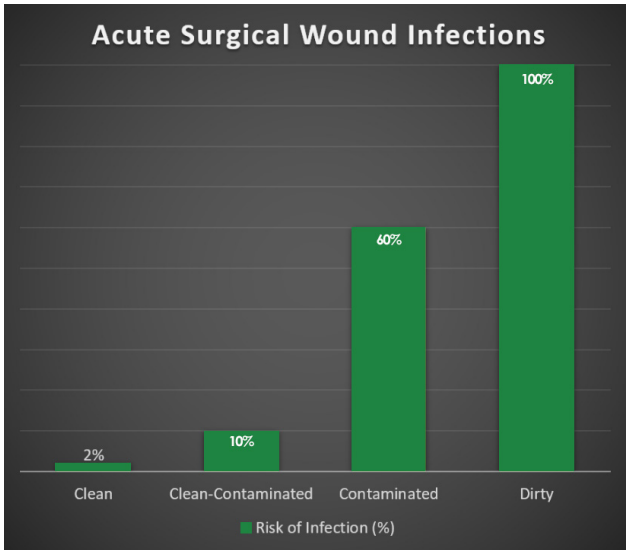
FROM THE LAB

Precision Wound Care Guided by Molecular Diagnostics

Acute Wounds & Infectious Risk

Acute wounds are characterized by a disturbance in the protective skin barrier. Outside of unintentional trauma, the most common etiology of an acute wound is a surgical incision. Even with the advent of sterile technique and minimally-invasive operating procedures, acute surgical wounds account for up to sixteen percent of all nosocomial infections in the United States.[1] Individuals who develop a wound infection following surgery spend on average more than ten days in the hospital compared to those who do not develop an infection.[2] The detriment of prolonged hospitalization is further compounded by an elevated risk of additional complications and a marked increase in both direct and indirect healthcare costs for the patient.[3]

Given these implications, acute wounds secondary to surgery are classified by their risk for infection as clean, clean-contaminated, contaminated, or dirty.[4] Clean surgical wounds are those performed in healthy, non-infected tissue without penetration of the viscera. Clean wounds carry the lowest risk of infection at approximately one to two percent.[5] Surgical incisions that require intentional entry into the viscera are considered clean-contaminated. Even with an intact sterile field, clean-contaminated wounds carry a risk of infection as high as ten percent.[5] Wounds are characterized as contaminated when entry into a visceral organ is unintentional, the incision was performed in an area of significant inflammation or infection, or when a major disruption of the sterile field occurs. Resultant infections occur in up to sixty percent of contaminated surgical wounds.[5] Finally, surgical wounds are dirty when incisions are performed in grossly purulent areas, a foreign body is present, or large-scale spillage occurs from an unintentionally perforated segment of the bowel. Dirty surgical wounds frequently progress to infections caused by a large burden of diverse pathogens, which can make them difficult to diagnose and treat.



The Vicious Cycle of Chronic Wounds & Infection

Chronic wounds persist for a prolonged duration or exhibit continued recurrence. Common forms of chronic wounds, such as pressure ulcers, diabetic foot ulcers, arterial insufficiency ulcers, and venous stasis ulcers, affect more than six million people in the United States alone.[6] These wounds are notoriously difficult to manage and are associated with a reduced quality of life, more frequent hospitalizations, and increased disability.[7] Furthermore, patients with chronic wounds require frequent utilization of the healthcare system, imparting an economic burden reported to exceed twenty-five billion dollars per year.[8]

Challenges associated with chronic wounds stem from a reciprocal relationship between underlying dysfunction of the normal healing process and the subsequent development of a complex microbiome within the wound. In contrast to acute wounds, chronic wounds exhibit impaired healing driven by progressive ischemia, necrosis, and fibrosis of the tissue.[9] As this process continues, the accumulation of necrotic tissue fosters the ideal environment for the unregulated growth of a diverse spectrum of microorganisms. Together, multiple pathogens may act synergistically to form a bacterial biofilm, which can confer antimicrobial resistance, escalate pathogenicity, and further impede the healing process of the

wound.[10] Ultimately, the unrelenting cycle of impaired healing and polymicrobial infection drives the significant increase in morbidity and mortality experienced by patients with chronic wounds.[11]

Microbiology of Wound Infections

Management of both acute and chronic wound infections is a multifaceted endeavor that requires a combination of surgical debridement, local antimicrobial measures, and antibiotic therapy. Ideally, these actions are driven by timely and accurate identification of causative pathogens as well as their susceptibility to antimicrobials. However, this process is complicated by the diverse and continually evolving bacterial composition underlying most wound infections.

Initially, the microbiome within a wound is primarily composed of normal skin flora. However, *Staphylococcus aureus* and *coagulase-negative Staphylococcal species* (e.g., *epidermidis*, *saprophyticus*) rapidly become the predominant microorganism in many acute wounds. Studies have shown *Staphylococcus aureus* is present in up to 88% of chronic lower extremity wounds in which there is a clinically apparent infection and up to 43% in those without obvious signs of infection.[12] Numerous other aerobic bacteria have been routinely identified in acute and chronic wounds, including *Pseudomonas aeruginosa*, group A *Streptococcus*, *Klebsiella species* (e.g., *aerogenes*, *michiganensis/oxytoca*), *Proteus mirabilis*, *Enterobacter cloacae*, and *Escherichia coli*. As the wound progresses, necrosis of deeper tissue also increases the likelihood that obligate anaerobes, such as *Bacteroides fragilis*, will be present. The volatile nature of the wound microbiome was thoroughly illustrated by a prospective study that showed chronic wounds acquired a new aerobic or anaerobic bacterium in 45% and 90% of cases over eight weeks.[13] As the microbial diversity broadens, wound infections become increasingly difficult to treat; therefore, early and accurate detection of pathogens becomes paramount.

