NIHR Health Technology Assessment programme

NHS National Institute for Health Research

NIHR, HTA programme

1 June 2012

The Health Technology Assessment programme is managed by NETSCC, HTA as part of the NIHR Evaluation, Trials and Studies Coordinating Centre at the University of Southampton.

Alpha House, University of Southampton Science Park
Southampton SO16 7NS
Suggest a topic for research via our online form at www.hta.ac.uk/suggest

tel: +44(0)23 8059 5586
fax: +44(0)23 8059 5639
email: hta@hta.ac.uk

www.hta.ac.uk

UNIVERSITY OF LEEDS

ctru University of Leeds

CODIFI

Comparing sampling methods in diabetic foot infections

CODIFI:

COncordance in DIabetic Foot Infection

Version	3.0
Date	12th September 2011
Sponsor	The University of Leeds
Sponsor Reference	CODIFI2010
REC Reference	11/YH/0078
NIHR HTA	09/75/01
Chief Investigator	Professor (Elizabeth) Andrea Nelson School of Healthcare University of Leeds Leeds LS2 9UT

1. KEY CONTACTS

Chief Investigator

Professor Andrea Nelson
Professor of Wound Healing
School of Healthcare
Baines Wing
Woodhouse Lane
University of Leeds
Leeds
LS2 9UT
Telephone: 0113 343 1373
Fax: 0113 343 7560
Email: e.a.nelson@leeds.ac.uk

Study Management

CTRU Principal Investigator

Professor Jane Nixon
Deputy Director
Clinical Trials Research Unit
University of Leeds
Leeds
LS2 9JT
Telephone: 0113 343 1488
Fax: 0113 343 1471
Email: j.e.nixon@leeds.ac.uk

Principal Statistician

Miss Sarah Brown
Clinical Trials Research Unit
University of Leeds
Leeds
LS2 9JT
Telephone: 0113 343 1475
Fax: 0113 343 1471
Email: medsbro@leeds.ac.uk

Dr Janine Gray
Clinical Trials Research Unit
University of Leeds
Leeds
LS2 9JT
Telephone: 0113 343 1481
Fax: 0113 343 1471
Email: j.c.gray@leeds.ac.uk

Study Statistician

Miss Alex Wright-Hughes
Clinical Trials Research Unit
University of Leeds
Leeds
LS2 9JT
Telephone: 0113 343 8016
Fax: 0113 343 1471
Email: a.wright-hughes@leeds.ac.uk

Clinical & Microbiology Co-investigators

Professor Christopher Dowson
Professor in Microbiology
Department of Biological Sciences
University of Warwick
Coventry
CV4 7AL
Telephone: 02476 523534
Fax: 02476 523568
Email: c.g.dowson@warwick.ac.uk

Dr Edward Jude
Consultant Diabetologist and Senior Lecturer
Diabetes Centres
Tameside Hospital NHS Foundation Trust
Fountain Street
Ashton-Under-Lyne
Lancashire
OL6 9RW
Telephone: 0161 331 6964
Fax: 0161 331 6442
Email: Edward.Jude@tgh.nhs.uk

Dr Carol Amery
Consultant Physician/Honorary Senior Lecturer
Diabetic Medicine
Department of Diabetes and Endocrinology
Leeds General Infirmary
Leeds
LS1 3EX
Telephone: 0113 206 5066
Fax: 0113 206 5065
Email: carol.amery@leedsth.nhs.uk

Dr Jill Firth (Investigator until July 2011)
Honorary Senior Research Fellow
School of Healthcare
Baines Wing
University of Leeds
Leeds
LS2 9UT
Telephone: 0113 343 7972
Fax: 0113 343 7560
Email: j.firth@leeds.ac.uk

Dr Michael Edmonds
Consultant Physician
Diabetic Foot Clinic, King's Diabetes
Kings College Hospital
Denmark Hill
London
SE5 9RS
Telephone: 0203 2993223
Fax: 0203 2994536
E mail: michael.edmonds@nhs.net

Professor Benjamin Lipsky
Professor of Medicine
University of Washington School of Medicine
VA Puget Sound HCS
1660 S. Columbian Way
Seattle, WA 96108
USA
Telephone: 206 277 1 1640
Fax: 206 764 2849
Email: balipsky@uw.edu

