The use of a point-of-care test for bacterial protease activity in chronic wounds

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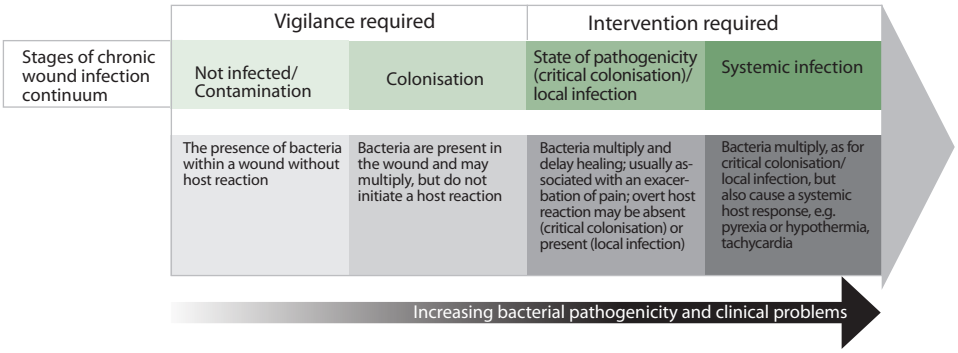
Most wounds contain micro-organisms, but heal successfully[1]. However, in some wounds the presence of bacteria may cause delayed healing and local and/or systemic infection, which may reduce quality of life by increasing morbidity, mortality, hospitalisation and socioeconomic burden[2, 3, 4]. It can be difficult to recognise when bacterial burden is affecting healing and requires intervention, particularly in chronic wounds[5]. A 2008 international consensus document recognised that diagnostic tests for wounds that are not healing as expected, such as tests that signal when bacteria are having harmful effects, have the potential to improve outcomes and to have economic benefits[6]. WOUNDCHEK™ Laboratories is developing a rapid point-of-care test to identify wounds in which bacteria may delay healing. A positive test result indicates that the wound contains levels of a group of enzymes — the bacterial proteases — that may be detrimental to healing and indicate imminent or current infection. This article explores the potential role and benefits of this test in the management of chronic wounds.

The effects of bacteria in chronic wounds may be viewed as a continuum of increasing clinical importance, ranging from contamination to colonisation to critical colonisation/local infection to systemic infection[3,7]. These stages can be characterised according to bacterial pathogenicity *[Box 1]*, host response and tissue effects, i.e. the patient's immune system response and resultant signs of inflammation and tissue damage[7, 8] *[Figure 1]*.

The term 'critical colonisation' was developed to convey the concept that bacteria in wounds may delay healing without causing overt wound infection and the classic signs of spreading inflammation[9]. The term continues to cause debate; it was first described as local infection by Cutting and Harding in 1994[10] and as localised infection by the World Union of Wound Healing Societies in 2008[7]; more recently, Serena et al have proposed the term 'state of pathogenicity'[11]. This is defined as the stage when bacteria begin to produce proteases (virulence factors).

Bacteria in wounds can be planktonic (i.e. mobile, non-attached, single), or sessile (i.e. immobile, attached, dormant) within a biofilm[12]. Many chronic wounds contain biofilm on at least some of the wound bed[13]. Biofilm comprises bacteria embedded in a self-generated matrix or 'slime' that adheres to the wound bed. The biofilm matrix protects the bacteria from the patient's immune defences. However, the biofilm can stimulate a chronic inflammatory response that may contribute to delayed healing and may release planktonic

Figure 1. The wound infection continuum in chronic wounds[3, 8, 11].



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Box 1. Definitions[16, 17, 18].

- **Pathogen:** a micro-organism that causes or is able to cause disease
- **Pathogenicity:** the ability of a micro-organism to cause disease
- **Quorum sensing:** interbacterial communication that regulates gene expression according to the population density of bacteria
- **Virulence:** a quantitative measure of the likelihood that a pathogen will cause disease
- **Virulence factor:** a molecule produced by bacteria to facilitate colonisation, replication and spread within a host

bacteria and biofilm fragments that can disperse to other parts of the wound bed or to other wounds[14; 15].

