across the wound bed. For wounds that are approximated such as surgical wounds that display signs of a surgical site infection, which may or may not have wound dehiscences, it is not possible to use either method. It is also not

achievable to use either method in cavity wounds where the base cannot be identified. Therefore the study only provides guidance in swabbing open wounds

Tables 8-10 & 11-16 illustrate the clinical signs of infection present and the organisms identified. Analysis was not conducted to determine which patients had one or more clinical sign of infection, a recommendation for further studies. The author could only report on each individual clinical sign of infection and organisms identified with both the Levine and the Z technique.

Recommendations

The author would recommend repeating the study with a larger size population. The study should also be replicated and compare the effectiveness of tissue biopsy with both methods of wound swab collection. Based on the study results it could be determined that the Levine method is more efficacious than the Z method and this should be a clinical consideration when collecting a wound swab.

It is also recommended that any further studies incorporates a method of data collection that allows for analysis of each of the clinical signs of infection present in individual patients. This would provide a comprehensive insight into the relationship between specific clinical signs of infection and organisms responsible for causing the wound infection.

As increasing evidence proposes bioflims are problematic in chronic wounds future studies could include sampling methods that identify the biofilms and organisms contained within them.

Conclusion

The study illuminates the clinical efficacy of the Levine and the Z methods for wound swab collection and semi-quantitative microscopy. The results from the study suggest that the Levine method is more reliable in determining the organisms in acute and chronic wounds when wound swabbing is the selected method for sampling and culture.

Consistent with the literature that chronic wounds are polymicrobial, this study also discovered that chronic wounds isolated more organisms. This makes the treatment of these wounds challenging, highlighting the need for accurate specimen collection and subsequent antimicrobial therapy.

The isolation of anaerobes is poorly reported, this study goes some way in validating that they are less likely to be cultured from acute wounds.

Biofilms are difficult to identify and contribute to delayed wound healing in chronic wounds. A discrepancy between the results from the Levine and Z techniques may be contributed to biofilm formation on the exposed extracellular matrix. The pressure applied to the wound bed when employing the Levine technique may be responsible for collecting planktonic bacteria released from the biofilm. However without microscopic analysis of the bacteria within the biofilm one can only hypothesize that this may be the case.

Overall the study provides the clinician with evidence of the superiority of the Levine technique over the Z technique, when wound swabbing is clinically indicated.

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Appendix I

Patient information sheet

Title

Comparison of two semi-quantitative wound-swabbing techniques to establish the clinical efficacy in identifying causative organism(s) in infected cutaneous wounds.

Wound Swabs

You are being asked to participate in a research study, which is being conducted by Donna Angel Nurse Practitoner, Royal Perth Hospital and a student at Curtin University of Technology. Please take the time to read this information. Please note that the Ethic's Committees have approved this study at both Royal Perth Hospital and Curtin University of Technology.

What is the study about?

When there is a suspected wound infection, a wound swab is collected and sent to the laboratory in order to find out what organisms or bugs are growing in the wound. This lets the Doctor know which antibiotics to prescribe if required.

The purpose of this study is to compare two different methods of collecting a wound swab and to determine which is the best method.

If you decide to be involved in the study it will mean that we will take a second swab from your wound. This will take approximately an extra 5 minutes. You will not experience any further discomfort as a result of the second swab being taken.

Who is conducting the study?

Donna Angel Nurse Practitioner, Royal Perth Hospital and a student at Curtin University of Technology, will be conducting the study and either

herself or one her colleagues, a Registered Nurse employed at Royal Perth Hospital will be collecting the wound swabs.

Who is invited to participate?

Any patient with a wound where infection is suspected and where a wound swab would usually be collected is invited to participate.

Voluntary participation

It is important for you to know that you do not have to take part in this project, and if you decide not to be involved your care will not be compromised in any way. However, a single wound swab will still be collected in the usual manner.

How will your privacy be protected?

If you decide to take part in this study, all information relating to you that is used, as part of this study will be kept strictly confidential. The research data and results of this study will be presented at conferences and reported in journal articles but this will not involve the reporting of your personal information. In accordance with The National Health and Medical Research Committee (NHMRC) guidelines, all study records will be stored for five years by Ms Angel and kept under lock and key in a secure location and then will be destroyed.