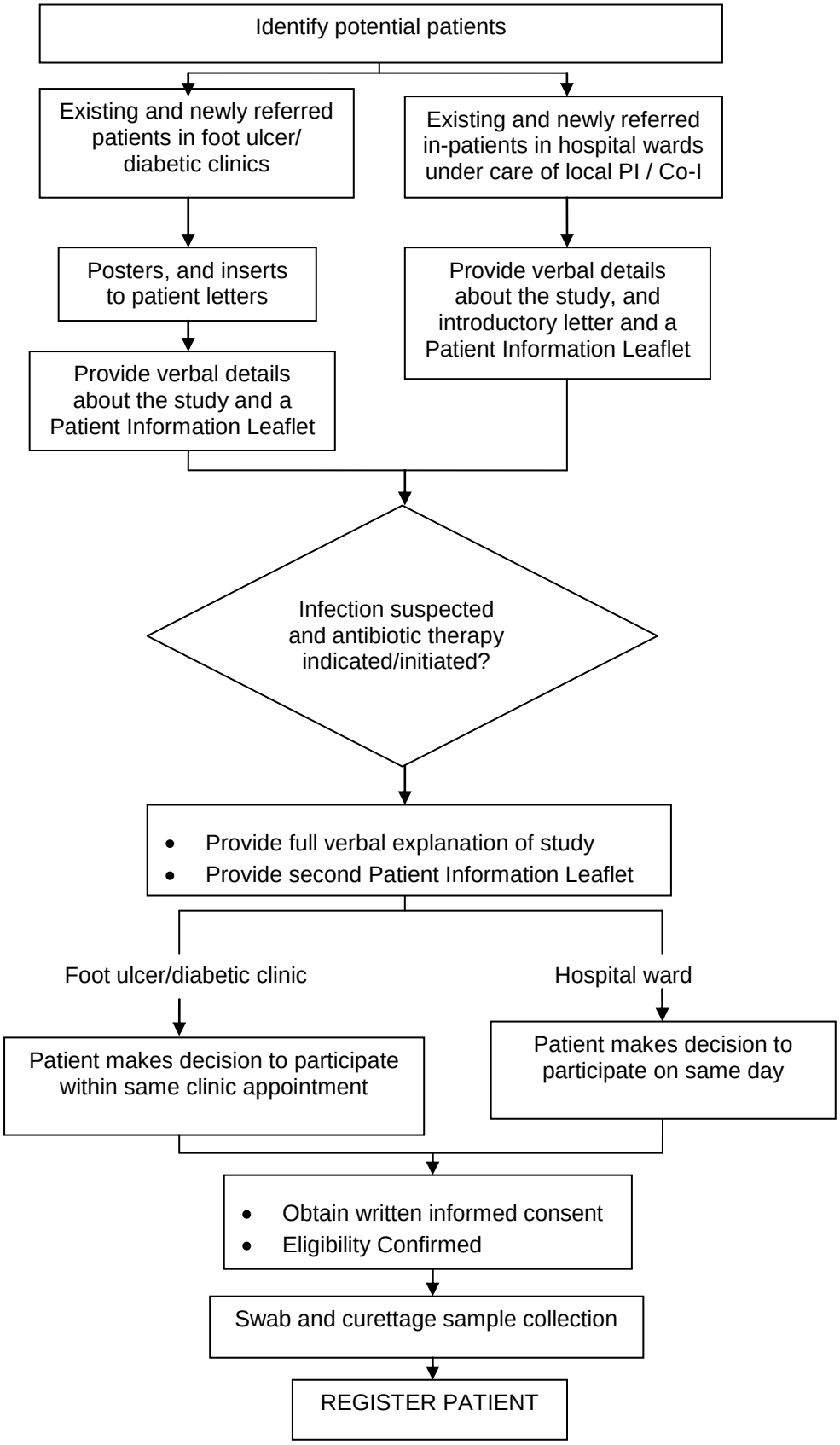
Mr Tom Dickie
Foot Health Manager
Foot Health Dept
B Floor
Martin Wing
Leeds General Infirmary
Great George Street
Leeds
LS1 3EX
Telephone: 0113 3923558
Fax: 0113 3923558
Email: thomas.dickie@leedsth.nhs.uk

Mrs Gill Sykes
Clinical Lead
Clinical Therapy and Rehabilitation Directorate
Brighouse Health Centre,
Lawson Road
Brighouse
West Yorkshire
HD6 1NY
Telephone: 01484 712515
Fax: 01484 718967
Email: gill.sykes@cht.nhs.uk

Professor Peter Vowden
Consultant Vascular Surgeon
Bradford Royal Infirmary
Duckworth Lane
Bradford
BD9 6RJ
Telephone: 01274 364466
Fax: 01274 364807
Email: peter.vowden@bradfordhospitals.nhs.uk

2. TABLE OF CONTENTS

3. STUDY FLOW DIAGRAM / STUDY SUMMARY



4. LIST OF ABREVIATIONS

AE	Adverse Event
CI	Chief Investigator
CODIFI	Concordance in Diabetic Foot Infection
CRF	Case Report Form
CRN	Clinical Research Nurse
CTRU	Clinical Trials Research Unit
DNA	Deoxyribonucleic Acid
GCP	Good Clinical Practice
HPA	Health Protection Agency
HTA	Health Technology Assessment
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDSA	Infectious Diseases Society of America
ISF	Investigator Site File
ITT	Intention To Treat
IWGDF	International Working Group on the Diabetic Foot
NHS	National Health Service
NIHR	National Institute of Health Research
NRES	National Research Ethics Service
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PIL/ICD	Patient Information Leaflet/Informed Consent Document
PIN	Personal Identification Number
REC	Research Ethics Committee
RNA	Ribonucleic Acid
RU SAE	Related Unexpected Serious Adverse Event
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SMG	Study Management Group
SSC	Study Steering Committee

5. BACKGROUND AND INTRODUCTION

Diabetes: prevalence and complications

Both type 1 diabetes (insulin dependent diabetes: about 1/10 cases), and type 2 diabetes (non-insulin dependent diabetes: 9/10 cases) can lead to significant health problems. Worldwide prevalence was estimated at around 2.8% in 2000 (Wild et al. 2004). Diabetes is known to affect around 3% of people in England and many more people may have diabetes yet are unaware that they are affected (Diabetes UK, 2009). An increase in obesity in industrialised countries will contribute to the increased prevalence of diabetes and is predicted to double by 2030 (Wild et al. 2004). Estimates from the US predict that Americans born in 2000 will have a 1 in 3 lifetime risk of developing diabetes (Narayan et al. 2003).

Complications of diabetes, especially in patients with poorly controlled blood sugar levels, include damage to the eyes, kidneys, nerves and arteries. In the feet complications lead to changes to foot architecture (hence increasing pressure on plantar surfaces, including those unaccustomed to load-bearing), reduced sweating (hence dry, cracking skin), poor sensation (hence susceptibility to trauma) and reduced circulation (hence reduced ability to heal wounds and fight infection). These neuropathic and arteriopathic changes, either alone or in combination, predispose the foot to damage and ulceration.

