EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR WOUNDCHEK Bacterial Status DECISION SUMMARY

A. De Novo Number:

DEN180014

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the WOUNDCHEK Bacterial Status

C. Measurand:

Bacterial Proteases

D. Type of Test:

Lateral flow chromatographic assay

E. Applicant:

Alere Scarborough, Inc.

F. Proprietary and Established Names:

WOUNDCHEK Bacterial Status
WCBS

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3231

2. Classification:

   Class II

3. Product code(s):

   QFA

4. Panel:

   Microbiology (83)

H. Indications For Use:

1. Indication(s) for use:

   WOUNDCHEK Bacterial Status (WCBS) is an in vitro diagnostic chromatographic test for the qualitative detection of bacterial protease activity directly from wound fluid samples collected with a swab. The WCBS test is intended for use in adult patients as an aid in assessing the risk for non-healing of chronic venous, diabetic foot, and pressure ulcers associated with wounds where there are no signs of wound infection and where patients are asymptomatic for clinical signs of infection. The test is intended for use with chronic wounds that are between 21 days and < 6 months of age and chronic wounds that are ≥ 6 months of age that are < 1cm^2 in size.

   This test is indicated for use solely by health care professionals whose clinical practice primarily or routinely involves the assessment and treatment of chronic wounds. WCBS results are intended for use in conjunction with the assessment of other known risk factors for wound healing that significantly contribute to the assessment of risk for non-healing chronic wounds such as wound age, wound size, and vascular status.

3. Special conditions for use statement(s):

   For prescription use only.

   For *in vitro* diagnostic use only.

   For use with wound fluid swab specimens only.

4. Special instrument requirements:

   Not applicable.

I. Device Description:

WOUNDCHEK Bacterial Status (WCBS) is a lateral flow assay that qualitatively detects bacterial protease activity in chronic wound fluid. Wound fluid is collected by rolling a swab over the cleansed wound surface until it is saturated. The swab is then incubated in the assay reagent which contains a substrate that can be cleaved by bacterial proteases and human neutrophil elastase (HNE) and an HNE inhibitor. The WCBS test card is used to detect cleavage products of the substrate. It consists of biotinylated[1] bovine serum albumin (BSA) (Test Line (T)), and a control system protein, (Control line (C)), on a nitrocellulose membrane support. Neutravidin, which is conjugated to visualizing particles, binds the biotinylated end of a synthetic peptide and the biotinylated BSA test line. The test result is based on the presence or absence of a pink-to-purple colored Test Line (T) which means bacterial protease activity was detected. A negative test result is defined by the absence of a Test Line (T) which means bacterial protease activity was not detected. If the control line (C) is not present, then the test result is invalid. The device is intended for use on venous leg ulcers (VLU), diabetic foot ulcers (DFU) and pressure ulcers (PU).

A control kit, consisting of negative and positive swabs, is also available for WOUNDCHEK Bacterial Status.

J. Standard/Guidance Document Referenced:

De Novo Classification Process (Evaluation of Automatic Class III Designation): Guidance for Industry and Food and Drug Administration Staff. October 2017.

Guidance for Industry Chronic Cutaneous Ulcer and Burn Wounds – Developing Products for Treatment. June 2006.

K. Test Principle:

The production of proteases from bacteria in chronic wounds can delay the healing of these wounds. The WCBS detects the presence of bacterial protease activity in chronic wounds that can indicate a delay in wound healing.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision
   The precision study was performed at three sites, with three operators per site, for a total of nine operators in the study. Positive panel members were made by combining representative bacterial proteases (i.e., V8, GelE, ZapA and LasB) in wound fluid.

[1] WCBS has not been evaluated for potential biotin interference. For more information see FDA Biotin Safety Communication

The concentration of each protease used in the mixture and tested in each panel member is provided in enzyme units (U) in Table1 below.

