

## General Questions

### 1. What is WOUNDCHEK™ Bacterial Status?

WOUNDCHEK™ Bacterial Status is the world's first rapid, point of care (POC) diagnostic test for the qualitative assessment of bacterial pathogenicity as indicated by virulence factors such as bacterial protease activity from the most common bacterium<sup>1</sup> in chronic non-healing wounds, *Staphylococcus aureus*. The product is indicated for wounds asymptomatic for clinical signs of infection.

1. Gardner SE, Frantz RA. Wound bioburden and infection-related complications in diabetic foot ulcers. *Biol Res Nurs*. 2008; 10(1): 44-53

### 2. What does the WOUNDCHEK™ Bacterial Status detect?

WOUNDCHEK™ Bacterial Status is an in vitro, visually read, chromatographic test for the detection of bacterial protease activity produced by a serine protease secreted by *S. aureus* directly from wound fluid swab samples collected from chronic wounds that are asymptomatic for clinical signs of infection, including venous, arterial and mixed etiology leg ulcers, diabetic foot ulcers and pressure ulcers.

### 3. What are bacterial pathogenicity and virulence?

Bacterial Pathogenicity is the ability of a bacteria to cause disease. Virulence is a quantitative measure of the likelihood that a pathogen (a micro-organism that is able to cause disease) will actually cause disease.

### 4. What is meant by a chronic wound?

A Chronic Wound is a wound that fails to progress through a normal, orderly, timely sequence of repair and where co-morbidities interfere with the normal healing process<sup>1</sup>. This encompasses wounds described as delayed, stalled, hard to heal, recalcitrant, difficult, complex, or failing to respond and could include acute wounds that have healing problems. Chronicity is not necessarily dependent on the time since the wound was first formed<sup>2</sup>.

1. Lazarus GS, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol*. 1994;130(4):489-493.

2. Using a Diagnostic Tool to Identify Elevated Protease Activity Levels in Chronic and Stalled Wounds: A Consensus Panel Discussion. *Ostomy Wound Management*. 2011; 57(12):36-46

### 5. What does "point of care" mean?

A test that is designed for use outside of a lab and for use at or near the patient.

### 6. How does WOUNDCHEK™ Bacterial Status work?

WOUNDCHEK™ Bacterial Status is an easy to use test that can be performed on a sample of wound fluid collected from chronic wounds (see definition under Q2) using a swab. The swab is inserted into a test card and following a simple test procedure either a positive or negative bacterial protease activity test result can be visually read. The positive test result is indicative of pathogenic behaviour of bacteria from *S. aureus* in a non-healing chronic wound. The result can then aid the clinician in determining the appropriate treatment / therapy more effectively.

### 7. What types of wounds can I test?

The test is indicated for the use on chronic wounds (see definition under Q2) such as leg ulcers (venous, arterial and mixed etiology), diabetic foot ulcers and pressure ulcers. Clear descriptions of how to perform sample collection can be found in the instructions for use (IFU). The sample collection procedure needs to be adhered to strictly.

**8. Is wound bed preparation needed prior to sample collection, what if I perform the test following sharp/surgical debridement? Is any specific patient care required for sample collection?**

The Instructions for use include clear descriptions of how to perform sample collection for best results, using swabs that are provided in each test kit. This includes guidance on cleansing of the wound, and it states that the wound should NOT be debrided before sample collection.

The sample collection technique is gentler than Levine's technique and is not accompanied by pain, such as what you would expect from a biopsy, so no specific patient care should be needed. The sample collection procedure needs to be adhered to strictly.

**9. What does it measure, which proteases?**

WOUNDCHEK™ Bacterial Status provides a qualitative assessment of bacterial protease activity from *S. aureus*. Studies show *S. aureus* secretes a serine protease and is the bacteria most commonly associated with non-healing wounds. The test tells the user whether protease activity is positive or negative in the chronic wound being tested. A positive test result is indicative of bacterial pathogenicity shown in a clinical study to be associated with non-healing chronic wounds. Although bacterial protease activity has been shown in many studies to be associated with non-healing chronic wounds, one study has shown that elevated protease activity is associated with ~49% of non-healing chronic wounds. Negative bacterial protease activity is either associated with wounds that are on a healing trajectory or non-healing chronic wounds where the underlying cause of them being stalled is not linked to the presence of bacterial proteases.