Foot ulcers affect many people with diabetes at some time in their life. They are associated with reduced quality of life, higher costs, increased mortality and morbidity (Iversen et al. 2009). It has been estimated that the proportion of people with diabetes in the UK who have ever had a foot ulcer is around 6% (Williams et al. 2000). The lifetime risk of a foot ulcer is estimated to be between 15% and 25% (Singh et al. 2005). Diabetic foot ulcers take weeks (often months) to heal, and while they are open they increase the risk of infection of the foot or lower limb (including osteomyelitis) and/or gangrene.

Foot infections in people with diabetes can be hard to manage because of the impaired arterial supply to the legs, as well as disruption of the immune system (especially of the function of polymorphonuclear leukocytes) that accompany diabetes. This means there is an increased risk of worsening infection (more damage to the tissues of the foot, including the bones), and extending infection (up the foot / leg and damaging more tissues), as well as systemic spread into the blood stream. Thus, when foot / ulcer infection spreads the treatment may need to include some level of lower extremity amputation (Williams et al. 2000), as a leg-saving and ultimately life-saving measure.

The vast majority of foot / leg amputations in people with diabetes are preceded by a diabetic foot ulcer infection. Amputation is a major operation, it dramatically reduces the quality of life, and is expensive for the patient and the healthcare system, therefore foot clinics have been set up to coordinate and deliver the various elements of foot care to prevent ulceration and amputation. The foot care of people with diabetes focuses on the prevention of foot ulcers (optimising diabetic control / foot and skin care / supplying special shoes), supporting the healing of foot ulcers (optimising diabetic control / off-loading pressure on the feet using footwear or prostheses / wound dressings / adjuvant treatments), and the prevention of amputation by the early identification, characterisation, and aggressive treatment of ischaemia and infection. Identifying infection and treating it is therefore very important in caring for people with diabetes and foot ulcers.

Wound Infection: definition, identification and characterisation

All chronic wounds, such as diabetic foot ulcers, have bacteria on their surface. These originate from the normal skin flora, as well as some opportunistic colonising bacteria, such as gut flora. The presence of bacteria is not a sufficient criterion to diagnose infection. When the host tissues (the patient‘s soft tissues) are not damaged by the bacteria the wound is described as ‘colonised’. At this stage there is a balance between the growth of the various bacteria and no single organism dominates.

Infection is a clinical diagnosis denoted by the presence of signs and symptoms, such as pyrexia, pus, pain, erythema, warmth, induration, and delayed healing. These signs and symptoms indicate that the balance between the host‘s defences and the bacteria within the ulcer has shifted, and that the bacteria have started to destroy host tissues. Infection can increase the size of an ulcer, change its appearance (more dead tissue, also called slough, on the ulcer surface), and / or delay healing. Antibiotics are used to stop the multiplication of bacteria (bacteriostatic drugs), or to kill them (bactericidal drugs). They help prevent infection spreading from the skin to deep soft tissue to bone and blood vessels. This is to help reduce the risk of osteomyelitis, septicaemia, gangrene, and possible amputation.

There is no agreed ‘gold-standard’ test for wound infection. Once a clinician suspects infection (from the presence of the cardinal signs of infection or a non-healing wound), then immediate treatment with antibiotics is commenced, based on empirical knowledge of the likely causative organisms. For wounds that have not been recently treated with antibiotics, then the likely cause of infection is a monoculture of a pathogen such as *Staphylococcus aureus*. Wounds that are chronic, especially if the patient has recently been treated with antibiotic therapy, are more likely to have several (usually 3-5) pathogens present. The clinician seeks to characterise the wound flora (type of organism[s] and their

antibiotic susceptibility) but will usually start treatment for infection before the results of analysis are available. This is because the potentially devastating consequences of infection mean that clinicians prefer not to risk delay and hence start treatment immediately. Microbiological evaluation of the microbiology of the wound usually includes a description of the number and types of species present (e.g., by Gram-stain characteristics), a determination as to which of the isolated organisms likely represent pathogens versus colonizers, and a determination of the sensitivity of potential pathogens to various antimicrobials. This allows the clinician to confirm a clinical diagnosis of infection (by the presence of known pathogens or a heavy bacterial load of less virulent organisms). Crucially, it also guides revision of the antibiotic regimen if the ulcer is not showing signs of improvement at an early review visit. The laboratory results take around 4 or 5 days to come back to clinicians and at this stage they can evaluate progress and amend antibiotics if necessary. The delay in reporting, however, combined with the antibiotic treatment given in the intervening period, means that the laboratory result is out of date and hence if the clinician is reviewing an ulcer that has not improved with treatment they cannot presume that the bacteria described in the microbiologists report are still the pathogens responsible for an infected ulcer 5 days later. Quicker techniques for microbiological analyses, such as molecular techniques that take a day, may help address this delay.
The microbiological analysis of specimens from the ulcer is only useful if the specimen is properly collected. The aim is to acquire a sample of any pathogens and to avoid sampling the colonising flora. First, the ulcer / wound site must be cleaned (and often debrided) to remove necrotic material or callus and undermining tissues. Next, a specimen is taken from the site of infection, using one of a number of specimen acquisition techniques: wound swabbing, fluid sampling using a fine-needle aspiration, cutting a sample of wound (biopsy) and wound curettage. Curettage involves scraping the base of a wound with a sharp-edged dermal curette / scalpel blade to obtain visible tissue (Senior, 2001).

