FAQ and Answers WOUNDCHEK™ Protease Status; Rev 7; Jan 02, 2020

General Questions

1. What is WOUNDCHEK™ Protease Status?

WOUNDCHEK™ Protease Status is the world's first rapid, point of care (POC) diagnostic test for the qualitative assessment of elevated human (host) protease activity (EPA) indicative of an out of control inflammatory response in a chronic wound.

2. What does the WOUNDCHEK™ Protease Status detect?

WOUNDCHEK™ Protease Status is an *in vitro*, visually read, chromatographic test for the qualitative assessment of human neutrophil-derived inflammatory protease activity directly from wound fluid swab samples taken from chronic wounds.

Performance claims are based on the presence of HNE and MMP activity only.

3. Why is protease status important?

Studies have shown that elevated protease activity, primarily of matrix metalloproteases (MMPs) and human neutrophil-derived elastase (HNE), is associated with chronic wounds and leads to degradation of the extracellular matrix and growth factors. Wound healing appears to be linked to the interaction of proteases and inhibitors of these enzymes in wound fluid.[1,2,3]

1. Moore, K. Compromised wound healing: a scientific approach to treatment. *British Journal of Community Nursing* 2003; 8(6): 274-278.
2. Yager DR, Chen SM, Ward SI, Olutoye OO, Diegelmann RF, Cohen IK. Ability of chronic would fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors. *Wound Repair & Regeneration* 1997; 5(1): 23-32.
3. Wlaschek M, Pees D, Achterberg V, Meyer-Ingold W, Scharfetter-Kochanek K. Protease inhibitors protect growth factor activity in chronic wounds. *Br. J. Dermatol*. 1997; 137: 646.

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[1]. 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[2].

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™ Protease Status work?

WOUNDCHEK™ Protease Status is an easy to use test that can be performed on a sample of wound fluid collected from chronic wounds (see definition under Q4) using a swab. The swab is inserted into a test card and following a simple test procedure either an elevated (↑E) or low (↓L) inflammatory human (host) protease activity test result can be visually read. The elevated test result is indicative of protease activity levels shown to be associated with non-healing chronic wounds. The result can then help you to determine the appropriate therapy more effectively.

7. What types of wounds can I test? 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?

WOUNDCHEK™ Protease Status is indicated for use on chronic wounds (see definition under Q4), as assessed by the clinician. This primarily includes leg ulcers, diabetic foot ulcers, or pressure ulcers, but can also include surgical or trauma wounds that have been assessed as chronic due to the underlying pathophysiology.

The instructions for use (IFU) 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.

8. What does it measure, which proteases?

WOUNDCHEK™ Protease Status provides a qualitative assessment of inflammatory human (host) protease activity, telling the user whether the protease activity is elevated (↑E) or low (↓L) in the chronic wound being tested. An 'elevated' test result (aka EPA) is indicative of protease activity levels (matrix-metalloproteases, MMPs, and/or human neutrophil-derived elastase, HNE) shown in a separate study to be associated with non-healing chronic wounds with at least a 90% probability of not healing.[1] Although this inflammatory human (host) protease activity has been shown in many studies to be associated with non-healing chronic wounds, a weighted average of 8 studies covering 503 patients has shown that elevated protease activity is associated with ~22% of non-healing chronic wounds.2

Low 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 may not be excess MMP and HNE activity.

1. *Serena T, et al, Protease activity levels associated with healing status of chronic wounds. Poster, Wounds UK 2011.*
2. *Weighted avg. of Serena 2011, Ivory Wounds UK 2013, Duteille EWMA 2013, Anichini EWMA 2013, Uccioli 2014, Haycocks 2012, Hodgson 2012, and Serena SAWC 2015 available from WCL data library.*

9. Why assess for elevated MMPs and HNE activity and what is their involvement in delayed healing?

Studies have shown that elevated activity levels of several MMPs (in particular MMP-9, MMP-8 and MMP-2), together with Elastase (HNE), are associated with a delay in wound healing, and wounds that respond to protease modulating treatments show a reduction in this protease activity, leading to a positive healing trajectory.