Table1. Protease Concentrations in Panel Members

Protease	Moderate Positive (3x LOD)	Low Positive (2x LOD)	High Negative (C5)
Endoproteinase Glu-C (V8)	(b) (4)		
Serralysin (ZapA)			
Gelatinase (GelE)			
Pseudolysin (LasB)			

Reproducibility

Testing was conducted over five days. Each day operators tested a blinded panel of (b) (4) swabs; three replicates each of a true negative (wound fluid), moderate positive (3x LOD), low positive (2x LOD) and high negative (C5) panel members.

Swabs were prepared fresh daily by pipetting (b) (4) of the bacterial protease mixture in wound fluid or protease-free wound fluid o e vertical center of the swab head. Once all solution was absorbed, swabs were assembled into blinded panels. Each test operator was blinded to the expected sample result.

One positive and one negative control swab were tested with the WOUNDCHEK Bacterial Status on each day of testing prior to performing study testing. All control swabs produced the expected results.

The results of the reproducibility study are in Table 2 below.

Table 2. Reproducibility Results by Site

Panel Member	Site 1		Site 2		Site 3		All Sites	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Moderate Positive	100% (45/45)	0.0% (0/45)	97.8% (44/45)	2.2% (1/45)	97.8% (44/45)	2.2% (1/45)	98.5% (133/135)	1.5% (2/135)
Low Positive	100% (45/45)	0.0% (0/45)	93.3% (42/45)	6.7% (3/45)	93.3% (42/45)	6.7% (3/45)	95.6% (129/135)	4.4% (6/135)
High Negative	6.7% (3/45)	93.3% (42/45)	8.9% (4/45)	91.1% (41/45)	4.4% (2/45)	95.6% (43/45)	6.7% (9/135)	93.3% (126/135)
True Negative	4.4% (2/45)	95.6% (43/45)	2.2% (1/45)	97.8% (44/45)	0.0% (0/45)	100% (45/45)	2.2% (3/135)	97.8% (132/135)

The moderate positive, low positive and true negative panel members had greater than 95% agreement with the expected results; this meets the acceptance criteria.

The negative results for the high negative panel member was less than 95%. Because of the nature of the high negative panel member, the percent agreement with expected results is more variable; the percent agreement with expected results observed in this study is acceptable.

There were no significant differences in detection rate by site, operator, or day. Acceptance criteria for the study was met.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Stability

External Controls

To monitor the assay performance, reagent performance, and procedural errors, positive and negative external controls must be run in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Control swabs should be tested with each new shipment received and once for each untrained operator.

WOUNDCHEK Bacterial Status comes with a Positive and Negative Control Swab. If additional control swabs are needed, then WOUNDCHEK Bacterial Swab Control Kits can be purchased separately. These swabs will verify the entire assay. If the correct control results are not obtained, do not report patient results.

Sample Stability

Positive swab samples were prepared by spiking representative bacterial proteases (V8, GelE, ZapA and LasB) near the assay LOD to wound fluid. Negative swab samples were prepared by adding wound fluid to swabs. Swabs were prepared by pipetting (b) (4) of the bacterial protease mix in wound fluid or protease- free wound fluid onto ertical center of the swab head.

The following sample storage conditions were evaluated:

- Time 0.0 hours
- After 0.5 hours of storage at 2-8°C and 30°C
- After 1 hour of storage at 2-8°C and 30°C
- After 1.5 hours of storage at 2-8°C and 30°C
- After 2 hours of storage at 2-8°C and 30°C
- After 4 hours of storage at 2-8°C and 30°C
- After 6 hours of storage at 2-8°C and 30°C
- After 9 hours of storage at 2-8°C and 30°C

Following the WOUNDCHEK Bacterial Status procedure, ten positive and negative swabs for each storage condition were tested. Results were then interpreted by two

operators (each blinded to each other's results) for a total of (b) (4) determinations per storage condition.

(b) (4) Positive control swab and (b) (4) Negative control swab were tested per the WOUNDCHEK Bacterial Status procedure on each day of the study. All daily control testing generated the expected results.