Diagnostic Modalities for Detecting Wound Pathogens

Cultures have historically been relied upon as the lone modality for the identification of wound pathogens and antibiotic sensitivity testing.[14] While routine cultures can detect numerous common wound pathogens and determine their susceptibility to various antibiotics, this method has drawbacks when used independently of other diagnostic tests. First, cultures are time-consuming to perform, and many pathogenic bacteria are difficult to culture using standard techniques.[15] Cultures have also been found to exhibit reduced sensitivity and specificity when employed for polymicrobial infections, which are common in both acute and chronic wounds. This results from the inability of cultures to quickly cultivate many of the organisms that contribute to biofilm formation and a lack of precision needed to determine the relative burden of competing organisms.[16] Incomplete and inaccurate culture results also frequently lead to the use of inadequate or inappropriate antibiotics in wound patients, which may prolong chronicity and promote bacterial resistance. For example, one study found that patients with chronic wounds received an average of 2.3 courses of antibiotic therapy per year versus 0.6 courses in patients without a chronic wound.[12]

Given these concerns, molecular diagnostic techniques have been increasingly used as an adjunct for the identification of causative pathogens in numerous types of infections, such as those involving the blood, cerebrospinal fluid, and joints. Real-time polymerase chain reaction (PCR) tests are the most frequently employed, as they provide a rapid, accurate, and affordable complement to cultures. In contrast to the lag-time required by cultures, PCR results can be readily available within four to six hours. The literature has also demonstrated the importance of PCR in ensuring all pathogens responsible for a chronic wound infection are accurately detected. One study reported the analytical sensitivity of PCR for detecting aerobic and anaerobic bacteria in chronic wound infections to be 100% and 90% when compared to quantitative and qualitative cultures, respectively.[17] The same study found that PCR detected an additional ten bacteria in the chronic wounds that were not identified by either quantitative or qualitative culture. Similar findings were reported by another study, which found that when used to analyze the microbiome of 168 wounds, culture identified only 17 different bacterial taxa compared to 338 bacterial taxa identified by PCR. Many of the bacterial species identified by only PCR in this study comprised a small proportion of the total bacterial burden; however, this sample included potentially pathogenic bacterial species such as *Bacteroides*, *Serratia*, and *Staphylococcus*.[18]

Role of PCR in Combatting Antibiotic Resistance

Polymicrobial infections affecting chronic wounds have become increasingly synonymous with antimicrobial resistance due to biofilm-protected growth and the free exchange of genetic material across a broad spectrum of microorganisms. Specimens collected from chronic wounds of patients in one hospital demonstrated methicillin-resistance in up to 50%

of *Staphylococcus aureus* (MRSA) isolates and ciprofloxacin resistance in up to 33% of *Pseudomonas aeruginosa* isolates.[19] Moreover, chronic wounds were responsible for the first two cases of vancomycin-resistance *Staphylococcus aureus* (VRSA) detected in the United States.[20] The idealistic breeding ground of chronic wounds for resistance can be further exacerbated by the haphazard and recurrent administration of broad-spectrum antibiotics. Another study demonstrated this by showing that patients that had previously been administered numerous rounds of antibiotic therapy for their chronic wounds had significantly more isolates of MRSA than those who were antibiotic-naïve.[21]

The use of adjunct PCR can also be useful in combatting the issue of antibiotic resistance within chronic wound infections. In addition to the identification of bacteria, real-time PCR is also capable of simultaneously detecting numerous common resistance markers. The presence of resistance against many of the most commonly used antibiotic classes for treating chronic wounds can be detected, including beta-lactams, fluoroquinolones, sulfonamides, penicillin-like drugs (e.g., mecA), and vancomycin (e.g., vanA, vanB). When used in combination with culture-based antimicrobial sensitivity testing, antibiotic resistance testing with PCR can allow providers to administer personalized and targeted antibiotic therapy to each patient. Initial therapy guided by diagnostic studies can reduce the need for frequent antibiotic administration. Rapid treatment with an appropriate antibiotic may result in a faster return to normal wound healing and improved patient outcomes. Moreover, it can lessen the push toward new and difficult to manage bacterial resistance.

Assurance Scientific Laboratories Panels for Wounds/Infections

Assurance Scientific Laboratories offers a real-time PCR-based wound/infection panel for the detection of bacterial pathogens in both acute and chronic wounds. Also, antibiotic resistance markers can be performed alongside the wound panel or independently. We also offer cultures with antimicrobial sensitivity testing for both gram-positive and gram-negative organisms. Beyond this, our lab is currently in the process of finalizing a PCR-based fungus panel aimed to guide the management of onychomycosis, another chronic and often difficult to treat infection.

General Guidelines

Laboratory test results should always be considered in the context of clinical observations and epidemiological data (such as local prevalence rates and current outbreak/epicenter locations) in making a final diagnosis and patient management decisions. With any test, the possibility of false-positive and false-negative results should always be considered, and the impact on patient management decisions and clinical outcomes should be carefully weighed.

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