Chronic wound assessment: challenges on the infection continuum

Clinicians trying to identify when bacteria are causing problems in chronic wounds, and which bacteria are responsible, are faced with a variety of difficulties that can lead to under- or over-diagnosis[19]. Clinicians rely largely on a combination of signs, symptoms and experience to decide when a wound is infected and intervention is required[20].

Clinical criteria

Overt wound infection may be characterised by the classic signs and symptoms that occur as a result of the immune response to bacteria. These signs and symptoms are pain, heat, swelling, redness and purulent exudate[21]. However, in chronic wounds, assessment for infection can be particularly challenging because the classic signs may not be present: the presence of comorbidities (such as diabetes, other immune modifying conditions, peripheral vascular disease or advanced age) may suppress the immune response[3, 7, 22]. Bacteriological studies of biopsies from apparently uninfected venous leg ulcers found that, microbiologically, about a quarter of wounds could be classified as infected, i.e. stating that the wound was uninfected was correct in only about 75% of wounds[23, 24].

Over the years, attempts have been made to clarify signs and symptoms that are indicative of problematic bacterial burden in chronic wounds[10, 21, 25, 26]. Increased exudation, malodour, delayed healing, friable granulation tissue and newly occurring or increased levels of pain are examples of criteria that have been used to describe the secondary signs and symptoms of wound infection[4].

Microbiological investigations

Microbiological analysis of wound samples may be undertaken to confirm a clinical diagnosis of wound infection and to indicate appropriate management[27, 28].

Most chronic wounds contain multiple bacterial species, meaning that interpreting microbiology reports may not be straightforward. As a result, microbiological reports should not replace clinical judgement, but be used to guide antimicrobial therapy if deemed clinically appropriate[29]. Depending on the method of sampling, microbiological analysis may provide information on the level of bacterial burden, in addition to the species present and antibiotic sensitivities[30].

Traditionally, a bacterial load of ≥1 x10^5 CFU/g (colony-forming units per gram of wound tissue) has been used as the threshold for diagnosing wound infection[31]. However, in non-healing wounds, this threshold may not be appropriate because the relationship between bacterial load and infection is not straightforward[4]. Bacterial burdens below the threshold may delay healing, particularly in patients with impaired immune defences and/or when particularly virulent bacteria are involved; some wounds with burdens well above the threshold may heal without antimicrobial intervention[29, 32].

In addition, samples obtained using wound swabs may be unrepresentative of the microbiology of the whole wound and may not sample bacteria below the wound surface or identify the species responsible for impaired healing. Although a biopsy is likely to produce a sample more representative of the bacterial profile through the full thickness of the wound, it is invasive, costly and may cause complications[22].

Whichever sampling method is used, microbiological examination is an additional expense, and results may not be available to clinicians for several days or more after sampling[3]. Debate about the best technique for acquiring samples and the precise role for microbiological analysis in chronic wound management is ongoing[31, 33].

Chronic inflammation

An additional challenge in the management of chronic wounds is that some wounds may be caught in a cycle of perpetuated chronic inflammation that may at most be only partially attributable to bacteria[34,35]. This state of chronic inflammation damages the extracellular matrix and degrades growth factors involved in repair. The damaged wound tissue stimulates further release of inflammatory mediators, such as cytokines and free radicals, to cause a heightened inflammatory response, further tissue damage and delayed healing *[Figure 2]*.

Differentiating the signs and symptoms of chronic inflammation from those of wound infection can be difficult[36]. These difficulties with differentiation, combined with the delay and problems inherent in microbiological analysis and interpretation, can potentially lead to both over- and under-usage of antimicrobial therapy[37]. Over-usage is of

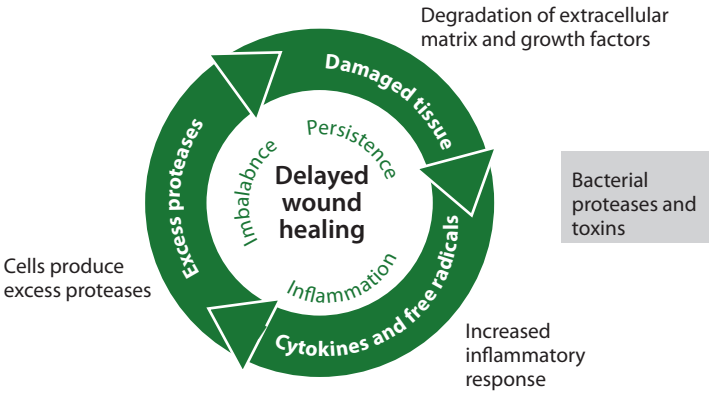


Figure 2. Cullen's circle: the role of chronic inflammation in delaying wound healing[39].

particular concern because of the rise in resistance to antimicrobials and especially to antibiotics[38]. A test that indicates when bacteria in a wound are likely to be causing or are about to cause detrimental effects may aid clinicians in the judicious and appropriate use of antimicrobial agents.