Testing of all positive and negative swabs generated the expected results, meeting the acceptance criteria of the study.

d. Detection limit:

The objective of this study was to establish the WOUNDCHEK Bacterial Status limit of detection (LOD) for four representative bacterial proteases individually and pooled. The LOD level is defined as the level of bacterial protease activity that generates a positive result approximately 95% of the time, when tested in multiple replicates by multiple operators (i.e., the C95 level). A second objective of this study was to identify the level of protease activity that generates a positive result approximately 5% of the time (i.e., the C5 level).

A range of concentrations for each of the representative bacterial proteases were spiked into protease-free wound fluid, coated onto swabs and tested to identify preliminary levels that generated a positive result 100%, 95%, and 5% of the time. Negative swabs were coated with protease-free wound fluid.

Once the bacterial protease levels producing approximately 95% and 5% positive results were identified, LOD test panels were prepared for each bacterial protease. Each LOD panel was blinded to the operators participating in the study. Each panel for each bacterial protease contained swabs with the preliminary LOD, swabs with dilutions flanking (above and below) the preliminary LoD, a negative and a positive swab. Preliminary LoD and flanking dilutions were tested in duplicate in each panel. Ten panels for each bacterial protease were prepared, 20 replicates of each concentration and tested by participants within 30 minutes of swab sample preparation.

The same approach was used in to identify confirm the LOD for pooled bacterial proteases.

Preliminary and confirmed LOD for individual and pooled bacterial proteases are presented in the tables below.

Table 3. Preliminary LOD –Individual Proteases

	Activity (mU/test)	**Number Detected** (+ result / total)	**% Detection**
Staphylococcus aureus (V8)	TRUE POSITIVE	(b) (4)	100%
	(b) (4)		100%
			97.5% (LOD)
			80%
			65%
			27.5%
			50%
			20%
			10%
			0%
			5%
			5%
	TRUE NEG		0%
Enterococcus faecalis (GelE)	TRUE POSITIVE	(b) (4)	100%
	(b) (4)		100%
			90% (LOD)
			95%
			95%
			75%
			65%
			65%
			55%
			30%
			5%
			10%
			5%
	TRUE NEG		0%

	Activity (mU/test)	Number Detected (+ result / total)	% Detection
Proteus mirabilis (ZapA)	TRUE POSITIVE	(b) (4)	100%
	(b) (4)		**95% (LOD)**
			80%
			65%
			70%
			65%
			40%
			40%
			25%
			10%
			25%
			10%
			5%
	TRUE NEG		0%
Pseudomonas aeruginosa (LasB)	TRUE POSITIVE	(b) (4)	100%
	(b) (4) TEST)		**95% (LOD)**
	(b) (4)		95%
			75%
			0%
			5%
			5%
	NEG		0%

Table 4. LOD – Individual Proteases

Protease	C95 (mU/test)	C5 (mU/test)	C50 (mU/test)
Endoproteinase Glu-C (V8)	9.0	(b) (4)	
Serralysin (ZapA)	12.6		
Gelatinase (GelE)	56.7		
Pseudolysin (LasB)	34.5		

Table 5. Preliminary LOD – Pooled Proteases

Protease	Activity (mU/test)				Number Detected	% Detection
	V8	GelE	ZapA	LasB		
Pooled: *Staphylococcus aureus* (V8) *Enterococcus faecalis* (GelE) *Proteus mirabilis* (ZapA) *Pseudomonas aeruginosa* (LasB)	(b) (4)					100% 100% 100% **95% (LOD)** 85% 50% 60% 30% 5% 35% 5% 10% 5% 15% 5%

Table 6. LOD – Pooled Proteases (mU/test)

Protease	C95 (mU/test)	C5 (mU/test)	C50 (mU/test)
Endoproteinase Glu-C (V8)	(b) (4)		
Serralysin (ZapA)			
Gelatinase (GelE)			
Pseudolysin (LasB)			

e. *Analytical specificity:*

Cross-reactivity - Human Proteases:

The objective of this study was to determine if the presence of human proteases in wound fluid interfere with the performance of WCBS.