It is important that a sample reflects an accurate profile of the bacterial environment in the ulcer. Failing to identify a true pathogen (in diagnostic test terms poor test sensitivity) or identifying a coloniser as a pathogen (poor specificity) can each worsen the outcome of treating an infected wound. Thus, it is important that the clinic staff use a technique which will give a valid account of the bacteria present and their number and sensitivity to antibiotics. The need for accuracy means that most published guidelines recommend obtaining a tissue specimen rather than a swab, in order to increase the chances of getting the characterisation of the infection correct at initial presentation. It is widely assumed that tissue samples are the best source of wound bacteria, as opposed to wound fluid (obtained via swabbing or aspiration). Accurate, timely characterisation of bacteria and their resistance should help reduce the overuse of broad spectrum antimicrobials that leads to increased antibiotic resistance, hence preserving the armoury of antimicrobials for life-threatening infections.

Most bacteriological samples from wounds are taken with a cotton swab to collect wound fluid, less often they are taken using a curette (known as curettage). The advantages of a wound swab include the almost universal availability of the equipment, relative ease of sampling, the low cost of the swab, and the fact that little training is needed to perform this correctly. Furthermore, there is a very small risk of harm using a swab to collect a tissue sample. The disadvantages of a swab include the concern that it will not collect those bacteria responsible for the infection deep within the tissues, that it will collect the innocuous colonisers on the wound surface, or that it will fail to provide an environment conducive to growth of obligate anaerobes and other fastidious organisms (i.e. those that may be present in the wound but die in a swab sample which does not provide an adequate medium for their survival). To counter this, the techniques for wound swabbing specify how to prepare the ulcer bed (by removing dead tissue which may contain irrelevant bacterial groups), and to get a sample from deep in the ulcer, by pressing to collect fluid from deep in the subcutaneous tissues, as well as storage and transport procedures (charcoal swab, transport medium, and swift delivery to laboratory to maintain the viability of fastidious organisms).

In contrast, curettage takes a piece of ulcer tissue for microbiological analysis. The reported advantage is that the sample is likely to contain the pathogens responsible for tissue destruction and infection. However curettage and biopsy techniques require disruption or cutting of the ulcer bed to obtain a sample and this may lead to bleeding or pain (although most diabetic foot ulcers are complicated by neuropathy reducing the ability to perceive pain). Some clinical staff (including nurses) may need extra training to be able to take these samples safely, using sharp sterile blades / curettes, or a biopsy cutter. Appropriate storage and transport procedures (transport medium, and swift delivery to laboratory to maintain the viability of fastidious organisms) are still required.
The characterisation of the bacterial flora depends on *both* the sample collection method *and* the processing method. Standard culture and plating techniques involves the multiplication of the bacteria in a medium, growing them on culture plates, and assessing their resistance to antimicrobial agents on different media. It is thought that some organisms do not survive collection and transport, and hence a swab or tissue sample does not fully reflect the organisms causing the wound infection. These ‘fastidious’ organisms remain undetected in the laboratory but may be important pathogens. It is generally believed that several bacterial species may be present in infected foot ulcers. Because some of these organisms cannot be easily cultured, proper identification is problematic and thus, appropriate treatment modalities cannot be applied. This may account for the fact that approximately 10–20% of diabetic foot wounds fail to respond to initial antibiotic treatment. Newer, and more rapid, techniques multiply the genetic material of the bacteria rather than growing them in culture. Genetic fingerprint techniques are then used to identify the bacteria group from its DNA / RNA signature. It has been found previously that culture-based methodology may not identify minor, though possibly important,

components of a mixed bacterial population whereas genetic fingerprinting techniques can (Redkar et al. 2000). Therefore this study will examine the identification of ulcer pathogens by using both conventional culture techniques, and the increasingly widely used molecular techniques, in a small sub-study so that we can report on the agreement between both analysis techniques. This will allow us to determine if the quicker molecular technique reflects the bacterial load captured by swabs and tissues samples in the foot ulcer and to further compare between swab and curettage (e.g. for those organism not identified via plating and culture). Culturing is also limited due to the length of time required to obtain results. Ideally wounds would be treated with antibiotics only after receiving results from microbial analysis (to limit over-prescription and ensure narrow spectrum antibiotics are used when possible). This is, however, only possible with rapid techniques such as polymerase chain reaction (PCR). Furthermore, traditional culturing methods may be biased as a diagnostic tool as they select for easily cultured organisms such as *Staphylococcus aureus* and against difficult to culture bacteria such as anaerobes (Dowd et al. 2005).

The need for a study

Both swab samples and tissue samples are used to provide information on the types of bacteria in a clinically infected wound, but it is often stated that tissue samples are preferred. If we found that wound swabs provided very similar information to tissue samples, then we could recommend use of the (less invasive) wound swab without reducing the amount of information that clinicians had for making decisions about antibiotic treatment. On the other hand, if we were to find that swabs provided so much less information that clinicians' conclusions about the important bacteria affecting the ulcer were changed, then it would be important to tell clinicians that swab results were not sufficient. Stopping clinicians from using poor tests and helping them choose reliable tests should reduce wasteful testing.

Numerous clinical guidelines and consensus documents recommend the collection of a tissue sample rather than a swab (Armstrong et al. 2004; IWGDF, 2003). Many cite the study by Pellizzer et al. (2001), of 29 people who were suspected of severe infection and were neither recently treated with antibiotics, nor hospitalised. Unfortunately, the analysis of these results in this study did not report agreement between swab and tissue sample, simply the number of bacterial colonies in each sample. Their conclusion that tissue samples are better can be traced to a comparison of the numbers of isolates in 21 people remaining in the study at 30 days; but this result may be due to chance as they performed 20 comparisons without adjustment for multiple testing. Furthermore, this selected population does not reflect many of the foot ulcers seen in foot clinics, who may have had recent antibiotics. More recently, Slater et al. (2004) report the results from swabs and a deeper tissue sample (obtained via needle aspiration), but their study only contains 30 people with ulcers (in a

sample of 60: other patients had deep abscesses etc) and it is not clear if the results are heterogeneous across tissue damage types or apply to curette samples. They found that in 62% of samples, there was a similar profile of organisms isolated from the swab and the deep tissue sample. In 20% of samples, the swab identified more organisms and in 18% of samples the deeper tissue sample picked up more organisms. This data was not stratified by presence or absence or ulcer, or ulcer type (neuropathic / ischaemic).