Who to contact if you have any questions about the study?

This study has been approved by the Curtin University Human Research Ethics Committee (Approval Number HR 103/2007). The committee is comprised of members of the public, academics, lawyers, doctors and pastoral carers. Its main role is to protect participants. If needed, verification of approval can be obtained either by writing to the Curtin University Human Ethics Committee, c/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784 or by emailing hrec@curtin.edu.au.

If you have any questions please contact Professor Nick Santamaria on 0414560929.

If you wish to make a complaint you can contact the Human Ethics Committee (Secretary) 9266 2784. hrec@curtin.edu.au or in writing C/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth WA 6845

Appendix II

Patient consent form

Study Title

Comparison of two semi-quantitative wound-swabbing techniques to establish the clinical efficacy in identifying causative organism(s) in infected cutaneus wounds.

I have been informed of and understand the purposes of the study. I have read and understood the patient information sheet related to the above study, and I have had the opportunity to ask any questions related to my involvement in the study. I understand I can withdraw at any time without prejudice. Any information, which might potentially identify me, will not be used in published material.

I freely consent to my involvement in the study and the use of my data as specified in the patient information sheet.

Patient

Print name: ----------

Signature: ----------

Date: ----------

Witness

Print name: ----------

Signature: ----------

Date: ----------

Appendix III

Data collection sheet

Title: The effectiveness of two semi-quantitative wound swabbing techniques for determining microbiological bioburden in clinically infected cutaneous wounds.

Patient sticker

1. Gender:
   - ☐ 1. Male
   - ☐ 2. Female

2. Wound aetiology:
   - ☐ 1. Arterial
   - ☐ 2. Venous
   - ☐ 3. Mixed (arterial/venous)
   - ☐ 4. Neuropathic
   - ☐ 5. Neuro ischaemic
   - ☐ 6. Pressure ulcer Stage (circle): I II III IV
   - ☐ 7. Surgical
   - ☐ 8. Other
   - 9. Specify:----------

3. Wound duration (days) _____

4. Anatomical location: _____

5. Wound Depth (please circle):
   1. Intradermal
   2. Involving, but not extending past the subcutis

3. Tendons and muscle on view
4. Bone on view

6. Acute/Chronic wound (a wound present for 6 weeks or longer will be considered chronic)

☐ 1. Acute (if yes complete Q7 then proceed)
☐ 2. Chronic (if yes complete Q8 then proceed)

7. Acute Wound: Signs and symptoms of wound infection (please circle all that apply)

1. Inflammation that persists for more than 5 days (i.e. warmth, redness, oedema, localised pain)
2. Purulent drainage
3. Core body temperature of >38^{o}C
4. Spontaneous dehiscence
5. Abscess present

8. Chronic wound: Signs and symptoms of wound infection (please circle all that apply)

1. Increased exudate
2. Presence of odour
3. Erythema >1-2cm
4. Warmth around wound
5. Poor quality granulation tissue (friable bright red, exuberant)
6. Pain or tenderness at the site
7. No improvement in healing in 2 weeks in a clean wound

9. Antibiotics:

☐ 1. No
☐ 2. Yes (if yes complete below)
☐ 3. Oral: Specify drug and dose duration of therapy----------
---

☐ 4. Intravenous: Specify drug and dose----------
---

10. Investigations:

Please attached each wound swab result from isoft

Wound swab Z technique:

☐ 1. No organisms
☐ 2. Organisms

Organisms Identified and amount

Levine technique:

☐ 1. No organisms
☐ 2. Organisms

Organisms identified and amount:

---

11. White Blood Cell Count

☐ 1. Normal (Reference range: 3.4-10x10^9/L
☐ 2. Elevated
☐ 3. Not performed

12. Erythrocyte Sedimentation Rate (please circle):

☐ 1. Normal (reference range: 3-10mm/hr)
☐ 2. Elevated
☐ 3. Not performed

13. C-Reactive Protein:

☐ 1. Normal (reference range <10mg/l)
☐ 2. Elevated
☐ 3. Not performed

14. Other: