BIOME study

North Cumbria Integrated Care NHS Foundation Trust

Prospective, single-centre, cohort study assessing the potential application of WOUNDCHEK™ diagnostics for ulcer management

(BIOME, Bacterial Infection: Observation & Management Evaluation)

V1, dd 18 November 2022

Chief Investigator's Statement of Ownership and Content.

I, Grace Messenger, confirm that this protocol is my work and is owned by me. The protocol conforms to standards outlined in the Declaration of Helsinki 1964.

Name (PRINT):_____

Signature:_____

Date: _____

RESEARCH PROTOCOL SUMMARY

TITLE:	**Prospective, single-centre, cohort study assessing the potential application of WOUNDCHEK™ diagnostics for ulcer management**
Short title:	BIOME, Bacterial Infection: Observation & Management Evaluation
IRAS number	314595
Device description	CE-marked point of care test: WOUNDCHEK™. WOUNDCHEK™ Bacterial Status – or WCBS - is able to detect bacterial EPA (elevated protease activity). WOUNDCHEK™ Bacterial Status will help clinicians establish within minutes which wounds may most benefit from a protease modulating therapy, ensuring appropriate and targeted use of these therapies.
Study design	Prospective, single-centre, controlled, non-randomised, prospective cohort comparative study Participants' index ulcer will be swabbed twice, at week 0 and at week 6, plus a medium-term assessment at week 12.
Primary objective	Determine if there is a difference in rate of positive outcome between clinical opinion (ie presence of infection based on wound symptoms) and WOUNDCHEK™ Bacterial Status test result for the same wound.
Secondary objectives	To define the degree of concordance between a clinical staff member's opinion and results from the WOUNDCHEK™ detection kit, concerning the assessment of infection presence in ulcer wounds. Determine what patient and/or wound characteristic(s) are significantly linked to a non-matching result between clinical opinion and WOUNDCHEK™ WCBS result. These outcomes are relevant for application of the WOUNDCHEK™ test in standard clinical practice. Two binary logistic regression analyses will be conducted for variables associated with the sample's proportion of non-matched outcome: - Clinical opinion negative ('no infection') vs WOUNDCHEK™ test positive or - Clinical opinion positive ('possible' or 'definite' infection) vs WOUNDCHEK™ test negative Evaluate if there are factors associated with infection rates and/or WOUNDCHEK™ result, including clinical indicators of infection such as erythema, purulence, odour, and patient characteristics (health status, co-morbidities, demographic). Assess if there are any trends in bacterial protease detection rates in ulcers when patients are followed up, ie result of first test versus second follow-up test of same ulcer Evaluate if clinical staff deviate from their initial treatment plan once the WOUNDCHEK result is available to them during a consultation

Inclusion & Exclusion criteria	Inclusion criteria:
	- Adult patients aged ≥ 18 years
	- Patients can be newly presenting to or existing users of the specialist service in question (eg podiatry, vascular surgery)
	- Patients with recurrent wounds, including multiple wounds, are eligible; largest ulcer to be index wound
	- If infection occurs and antibiotics applied, whilst in study, then this is not deemed an exclusion criterion.
	- Prophylactic systemic antibiotic use is not an exclusion criterion
	- Chronicity: clinical diagnosis of ulcer with wound duration > 30 days.
	- Wound type:
	  o Leg ulcer (can be venous, mixed or arterial in nature)
	  o Foot ulcer (can be diabetic or non-diabetic in nature)
	
	Exclusion criteria:
	- Aged < 18 years
	- Any reasons for the patient being unable to follow the protocol, including lack of mental capacity to consent to taking part in the study.
	- The patient has concurrent (medical) conditions that in the opinion of the investigator may compromise patient safety or study objectives
	- Confirmed and ongoing wound infection at baseline which is already being treated with systemic antibiotics (within 3 weeks of first study visit).
	- Previous participation in BIOME study

Sample size	258 samples required, taken from minimum of 129 patients at week0 and week6 visits. This study element is powered to detect a 4% difference in detection of clinician-specified wound infection and a positive WOUNDCHEK™ Bacterial Status test result. (Chi-squared test, 80% power, p-value 0.05, 10% attrition rate). This is provided the incidence of infection detected equals 9% and 5% per rater, respectively. Furthermore, the study is also sufficiently powered to detect an inter-rater concordance level (Kappa) of 0.9, at 80% power and an infection detection rate of 10%.
Manufacturer & provider of material	WOUNDCHEK™ Laboratories Airebank Mill, Gargrave, BD23 3RX North Yorkshire, United Kingdom T: +44 (0) 1756 639111 E: Simon.Bayliff@woundchek.com
Chief Investigator	Grace Messenger, Head of Podiatry, North Cumbria Integrated Care NHS Foundation Trust, grace.messenger@ncic.nhs.uk
Co-investigators	Jane Todhunter, specialist vascular nurse, North Cumbria Integrated Care NHS Foundation Trust jane.todhunter@ncic.nhs.uk

	Dr Leon Jonker PhD, Science & Innovation Manager, North Cumbria Integrated Care NHS Foundation Trust Leon.jonker@ncic.nhs.uk
Sponsor and organisation where research will take place	North Cumbria Integrated Care NHS Foundation Trust R&D department Workington Ann Burrow Thomas Centre CA14 2ED
Planned timeline	Recruitment start date (first patient, first visit): 1 Feb 2023, Recruitment end date (last patient, last visit): 30 Apr 2024 Follow-up end date (last patient, last visit): 30 Jul 2024 Study end date: 30 Aug 2024
Protocol version, date	Version 1, dd 18 November 2022

Contents

Lay Summary

Chronic ulcer wounds of the legs and feet can be challenging for healthcare staff to manage. Patients often have co-morbidities and there is a considerable likelihood of non-healing and recurrence of ulcers, plus infection can occur. Apart from having a significant negative impact on patients' lives, it is also a huge economic burden to the National Health Service. Clinical opinion – by checking for hallmark signs of infection - is the main way to determine if a wound is infected. Microbiology testing offers information on what type of antibiotic may help to treat the infection. Different companies have developed point-of-care tests that assess something that cannot be readily observed: bacterial protease activity. Its presence may be indicative of infection since bacteria use said protease enzymes to break down protein structures in a wound that are needed for a wound to heal. Due to logistic and cost reasons it would not be practical to apply a protease point-of-care test for all patients' wounds. This study investigates to what degree clinical opinion and results of the WOUNDCHEK Bacterial Status test align, and also what factors and variables may be associated with non-matching results. Furthermore, the degree of influence the WOUNCHEK test result may have on clinical management of chronic lower limb ulcers will be explored. For this purpose a total of 258 wounds (minimum of 129 patients) will be assessed at baseline and then six and twelve weeks later.

1 BACKGROUND AND RATIONALE

Leg and foot ulcers can occur for various reasons, see Figure 1. Though venous leg ulcers (VLUs) are the most common type of leg ulcers, contributing to the majority of leg ulcers, there are many patients who have ulcers due to a mixed venous-arterial or other underlying condition (SIGN 2010, Graham *et al* 2003). The natural history of the disease – particularly in those patients who have venous insufficiency and or other chronic disease affecting the vasculature such as diabetes and peripheral arterial disease - is a continuous cycle of healing and breakdown over decades. VLUs are associated with considerable expense, morbidity and impaired quality of life (Persoon *et al* 2004). A positive relationship has been observed between VLU occurrence and specific modern lifestyle risk factors such as sedentary lifestyles and obesity (Brand *et al* 1998). Table 1 shows annual NHS cost for treating VLUs compared to other chronic wound treatments. Similar to VLUs, the occurrence of diabetic foot ulcer (DFU) is a common complication of diabetes with enormous cost implications, totalling £650 million per year once associated morbidity is taken into account (NHS Diabetes report).