Bacterial pathogenesis: role of virulence factors

Chronic wounds often contain multiple species of bacteria and so are at increased risk of infection[40]. Bacterial pathogens are those species of bacteria that cause, or are capable of causing disease or harm, such as delayed healing or overt wound infection[17]. However, an individual species of bacterium can exist in non-pathogenic and pathogenic states[17, 41].

Conversion to a pathogenic state is largely dependent on the interaction between the bacteria and the host[17]. Some bacteria are reliant on a breach in a host barrier, e.g. a wound, to enter tissues and cause infection, while others have evolved mechanisms to cross host barriers, such as intact skin, or to overcome the immune system to cause disease[41].

Pathogenicity may also arise when the immune response to bacterial invasion is insufficient and allows bacteria to multiply and spread. Disease may also occur when there is an exaggerated immune response to the presence of bacteria, e.g. excessive inflammation, which itself causes tissue damage or aids further disruption of host barriers[17, 41].

Virulence factors

Bacteria in a pathogenic state produce a range of molecules known as virulence factors [16] *[Box 1]* to aid the process of infection[12, 42–46].

Bacteria produce virulence factors at a variable rate in response to changes in the environment, the stage of infection and the host defence mechanisms faced[41, 42, 45]. Some bacteria, e.g. *Pseudomonas aeruginosa*, also use quorum sensing *[Box 1]* to trigger production of virulence factors once a critical concentration of bacteria has been reached[46, 47].

Bacterial protease virulence factors

Bacterial proteases are regarded as the most important of all bacterial virulence factors in the establishment of infection[48]. Proteases are enzymes that act on proteins, usually by splitting a protein molecule into shorter fragments. The effects of proteases include inactivation or breakdown of proteins.

Bacterial proteases are known to be produced by a number of the bacteria found in chronic wounds, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis* and *Enterococcus faecalis*[49]. There are many different types of intracellular and extracellular bacterial proteases, including serine, cysteine and metallo- proteases[12]. Some have non-specific actions and are capable of degrading a wide range of proteins; others have highly specific targets[50].

The main role of extracellular bacterial proteases is to degrade host tissue proteins to provide nutrients for the bacteria[48]. However, bacterial proteases also aid the process of infection by assisting with evasion and destruction of host immune defences, and local and systemic spread[44, 45, 49].

Bacterial proteases interfere with immune function in a number of ways, including breaking down antibody molecules and impeding immune cell function by preventing phagocytosis, suppressing chemotaxis and hindering immune cell communication[49]. Some also degrade enzymes involved in activation of the complement system, a component of the immune system that aids recognition of foreign material (including bacteria) in host tissues[49].

Bacterial proteases may also induce the host to produce an excessive and prolonged inflammatory response. The inflammatory response increases host protease production and interferes with host protease regulatory mechanisms. This contributes to the vicious circle of delayed healing in which chronic wounds may become trapped[35, 49] *[Figure 2]*. In addition, bacterial proteases can contribute to problems with wound healing by degrading growth factors and their receptors[51].

The overall effect of bacterial protease production for the wound is tissue damage leading to delayed healing, with increased risk of local and systemic infection[52] *[Figure 3]*.

Clinical value of testing for bacterial protease activity

Misdiagnosis of wound infection versus chronic inflammation can occur due to subtle signs and symptoms. Consequent implementation of inappropriate management and lost opportunities for effective treatments may result in unnecessary economic, clinical and psychosocial costs.

The presence of bacterial proteases in a chronic wound signals impending or active infection, whether or not clinical signs of infection are present[52, 53]. Detection of bacterial protease activity (BPA) therefore has the potential to allow recognition of when bacteria are behaving pathologically in wounds where infection is not obvious and has the potential to allow prompt action to reduce bacterial load in the wound.