10. How accurate is WOUNDCHEK™ Protease Status? How does this compare to a normal lab test?

There are no visual signs to detect elevated inflammatory human (host) protease activity.

Currently there are no laboratory tests approved for clinical use to measure inflammatory human (host) protease activity. The performance of WOUNDCHEK™ Protease Status has been established by comparing to healing status (healing / non-healing). 80% of the time that the WOUNDCHEK™ Protease Status test generated an elevated protease activity result, the wound was also assessed as non-healing (PPV), meaning you can have confidence in detecting which wounds that are non-healing due to the presence of EPA and helping you identify those that would most benefit from treatment with a protease modulating dressing. In the development of the test, using commercially available tests for protease activity available for research use only, an overall agreement of 82% was shown when the WOUNDCHEK™ Protease Status test was compared to a reference method that assessed protease activity as elevated if MMP activity exceeded 48U/110uL of sample and/or HNE activity exceeded 25mU/110uL of sample[1.]

1. *Protease Status package insert PN350000 Rev. 6, 2014/12*

11. What data is there, to support this? Is there any variability in testing results?

A study was conducted on wound fluid samples taken from 215 chronic wounds. All samples were assessed using WOUNDCHEK™ Protease Status and compared to the clinical healing status. WOUNDCHEK™ Protease Status was shown to have a positive predictive value of 80% which is consistent with information in the literature indicating that wounds containing elevated levels of inflammatory protease activity have a high probability of being non-healing.[1]

[1] *Protease Status package insert IN350002 Rev. 1, 2018/03*

12. What are proteases and what are their roles in wound healing?

Human (host) proteases are enzymes present in normal wound healing as part of the initial inflammatory response; they break down old / damaged proteins so that new tissue can form. However, if inflammatory protease activity levels are elevated for too long in a wound they can impair healing by breaking down new proteins, such as growth factors, that are essential for healing. The management of these inflammatory proteases such as MMPs (matrix metalloproteinases) and Elastase (HNE) may help in starting the healing process of your wound.

13. What are the main reasons that human (host) proteases get out of control?

Due to the destructive nature of inflammatory proteases they are normally subject to regulation by inter-cellular substances called 'Tissue Inhibitors of Metalloproteases' (TIMPs) among others. When the critical balance between inflammatory proteases and their control systems are disrupted, for example due to underlying co-morbidities, wounds may become chronic.

14. What is the relationship between inflammatory proteases and infection? How do I know that an 'elevated' test result isn't indicative of an infected wound rather than a stalled, non-healing chronic wound and that I shouldn't treat with antimicrobial or antibiotic treatments?

Non-healing chronic wounds may have elevated inflammatory protease activity and, if infected, may additionally include bacterial proteases. However, WOUNDCHEK™ Protease Status is designed to detect human neutrophil-derived inflammatory proteases, not bacterial proteases. A WOUNDCHEK™ Protease Status test result will not tell clinicians anything about the infection status of a wound. A separate bacterial protease test WOUNDCHEK™ Bacterial Status is currently commercially available in countries accepting CE mark for distribution and will be made available in other countries as regulatory requirements are fulfilled.

Low inflammatory protease activity is either associated with chronic wounds that are on a healing trajectory or non-healing chronic wounds where the underlying cause of them being stalled may not be excess inflammatory human (host) protease activity. This test result needs to be considered in conjunction with clinician assessment and treated accordingly. For more information, we suggest that you review the publications offered at http://www.woundchek.com/protease-status. Specifically, we'd suggest: "EPA made easy" and "The Role of Proteases in Wound Diagnostics".

15. 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 can also be performed in a laboratory by laboratory professionals but, as it is easy enough to be used at the point of care, it is not necessary to wait the extra time to transport the sample to the laboratory, schedule the test, and deliver the results back to the clinician, which can take more than 1-2 days in some cases due to the processes in place. Further the sample, once collected, can only be stored refrigerated for a maximum of 4 hours before being assessed using WOUNDCHEK™ Protease Status, so laboratory use may not be very practical.

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, a simplified procedure card is provided to help remember the steps of the test.