Table 1. Chronic wound treatment costs to the NHS (Posnett & Franks 2008)

	Annual incidence	Cost per patient	Annual NHS cost (2005–2006)
Venous leg ulcers	108,600	£1,500–1,800	£168–198m
Foot ulcers	57,000	£5,200	£300m
Pressure ulcers	410,000	£4,300–6,400	£1.8–2.6bn
TOTAL	575,600	£4,000–5,400	£2.3–3.1bn

Figure 1, Different causes of leg ulcers (Agale 2013)

Vascular	Venus
	Arterial
	Mixed
Neuropathic	Diabetes
	Tabes
	Syringomyelia
Metabolic	Diabetes
	Gout
	Prolidase deficiency
Haematological	Sickle cell disease
	Cryoglobulinemia
Trauma	Pressure
	Injury
	Burns
Tumors	Basal cell carcinoma
	Squamous cell carcinoma
Infection	Bacterial
	Fungal
	Protozoal
Panniculitis	Necrobiosis lipoidica
	Fat necrosis
Pyoderma	Gangrenosum
Special	Hypertensive ulcer

Bacterial infection of wounds carries the risk of further degenerative complications including cellulitis, necrotising fasciitis, and sepsis (Grothier, 2015). Specific wounds, such as diabetic foot ulcers may lead to amputation if osteomyelitis develops. An additional undesirable effect of infection of wounds is that it delays – or stops altogether – the wound healing process (Halbert et al, 1992).

Detection of chronic leg/foot ulcer infection remains reliant on clinical judgement. For example, imaging modalities such as MRI do not perform better than clinical appraisal in terms of sensitivity and specificity when it comes to detecting osteomyelitis (Dinh et al, 2008). Quantitative detection of infection is still undertaken by swabbing the wound and then culturing the pathogens in a microbiology laboratory. Obtaining these results generally takes days; even molecular profiling does not give an instant result. Microbiological counts and species identification do not necessarily reflect infection as defined by other assessments, as demonstrated by Gardner et al (2014).

Clinical guidelines stipulate that the only available laboratory-based diagnostic option, microbiological testing, should only be used to identify the pathogen strain in clinically confirmed infection. Therefore, clinical opinion is the mainstay of predicting and diagnosing infection (NICE, 2008, 2015). The lack of a simple cost-effective and repeatable testing method may have three consequences: 1) lack of uniformity in diagnosis, due to differences in clinical judgement, which in turn may result in 2) over-diagnosis of infection with inappropriate prescription of antibiotics or antimicrobial dressings, or 3) late presentation of patients with systemic signs and spreading cellulitis or osteomyelitis requiring hospital admission and treatment with intravenous antibiotics or emergency surgery.

O'meara and colleagues (2006) concluded from a systematic review on clinical examination, sample acquisition and sample analysis in DFUs that there is a lack of evidence regarding what samples should be taken and how they should be analysed. They did suggest that semi-quantitative sample analysis – a category that the WOUNDCHEK™ protease detection kit (called WOUNDCHEK™) would fall under – may be a useful alternative to quantitative analysis. The provision of a rapid, reliable, sensitive and relatively low cost infection detection test kit for bacterial protease presence (which can be highly indicative of infection), which can be used at point-of-care, has the potential to provide the NHS with significant cost savings as well as improving the outcomes for patients.

Outcome data from another study which appraised the Glycologic bacterial protease detection kit showed an agreement in terms of wound assessment for 'infection' – between podiatrists' clinical opinion and Glycologic test result - in 79% of samples (301 out of 383 wound assessments), where podiatrists identified more (possible) infections than the Glycologic kit (55 [15%] vs 14 [4%] swabs respectively). Regression analysis and primary component analysis shows that clinical signs of wound infection, namely erythema, purulence and odour, are all significantly associated with both a positive clinical opinion and Glycologic test result (Jonker et al 2022a, Jonker et al, 2022b). The same study also corroborated earlier research that bacterial load of a wound is not indicative of bacterial protease activity and clinical opinion of infection. Initial research involving the WOUNDCHEK™ Bacterial Status test also suggests that the degree of bacterial load does not correlate with the degree of pathogenesis exhibited by said bacteria (Serena et al, 2022; Benson et al 2017). On the other hand, unlike with Glycologic, the WOUNDCHEK™ Bacterial Status test did not find a strong association between clinical signs of infection and a positive WCBS test. This study did, however, not report on individual hallmark signs of infection such as erythema, purulence and odour (Serena et al 2022).

2 OBJECTIVES

2.1 PRIMARY OBJECTIVE

Determine what patient and/or wound characteristic(s) are significantly linked to a non-matching result between clinical opinion and WOUNDCHEK™ Bacterial Status (WCBS) result. These outcomes will be relevant for application of WOUNDCHEK™ test in standard clinical practice. See Table 2.

Table 2. Outcome options for WOUNDCHEK™ and clinical inspection, with the two non-matching outcome options shaded in green and blue respectively.

	WOUNDCHEK™ Bacterial Status	WOUNDCHEK™ Bacterial Status
Clinical opinion: **no infection**	negative	Positive
Clinical opinion: **possible infection**	negative	Positive
Clinical opinion: **infection**	negative	Positive

2.2 SECONDARY OBJECTIVES

The level of concordance between a clinical staff member's opinion and results from the WOUNDCHEK™ detection kit defining the assessment of infection presence in lower limb ulcers.

Evaluate if there are factors associated with infection rates and/or WOUNDCHEK™ result, including clinical indicators of infection such as erythema, purulence, odour, and patient characteristics (health status, co-morbidities, demographic). These include, but are not restricted to:

- Type of wound
- Wound size
- Chronicity of wound
- Clinical indicators of infection, including degree of erythema, purulence, and pain
- Presence of co-morbidities
- Patient health status
- Clinical rater

Assess if there are any trends in bacterial protease detection rates in ulcers when patients are followed up, ie result of first test versus second follow-up test of same ulcer

Evaluate if clinical staff deviate from their initial treatment plan once the WOUNDCHEK result is available to them during a consultation.

3 INVESTIGATIONAL PLAN

3.1 TRIAL DESIGN AND TIMELINE

The study is a prospective, single-centre, non-randomised, observational design. There is no 'gold standard' accepted detection test for infection available. Clinical guidelines stipulate that clinical judgement concerning the presence or absence of infection is the recommended clinical practice. Samples should only be sent off to a microbiology department to confirm the causative pathogen and inform choice of antibiotics. Therefore, the results of the novel detection infection test kit will be compared with the clinical staff member's judgement of infection status. Table 2 shows the anticipated timeline for the study. Appendix 3 summarises the patient pathway for the study.

For this study, appropriate clinical staff (vascular nurses, practice nurses, tissue viability or podiatry staff) will provide judgements on leg/foot wound infection status. This (ultimately subjective) evaluation can potentially contribute to variability in the detection of infection.

During the study period, all patients can continue to be managed and receive their standard treatment regime by their usual clinical team. Wound dressing selection, dressing frequency and all other aspects of their wound care will continue to be the responsibility of the clinical team. This study will, however, record if the clinical staff member was influenced by the WOUNDCHEK result in terms of dressing choice, antibiotics use, microbiological test swabbing, and interaction with the multidisciplinary team. The latter, MDT, is a group of clinical staff who discuss optimal management of patients with high-risk and more complicated wounds.

Table 3, Anticipated study timeline

Month	Setup	Cohort	Analysis
Nov 2022	Submission to NRES and HRA		
	NIHR portfolio adoption		
Feb 2023	HRA and Trust approval	Start recruitment	
Apr 2024		Finish all recruitment	
Jul 2024		Final visit, last patient	
Aug 2024			Finalise analysis & report

3.2 PRIMARY & SECONDARY OUTCOMES

Outcomes used for all analyses:

- Swab results from the WOUNDCHEK™ Limited kit, with specimen taken at dressing removal, prior to deep wound cleansing.
- Clinical staff member's assessment of infection, with opinion given prior to result of the WOUNDCHEK™ result being known.

4 PARTICIPANTS

4.1 TRIAL PARTICIPANTS & LOCATIONS

Patients with chronic leg or foot ulcers will be recruited into the study. Patients will be recruited from the adult population seen routinely by the evaluation clinical staff.

Identification of potentially eligible patients will be identified by the clinical staff - who themselves form part of the research. Therefore, the patient – carer of / responsible person for the patient - is approached initially by the clinical team caring for them. Only once the patient has given verbal consent to be approached for the study can another member of the study team conduct the consent process and study activities. The clinical staff can also opt to do these activities themselves.

The study will take place in the podiatry, vascular and tissue viability clinics of North Cumbria Integrated Care NHS.

4.2 INCLUSION & EXCLUSION CRITERIA

Inclusion criteria:

- Adult patients aged ≥ 18 years
- Patients can be newly presenting to or existing users of the specialist service in question (eg podiatry, vascular surgery)
- Patients with recurrent wounds, including multiple wounds, are eligible; largest ulcer to be index wound
- If infection occurs and antibiotics applied, whilst in study, then this is not deemed an exclusion criterion.
- Prophylactic systemic antibiotic use is not an exclusion criterion
- Chronicity: clinical diagnosis of ulcer with wound duration > 30 days.
- Wound type:
    - Leg ulcer (can be venous, mixed or arterial in nature)
    - Foot ulcer (can be diabetic or non-diabetic in nature)

Exclusion criteria:

- Aged < 18 years
- Any reasons for the patient being unable to follow the protocol, including lack of mental capacity to consent to taking part in the study.
- The patient has concurrent (medical) conditions that in the opinion of the investigator may compromise patient safety or study objectives
- Confirmed and ongoing wound infection at baseline which is already being treated with systemic antibiotics.
- Previous participation in BIOME study

5 STUDY PROCEDURES

5.1 INFORMED CONSENT

Before being recruited to the clinical evaluation, the patient must have consented to participate, after the nature, scope and possible consequences of the evaluation have been explained in an understandable form. A patient information leaflet and informed consent form will be provided to the patient. Consent to take part in this research is obtained from adult patients, where they possess mental capacity.

During the consent procedure the following information will be outlined in writing, which will also be relayed verbally: a) The evaluation involves research, a description of the aims of the evaluation and how it will be organised and the expected duration of the patient's participation; b) Any negative effects possibly attributable to the novel infection detection test kit; c) The freedom to ask for further information, and to withdraw from the study, at any time; d) The extent, if any, to which confidentiality of records identifying the patients will be maintained and that the Regulatory Authorities may inspect the records.

Although the clinical staff will be trained in obtaining informed consent as part of professional development, members of staff involved in the consent process will be encouraged to have current ICH Good Clinical Practice training.

5.2 STUDY PROCEDURES

Initial assessments at visit 1 (week 0)

After completing informed consent, the nature of the patient's wound will be recorded as well as the other relevant clinical parameters and demographics. The first WOUNDCHEK™ test will also be conducted. See Table 3 for an overview. Dressing selection, dressing frequency and all other aspects of their wound care will continue to be the responsibility of the clinical team – however, if they are influenced in this decision by the result of the WOUNDCHEK™ test then they are asked to record this in the clinical report form.

All visit assessments

Study visits will coincide with standard clinic attendance by patients. At the study visits week0 and week6, a diagnostic swab using the WOUNDCHEK™ infection detection test kit is performed in accordance with the SOP in Appendix 5.

The clinical staff treating the patient will ascertain their opinion regarding infection status of the wound prior to completing the WOUNDCHEK™ detection test, and will therefore be blinded to at least the first result of using the kit. Where possible, a member of the research team plus the clinical assessor will assess result of the WOUNDCHEK™ test.

Table 4, Overview of study activities per time point for different study participants.

Weeks	Visit 1 (wk 0)	Visit 2 (wk 6)#	Visit 3 (wk 12)#
Treatment deviation question	X	X	
WOUNDCHEK™ test (method, see appendix 6)	X	X	
Wound healed status~	n/a	X (if healed, no other outcome measures)	X (if healed, no other outcome measures)
Clinical opinion of ulcer re. infection	X	X	X
PUSH score ulcer size (appendix 3)	X	X	X
VAS pain scale (appendix 3)	X	X	X
SINBAD score (foot ulcers only, appendix 4)	X	X	X
QoL EQ-5D-5L (appendix 5)	X	X	X
Wound measurement*(if standard care activity)	X	X	X

~Wound status (healed vs non-healed), definition is: complete epithelial cover in the absence of a scab (eschar) with no dressing required bar any protective dressing. There is no minimum length of time that the index site has to have been in said condition for when participant present for follow-up study visit.
 # Can be ± two weeks.
**Using 'Minuteful for Wound' application (by Healthy.io), wound tracing sheet, or ruler measurement; needs to be consistency in method between different visits.*

5.3 DEFINITION OF END OF STUDY

The end of trial is the date of the last assessment in relation to the study or wound closure, whichever occurs first. If a wound is healed and any follow-up visit(s) were to take place thereafter, then these do not have to take place.

5.4 DISCONTINUATION OR WITHDRAWAL OF PARTICIPANTS

Each participant has the right to withdraw from the study at any time – withdrawal of patients has been anticipated in the sample size calculation. In addition, the Investigator may discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation/violation
- An adverse event which requires discontinuation due to inability to continue to comply with trial procedures (eg hospitalisation, death)
- Any event which results in inability to continue to comply with trial procedures
- Withdrawal of Consent
- Loss to follow-up

Data already collated as part of the study will be retained if a subject withdraws from the study.

5.5 SOURCE DATA

Source data will include patient's hospital records (for wound type and classification of infected wounds) and the Case Record Form for the results of the novel infection detection test. WOUNDCHEK™ will have no access to patient data other than pseudo-anonymised data for the test results and associated medical opinion for the relevant wounds.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study number.

6 EVALUATION PRODUCT

6.1 DESCRIPTION OF WOUNDCHEK™ LIMITED DETECTION KIT

The WOUNDCHEK™ infection detection test kit is CE-marked. WOUNDCHEK™ Bacterial Status is able to detect EPA (elevated protease activity). WOUNDCHEK™ Bacterial Status will help clinicians establish within minutes which wounds may most benefit from a protease modulating therapy, ensuring appropriate and targeted use of these therapies.

6.2 DISTRIBUTION & ACCOUNTABILITY

Delivery of kits to the centre will be arranged by WOUNDCHEK™ Limited. Records will be retained for kits received and on which dates.

7 SAFETY

7.1 SAFETY DEFINITIONS

Table 5, Description of different adverse event reporting definitions.

Adverse Event (AE)	Any untoward medical occurrence in a patient or other clinical investigation participant taking part in a trial of a medical device, which does not

	necessarily have to have a causal relationship with the device under investigation. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the device, whether or not considered related to the device.
Serious Adverse Event	A serious adverse event is any untoward medical occurrence that: - results in death - is life-threatening - requires inpatient hospitalisation or prolongation of existing hospitalisation - results in persistent or significant disability/incapacity - consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

7.2 PROCEDURES FOR RECORDING ADVERSE EVENTS

All SAEs need to be reported to the sponsor/host Trust R&D **within one working day** of the investigator team becoming aware of them. WOUNDCHEK™ will be updated on any SAE incidences once every two months or 20 recruited patients (whichever occurs sooner)

The only device to be in contact with the patient is the sterile swab provided by the hospital and employed by hospital staff only.