The acceptance criteria for this study was that positive swab samples must produce a positive result in the presence of any host protease. Negative swab samples must produce a negative result in the presence of any host protease.

Positive swab samples were prepared fresh on each study day at the LOD (as defined in by Analytical Sensitivity Study) in wound fluid. Negative swab samples were

prepared fresh on each study day using protease-free wound fluid. Swabs were prepared by pipetting 30µL of the bacterial protease mixture in protease-free wound fluid or protease-free wound fluid onto the vertical center of the swab head.

Human protease stocks were diluted (b) in wound fluid and tested. Each of the host proteases were tested by pipettin (b) (4) of each dilution in wound fluid to the top of the swab well through the top hol e card. Five negative swabs and five positive swabs were tested following the WCBS procedure. Each result was interpreted by two operators (each blinded to each other's results) for a total of (b) determinations per host protease. Any discrepant results between the operators for a given sample type was clarified by a third operator.

If any of the host protease replicates did not meet acceptance criteria, the protease stock was diluted in wound fluid in (b) (4) increments and tested as above until a level that met acceptance criteria was identified.

One positive control swab and one negative control swab was tested per the WCBS procedure on each day of the study. All daily and experimental control testing generated the expected results on each day of testing.

Table 7. Human Proteases – Lowest level at which interference with WCBS is not observed

Protease	Activity (per test)
MMP-13 Catalytic Domain	16.5 U
MMP-9 Catalytic Domain	10.0 U
MMP-8 Catalytic Domain	127.5 U
MMP-2 Catalytic Domain	3.7 U
Cathepsin	0.5 – 1 mU
Thrombin	10.3 U
Human Neutrophil Elastase (HNE)	82.0 mU
Plasmin	9.2 mU

Interference - Microbial:

The objective of this study was to determine if fungi, mold and viruses that may be present in chronic wounds interfere with the performance of WCBS.

The acceptance criteria for this study was positive swabs (i.e., swabs with analyte) must produce a positive result in the presence of the tested microorganisms. Negative swabs must produce a negative result in the presence of the tested microorganisms.

Fungus and mold were grown according to their individual requirements. Then harvested, suspended in (b) (4) nd diluted to an appropriate concentration then stored at (b) (4) were provided by the vendor. Viruses were stored at (b) (4) prior to testing. All organisms were tested live.

Positive swabs were prepared fresh each day with representative bacterial proteases at the LOD in wound fluid. Negative were prepared each day using wound fluid. Swabs were prepared by pipetting (b) (4) f the bacterial protease dilution in wound fluid or protease-free wound fluid e vertical center of the swab head.

Each microorganism was tested by pipetting 10µl of each stock solution to the top of the swab well through the top hole in the card. Five negative swabs and five positive swabs were tested following the WCBS procedure. Each result was interpreted by two operators (each blinded to each other's results) for a total of 10 determinations per microorganism. Any discrepant results between the operators for a given sample type was clarified by a third operator (i.e. 2/3 operators determined some test results).

If interference was observed with stock solutions, log dilutions of the organism stock solutions were tested until the acceptance criteria were met.

One Positive control swab and one negative control swab were tested on each day of the study. All controls generated expected results.

Table 8. Microbial Interference - Levels below which microbial interference is not observed

	Organism	ATCC Identifier	Activity (per test)
FUNGUS/MOLD	Candida parapsilosis	(b) (4)	2.9 X 10^6 cells/mL
	Candida albicans		2.4 X 10^6 cells/mL
	Candida tropicalis		9.5 X 10^5 cells/mL
	Aspergillus fumigatus		6.4 X 10^5 cells/mL
	Mucor indicus		2.9 X 10^6 cells/mL
	Rhizopus oryzae		2.0 X 10^6 cells/mL
	Apophysomyces elegans		3.2 X 10^5 cells/mL
VIRUSES	Herpes Simplex 1/Herpesvirus 1		1.6 X 10^6 $TCID_{50}$/mL
	Herpes Simplex 2/Herpesvirus 2		2.8 X 10^4 $TCID_{50}$/mL
	Varicella Zoster/Herpesvirus 3		8.9 X 10^2 $TCID_{50}$/mL
	Cytomegalovirus/Herpesvirus 5		1.6 X 10^4 $TCID_{50}$/mL

Interference – Healthy Skin Flora

The objective of this study was to determine if the presence of proteases present in normal skin flora interfere with the performance of WCBS.