1. Serena TE, et al. Bacterial Protease Activity in Chronic Wound Fluid, a Potential Indicator of Pathogenicity even in the Absence of Overt Signs of Infection. Presentation @ EWMA, May 2015; Serena TE, et al. Bacterial Proteases: A Marker for a 'State of Pathogenesis' in Chronic Wounds. Poster @ Wounds UK, Nov 2015.

**10. What types of bacteria does the test respond to?**

The Bacterial Status test can detect a serine protease produced by *S. aureus*. The test is indicated for the detection of V8 from *Staphylococcus aureus* (the most common bacterium in chronic non-healing wounds).

Analytical testing has shown it to be reactive to proteases produced by *Enterococcus faecalis*, *Pseudomonas aeruginosa* & *Proteus mirabilis*. Additionally, clinical studies have demonstrated that proteases can be detected from wounds that culture a large variety of different species including gram positive, gram negative and anaerobes.<sup>1</sup>

1. WOUNDCHEK™ Bacterial Status, Background and Data Summary on file, July 2015

**11. What are bacterial proteases?**

Proteases are enzymes that act on protein molecules, breaking them down to peptides and amino acids. Bacterial proteases are virulence factors known to be secreted by a number of bacteria commonly seen in chronic wounds.

**12. How do bacterial proteases influence the development of infection?**

Bacterial proteases degrade host tissue proteins, impair host immune defences and promote local and systemic spread of bacteria. Bacterial proteases hinder immune cell function by suppressing chemotaxis, preventing phagocytosis and impeding immune cell communication. In addition, bacterial proteases can stimulate the production of human proteases via immune system activation.

**13. Who can perform the test? What training is needed to perform the test?**

The test is not intended for use by patients or their family members.

The test is intended for use by Healthcare Professionals. This could be a physician, a nurse, or a pharmacist, among others, who is qualified to perform the sample collection. The test should be performed at the point of care on a fresh sample.

The test is simple enough that it can be performed by reading and following the instructions for use provided, but it is strongly advised that each user train themselves initially prior to performing the test for the first time on a patient sample by following the test procedure 1-2 times using user training swabs that can be obtained from the local sales representative. Additionally, WOUNDCHEK™ Laboratories has made available a visual on-line user training module to further enhance the training effectiveness. This can be followed in addition to reading the instructions for use, but should not replace reading of all the information contained in the instructions for use provided with each test kit. Finally, once a user has trained themselves, simplified Quick Reference Instructions are provided to help remember the steps of the test.

**14. What happens if I don't clean and moisten the wound appropriately?**

If the wound is not moistened appropriately or the swab collects too much slough, blood, or debris, false results may occur. Additionally, slough, blood, or debris collected on the swab may interfere with the flow of the assay, producing an invalid test result.

**15. What is the appropriate amount of saline to clean the wound with?**

Wounds should be cleaned according to standard procedures. (Demonstration videos are available on [www.woundchek.com](http://www.woundchek.com) under "Products".) Enough sterile saline should be used to remove all loose debris, remains of therapeutic agents (e.g. enzymatic debriders, gels, dressings, etc.) and necrotic tissue. Do not perform sharp wound debridement prior to sample collection, as this may cause the wound to bleed. Ensure that complete haemostasis has been achieved before obtaining the specimen.

**16. What happens if I add more than 5 drops of the test reagent to the reagent well in the test card?**

Adding additional reagent dilutes the specimen, risking the possibility of a false negative result.

**17. What happens if blood is on the sample swab?**

The WOUNDCHEK™ Bacterial Status test result may be affected by the presence of blood in the sample, either by the blood interfering with the protease enzyme activity in the sample, potentially leading to a false negative result, or by the blood causing the test strip background color to appear pink, which may make result interpretation more difficult. To avoid testing samples containing blood, follow the instructions in the Specimen Collection and Handling section closely.

**18. What happens if I twist the swab the wrong way in the test card?**

The user is instructed to rotate the swabs five times to the right for consistency over time and across different operators. The sterile patient swabs are manufactured of foam, and no ill effects should occur if twisted the opposite direction.

**19. How long can I wait between taking the sample and running the assay?**

The sample swab should be tested as soon as possible after collection. If immediate testing is not possible, samples may be stored at room temperature for up to thirty (30) minutes before testing.