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

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

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

    WOUNDCHEK™ Protease Status has been developed for optimal performance and accuracy, so adherence to the 10-minute incubation step is of paramount importance. Results read before or after 5 minutes may be inaccurate. (Note: Read results in a well-lite area.) Test cards should be discarded after the result is recorded.

18. **How do I go about trying out / evaluating WOUNDCHEK™ Protease Status in my hospital / institution?**

    Clinicians are encouraged to talk to their local sales representative about evaluating WOUNDCHEK™ Protease Status in their facility. WOUNDCHEK™ Laboratories is keen to support user evaluations and can provide guidance on how to structure these and tools, such as evaluation forms, to ensure appropriate recording, so that clear conclusions can be drawn about the potential clinical value and impact of implementing a testing regime.

Questions regarding Product Configuration, Use, Shelf-life, etc.

1. **What are the kit types & accessory packs?**

    350006 WOUNDCHEK™ Protease Status 6 Test Kit

    Contents:

    6 tests each within a pouch with all other materials needed

    350010 WOUNDCHEK™ Protease Status Control Kit

    Contents:

    3 Elevated Result Control Swabs
    3 Low Result Control Swabs

    NOTE: Not yet available in many countries, including the United States.

2. **What is the purpose of the Control Kit?**

    The WOUNDCHEK™ Protease Status Control Kit is available for use as facility controls for those facilities requiring it. It contains three (3) Low Result Control Swabs comprised of biologically buffered saline containing protein dried onto a swab, representing a low protease activity result and three (3) Elevated Result Control Swabs comprised of biologically buffered saline containing protein and proteases dried onto a swab, representing an elevated protease activity result.

3. **What is the shelf life of the WOUNDCHEK™ Protease Status Kit?**

    The current shelf life is 21 months from the date of manufacture.

4. **What are the storage requirements for the WOUNDCHEK™ Protease Status kit?**

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

5. **Am I able to use my kit if it's been frozen?**

   It is not recommended to use the kit if it's been frozen. The long-term impact of freezing has not been established.

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

   Testing has demonstrated that the test must be run at temps between 15 - 30°C and that not doing so can generate false results.

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

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

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

   Reagent, swabs and controls are not lot specific and may be used interchangeably between lots. The reference strips should not be interchanged between lots.

9. **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.

Questions regarding Controls

1. **What is the internal control line?**

   Each WOUNDCHEK™ Protease 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.

2. **If the internal control line signal is weaker than the test line, are the results still valid?**

   The internal control line is independent from the test line. The control line is not predictive of the performance characteristics of the test. Therefore, a test when the control line that is weaker than the test line is still a valid result.

3. **If the internal control line signal is weaker than the reference strip, are the results still valid?**

   The internal control line is independent of the reference strip. If the control line signal is weaker than the reference strip, the results are still valid. The reference strip is used to determine an elevated or low result only. It should only be used to judge the intensity of the test line.

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

   WOUNDCHEK™ Protease Status Controls (available separately) should be tested in accordance with:
   - local and/or federal regulations,
   - accrediting groups, and/or,
   - your lab's standard Quality Control procedures.

   These swabs will verify the entire assay.

5. **Why do the external controls require 6 drops of reagent to perform the test, while patient swabs only require 4 drops of reagent?**

   Two additional drops of reagent are required to test the control swabs, as the control swabs are dry when used in the WOUNDCHEK™ Protease Status device. Patient swabs have been moistened with wound fluid, and therefore require less reagent to achieve flow through the test strip. They are also comprised of different, more absorbent material.

6. **If the elevated external control swab generates a result where the test line is not visible at all, is the result valid?**

   If the elevated external control does not produce a test line, but the internal control line is visible, the swab has produced an acceptable elevated result.

7. **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.

SAMPLING THE WOUND

1. **What happens if I don't clean the wound appropriately?**

   If the wound is not cleaned appropriately, false results may occur. Additionally, the material that is not cleaned out of the wound may interfere with the flow of the assay, producing an invalid test result.

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

   Wounds should be cleaned according to standard procedures. 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 hemostasis has been achieved before obtaining the specimen.