Using the same swabs provided with WCBS, (b) (4) apparently healthy skin swab samples were collected by study trained personnel. One swab per unique, consented individual was collected from intact skin (i.e., no visible wounds or lesions) on the lower leg of each volunteer. Prior to swabbing, the area was gently cleansed with sterile saline to remove all loose debris and confirmed to be visibly moist without any pooling. The head of the swab was pressed flat against the cleansed area and gently rolled back and forth several times while applying pressure until it was fully coated. Swabs were tested within 30 minutes of collection following the WCBS procedure.

Each result was interpreted by two operators (each blinded to each other's results) for a total of 100 determinations.

One positive control swab and one negative control swab were tested per the WCBS procedure each day. All daily control testing generated the expected results on each day of testing.

Results from twenty swabs were interpreted as positive by both operators. Operator 1 interpreted an additional four swabs as positive. In the study there were a total of 44 false positives out of 102 readings. See Table 9 below for results.

Table 9. Interference – Healthy Skin Flora

Sample	Operator 1 Result	Operator 2 Result	Sample	Operator 1 Result	Operator 2 Result
(b) (4)					

This study demonstrates that the performance of WCBS is affected by normal skin flora. This is not unexpected as skin flora reportedly can secrete exogenous proteases (c.f. Koziel, J., & Potempa, J. (2013). Protease- armed bacteria in the skin. *Cell and Tissue Research*, *351*(2), 325–337). The Sample Collection and Handling section of the Instructions for Use includes instruction that states "**Do not swab intact skin**."

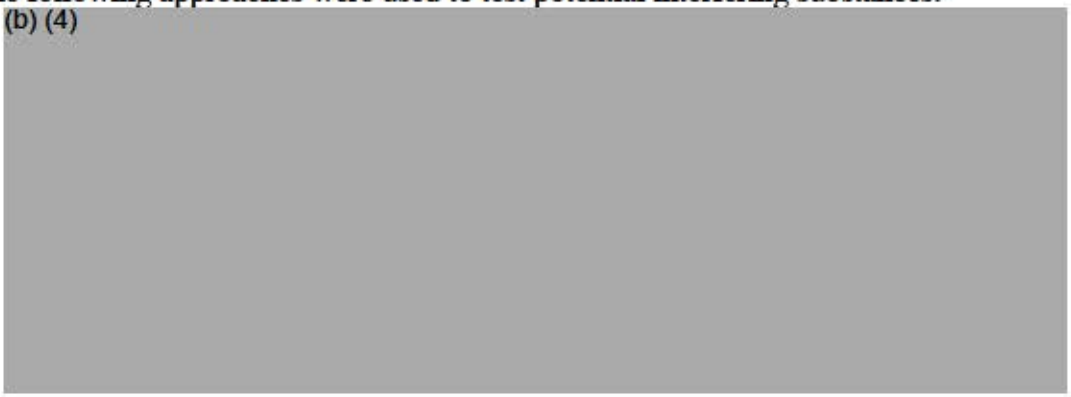
Interfering Substances

The purpose of this study is to determine if substances potentially found in wounds or used for wound treatment interfere with the performance of WCBS.

The acceptance criteria for this study was that positive swabs must produce a positive result in the presence of the interfering substance being tested. Negative swabs must produce a negative result in the presence of the interfering substance being tested.

The following approaches were used to test potential interfering substances.

(b) (4)



Positive swabs were prepared daily and tested in parallel with pooled representative bacterial proteases (i.e., V8, GelE, ZapA and LasB) near the LOD in wound fluid.