**20. What happens if I don't incubate the test for 10 minutes?**

The 10-minute incubation prior to closing the device is critical as this allows the conjugate to rehydrate and the conjugate, reagent and patient sample time to mix and incubate prior to the sample solution running up the test strip.

**21. Why does the test take 15 minutes? Do I really need to adhere to these timings? What if I attempt to read the result later than the 5-minute read time?**

WOUNDCHEK™ Bacterial Status has been developed for optimal performance and accuracy, so adherence to the 10-minute incubation step and the 5-minute read time is of paramount importance. Any deviation from these timings could lead to inaccurate results. If necessary, WOUNDCHEK™ Laboratories can provide users with a free timer to help adhere to the test procedure. The result

should be read at 5 minutes; not prior to or any time after. Test cards should be discarded after the result is recorded.

**22. How do I know that I ran the test correctly?**

Each WOUNDCHek™ Bacterial Status test card has an internal control line that is used to ensure the individual test is processed correctly. The internal control line in the test card is a processing control. This internal control line responds to the procedural steps and tells the user that the test procedure was performed correctly and that the sample flowed completely up the test strip. If the proper amount of reagent is used, the reagents in the well are mixed sufficiently and the test flows correctly this line will always appear. So you can be reassured that as long as you followed the swab sampling procedure correctly and a control line appears in the card's reading window, you ran the test correctly.

**23. What if I have difficulties reading / interpreting the test result? Why can't results be indicated by different colour lines?**

The Instructions for Use and the Procedure Card contained within each WOUNDCHek™ Bacterial Status test kit contains clear instructions and guidance on how to interpret each test result. User testing has been performed to validate the robustness of the test result interpretation, so users can be confident in their test result interpretation.

**Product Configuration, Shelf-life, Controls, etc. Questions**

**1. What are the product packaging configurations?**

- a. #360006 - WOUNDCHek™ Bacterial Status Test Pack
  - i. 6 individual sealed pouches each containing:
    - 1 sealed test card
    - 1 bottle of reagent
    - 1 sterile sample swab
    - 1 multi-language instruction for use with procedure card
- b. #360010 - WOUNDCHek™ Bacterial Status Control Kit
  - i. 3 positive control swabs and 3 negative control swabs
- c. #360024 - WOUNDCHek™ Bacterial Status User Training Pack
  - i. 6 positive swabs (positive control swabs intended for demonstration use)

Note: this product is not yet available in many countries including the United States

**2. What controls are available for this product?**

Each WOUNDCHek™ Bacterial Status test card has an internal control line that is used to ensure the individual test is processed correctly. The internal control line in the test card is a processing control. This internal control line responds to the procedural steps and tells the user that the test procedure was performed correctly and that the sample flowed completely up the test strip. If the proper amount of reagent is used, the reagents in the well are mixed sufficiently and the test flows correctly this line will always appear.

The WOUNDCHek™ Bacterial Status Control Kit is also available for use as facility controls for those facilities requiring it. It contains three (3) positive control swabs: biologically buffered saline containing protein and a bacterial protease from *Staphylococcus aureus* dried onto a swab and three (3) negative control swabs: biologically buffered saline containing protein dried onto a swab.

**3. How often should external controls be run?**

Given that each test card has an internal control, the frequency of running facility controls is a function of the local requirements, such as national regulations, accrediting groups, and/or your facilities' Quality Control requirements.

**4. Can you use external controls from other manufacturers?**

Externals controls from other manufactures cannot be used with the assay. The use of controls manufactured by another source may not produce the required results, and therefore, will not meet the requirements for an adequate quality assurance program.

**5. What is the purpose of the User Training Pack?**

The WOUNDCHEK™ Bacterial Status User Training Pack is available for training and demonstration purposes. The User Training Pack contains 6 positive control swabs intended for demonstration and training use and should not be used as a control.

**6. What is the shelf life of the WOUNDCHEK™ Bacterial Status Kit?**

The current shelf life is 11 months from the date of manufacture.

**7. What are the storage requirements for the WOUNDCHEK™ Bacterial Status kit?**

Storage requirements are indicated on the kit box and the Product Insert. The current labelled storage requirement is 2-30 degrees C.