If bacterial burden is not reduced, there is a risk that the wound will progress along the infection continuum *[Figure 1]* towards more overt infection, potentially increasing morbidity and mortality. Reducing bacterial burden in wounds that are BPA positive, and so reducing bacterial protease production and the potential for infection, is likely to be associated with improved clinical outcomes, cost savings and other economic benefits[52, 54].

Point-of-care test for BPA

A new test is being developed by WOUNDCHEK Laboratories to allow clinicians to determine non-invasively, and in 15 minutes, whether a wound contains bacteria that are acting pathogenically and are, therefore, likely to be causing tissue damage. Another point-of-care test has been developed that looks at elevated protease activity (EPA) in wounds, which may be another potential cause of delayed healing[35].

The test will use chronic wound fluid collected with a swab from the surface of the wound using a specific collection technique known as the Serena Technique®, in which the wound is covered with saline and the entire wound surface is swabbed[55]. A positive result will indicate that BPA is present in the sample.

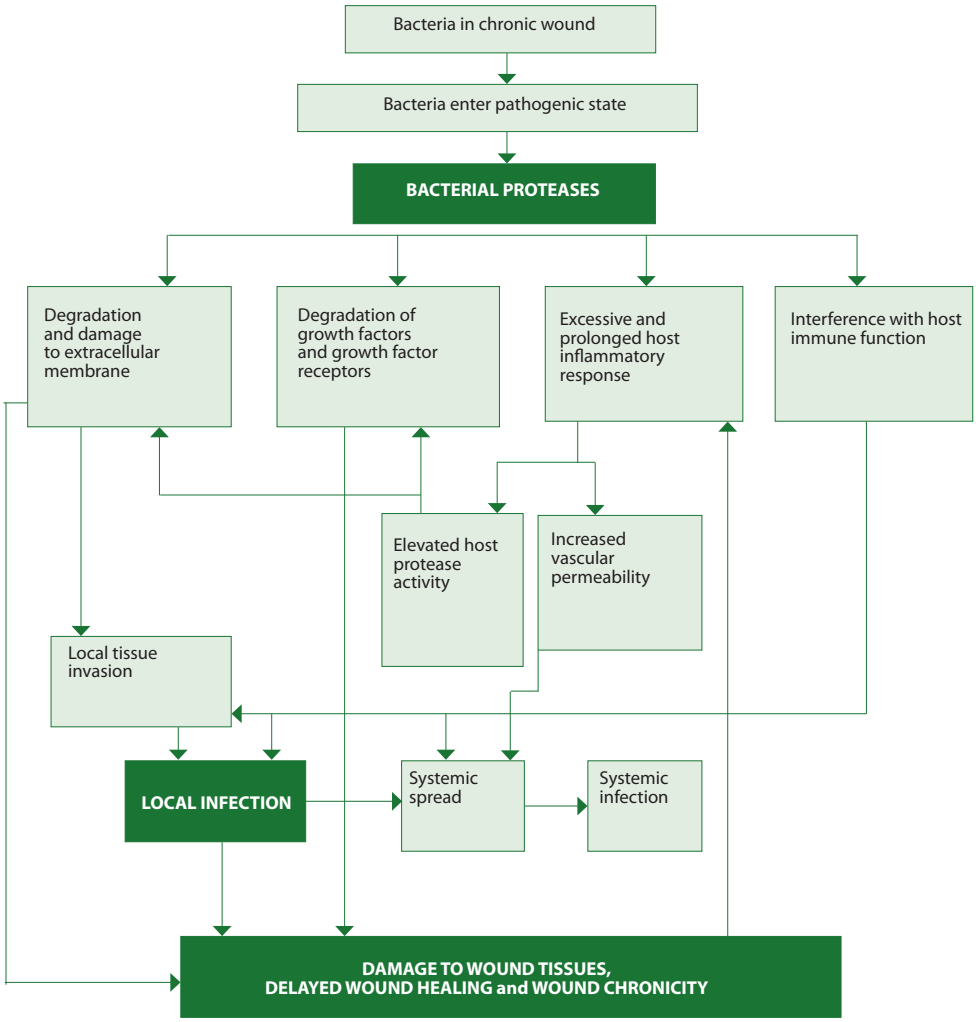


Figure 3. Contribution of bacterial proteases to the development of wound infection and delayed healing.