In a systematic review of the diagnosis and management of infection in diabetic foot ulcers (O'Meara et al. 2006), only one study was identified that evaluated sample acquisition and reported agreement in sufficient detail to allow full analysis. In this one study (Bill et al. (2001), including 18 pressure ulcers, 10 diabetic foot ulcers, 5 venous leg ulcers, and 5 arterial ulcers), a punch biopsy taken from the centre of the wound was compared with a wound swab with quantitative analysis. The presence of infection was defined by bacterial load (one million bacteria per gram of tissue). The sensitivity for wound swabbing was 79%, meaning that the swab failed to detect approximately one in five wound infections as defined by punch biopsy. The derived likelihood ratios suggested that the wound swab was not a useful method of identifying infection in chronic wounds. Interpretation of study findings is impeded by small size and heterogeneity in the ulcer population. It may be that this data is not directly transferable to a diabetic foot ulcer population all of whom have a diagnosis of ulcer infection. In addition there were potential sources of bias (such as no description of blind test verification and lack of clarity as to whether the same clinical data were available when test results were interpreted as would be available when the test is used in practice). Furthermore, the question we are addressing in this proposed study is not about 'diagnosis of infection', i.e. would both tissue sample and swab agree for an arbitrary bacterial load of $>10^5$ colony forming units bacteria per gram of tissue for tissue biopsy and greater than $>10^5$ colony forming units bacteria per cm^2 for swab, as this is a clinical decision. Rather the proposed study is about characterisation of the bacterial flora, not only for outcomes of bacterial load, but about relative presence of these bacteria and their sensitivity to antimicrobials. This means that the study by Bill et al. (2001) cannot conclude that swabs of clinically infected diabetic ulcers do not provide similar information as tissues samples obtained by curettage. People with a bacterial load of less than 10^5 colony forming units bacteria per gram of tissue did not have a wound swab, and it is not clear, therefore, if the swab provides additional information, as suggested by Pellizzer et al. (2006).

6. AIMS AND OBJECTIVES

The aim of this study is to assess the concordance between culture results from specimens taken by both surface swabs and by curettage, in patients with a diabetic foot ulcer with suspected infection requiring antibiotic therapy. The study also aims to evaluate whether any changes in bacterial profiles obtained from swabs and tissue samples are clinically relevant by ascertaining from a panel of clinicians whether the reports from swabs or tissue samples would have resulted in a change in clinical management or not.

In addition, via a sub-study, the study aims to assess the concordance between results from specimens taken by conventional culture techniques and by molecular techniques.

6.1 Primary Objectives

The main objective of this study is to evaluate concordance between culture results of surface swabs and tissue samples from diabetic foot ulcers requiring antibiotics for suspected infection. Agreement between the two techniques for the following three parameters of microbiological characterisation will be determined by:

- Reported presence or not of the following likely pathogens, identified by the UK Health Protection Agency (HPA) as likely isolates from limb-threatening diabetic foot ulcers (HPA 2009a)
    - *Staphylococcus aureus* (methicillin sensitive / resistant)
    - *Streptococcus*
    - *Enterobacter aerogenes*
    - *E.coli*
    - Pseudomonads
    - *Corynebacterium* species
    - *Anaerobic cocci (i.e.mixed anaerobes)*
    - *Fusobacterium species*
    - *Bacteroides fragilis*
    - *Prevotella bivia*
- Presence of antimicrobial resistance among likely pathogens as reported by standard techniques
- Number of species reported per specimen (swab / tissue sample).

6.2 Secondary Objectives

Secondary objectives are to:

- Compare the proportions of patients for whom empirical antibiotic therapy was 'appropriate' based on culture and sensitivity results of swab or tissue samples, assessed by a blinded clinical panel review (with record of antimicrobial therapy prescribed)
- Compare the number of species reported in both swab and tissue samples by conventional plating and culture against molecular techniques that identify the nucleic acids of bacteria in the wound
- Report and compare rates of adverse effects with the two techniques
- Report and compare costs of sampling with the two techniques.

7. STUDY DESIGN

This is a multi-centre, cross-sectional study involving 400 patients with a diabetic foot ulcer with suspected infection requiring antibiotic therapy. Consenting patients will have both a swab and curettage samples taken from the diabetic foot ulcer for conventional plating and culture.

Twenty patients will be included in a sub-study in which a second swab sample and half of the curettage sample will be processed using molecular (PCR) techniques for comparison with the conventional plating techniques.

8. ELIGIBILITY

All patients at least 18 years of age with a diabetic foot ulcer in which the clinician suspects ulcer infection, either a new case of infection or a chronic infection, will be screened for enrolment and must meet the eligibility criteria below. A diabetic foot ulcer will be considered to be any open wound on the foot (below the malleoli / ankle) in a patient with a diagnosis of diabetes mellitus.

8.1 Inclusion Criteria

- Patient has a diagnosis of diabetes (type 1 or type 2)
- Patient has a suspected ulcer infection with or without bone infection, based on clinical signs and symptoms using Infectious Diseases Society of America / International Working Group on the Diabetic Foot (IDSA / IWGDF) criteria and the judgement of the investigator

- The clinical plan is to treat the patient with antibiotics for their infected ulcer
- Patient is at least 18 years of age at the time of signing the consent form

8.2 Exclusion Criteria

- The clinician deems it inappropriate to take a curette sample or a swab sample for any reason
- The patient has already been recruited to the study.