Negative swabs were prepared daily using protease-free wound fluid. Swabs were prepared by pipetting (b) (4) of the pooled bacterial protease in wound fluid or protease-free wound fluid onto the vertical center of the swab head and stored until tested.

Each interfering substance was tested with five positive swabs following the WCBS procedure. Each result was interpreted by two operators (each blinded to each other's results) for a total of 10 determinations per interfering substance.

If a substance failed to meet acceptance criteria at the initial level, it was diluted two-fold in wound fluid until acceptance criteria were met.

One positive control swab and one negative control swab were tested according to the WCBS procedure each day of the study. All daily and experimental control testing generated the expected results.

Table 10. Interfering Substances – Interference not observed below levels listed

Substance	Concentration	Substance Type	Treatment Component	Dressing Type* (sub-type(s), if applicable)
Acetic acid	0.026%	Wound Cleanser	Acid	N/A
Antimicrobial Barrier Dressing with nanocystalline silver	N/A	Dressing	Silver	Medicated
Activated charcoal dressing with silver	N/A	Dressing	Activated Charcoal- Silver	Medicated
Manuka honey dressing	1.053%	Dressing	Hydrogel, Honey	Modern (Hydrogel)
				Medicated
Antimicrobian foam dressing with PHMB	N/A	Dressing	PHMB	Modern (Semi-permeable foam)
				Medicated
Hydrofiber dressing with silver	N/A	Dressing	Hydrogel, Silver	Modern (Hydrogel)
				Medicated
Mupirocin 2%	1.053%	Dressing	Antibiotic	Medicated
Blood	7.14%	Endogenous	N/A	N/A
Bromelain	0.01 U	Dressing	Enzyme	Medicated
Menthol/zinc oxide ointment	1.053%	Dressing	Zinc oxide, menthol	Medicated
Clindamycin 1%	1.053%	Dressing	Antibiotic	Medicated
Dermal allograft	N/A	Dressing	Skin Substitute	Tissue engineered skin substitute
Allograft	N/A	Dressing	Skin Substitute	Tissue engineered skin substitute
Alginate gel with glucose oxidase/lactoperoxidase	1.053%	Dressing	Hydrogel, Alginate, Enzyme	Modern (Hydrogel, Alginate)
				Medicated
Antibacterial foam dressing with methylene blue and gentian violet	N/A	Dressing	Antimicrobial dyes	Modern (Semi-permeable foam)
				Medicated
Non-adherent povidone iodine dressing	N/A	Dressing	Iodine	Traditional
				Medicated
Bacitracin/Neomycin/ Polymixin B	1.053%	Dressing	Antibiotic	Medicated
Hydrogel with alginate	0.527%	Dressing	Hydrogel, Alginate	Modern (Hydrogel, Alginate)
Nystatin	1.053%	Dressing	Antifungal	Medicated
Porcine small intestine submucosa	N/A	Dressing	Skin Substitute	Tissue engineered skin substitute

Substance	Concentration	Substance Type	Treatment Component	Dressing Type* (sub-type(s), if applicable)
Bacitracin zinc/Polymixin B sulphate	1.053%	Dressing	Antibiotic	Medicated
Promogran Prisma	N/A	Dressing	Collagen, Silver	Bioactive Medicated
Polyaminopropyl Biguanide 0.1% Wound Irrigation Solution	5.26%	Wound Cleanser	PHMB, Betaine	N/A
Becaplermin 0.01%	1.053%	Dressing	PDGF	Medicated
Collagenase 250units/g	1.053%	Dressing	Enzyme	Medicated

*As defined by: Dhivya S, Padma V, Santhini E. Wound dressings – a review. BioMedicine. 2015;5(4):22. doi:10.7603/s40681-015-0022-9.