BPA and bacterial burden in chronic wounds

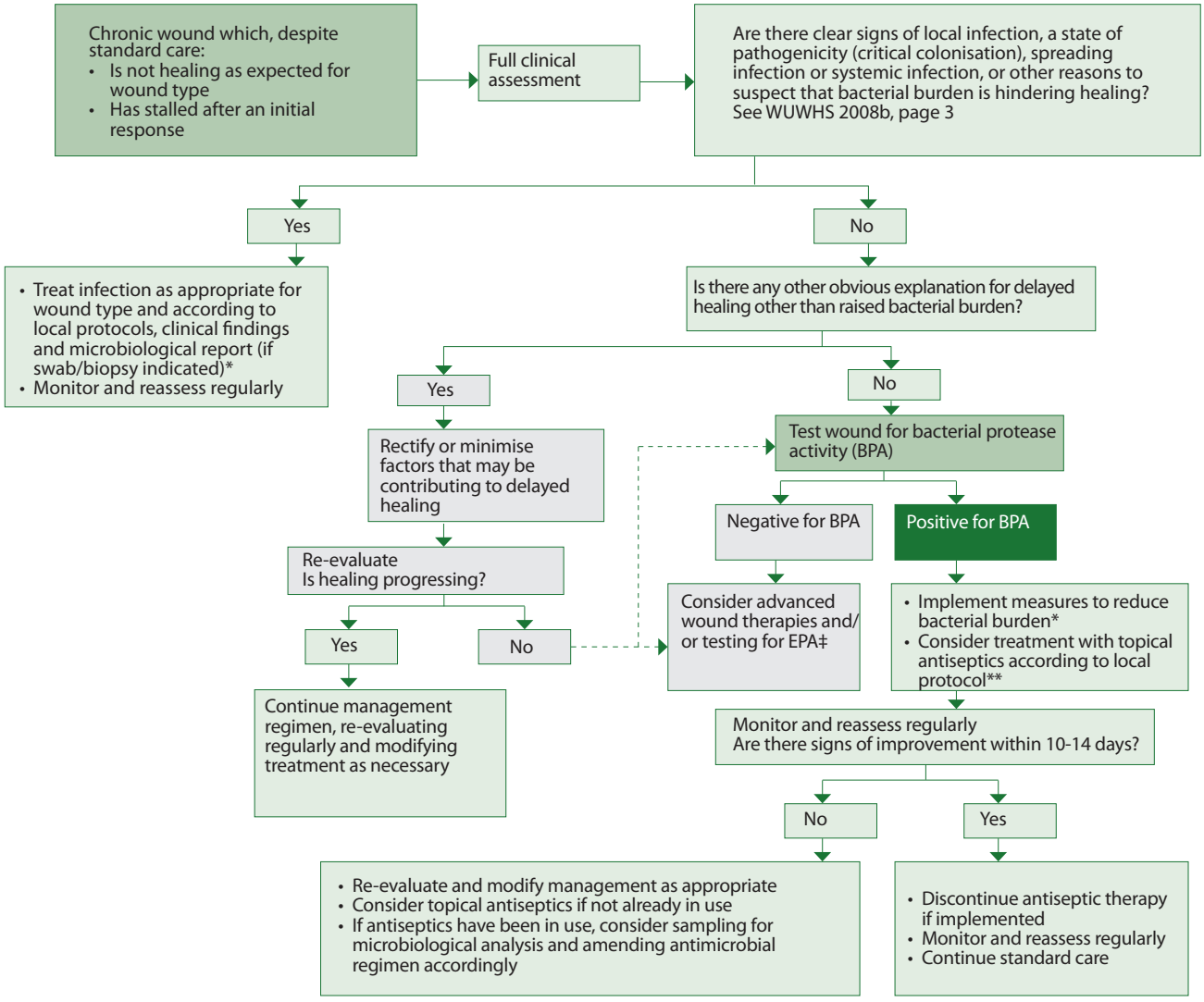
A multicentre study of 366 patients with a range of chronic wound types was carried out in the US[56]. Patients were assessed for signs of infection using validated assessment criteria[26] and wounds were swabbed to test for BPA and to assess quantitative bacterial load. The study found that 72% of wounds had >10^5 CFU/ml, but only 18% had signs of clinical infection. In contrast, 49% of wounds were positive for BPA. Of the BPA positive wounds, 77% did not have signs of infection. Overall, 38% of the wounds assessed were positive for BPA, but did not show clinical signs of infection. The investigators concluded that these wounds represented those in which the bacteria were behaving pathogenically but that overt infection was not yet present[56].