**8. At what temperatures can I run the test?**

Testing has demonstrated that the test must be run at temps between 20 - 30°C and that not doing so can generate false results.

**9. What are the approved swab types?**

The sterile sample swabs provided in the kit are the only swabs approved for use with the assay.

**10. Can I mix components between kit lots?**

Reagent, swabs and controls are lot specific and should not be used interchangeably between lots.

**11. The expiration date on the pouch or reagent vial is different than the expiration date on the kit. Which one is correct?**

The kits are dated by the kit component with the shortest Expiration date. The expiration date on the box mirrors this. The kit components also have unique lot numbers. This is because the components may be manufactured at different points in time, and the kit lot represents the combination of all the different component lots.

## **Product Performance Questions**

**1. How accurate is WOUNDCHEK™ Bacterial Status? How does this compare to a normal lab test?**

There are no visual signs to detect elevated bacterial protease levels. Normally, clinicians make observations of the wound and use observational criteria such as 3 or more NERDS (Non-healing, Exudate, Redness, Debris, Smell) to decide that there is an infection. There is published data that approximately 49%<sup>1</sup> of non-healing wounds test positive for bacterial proteases indicative of pathogenicity (infection) while only 18%<sup>1</sup> would be declared infected based on NERDS criteria.

Currently there are no laboratory tests approved for clinical use to measure bacterial protease activity. The performance of WOUNDCHEK™ Bacterial Status has been established by comparing healing over 12 weeks between positive and negative tests using a Kaplan-Meier time-to-event model. A significantly higher chance of healing is observed for wounds that are negative using the

WOUNDCHEK™ Bacterial Status test. Approximately 71% of the time that the WOUNDCHEK™ Bacterial Status test generated a positive bacterial protease activity result, the wound was also non-healing after 12 weeks (PPV). Detecting which wounds are non-healing due to the presence of bacterial proteases helps identify those that would most benefit from treatment to reduce bioburden, such as debridement and antimicrobials.

1. Serena TE, et al. Bacterial Protease Activity in Chronic Wound Fluid, a Potential Indicator of Pathogenicity even in the Absence of Overt Signs of Infection. Presentation @ EWMA, May 2015; Serena TE, et al. Bacterial Proteases: A Marker for a 'State of Pathogenesis' in Chronic Wounds. Poster @ Wounds UK, Nov 2015.

## 2. Are there any interfering substances?

The WOUNDCHEK™ Bacterial Status test was evaluated with several common dressings and ointments used in wound care and found to not interfere with the test. (See the IFU for a list of substances that were tested.)

## 3. Are there any microbial interferences?

Eleven (11) non-bacteria (yeast, fungus, virus) that may be present in wound fluid were tested on WOUNDCHEK™ Bacterial Status and found to not interfere with the test. (See the IFU for a list of microbes that were tested.)

## 4. What are the positive and negative predictive values and the time to event analysis of the assay?

Positive predictive value: 71% (73/103, 95% CI = 61% - 79%)

Negative predictive value: 50% (82/163, 95% CI = 43% - 58%)

Kaplan-Meier analysis showed that there was a significant difference between wounds that tested positive and those that tested negative using WOUNDCHEK™ Bacterial Status in regards to time to heal ( $p=.001$ ) when treatment decisions continue to be based on clinical assessment alone. A mean time to heal of 65 days (95% CI = 61 - 69) was observed for wounds reporting a negative result for bacterial protease activity using the WOUNDCHEK™ Bacterial Status test versus 74 days (95% CI = 70 - 78) for wounds that tested positive.

## 5. What can cause a false negative test?

- Improper swabbing technique
- Incorrect swab type used
- Blood on the swab
- Too much reagent is added
- Samples stored at room temperature more than 30 minutes (not tested immediately)
- Testing performed outside of 20-30 degree C temperature range

## 6. What can cause a false positive test?

- Reagent added to the test strip (not to top well)
- Incomplete mixing of swab in swab well
- Too much reagent added (7-10 drops diluting peptide)
- Too few swab twirls (1-2 twirls; insufficient mixing of swab and well components)
- Testing performed outside of 20-30 degree C temperature range

## 7. What can cause an invalid test?

- Reagent added to test strip (not to well)
- Wrong swab type used
- Too little reagent added (2-3 drops resulting in no flow)
- Sample interferes with the flow of the assay (slough, debris, blood)