f. High Dose Hook Effect Study

Not applicable

g. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Prospective Study

Clinical Study - WOUNDCHEK Bacterial Status compared to Clinical Healing Status:
The clinical performance characteristics of WOUNDCHEK Bacterial Status were evaluated in a blinded, prospective observational study conducted in 2016 at seven (7) sites in the U.S. Wound fluid swab samples, collected from adult patients presenting at the study sites with chronic wounds that did not show clinical signs of infection (i.e. had less than three clinical signs of infection) were tested using the

WOUNDCHEK Bacterial Status, and the results were compared to the clinical healing status of the wounds. The healing status of each wound (healed or not healed) was assessed within a time frame of up to 12 weeks after enrollment in the study. Per the study definition, a healed wound was one that had achieved "complete wound closure", which was defined as "skin re-epithelialization without drainage or dressing requirements" (i.e., 100% of wound is covered and surface is intact), as assessed by the treating clinician. The clinical performance as an aid in the risk assessment for non-healing of chronic venous, diabetic foot, and pressure ulcers associated with wounds where there are no signs of wound infection and where patients are asymptomatic for clinical signs of infection, is stated as:

Positive Likelihood Ratio (PLR) = Sensitivity / 1 – Specificity and;

Negative Likelihood Ratio (NLR) = 1 – Sensitivity / Specificity

Three hundred fifty wounds were enrolled in the study. Of these, one hundred and forty-seven (147) unique wound fluid samples were eligible for inclusion in evaluation of device performance. The table below summarizes the reasons why wounds were excluded from the performance analysis.

Reason for Exclusion from Initial Performance Analysis	**Number of Wound Fluid Samples**
Total number of wounds enrolled	**350**
Eligibility Violation	-85
Enrollment Wound Image Violation	-1
Subject Withdrawal	-6
Surgery – unrelated to wound, but wound removed	-4
Surgery – conducted on the wound to close or treat it, making it unacceptable for further use in the study.	-5
Subject Expired (prior to study endpoint)	-6
Unable to Source Verify Wound	-3
Insufficient numbers of MLU and ALU to demonstrate performance	-8
Lost to Follow-Up	-19
Total Number of Evaluable Wounds in the Initial Analysis per Study Protocol	**213**
Correlation with healing status was not observed in a subpopulation of wounds	-66
Total Number of Evaluable Wounds in the Final Analysis	**147**

Three hundred fifty wounds were enrolled in the study. Two hundred and thirteen (213) wound fluid samples (87 venous leg ulcers, 104 diabetic foot ulcers, and 22 pressure ulcers) were collected from the distinct chronic wounds of 200 adult subjects. Note, thirteen subjects consented to enroll two wounds in the study.

Sixty-six (66) evaluable subjects enrolled with wounds ≥ 1 cm^2 and ≥ 6 months of age were excluded from the results reported in the table below. Risk assessment claims for these types of wounds were not supported due to a low correlation of the test result with wound healing in most subjects in this cohort.

The remaining 147 wound samples were collected from 139 unique subjects; four subjects consented to enroll two wounds in the study. Included in the final analysis were 51 venous leg ulcers, 82 diabetic foot ulcers, and 14 pressure ulcers.

The table below summarizes device performance for the remaining 147 wounds.

Performance in the Intended Use Population: Patients with either wounds < 6 months old or wounds ≥ 6 months old that are <1 cm^2 in area*				
		No-healing	Healing	
WOUNDCHEK Bacterial Status	Pos	38	15	53
	Neg	43	51	94
		81	66	147
PLR= 2.06, 95% CI =1.25 - 3.41 NLR= 0.69, 95% CI =0.54 - 0.88 * Excludes 66 evaluable subjects with wounds ≥ 1 cm and ≥ 6 months of age.				

Likelihood ratios provide information on the pre- and post-test probability of a disease or condition, in this case healing. In the pivotal study, the pre-test risk of not-healing was 55.1%, the post-test risk of not-healing for wounds with a positive WCBS result was 71.7%, a 16.6% increase risk of not-healing. The post-test risk of not-healing for wounds with a negative WCBS result is 45.7%, a decrease in risk of not healing of 9.4%.