A further study investigated the relationship between BPA status and the production of two markers of a host inflammatory response, IL1-β and TNF-α. This found that production of both markers was significantly higher in wounds that are BPA positive when compared to wounds that are BPA negative[11].

Potential pathway for the use of a point-of-care test for bacterial protease activity (BPA)

A positive result from a BPA test alerts clinicians to pathogenic behaviour by bacteria in a wound. Therefore, the potential roles of the point-of-

Figure 4. Potential pathway for the use of a point-of-care test for bacterial protease activity (BPA).



*Incorporate into management plan:
- Optimisation of host response: nutrition, hydration, glycaemic control, tissue perfusion
- Reduction of bacterial load: prevent further contamination or cross-contamination, facilitate wound drainage, debride wound, increase dressing change frequency, cleanse wound at every dressing change, manage excess exudate, manage malodour, topical antiseptic +/- systemic antibiotic(s)
- General measures such as management of symptoms, patient and carer education, optimise patient cooperation, ensure psychosocial support [WUWHS, 2008b]

**Systemic antibiotics are usually reserved for patients with spreading or systemic infection; avoid use of topical antibiotics [WUWHS, 2008b]

‡ If positive for elevated protease activity (EPA), consider incorporating protease-modulating interventions into management [International Consensus, 2011; Dissemond et al, 2013]

Box 2. Questions for further research.

- Is there a correlation between a BPA positive test result and the presence of biofilm in a wound?
- Can retesting for BPA be used to monitor treatment?
- Does early intervention to reduce bacterial burden in a wound that is BPA positive improve outcomes?
- Does BPA testing help to determine which chronic wounds are suitable for antibiotic treatment?
- Is there a role for BPA testing in acute wounds?

care test for BPA are as an adjunct to wound assessment and to guide the management of chronic wounds with delayed healing that are not obviously infected *[Figure 4]*. BPA testing may also prove useful in indicating which wounds could be considered for advanced wound therapies, including protease modulation. As a result, initially the BPA test will be most suited for use at referral centres, such as specialist wound clinics.

Approaches to reducing bacterial burden include optimising host response, preventing further contamination, debridement, more frequent dressing changes, cleansing, and managing excess exudate and the use of topical antiseptics (e.g. iodine, silver, polyhexamethylene biguanide [PHMB]) in dressings and/or during cleansing[2, 7, 58].

Wounds that are positive for BPA may also contain biofilm[12,14]. It is not known, however, to what extent the test could be used to indicate the presence of biofilm. Even so, the management of a wound that is BPA positive includes measures (e.g. barrier dressings, debridement and the use of antiseptics) that are also indicated for the treatment of biofilm[15, 59, 61].

Overuse of antibiotics leading to resistance is a cause for concern worldwide, and particularly so in the management of chronic wounds. The polymicrobial nature of wounds increases the chance of genetic material that codes for resistance being exchanged between bacteria[62]. BPA testing may be valuable in reducing the use of systemic antibiotics by indicating which wounds may benefit from antibiotic therapy.

Further research is needed to fully determine the role of a test for BPA in the management of chronic wounds and the impact of early intervention following a positive test result *[Box 2]*.

Conclusions

Chronic wounds contain a variety of bacteria that do not necessarily cause problems or delay healing. An indication that bacteria have become pathogenic is the expression of virulence factors such as bacterial proteases. These proteases may damage wound tissue and stimulate an excessive host inflammatory response, delaying healing and facilitating spread and local or systemic infection.

A new test for BPA in wounds may assist clinicians in determining when interventions to reduce bacterial burden are indicated, particularly in chronic wounds with no obvious signs of infection. The aim of the test is to allow clinicians to target antibiotic therapies, reduce usage of systemic antibiotics, while providing clinical and economic benefits. WINT

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