9. RECRUITMENT AND REGISTRATION

9.1 Recruitment

Research centres will be required to have obtained local ethical and management approvals and undertake a site initiation meeting with the Clinical Trials Research Unit (CTRU) prior to the start of recruitment into the study.

The recruitment target requires 400 patients over a 15 month period. Patients will be recruited from multi-disciplinary primary and secondary care based foot ulcer/diabetic clinics and hospital wards.

9.1.1 Recruitment from clinic

Alerting patients to the study:

We will provide posters in the clinics and insert a patient introductory information letter into clinic appointment letters for existing and newly referred patients to alert them to the study. Existing and newly referred patients will be approached during foot ulcer clinic visits by a member of the attending clinical team, and will be provided with further information about the study, whilst not alarming them in relation to the risk of infection. Patients will have the opportunity to ask further questions at subsequent clinic visits.

Recruiting eligible patients:

Where infection is suspected and antibiotic therapy is an indicated treatment by the attending clinical team, a full verbal explanation of the study and a Patient Information Leaflet (PIL) will be provided by either the attending clinical team or the CODIFI CRN for the patient to consider. This will include detailed information about the rationale, design and personal implications of the study. Following information provision, patients will make a decision on whether to participate within the clinic appointment on the same day.

9.1.2 Recruitment from hospital wards

Alerting patients to the study:

Existing and newly referred in-patients under the care of local principal investigators/co-investigators, with diabetic foot ulcers will be provided with an introductory patient information letter at the discretion of the attending clinical team. They will be provided with further information about the study, whilst not alarming them in relation to the risk of infection. Patients will have the opportunity to ask further questions.

Recruiting eligible patients:

Where infection is suspected and antibiotic therapy indicated/initiated by the attending clinical team, a full verbal explanation of the study and a Patient Information Leaflet (PIL) will be provided by either the attending clinical team or the CODIFI CRN for the patient to consider. This will include detailed information about the rationale, design and personal implications of the study. To prevent a delay in normal sample collection, following information provision, patients will make a decision on whether to participate the same day.

9.2 Informed Consent Process

Assenting patients will be formally assessed for eligibility and invited to provide informed, written consent. The assessment of eligibility and the informed consent process will be undertaken by the CRN or by a member of the attending clinical team who are qualified by training and / or experience in taking informed consent to good clinical practice (GCP) standards. Informed, written consent for entry into the study must be obtained prior to registration.

The right of the patient to refuse consent without giving reasons will be respected. Further, the patient will be free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment/care.

We will alert patients attending clinics that this study is happening and should the attending clinical team think that antibiotics are required for an infection, then we will ask them to consider taking part in the study. Ideally samples need to be taken prior to newly initiated or a change in antibiotic treatment, and the antibiotics cannot be delayed. The interval between determining eligibility and the start time for the antibiotic treatment is a matter of hours therefore we need to have a short time to consider study

participation. As study participation requires an additional sample collection, the interval from eligibility and the second level information provision is short (i.e. within the period of the clinic visit).

A record of the consent process detailing the date of consent and all those present will be kept in the patient's notes. The original consent forms will be filed in the Investigator Site File, a copy of the consent forms will be given to the patient and a copy will be returned to the CTRU, at the University of Leeds.

9.3 Registration

Patients who are both eligible for participation in the study and have provided written informed consent will be registered. Informed consent for entry into the study must be obtained prior to registration. Following confirmation of eligibility, written informed consent, baseline assessment and sample collection, patients will be registered into the study by an authorised member of staff.

Registration will be performed centrally using the CTRU automated 24-hour telephone registration system. Authorisation codes and Personal Identification Numbers (PIN), provided by the CTRU, will be required to access the registration system.

The following information will be required at registration:

- Unique authorisation code and PIN
- Name of person registering patient
- Patient's name
- Patient's date of birth

- Confirmation of eligibility
- Date of written informed consent

Direct line for 24-hour Registration: 0113 343 2608

The CTRU will then issue an individual patient study number. This number should be used on all study documentation.

Following registration, the CTRU will send confirmation of registration to the research site.

9.4 Screening

Participating research sites will be required to complete a log of all patients screened for eligibility including those who are not registered either because they are ineligible or because they decline participation. Anonymised information will be collected including:

- age
- gender
- ethnicity
- date screened
- the reason not eligible for participation in the study OR
- eligible but declined and reason for this OR
- other reason for non registration

10. ASSESSMENT, SAMPLES AND DATA COLLECTION

10.1 Sample Acquisition

After wound cleansing (using sterile saline and gauze) and debridement (removal of necrotic tissue, foreign material, callus, undermining), a physician, nurse or podiatrist will obtain specimens for aerobic and anaerobic cultures by

- Firstly using a cotton-tipped swab rubbed over the wound surface to sample superficial wound fluid and tissue debris. The swab will be placed with sufficient pressure on the wound bed to capture expressed wound fluid, and will be positioned deep in the ulcer to collect from likely infected areas.
- Immediately after the cotton swab has been collected, curettage from the same area of the ulcer bed will be done. This procedure will be done using sterile equipment and an aseptic technique. It will involve the removal of a small piece of wound tissue at the base of the wound by scraping or scooping using a dermal curette or sterile scalpel blade. For the sub-study of culturing versus molecular techniques, in 20 patients, two wound swabs will be collected, one for culture by conventional techniques, and one for microbiological analysis.