Analysis of the impact of wound treatment was not evaluated in the observational study. An evaluation of the clinical impact of the result was not possible due to the observational design of the study. The observational clinical study did not evaluate correlation of results to a clinical diagnosis of infection or evaluate clinical intervention in response to WOUNDCHEK results. The observational study did not control for numerous clinical factors which impact healing therefore the clinical correlation of the WOUNDCHEK Bacterial Status result with wound healing cannot be clearly established. Differences in clinical practice may affect the numbers of days to wound healing associated with WOUNDCHEK results.

b. *Clinical specificity:*

See section L.3a above.

c. *Other clinical supportive data (when a. and b. are not applicable):*

N/A

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   Not Applicable

M. Instrument Name:

Not applicable. The device does not utilize an instrument for result generation.

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes _____ or No ____X_ ___

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes _____ or No ____X____

   The device does not contain any software or instrument components.

3. Specimen Identification:

   Not applicable.

4. Specimen Sampling and Handling:

   Prior to swabbing, the wound should be gently rinsed with sterile saline to remove debris, therapeutic agents and necrotic tissue, but pooling of saline in the wound should be avoided. Sharp wound debridement should not be performed, and wound should be free of blood prior to sample collection. Apply gentle pressure as you roll the swab back and

forth over the wound until saturated. Care should be taken not to swab blood or intact skin as this can cause false results.

5. Calibration:

   Not applicable.

6. Quality Control:

   WCBS contains an internal control which verifies the sample is flowing properly through the test strip. Additionally, each kit comes with a positive and negative external control swab. External controls should be tested, and the expected results obtained, prior to testing patient samples with a new kit and before an untrained user performs the test. Additional external control swabs can be purchased separately.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None.

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Risks to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
Risk of false test results	Use of certain specimen collection and transport devices identified in special control (1) Certain labeling information identified in special control (2) Certain design verification and validation activities identified in special control (3)
Failure to correctly interpret test results	Certain labeling information identified in special control (2)
Failure to correctly operate the device	Certain labeling information identified in special control (2)

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

The primary benefit associated with use of the WOUNDCHEK Bacterial Status Assay is identification of a risk factor for wound non-healing. The WOUNDCHEK Bacterial Status Assay can be used in chronic venous, diabetic foot, and pressure ulcers between 21 days and six months of age, or wounds more than six months of age that are less than 1 cm^2 in which there are no

signs of wound infection and where patients are asymptomatic for clinical signs of infection. Clinicians may change their management of chronic wounds based upon the results of the WOUNDCHEK Bacterial Status assay with subsequent decrease in unnecessary therapy for wounds likely to heal and increased therapeutic interventions for wounds not likely to heal.

Summary of the Assessment of Risk

The primary risk associated with use of the WOUNDCHEK Bacterial Status assay is erroneous results in which wounds that are identified as being at risk for non-healing may heal and wounds identified as being likely to heal may not heal. Clinicians may change their clinical management of chronic wounds due to the presumption that a wound will or will not heal based upon their perception of increased or decreased risk due to the WOUNDCHEK Bacterial Status assay result, with subsequent increases in patient morbidity.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

Summary of the Assessment of Benefit-Risk

The probable benefits of the WoundChek Bacterial Status Assay as an assay that identifies a risk factor for wound non-healing outweigh the potential risks in light of the listed special controls and applicable general controls. The proposed special controls, including the description of the recommended training (e.g., knowledge and experience) for safe and effective use of the device, are necessary to ensure safe use of the assay and mitigate the risks associated with use of the device. The proposed labeling and quick reference guide will communicate the limitations of the device to users. Overall, the probable benefits of the WOUNDCHEK Bacterial Status outweigh the probable risks for the proposed indications for use in light of the special controls for this type of device and in combination with the general controls.

S. Conclusion

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): QFA
Device Type: Device to detect bacterial protease activity in chronic wound fluid
Class: II (special controls)
Regulation: 21 CFR 866.3231