Introduction
The mere presence of bacteria in chronic wounds may not indicate active infection or impair wound healing. Contaminated wounds contain bacteria but there may or may not be a host reaction. However, there is a point at which bacteria do begin to inhibit healing, resulting in local and, eventually, systemic infection if left untreated. This point of pathogenicity is when the bacteria begin to secrete proteases. This Made Easy document discusses bacterial protease activity (BPA) as an objective marker of pathogenicity and a means of identifying wounds progressing towards infection.

What are bacterial proteases?
Proteases are enzymes that act on protein molecules, breaking them down into peptides and amino acids. Bacterial proteases are virulence factors known to be secreted by a number of bacteria commonly seen in chronic wounds, including Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis and Enterococcus faecalis.

How do bacterial proteases influence the development of infection?
Bacterial proteases degrade host tissue proteins, impair host immune defenses and promote the local and systemic spread of bacteria. Bacterial proteases hinder immune cell function by suppressing chemotaxis, preventing phagocytosis and impeding immune cell communication (Figure 1). In addition, bacterial proteases can stimulate the production of human/host proteases via immune system activation (Box 1).

What is the wound infection continuum?
While many wounds heal successfully despite the presence of bacteria, for other wounds, bacteria can cause various complications, such as:
- Tissue breakdown
- Pain
- Impedance of the wound’s healing ability and delayed healing
- Life-threatening complications, such as systemic infection.

The influence of bacteria in a wound can be described on a continuum of clinical importance (Figure 2), with increasing levels of vigilance or intervention required where the wound is in a period of pathogenicity, local infection or systemic infection. The different stages of clinical importance are determined by bacterial pathogenicity, as well as the host response and signs of inflammation or tissue damage.

What challenges could be faced when assessing wounds affected by bacteria?
Lack of clinical signs:
The host response to bacteria and their proteases often includes inflammatory markers such as interleukin-1 beta (IL-1β) or tumour necrosis factor-alpha (TNF-α). However, the typical clinical signs of infection may not always be present if the patient’s inflammatory response is weakened, such as where comorbidities like diabetes or immunosuppressive conditions are present.

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Figure 1: Contribution of bacterial proteases to the development of wound infection and delayed healing (Läuchli et al, 2015)

Bacterial proteases:
- Degradation and damage to extracellular membrane
- Degradation of growth factors and growth factor receptors
- Excessive and prolonged host inflammatory response
- Interference with host immune function

LOCAL INFECTION:
- Local tissue invasion

DAMAGE TO WOUND TISSUES, DELAYED WOUND HEALING and WOUND CHRONICITY

Vigilance required:
- Not infected/Contamination
- Colonisation
- State of pathogenicity
- Systemic infection

Intervention required:
- The presence of bacteria within a wound without host reaction
- Bacteria multiply, express proteases that attack tissue, and delay healing; usually associated with an exacerbation of pain; overt host reaction may be absent or present
- Bacteria multiply, as for critical colonisation/local infection, but also cause a systemic host response, e.g., pyrexia or hypothermia, tachycardia

Figure 2: The wound infection continuum in chronic wounds (Adapted from: Siddiqui and Bernstein, 2010; Collier, 2004; Kingsley et al, 2004; WUWHS, 2008)
Difficulty interpreting microbiological investigations:
While a bacterial load of >1x10^5 CFU/ml is typically used for diagnosing wound infection, this threshold may not be appropriate for all wounds; for example, healing may be delayed below this threshold in patients with impaired immune defences or if particularly virulent bacteria are involved, whereas other wounds with bacterial bioburden above this threshold may heal without intervention. Moreover, in some instances, microbiological examinations can be difficult to interpret, especially when multiple bacteria are present. As such, culture results should not replace clinical judgement, but can be used to guide choice of antimicrobial therapy, where appropriate.12,13

Chronic inflammation versus infection:
Wounds may be stuck in a perpetual cycle of inflammation partially attributable to bacteria; this damages the extracellular matrix and degrades growth factors, which stimulate release of inflammatory mediators to cause a heightened inflammatory response, further damaging tissue and delaying healing. As such, it is important to differentiate between inflammation and infection.14,15

Could bacterial protease activity be a useful marker for pathogenicity?
Bacteria in chronic wounds can be non-pathogenic or pathogenic in nature. One indication of the pathogenicity of bacteria is expression of enzymatic virulence factors, such as bacterial proteases, which may stimulate excessive inflammation in the host and, eventually, damage wound tissue and hinder immunologic response.

The results of two recent studies conducted by Serena et al (2015) demonstrate that bacterial protease activity (BPA) can be used as a marker for a ‘period of pathogenesis’ in chronic wounds (Study 1 and Study 2). As such, testing wound fluid for BPA could be a useful method to detect pathogenic bacteria that are capable of causing infection prior to the appearance of clinical signs.
Chronic wound which, despite standard care:
• Is not healing as expected for wound type
• Has stalled after an initial response

Are there clear signs of local infection, a period of pathogenicity (critical colonisation), spreading infection or systemic infection, or other reasons to suspect that bacterial burden is hindering healing? (WUWHS 2008)

Yes

Is there any other obvious explanation for delayed healing other than raised bacterial burden?

Yes

Rectify or minimise factors that may be contributing to delayed healing

Re-evaluate: Is healing progressing?