7.3 CAUSALITY

The relationship of each adverse event to the trial must be determined by a medically qualified individual according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from swabbing. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

8 Statistical consideration and data analysis plan

8.1 GENERAL AND BASELINE CHARACTERISTICS

The numbers of patients entering the study will be recorded. Reasons for treatment and study discontinuation, withdrawal and loss to follow-up will be recorded. The date the first patient recruited and last patient completed or termination will also be noted. Protocol deviations including failure to meet inclusion and exclusion criteria will be recorded. Adverse events will also be recorded.

Serious protocol deviations defined as breach of the conditions and principles of ICH GCP or the study protocol, which is likely to effect to a significant degree the safety or physical or mental integrity of the subjects of the study, or the scientific value of the study will be recorded. Patients not withdrawn with a serious device related adverse event will be recorded as a serious protocol deviation.

In order to describe the sample and facilitate analysis of secondary objectives, the following baseline characteristics and parameters will be collated, either from the CRF or the patients' records (using EMIS patient clinical record system), see also Appendix 2:

- Patient demographics, including age and sex.
- Pre-existing co-morbidities, including peripheral arterial disease, diabetes, heart failure.
- Age of and size of foot/leg ulcer wound at baseline
- Dressing used before presentation at baseline.
- Presence or absence of infection

8.2 SAMPLE SIZE CALCULATION

The primary outcome, presence of bacterial infection is used for sample size calculation. Data from studies involving another point-of-care test (Jonker et al, 2020 and Jonker et al 2022) gives some guidance on the incidence of wound infection as defined by clinical staff. Circa 5% of samples were deemed as infected by clinical staff. A hypothetical difference in opinion/outcome of 4% is used to determine the required sample size that has sufficient power to detect such a difference.

Power calculations for sample size, 80% power and 5% significance, based on two-sided Chi-squared test. A priori power calculations using GPower 3.1 software, result in the following sample size summarized in Table 6. The raters are the WOUNDCHEK WCBC and the clinical staff. Patients will be recruited from the adult (age 18+) population routinely seen by the evaluating clinical staff members. Attrition rate – determined in other similar studies – is anticipated to be 10%.

Table 6, Sample size calculation for BIOME study

	No infection	**Infection**
Rater A (*hypothetical*)	*0.95*	*0.05*
Rater B (*hypothetical*)	*0.91*	*0.09*
	Power beta of 80%, Alpha p-value of 0.05, Degrees of freedom = 1, Effect size 0.184 Sample size required without any attrition: 234 samples. Sample size required with 10% attrition: 258 samples This equates to: 129 patients if they attend for the planned two research visits at week 0 and week 6 (258 samples). If not all participating patients attend twice, eg due to a wound healing before week6, then more patients will be recruited to get to the required number of samples.	

The above sample size will also allow to assess the secondary objective, ie concordance between the two raters – namely clinical opinion and WOUNDCHEK™ test result - at a confidence level of 90% and Kappa concordance level of 0.80 (calculated via https://wnarifin.github.io/ssc/sskappa.html).

8.3 PRIMARY OUTCOME STATISTICS

The WOUNDCHEK™ kit result will be cross tabulated with the clinical staff member's assessment of wound infection. Two tests will explore the relationship in outcomes between clinical judgement and WOUNDCHEK™ kit detection testing:

- Chi-squared test (cross-tabulation of results); primary outcome measure
- Kappa inter-rater concordance level.

8.4 SECONDARY OUTCOME STATISTICS

Binary logistic regression will be applied to evaluate if any parameters are associated with either clinically judged infection or WOUNDCHEK™ positive tests. To allow logistic regression, the following dependents are used: clinical opinion positive ('possible infection' and 'definite infection' combined) vs clinical opinion negative ('no infection'), and positive WOUNDCHEK™ result vs negative WOUNDCHEK™ result.

Two binary logistic regression analyses will be conducted for variables associated with the non-matched outcome:

- Clinical opinion negative vs WOUNDCHEK™ test positive (as a proportion of all results, non-matched plus matched results combined)
  or
- Clinical opinion positive vs WOUNDCHEK™ test negative (as a proportion of all results, non-matched plus matched results combined)

To determine if the intended sample size gives sufficient power for binary logistic regression analysis, the paper by Peduzzi et al. (1996) is used as a guide regarding achieved power. The formula for the sample size is N = 10k / p, where p is the proportions of (non-matching) negative or positive cases in the population and k the number of covariates (the number of independent variables). If with backwards elimination approach a total of five covariates makes up the final regression model (k = 5), and the non-matched proportion is 20% (i.e 0.16) then N = 10 x 5 / 0.16 = 250 samples.

Odds ratios (logistic) will be expressed as Beta with 95% confidence intervals. The variables included are: type of wound, size of wound, age of wound, patient age, patient sex, patient pain level for wound, purulence level, erythema level, odour level, presence/absence of co-morbidities (including diabetes, peripheral arterial disease, heart failure), patient mobility, patient quality of life.

Parameters collated at baseline, such as wound type, wound size (including PUSH score) dressing type, patient age, sex and BMI will be recorded and presented in a tabulated format. No identifiable data will be presented, only averages and totals. Furthermore, vasculature-related co-morbidities will be recorded and graded, with the most severe co-morbidity defining the grading. Classed as mild-moderate will be type I diabetes, hypertension, chronic kidney disease (CKD) up to and including stage 3, retinopathy,

varicose veins, atrial fibrillation, history of deep venous thrombosis, heart failure. Moderate-severe co-morbidities will be history of myocardial infarction, stroke or transient ischaemic attack, CKD stage 4 or higher, peripheral vascular disease, history of amputation. Any other significant non-vascular conditions will be recorded too (e.g. COPD, cancer, auto-immune disease).

Treatment Deviation outcome data will be collated and presented with descriptive statistics. This will be in the form of the number of treatment deviations that occurred as a result of the WOUNDCHECK test result, the nature of the deviation and the longer term patient/wound outcomes at week 6 and/or week 12.

Ulcer healing and more general health measures is assessed by the following parameters:

- Ulcer status (healed vs non-healed)
- Ulcer size (cm^2)
- PUSH score
- SINBAD score
- Visual analogue pain score
- EQ-5D-5L

To assess the Ulcer size, PUSH score, visual analogue pain score, which are measured every 6 weeks, the average difference between groups (ie negative or positive Woundchek test outcome) will be calculated and Mann-Whitney U-test will be applied.

To measure patient-reported outcome measures on quality of life (EQ-5D-5L) at baseline, week6 and week 12; the the Mann-Whitney U-test will be performed between Woundchek test outcome groups.

Analysis will be performed on a per protocol basis, and inferential statistics will be performed on pooled data. Data will first be collated in Microsoft Excel, followed by analyses performed using SPSS v24.

9 Data handling and Monitoring

Data arising from this study is confidential. Identifiable information can only be accessed by delegated members of the study team. Anyone in the research team who will work on Trust premises and see patients, and does not have a substantive contract with NCIC, will need to apply for a letter of access via the NIHR research passport scheme.

Participants will be pseudo anonymised by allocating a study ID to each of them. Patient identifiable data will only be used within North Cumbria Integrated Care NHS Foundation Trust; if applicable, only anonymised data are shared with the wider members of the study team. All identifiable data is stored on password protected NHS computer systems. Anonymised data will be shared and stored using security-enabled systems such as password-protection and encryption of e-mails and files. The requirements of the Data Protection Act and NHS Code of Confidentiality will be followed at all times. All researchers will be fully trained in NHS Confidentiality and GCP.