These sampling techniques will be supported by standard operating procedures (SOP) in the sites from the HPA documents (Health Protection Agency, 2009a/b). We will not be altering current swabbing practice significantly by instituting training at sites as swabbing is a routine procedure with established patterns of practice from the HPA etc.

10.2 Training and Auditing of Swab and Sampling Techniques

Clinicians in the participating sites will participate in a study information session to update their technique for sample acquisition. In order to ensure that clinicians from all sites are acquiring samples as per the national guidance, there will be an audit of swabbing and curetting practice. This will be accomplished by the clinical coordinator visiting recruiting sites to observe sample acquisition in realistic settings and providing feedback, if required, on any deviation from the standardised protocol. Any deviations will be noted in order to allow the investigators to report those elements of sample acquisition associated with higher non-compliance.

10.3 Sample Transport

10.3.1 Sample Transportation for Culture (all patients)

Both samples will be placed in transport medium suitable for both aerobic and anaerobic organism identification and delivered to the local medical microbiology laboratory within 2 hours, in accordance with standard practice. A national standard method will be used for collecting and processing samples (Health Protection Agency, 2009a/b). Both samples will be processed in the same laboratory.

10.3.2 Sample Transportation for Molecular Analysis (20 patients)

One swab sample and half of the tissue sample from curettage taken for molecular analysis, will be identified by study number, patient‘s date of birth and date taken, will be sent by first class post at ambient temperature to Micropathology Ltd. Upon receipt, samples will be stored at –70^{o}C. Batches will be defrosted before being processed. Further details on the methods for the molecular analysis will be developed into a study standard operating procedure (SOP).

10.4 Data Collection

Study data will be recorded by clinical research staff on Case Report Forms (CRFs) and submitted to the CTRU at the University of Leeds. Details on the schedule of CRFs, data to be collected and guidance on the completion of CRFs will be given to the clinical research staff when all local approvals to run this study are obtained.

Participating sites will maintain a file of essential study documentation (Investigator Site File (ISF)) provided by CTRU, and keep copies of all completed CRFs for the study.

10.4.1 Assessments

10.4.1.1 Baseline and Sampling Assessments

Demographic Data: date of birth, ethnicity, gender

Personal Data: Initials, National Health Service (NHS) ID and Hospital number

Clinical History: duration of diabetes, number of ulcers on infected foot / both feet, first or recurrent ulcer, duration of ulcer, site of ulcer, ischaemic/neuropathic aetiology (clinical assessment)

Treatment: current and proposed antibiotic treatment (agent, dose and dates), current diabetes drugs

Clinical Assessment: Temperature in °C, heart rate, respiratory rate,

Ulcer Classification/Grading:, PEDIS (Perfusion, Extent, Depth, Infection, Sensation: Schaper 2004), Clinical Signs and Symptoms Classification (for infection: Gardner et al 2001) and Wagner Scale (Wagner 1987 as modified by Fykberg 2003)

Pain Score using a Verbal Rating Scale, VRS

Adverse Events

Details of the ulcer classification scales, and the data items to be collected, can be found in appendix 2.

10.4.1.2 Microbiology results (hospital notes)

Microbiology results and changes in antibiotic therapy will be obtained from the healthcare record.

10.4.2 Assessment of empirical antibiotic therapy

Appropriateness of empirical antibiotic therapy will be judged against both swab and curettage findings by a central panel. The panel will be blind to source of sample (curettage or swab) and samples will be unpaired and mixed up for judging purposes to eliminate bias. Panel members will be asked to reach a consensus on the following codings: no change to therapy required, possible change of therapy following review of patient, definite change of therapy required.

10.5 Withdrawal

In line with usual clinical care, cessation or alteration of protocol research at any time will be at the discretion of the clinical staff or the patients themselves. Withdrawal from protocol research will be documented in the corresponding CRF.

Withdrawal may refer to the following situations: consent has been obtained and the participant withdraws agreement for microbiological sample collection, or consent has been obtained and the participant then withdraws agreement for their samples to be used for research purposes.

Loss to follow up may occur when there is no data available on a patient, for example, if microbiological results are declared missing. This will be minimized by making links with the microbiology laboratories to ensure that staff are aware of the study and are assured of the appropriateness of providing any duplicate results when originals are lost in transit.

11. SERIOUS ADVERSE EVENTS PROCEDURES

11.1 General Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study's subject which does not necessarily have a causal relationship with this device/procedure and can include:

- any unintentional, unfavourable clinical sign or symptom
- any new illness or disease or the deterioration of existing disease or illness
- any clinically relevant deterioration in any laboratory assessments or clinical tests.

A Serious Adverse Event (SAE) is defined in general as an untoward (unfavourable) event which is:

- fatal or life threatening*
- requires or prolongs hospitalisation
- is significantly or permanently disabling or incapacitating
- constitutes a congenital anomaly or a birth defect
- may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

* The term life-threatening in the definition of a SAE refers to an event in which the patient 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 was more severe.

A SAE occurring to a patient which, in the opinion of the Chief Investigator, is Related and Unexpected will be reported to the main Research Ethics Committee (main REC).

The National Research Ethics Service (NRES) defines related and unexpected SAEs as follows:

- 'related' – that is, it resulted from administration of any research procedures; and
- 'unexpected' – that is, the type of event is not listed in the protocol as an expected occurrence.

11.2 Operational Definitions and Procedures

11.2.1 Expected AEs / SAEs – Not Reportable

This is an observational study in a patient population with high levels of morbidity and co-morbid diseases and as such in this patient population, acute illness resulting in hospitalisation, new medical problems and deterioration of existing medical problems are expected.