Yes

Test wound for bacterial protease activity (BPA)

Negative for BPA

Positive for BPA

Consider advanced wound therapies and/or testing for EPA‡

• Implement measures to reduce bacterial burden*
• Consider treatment with topical antiseptics according to local protocol**

Monitor and reassess regularly

Are there signs of improvement within 10–14 days?

• Discontinue antiseptic therapy if implemented
• Monitor and reassess regularly
• Continue standard care

No

Continue management regimen, re-evaluating regularly and modifying treatment as necessary

• Re-evaluate and modify management as appropriate
• Consider topical antiseptics if not already in use
• If antiseptics have been in use, consider sampling for microbiological analysis and amending antimicrobial regimen accordingly

No

NOTE: Some patients with a DFU and bacterial bioburden may not show clinical signs of infection due to neurological, immunological or vascular conditions. It may be beneficial to test earlier than 15 days in such cases.

*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, 2008)

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

† If positive for elevated protease activity, consider incorporating protease-modulating interventions into management (Wounds international, 2011; Dissemond et al, 2013)
What is the WOUNDCHEK™ Bacterial Status test?

WOUNDCHEK™ Bacterial Status* is an innovative lateral flow test for the qualitative assessment of BPA directly from a swab sample from a chronic wound. WOUNDCHEK™ Bacterial Status is intended for diagnostic use, at the point of care, as an aid in the healthcare professional’s assessment of whether a wound may become non-healing due to bacterial pathogenesis as indicated by the presence of BPA.

The WOUNDCHEK™ Bacterial Status test uses chronic wound fluid from the wound, collected using a method known as the Serena Technique*, whereby the cleansed wound is moistened with saline and the surface is swabbed with a rolling action until the entire swab foam tip is coated15. A positive result of the test indicates the presence of BPA in the wound. This detection may allow for earlier clinical intervention to prevent the wound from continuing along the infection continuum to more serious clinical infection or sepsis.

What do results of the WOUNDCHEK™ Bacterial Status test mean in practice?

Since presence of BPA is indicative of impending or active infection, its detection could allow recognition of bacteria behaving pathologically, even without clinical signs of infection, prompting treatment to reduce bacterial burden. A positive result on the BPA test alerts the clinician to the presence of bacteria in a wound behaving pathogenically, allowing them to determine in an objective manner if bacterial burden needs to be addressed when clinical signs may not be immediately apparent, and intervene prior to the development of an obvious infection.

As seen in the pathway for use of the point-of-care bacterial protease test provided in Figure 3, the test result allows the clinician to improve care of the patient, by:

- Implementing measures to reduce the wound’s bacterial burden, such as optimising host response, preventing further wound contamination, debridement, increasing the frequency of dressing changes or using topical antiseptics like silver or PHMB
- Monitoring and reassessing the wound regularly
- Continuing standard care, as appropriate.

If healing is delayed but the result is negative and clinical signs of infection are absent, the clinician is able to choose an alternative approach to care, such as testing for elevated host protease activity or considering protease-modulating interventions.

Bacteria in biofilms can secrete proteases and have been shown to produce them at greater levels than when in the planktonic state17. Wounds that test positive on the BPA test may also contain a biofilm on at least some of wound bed18. Although it is not known whether the BPA test could be used to indicate biofilm presence, many of the treatments used for a BPA-positive wound are also used for biofilm management, such as barrier dressings, debridement and antiseptics19–21.

As an adjunct to existing wound assessment techniques, which are generally subjective in nature, use of this test could have a number of benefits, both clinical and economic. In an era of increasing antibiotic resistance, solutions that could reduce their overuse and support the targeted use of antibiotics are valuable to the wider healthcare system1. Indeed, where diagnostic tests are able to successfully guide clinician decisions, this could lead to the ability to monitor progress of the therapy selected, reduction in long-term complications (for example, loss of limb) and improvements in patient quality of life (such as reduction in pain).

*Not yet available in all countries

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USE OF WOUNDCHECK BACTERIAL STATUS TEST: CASE STUDY

BACKGROUND
This patient had a diabetic foot ulcer to the right plantar aspect. The wound had no signs of clinical infection when reviewed at Week 1 and Week 4, but tested positive on the test for BPA on both occasions. By Week 5, the patient had a number of signs of infection and had been referred to surgery for amputation. NB: This case was part of a clinical study protocol requiring the treating clinician to be ‘blinded’ to the BPA test results until study completion.

Week 0:
- No clinical signs of infection
- However, the wound tested positive for BPA
- Treatment provided: silicone polyurethane foam dressing and silicone non-adherent contact layer

Week 4:
- No clinical signs of infection
- However, the wound tested positive for BPA
- Treatment provided: absorbent gelling fibre dressing

Week 5:
- Patient was bedridden with chills and pains
- Foot was swollen and there was increased odour
- Third digit purple with a 9cm x 8cm area of redness on dorsal foot
- Patient referred to surgery for amputation

References

Summary
The presence of bacteria on the surface of a wound may not indicate infection or impaired healing. Indeed, in many instances healing still occurs despite the existence of bacteria. However, when bacteria start to behave pathogenically complications can occur, including local or systemic infection. Recent studies have demonstrated that BPA can be used as an objective marker for bacterial pathogenicity, even when typical clinical indicators of infection are not apparent. The innovative WOUNDCHEK™ Bacterial Status lateral flow test provides qualitative assessment of BPA directly from a chronic wound swab sample. This detection may allow for earlier clinical intervention to prevent wounds from continuing along the infection continuum to more elevated infection status.