Participants' GP practices will not be informed that they are taking part in the study.

All paper data will be held in secure locked environments in the office of the Research & Development department in Cumberland Infirmary, Carlisle, North Cumbria Integrated Care NHS Foundation Trust.

Electronic data will be saved on the patient management system such as EMIS, and also a password protected research database. Data released (e.g. by publication) will contain no information that could lead to the identification of an individual participant. Upon completion of the study the site files will be archived for a period of 10 years in line with local archiving policy and procedures.

Direct access to anonymised data only will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

Final data, will be shared with WOUNDCHEK™ Ltd in pseudo-anonymised form. The final anonymised dataset will be shared with WOUNDCHEK™ in pseudoanonymised form.

10 Governance of study

10.1 APPROVALS

This study will be conducted in compliance with the protocol approved by the Health Research Authority, National Research Ethics Service, and local Trust R&D Approval, and according to Good Clinical Practice standards including the Declaration of Helsinki (1964, Amended Oct 2013). No deviation from the protocol will be implemented without the prior review and approval of the aforementioned review bodies, except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported according to policies and procedures.

10.2 SPONSOR & INDEMNITY

North Cumbria Integrated Care NHS Foundation Trust is the sponsor of this study and therefore NHS indemnity applies for design, conduct and management of the study. WOUNDCHEK™ has provided a grant for this study by means of provision of the WOUNDCHEK™ test kits free of charge.

Patients will not be given financial incentives for taking part in the study. Travel expenses are not offered in this study since patients are seen when they attend their regular clinical appointment with the podiatry and/or tissue viability services. If indicated as per clinical routine practice, patients will be seen in their homes.

11 Publication and data-sharing policy

The results of this study will potentially be disseminated through:

- Peer-reviewed scientific journal (this will be subject to agreement from WOUNDCHEK™ Ltd, following completion of the study)
- Internal report

A summary of the main findings can be supplied to participants on request and this will be stated in the patient information leaflet.

12 References and Further Reading

Agale SV. Chronic leg ulcers: epidemiology, aetiopathogenesis, and management. Ulcers. 2013 Apr 22;2013.

Armstrong DG, Meyr AJ. Compression therapy for the treatment of chronic venous insufficiency. U: UpToDate, Mills JL, Eidt JF. ur. UpToDate [Internet]. 2017.

Back, T.L., Padberg, F.T., Araki, C.T., Thompson, P.N. and Hobson, R.W., 1995. Limited range of motion is a significant factor in venous ulceration.*Journal of vascular surgery*, *22*(5), pp.519-523.

Benson R, Serena TE, Simmons R, Bayliff S, Cullen B, Digby L. Bacterial protease activity, an indicator of bacterial pathogenicity in chronic wounds even in the absence of overt clinical signs (2017). Retrieved from Woundchek website, https://wounddiagnostics.com/wp-content/uploads/2017/01/Bacterial-protease-activity-an-indicator-of-bacterial-pathogenicity-in-chronic-wounds-even-in-the-absence-of-overt-clinical-signs.pdf (accessed 1 Sep 2022).

Bergan JJ, Schmid-Schönbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. New England Journal of Medicine. 2006 Aug 3;355(5):488-98.

Brand, F., Dannenberg, A.I., Abbott. R.D., Kannel, W.B. (1988) 'The epidemiology of varicose veins: the Framington Study', *American Journal of Preventative Medicine,* 4(2) pp.96-101.

Davies, J.A., Bull, R.H., Farrelly, I.J. and Wakelin, M.J., 2007. A home-based exercise programme improves ankle range of motion in long-term venous ulcer patients. *Phlebology*, *22*(2), pp.86-89.

Dinh MT, Abad CL, Safdar N. Diagnostic accuracy of the physical examination and imaging tests for osteomyelitis underlying diabetic foot ulcers: meta-analysis. Clinical Infectious Diseases. 2008 Aug 15;47(4):519-27.

Dixy, F.P., Brooke, R. and McCollum, C.N., 2003. Venous disease is associated with an impaired range of ankle movement. *European journal of vascular and endovascular surgery*, *25*(6), pp.556-561.

Dunn DT, Copas AJ, Brocklehurst P. Superiority and non-inferiority: two sides of the same coin?. Trials. 2018 Dec;19(1):1-5.

Eberhardt, R.T,& Rafetto, J.D. (2005) 'Chronic venous insufficiency', *Circulation,* 111(18) pp.2398-2409.

Edwards LM. Why patients do not comply with compression bandaging. British journal of nursing. 2003 Jun 12;12(Sup2):S5-16.

Eklof B, Rutherford RB, Bergan JJ, Carpentier PH, Glovicski P, Kistner RL, et al. Revision of the CEAP classification for chronic venous disorders: consensus statement. J Vasc Sur 2004;40:1248-52

Flight, L. orcid.org/0000-0002-9569-8290 and Julious, S.A. (2016) Practical guide to sample size calculations: non-inferiority and equivalence trials. Pharmaceutical Statistics, 15 (1). pp. 80-89.

Gardner SE, Frantz RA, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair and Regeneration. 2001 May;9(3):178-86

Gardner SE, Frantz RA, Faan RN, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Rep Reg. 2001; 9: 178-186.

Gardner SE, Haleem A, Jao YL, Hillis SL, Femino JE, Phisitkul P, Heilmann KP, Lehman SM, Franciscus CL. Cultures of diabetic foot ulcers without clinical signs of infection do not predict outcomes. Diabetes Care. 2014 Oct 1;37(10):2693-701.

Gonzalez-Consuegra, R.V. & Verdu, J. (2011) 'Quality of life in people with venous leg ulcers', *Journal of Advanced Nursing,* 67 (5), pp. 926-944.

Graham, I.D., Harrison, M.B., Nelson, E.A., Lorimer, K. & Fisher, A. (2003) 'Prevalence of lower limb ulceration: a systematic review of prevalence studies', *Advanced Skin Wound Care,* 16(6) pp.305-316.

Grothier, L., & Stephenson, J. (2015). An audit to determine the clinical effectiveness of a pathway for managing wound infection. *Wounds UK*, *11*(2), 32-40.

Halbert, A.R., Stacey, M.C., Rohr, J.B. and Jopp-Mckay, A., 1992. The effect of bacterial colonization on venous ulcer healing. *Australasian journal of dermatology*, *33*(2), pp.75-80.

Hanna R, Bohbot S, Connolly N. A comparison of inferface pressures of three compression bandage systems. British Journal of Nursing. 2008 Nov 13;17(Sup9):S16-24.

Harding K, Gottrup F, Jawień A, Mikosiński J, Twardowska-Saucha K, Kaczmarek S, Sopata M, Shearman C, Pieronne A, Kommala D. A prospective, multi-centre, randomised, open label, parallel, comparative study to evaluate effects of AQUACEL® Ag and Urgotul® Silver dressing on healing of chronic venous leg ulcers. International Wound Journal. 2012 Jun;9(3):285-94.

Hareendran, A., Bradbury, A., Budd, J., Geroulakos, G., Hobbs, R., Kenkre, J., & Symonds, T. (2005). Measuring the impact of venous leg ulcers on quality of life. *Journal of Wound Care*, *14*(2), 53-57.

Heinen MM, van der Vleuten C, de Rooij MJ, Uden CJ, Evers AW, van Achterberg T. Physical activity and adherence to compression therapy in patients with venous leg ulcers. Archives of dermatology. 2007 Oct 1;143(10):1283-8.

Hon J, Lagden K, McLaren AM, O'Sullivan D, Orr L, Houghton PE, Woodbury MG. A prospective, multicenter study to validate use of the PUSH in patients with diabetic, venous, and pressure ulcers. Ostomy/wound management. 2010 Feb;56(2):26-36.