3. **What happens if I add too much saline to moisten the wound?**

   Per the product insert, apply additional saline to the wound area to be swabbed such that the area is visibly moist. Care should be taken not to flood the wound with excessive saline. Avoid pooling of saline.

4. **What happens if I don't sample the wound appropriately?**

   Sampling the wound inappropriately may produce false results or an invalid assay.

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

   The WOUNDCHEK™ Protease 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 low 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.

6. **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, the swab can be held refrigerated for up to 4 hours before testing.

7. **How do I store the swab prior to running the test?**

   After the sample is collected and prior to running the assay, the swab head should not come in contact with another surface. Users should use a rack or similar method to hold swabs if necessary between sampling and performing the assay.

RUNNING THE ASSAY

1. **What happens if I add the reagent to the bottom hole instead of the top hole?**

   If the reagent is added to the bottom hole instead of the top hole of the swab well, false results may occur due to incomplete dissolution of the conjugate pellet.

2. **What happens if I add the swab to the swab well before adding the reagent?**

   If the swab is added to the swab well prior to the reagent, false results or invalid results may occur.

3. **What happens if I add the incorrect number of reagent drops to the assay?**
   Adding too few drops of reagent may produce an invalid result. Adding too many drops of reagent may produce false results or an incorrect result if the control swabs are being tested.

4. **What happens if I twist the swab the wrong way?**

   The user is instructed to rotate the swabs five times to the right. This is noted as there is a low risk that the polyester control swabs may unwind if rotated to the left. The sterile patient swabs are manufactured of foam, and no ill effects should occur if twisted in the opposite direction.

5. **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 prior to the sample flowing up the test strip.

6. **What if I don't read the test right at the read time?**

   WOUNDCHEK™ Protease Status performance was validated at a 5-minute read time. Reading the device before or after 5 minutes may give erroneous results.

7. **Why are the Reference Strips single use only?**

   The Reference Strips are single use only, as the intensity of the Reference Strip may change over time due to exposure to light and the environment. Discard each strip immediately after using to interpret test results. Keep all unused Reference Strips in the bag in which they are supplied to protect them from light.

8. **If the intensity of the Reference Strip and test line are equivalent, how do I interpret the test result?**

   If the color intensity of the Test Line (T) is equal to the color of the Reference Line (R), then the sample contains low levels of inflammatory protease activity.

9. **How is a test that has variable intensity across the test line interpreted?**

   If the color intensity of the test line is variable across the test strip, use the intensity that is present on the majority of the line to interpret the test result.

10. **Should the test be run under a safety hood?**

    Laboratories and clinics should follow their in-house safety procedures for handling potentially infectious material.

PERFORMANCE CHARACTERISTICS

1. **Are there any interfering substances?**

   The WOUNDCHEK™ Protease Status test result may be affected by the presence of Acticoat Absorbent, Iodoflex Pads, bromelain, trypsin, Activon Gel, Flaminal Hydro Gel or Nu-Gel Hydrogel in the sample. To avoid testing samples containing these substances, follow the instructions in the Specimen Collection and Handling section closely.[1]

   The WOUNDCHEK™ Protease 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 low 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.[1]

[1] *Protease Status package insert PN: IN350002 Rev. 1, 2018/03*

2. Is there any microbial interference?

Fourteen (14) bacteria that may be present in wound fluid were tested on WOUNDCHEK™ Protease Status. None of the following bacteria affected test performance when tested at a concentration of 10^6 organisms per test.

Acinetobacter anitratus
Bacteroides fragilis
Corynebacterium diphtheria
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Klebsiella pneumoniae (subsp. pneumoniae)
Peptostreptococcus anaerobius
Proteus mirabilis
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pyogenes

3. **What is the positive and negative predictive value as compared to clinical healing status?**

		WOUNDCHEK™ Protease Status	
		Elevated (E↑)	**Low (L↓)**
Clinical Healing Status	Non-Healing	32	107
	Healing	8	68
		40	175

Positive predictive value: 80% (32/40, 95% CI = 65.1% - 89.4%)
Negative predictive value: 39% (68/175, 95% CI = 31.9% - 46.3%)