Jonker L, Mark E, Singleton L, Smith D, Fisher S, Gratwohl D. Evaluation of Glycologic Point-of-Care Infection Test Kit for Diabetic Foot Ulcers in Relation to Bacterial Presence: A Prospective Cohort Study. Journal of the American Podiatric Medical Association. 2022 Jan 1;112(1).

Jonker L, Smith D, Mark E, Schutter J, Thornthwaite S, Johnston S. Point-of-care testing for bacterial infection in diabetic foot ulcers: a prospective cohort study. Journal of Wound Care. 2020 Nov 2;29(11):649-57.

Jonker L, Todhunter J, Robinson L, Fisher S. Open-label, randomised, multicentre crossover trial assessing two-layer compression bandaging for chronic venous insufficiency: results of the APRICOT trial. British Journal of Community Nursing. 2020 Jun 2;25(Sup6):S6-13. (b)

Jonker L, Todhunter J, Schutter J, Halliday C, Fisher S. A multi-centre, prospective, randomised controlled feasibility study of plantar resistance exercise therapy for venous leg ulcers–Results of the PREVUE study. Phlebology. 2020 May;35(4):237-46. (a)

Kan, Y.M. & Delis, K.T. (2001) 'Haemodynamic effects of supervised muscle exercise in patients with venous leg ulceration: a prospective controlled study', *Archives of Surgery,* 136 pp.1364-1369.

Lamping DL, Schroter S, Kurz X, Kahn SR, Abenhaim L. Evaluation of outcomes in chronic venous disorders of the leg: development of a scientifically rigorous, patient-reported measure of symptoms and quality of life. Journal of vascular surgery. 2003 Feb 1;37(2):410-9.

Launois R. Health-related quality-of-life scales specific for chronic venous disorders of the lower limbs. Journal of Vascular Surgery: Venous and Lymphatic Disorders. 2015 Apr 1;3(2):219-27.

Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA. Risk factors for foot infections in individuals with diabetes. Diabetes care. 2006 Jun 1;29(6):1288-93.

Levine NS, Lindberg RB, Mason JA, Pruitt JB. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. The Journal of trauma. 1976;16(2):89-94

Mayberry JC, Moneta GL, Taylor JL, Porter JM. Fifteen-year results of ambulatory compression therapy for chronic venous ulcers. Surgery. 1991 May;109(5):575-81.

Medina, A., Scott, P.G., Ghahary, A., Tredget, E.E. The pathophysiology of chronic nonhealing wounds. J Burn Care 2005. Rehabil 26:306-319.

Moffatt C, Marston WA, Stacey MC, Woodward MC, Franks P, Quere I. An examination of PROGUIDE® in compression therapy (EXPECT): a multi-centre randomised non-inferiority trial of two compression systems in the treatment of venous leg ulcers. Journal of Wound Care. 2019 Apr 26.

Moffatt C, Murray S, Keeley V, Aubeeluck A. Non-adherence to treatment of chronic wounds: patient versus professional perspectives. International wound journal. 2017 Dec;14(6):1305-12.

Moffatt CJ, Edwards L, Collier M, Treadwell T, Miller M, Shafer L, Sibbald G, Brassard A, McIntosh A, Reyzelman A, Price P. A randomised controlled 8-week crossover clinical evaluation of the 3M™ Coban™ 2 Layer Compression System versus Profore™ to evaluate the product performance in patients with venous leg ulcers. International wound journal. 2008 May;5(2):267-79.

Moffatt, C.J., Franks, P.J., Doherty, D.C., Smithdale, R. & Martin, R. (2006) 'Sociodemographic factors in chronic leg ulceration', British Journal of Dermatology, 155(2) pp.307-312.

Morgan PA, Murray S, Moffatt CJ, Young H. The experience of patients with lymphoedema undergoing a period of compression bandaging in the UK and Canada using the 3M™ Coban™ 2 compression system. International wound journal. 2011 Dec;8(6):586-98.

NHS Diabetes report. https://www.networks.nhs.uk/news/new-foot-care-report-shows-ps650m-cost-of-ulcers-and-amputations last accessed 1 September 2022

NICE Clinical Guideline 15, Oct 2008, Surgical site infections: prevention and management, https://www.nice.org.uk/guidance/ng19 last accessed 1 September 2022.

NICE Guideline 15, Aug 2015, Diabetic foot problems: prevention and management, https://www.nice.org.uk/guidance/ng19 last accessed 1 September 2022.

O'Meara, S., Cullum, N.A. & Nelson, E.A. (2009) 'Compression for venous leg ulcers', The Cochrane Database Systematic Review, 1.

Oczkowski SJ. A clinician's guide to the assessment and interpretation of noninferiority trials for novel therapies. Open Medicine. 2014;8(2):e67.

O'meara S, Nelson EA, Golder S, Dalton JE, Craig D, Iglesias C. Systematic review of methods to diagnose infection in foot ulcers in diabetes. Diabetic medicine. 2006 Apr 1;23(4):341-7.

Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR (1996) A simulation study of the number of events per variable in logistic regression analysis. Journal of Clinical Epidemiology 49:1373-1379

Peleg, A.Y., Weerarathna, T., McCarthy, J.S., Davies, T.M.E. Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. Diabetes Metab Res Rev. 2007. 23 3-13

Persoon, A., Heinen, M.M., Van Der Vleuten, C.J., De Rooij, M.J., Van De Kerkhof, P.C.M. and Van Achterberg, T. (2004) 'Leg ulcers: a review of their impact on daily life', *Journal of Clinical Nursing* 13 (3) pp.341–354.

Pillen, H., Miller, M., Thomas, J., Puckridge, P., Sandison, S. & Spark, J.I. (2009) 'Assessment of wound healing: validity, reliability and sensitivity of available instruments', *Wound Practice and Research,* 17(4) pp. 208-217.

Porter JM, Moneta GL. Reporting standards in venous disease: an update. International Consensus Committee on Chronic Venous Disease. J Vasc Surg 1995;21:635-45

Posnett J, Franks PJ, (2008). The burden of chronic wounds in the UK. Nursing Times; 104: 3, 44–45, link https://www.nursingtimes.net/Journals/2015/06/05/q/f/y/The-burden-of-chronic-wounds-in-the-UK.pdf

Ratliff, C.R. & Rodeheaver. G.T. (2005) 'Use of the PUSH tool to measure venous ulcer healing', *Ostomy Wound Management,* 51(5) p.58.

Ratliff, C.R. & Rodeheaver. G.T. (2005) 'Use of the PUSH tool to measure venous ulcer healing', *Ostomy Wound Management,* 51(5) p.58.

Reich-Schupke S, Murmann F, Altmeyer P, Stücker M. Quality of life and patients' view of compression therapy. International Angiology. 2009 Oct 1;28(5):385.

Roaldsen, K.S., Rollman, O., Torebark, E. Olsson, E. & Stanghelle. J.K. (2006) 'Functional ability in female leg ulcer patients- a challenge for physiotherapy', *Physiotherapy Research International,* 11(4) pp191-203.

Rubin JR, Alexander J, Plecha EJ, Marman C. Unna's boot Vs polyurethane foam dressings for the treatment of venous ulceration: A randomized prospective study. Archives of Surgery. 1990 Apr 1;125(4):489-90.

Schulz, K.F., Altman, D.G. & Moher, D. (2010) 'CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials', British Medical Journal, 340 p.332.

Schuren J, Bernatchez SF, Tucker J, Schnobrich E, Parks PJ. 3M Coban 2 layer compression therapy: intelligent compression dynamics to suit different patient needs. Advances in wound care. 2012 Dec 1;1(6):255-8.

Serena TE, Bayliff SW, Brosnan PJ. Bacterial protease activity: a prognostic biomarker of early wound infection. Journal of Wound Care. 2022 Apr 2;31(4):352-5.

Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Diabetes care. 2003 Jun 1;26(6):1879-82.

Shohrati M, Davoudi M, Almasi M, Sadr B, Peyman M. Comparative Study of Unna's Boot and Betamethasone Cream in the Treatment of Sulfur Mustard–Related Pruritus. Cutaneous and ocular toxicology. 2007 Jan 1;26(4):303-9.

SIGN (2010) Management of Chronic Venous Leg Ulcers. Edinburgh: SIGN. last accessed 1 September 2022

Smith, J.J., Guest, M.G., Greenhalgh, R.M., Davies, A.H. (2010) 'Measuring the quality of life in patients with venous ulcers', *Journal of Vascular Surgery,* 31 pp.642-649.

Stalvey, B. T., Owsley, C., Sloane, M. E., & Ball, K. (1999). The Life Space Questionnaire: A measure of the extent of mobility of older adults. *Journal of Applied Gerontology*, *18*(4), 460-478.

Stotts, N. A., Rodeheaver, G. T., Thomas, D. R., Frantz, R. A., Bartolucci, A. A., Sussman, C., ... & Maklebust, J. (2001). An instrument to measure healing in pressure ulcers development and validation of the Pressure Ulcer Scale for Healing (PUSH). *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*(12), M795-M799

Stotts, N. A., Rodeheaver, G. T., Thomas, D. R., Frantz, R. A., Bartolucci, A. A., Sussman, C., ... & Maklebust, J. (2001). An instrument to measure healing in pressure ulcers development and validation of the Pressure Ulcer Scale for Healing (PUSH). *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*(12), M795-M799.

Thomas S. Compression bandaging in the treatment of venous leg ulcers. 1997, http://www.worldwidewounds.com/1997/september/Thomas-Bandaging/bandage-paper.html (last accessed 2 Sep 2018).

Walshe C. Living with a venous leg ulcer: a descriptive study of patients' experiences. Journal of advanced nursing. 1995 Dec;22(6):1092-100.

White GH. Chronic venous insufficiency. In: Veith F, Hobson RW, Williams RA, Wilson SE, editors. Vascular surgery. 2nd ed. New York: McGraw-Hill, Inc; 1993. p. 865-88.

White, J.V. & Ryjewski, C. (2005) 'Chronic venous insufficiency', Perspectives in Vascular Surgery and Endovascular Therapy, 17(4) pp319-327.

Yoshikawa TT, Livesley NJ, Chow AW. Infected pressure ulcers in elderly individuals. Clinical infectious diseases. 2002 Dec 1;35(11):1390-6.

Appendix 1, Clinic Report Form week 0, week 6, and week 12

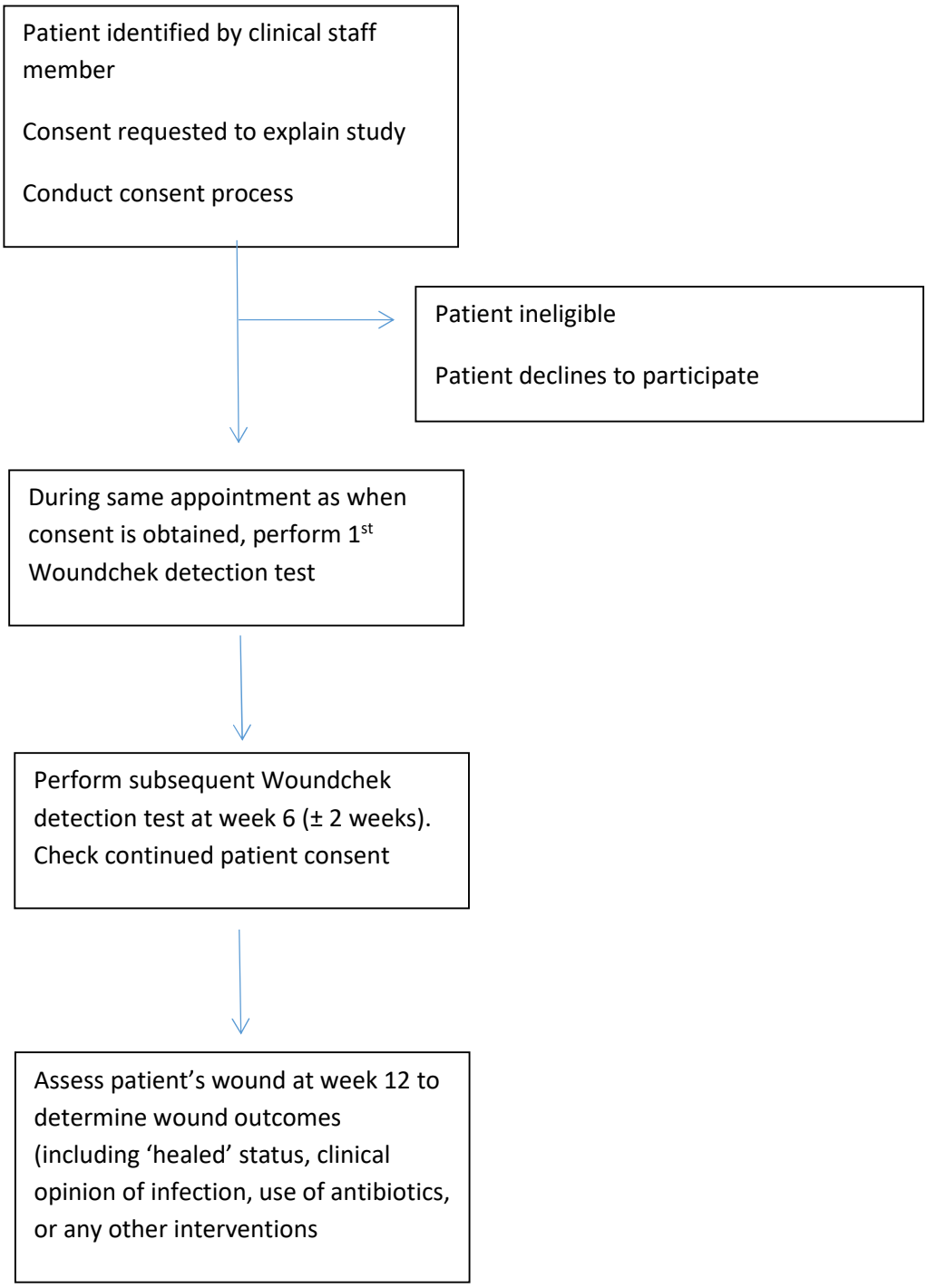
Patient Code: BIOME-……. Researcher completing form: …….

Please note: complete clinical opinion before testing with WOUNDCHEK™ test kit, and swab for WOUNDCHEK test prior to any debridement.

Patient visit #	**1 (study week 0)**	**2 (study week 6)**	**1 (study week 12)**
Patient visit date			
Week 6/12 only: Wound healed? (if yes, end of study for participant, no more data collection)		Yes / no	Yes / no
Week 6/12 only: Patient withdrawn? (if yes, end of study for participant, no more data collection)		Yes / no If yes: LFU, death, active withdrawal, hospitalization, other: ……	Yes / no If yes: LFU, death, active withdrawal, hospitalization, other: ……
Clinical opinion (no infection/ possible infection/ infected)			
Erythema around DFU (-/+/++)			
Purulence (-/+/++)			
Odour (none, low, moderate, high)			
WOUNDCHEK™ test result at 10 mins (+ve or -ve)			
Patient on oral/iv non-prophylactic antibiotics within 3 weeks (week0) or since last study visit (week6/12)? (yes/no)	*If yes, patient not eligible!*		
Oral/iv non-prophylactic antibiotics prescribed at this clinic visit? (yes/no)			
Swab sent for microbiology testing? (yes / no)			
Was wound debridement carried out? (yes / no)			
PUSH wound size score completed (yes / no)			
Patient VAS pain score completed (yes / no)			
QoL EQ-5D-5L completed (yes / no)			

PLEASE TURN OVER SHEET FOR MORE QUESTIONS			
Patient visit #	**1 (study week 0)**	**2 (study week 6)**	
Any treatment deviation as a result of WOUNDCHEK result?	YES / NO	YES / NO	
If YES to above question	Wound dressing - Antimicrobial instead of standard - Standard instead of antimicrobial Microbiology sample - Sent off when initially not planned - Not sent off when initially planned Antibiotics - Prescribed when initially not planned - Not prescribed when initially planned MDT - Referred to MDT when initially not planned - Not referred to MDT when initially planned.	Wound dressing - Antimicrobial instead of standard - Standard instead of antimicrobial Microbiology sample - Sent off when initially not planned - Not sent off when initially planned Antibiotics - Prescribed when initially not planned - Not prescribed when initially planned MDT - Referred to MDT when initially not planned - Not referred to MDT when initially planned.	

Appendix 2, Study participant flowchart



Appendix 3, PUSH score and VAS pain scale

The Pressure Ulcer Scale for Healing (PUSH) tool is a standardised method of assessing and monitoring the severity and healing of both pressure ulcers and venous leg ulcers (Stotts et al, 2001; Ratliff & Rodeheaver 2005). The Pressure Ulcer Scale for Healing (PUSH) is a valid, responsive, evaluative tool to monitor and document wound progress of foot ulcers (Hon, 2010).

NATIONAL PRESSURE ULCER ADVISORY PANEL

Pressure Ulcer Scale for Healing (PUSH)
PUSH Tool 3.0

Patient Name_____ Patient ID# _____

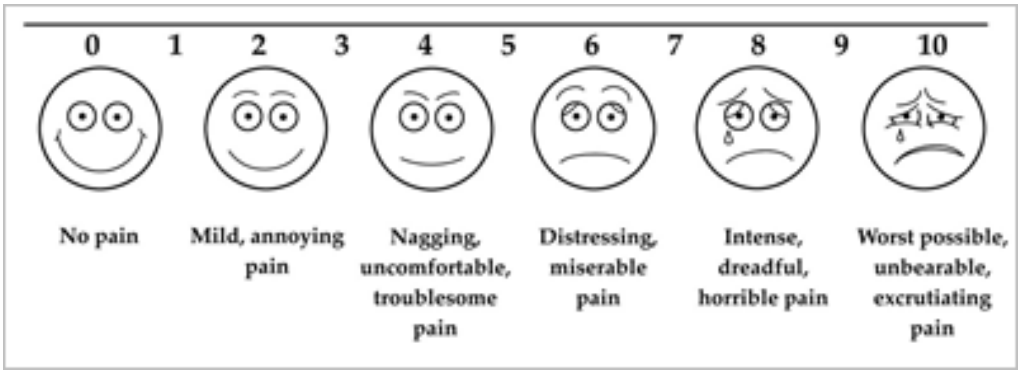
Ulcer Location _____ Date _____

Directions:

Observe and measure the pressure ulcer. Categorize the ulcer with respect to surface area, exudate, and type of wound tissue. Record a sub-score for each of these ulcer characteristics. Add the sub-scores to obtain the total score. A comparison of total scores measured over time provides an indication of the improvement or deterioration in pressure ulcer healing.

LENGTH X WIDTH (in cm^2)	0 0	1 < 0.3	2 0.3 – 0.6	3 0.7 – 1.0	4 1.1 – 2.0	5 2.1 – 3.0	Sub-score
		6 3.1 – 4.0	7 4.1 – 8.0	8 8.1 – 12.0	9 12.1 – 24.0	10 > 24.0	
EXUDATE AMOUNT	0 None	1 Light	2 Moderate	3 Heavy			Sub-score
TISSUE TYPE	0 Closed	1 Epithelial Tissue	2 Granulation Tissue	3 Slough	4 Necrotic Tissue		Sub-score
							TOTAL SCORE

Visual analogue pain scale, for pain currently experienced from site of wound (enter in table, not on the scale itself):



Appendix 4, Quality of life: EQ-5D-5L

Table 1. SINBAD Wound Classification system (Ince et al, 2008).		
Category	**Definition**	**SINBAD score**
Site	Forefoot	0
	Hindfoot	1
Ischaemia	Pedal blood flow intact (at least one pulse palpable)	0
	Clinical evidence of reduced pedal blood flow	1
Neuropathy	Protective sensation intact	0
	Protective sensation lost	1
Bacterial infection	None	0
	Present	1
Area	Ulcer $<1\ cm^2$	0
	Ulcer $\geq 1\ cm^2$	1
Depth	Ulcer confined to skin and subcutaneous tissue	0
	Ulcer reaching muscle, tendon or deeper	1
Total possible score		6

Ince P, Abbas ZG, Lutale JK, Basit A, Ali SM, Chohan F, Morbach S, Möllenberg J, Game FL, Jeffcoate WJ. Use of the SINBAD classification system and score in comparing outcome of foot ulcer management on three continents. Diabetes Care. 2008 May 1;31(5):964-7.

Appendix 5, Quality of life: EQ-5D-5L

Under each heading, please tick the **ONE** box that best describes your health **TODAY**

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work ,study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

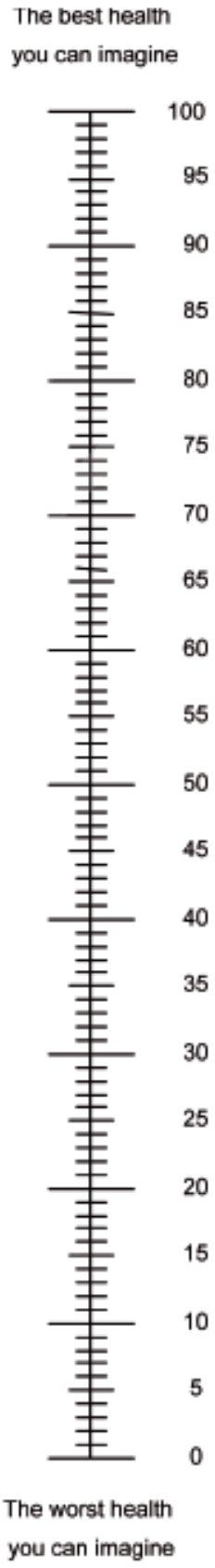
I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderate anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

EQ-5D

- We would like to know how good or bad your health is **TODAY.**
- This scale is numbered from **0** to **100**.
- **100** means the best health you can imagine.

  0 means the worst health you can imagine
- Mark an **X** on the scale to indicate how your health is **TODAY.**
- Now, please write the number you marked on the scale in the box below

YOUR HEALTH TODAY = ☐



©EuroQoL Group 1990

Appendix 6, WOUNDCHEK Specimen Collection

Source: https://www.woundchek.com/specimen-collection/SC-English.html

1. Prior to swabbing, gently cleanse the wound with sterile saline 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.

2. Ensure that complete hemostasis has been achieved before obtaining the specimen.

3. 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. Avoid swabbing areas that contain blood, necrotic material, thick slough or fibrinous tissue.

5. Press the head of the swab flat against the base of the wound and gently roll it back and forth several times while applying pressure. Continue rolling the swab head until fully coated and discoloured (tan/yellow) by wound fluid.

6. Test the sample swab as soon as possible after collection.