In addition, patients will be seen only once for baseline and sample acquisition. In recognition of this, events fulfilling the definition of an adverse event or serious adverse events will not be reported in this study unless they are classified as 'related'.

11.2.2 Related and Expected AEs/SAEs - Reportable

It is expected that there will be minimal risks associated with the procedures of this study.
The following AEs/SAEs are expected within the study population and will be reported by the clinical research team using standardised CRFs including:

- Pain or bleeding relating to the swab or curettage sampling

These events are expected within the study population and will not be subject to expedited reporting to the main REC. They should be reported to CTRU within 1 week of recording. They will however, be included in the annual safety report provided to the main REC.

11.2.3 Related and Unexpected SAEs (RU SAEs)

Any SAE which is considered as 'Related' to the research and is Unexpected must be recorded on the Related Unexpected Serious Adverse Event Form and faxed to the CTRU **within 24 hours** of the research staff becoming aware of the event. The original form should also be posted to the CTRU in real time and a copy retained at site.

For each RU SAE the following information will be collected:

- full details in medical terms with a diagnosis, if possible
- date of SAE
- its duration (start and end dates; times, if applicable)
- action taken
- outcome

Any follow-up information should be faxed to CTRU as soon as it is available. Events will be followed up until the event has resolved or a final outcome has been reached.

Fax Number for Reporting RU SAEs: 0113 343 1487

All Related / Unexpected SAEs will be reviewed by the Chief Investigator and subject to expedited reporting to the main REC and Sponsor by the CTRU on behalf of the Chief Investigator within 15 days.

11.3 Reporting

Safety issues will be reported to the Main REC in the annual progress report. An annual summary of all events will also be reported to a Study Steering Committee (SSC) and Sponsor. Expedited reporting of events to the main REC and the Sponsor will be subject to current NRES guidance, CTRU SOPs and Sponsor requirements.

11.4 Responsibilities

11.4.1 Clinical Co-Investigators / Authorised Personnel

- Checking for SAEs when patients attend for study research
- Judgment in assessing:
  - Seriousness
  - Causality
  - Expectedness
- To ensure all RU SAEs are recorded and reported to the CTRU within 24 hours of becoming aware and to provide further follow up information as soon as available
- To report RU SAEs to local committees in line with local arrangements.

11.4.2 Chief Investigator (or nominated individual in CI's absence)

- Assign relatedness and expected nature of SAEs where it has not been possible to obtain assessment by authorised personnel
- Undertake SAE review
- Review all events assessed as Related & Unexpected in the opinion of authorised personnel. In the event of disagreement between authorised personnel's assessment and the Chief Investigator, authorised personnel's assessment may be upgraded or downgraded by the Chief Investigator prior to reporting to the main REC.

12.4.3 CTRU

- Expedited reporting of RU SAEs to the main REC and Sponsor within required timelines
- Preparing safety reports at least annually to the main REC and Sponsor. Safety may be reported more frequently if appropriate
- Expedited reporting of other safety issues, including an increase in the rate of occurrence in severity of RU SAEs, to the main REC and Sponsor within required timelines.

12. ENDPOINTS

12.1 Co-Primary Endpoints

- Reported presence of likely pathogens (e.g. *Staphylococcus aureus*, *Streptococcus, Enterobacter aerogenes*, Psuedomonads, *Corynebacterium* species, *Fusobacterium* species, Bacteriodes *fragilis, Prevotella bivia*)
- Reported presence of antimicrobial resistance among likely pathogens
- Number of species reported per specimen

12.2 Secondary Endpoints

- Appropriateness of empirical antibiotic therapy
- Number and presence of pathogens reported (conventional plating and molecular techniques)
- Adverse events

13. STATISTICAL CONSIDERATIONS

13.1 Sample Size

The sample size is based on the outcome reported ‘presence or absence of pathogen’ for the whole sample overall. To be confident that swabs adequately sample wound flora, then it is assumed that the chance corrected agreement between swabs and tissue samples needs to be at least 'good': usually defined as a *kappa larger than 0.6 (*Landis et al. 1977*)*.

A sample size of 399 patients will provide 80% power for detecting a difference of 3% in the reported presence of an organism, assuming an overall prevalence of 10%, discordance of 5%, and a two-sided test at the 5% level of significance. This amount of agreement would provide a kappa of ~ 0.7. This is based on less prevalent organisms, such as *Pseudomonas* (present in 10% of samples in Pellizzer et al. 2001). A total of 400 patients will be recruited.

As Kappa alone does not convey the distribution of disagreement between swabs and tissue samples and that good overall agreement, with balanced disagreement around the central axis of a table of distributions, would be clinically important if tests were to be regarded as interchangeable, the total sample size has been based on there being good agreement and reasonably balanced discordance, for clinically important and less prevalent organisms.

The sub-study (culture vs molecular techniques) will collect samples from 20 patients, based on feasibility, to allow an evaluation of the level of agreement and inform a powered, definitive study.

13.2 Planned Recruitment Rate

All sites are expected to see an estimated 50 infected diabetic foot ulcer patients per week; approximately 2000 unique patients per annum (assuming ¼ are repeatedly affected with infection). Assuming 50% will be eligible and a 40% consent rate, a total of 15 months recruitment is expected, allowing for staggered starts and variation in recruitment.

14. STATISTICAL ANALYSIS

14.1 General Considerations

Statistical analysis is the responsibility of the CTRU Statistician. A full statistical analysis plan will be written before any analyses are undertaken.

All analyses will be conducted on all patients recruited with at least one sample result, and the per-protocol population (if required).

All significance tests will be two-sided. P-values will be quoted and 95% confidence intervals will be generated where appropriate. The results will be reported in line with the STARD guidelines.

14.2 Primary Endpoint Analysis

Reported presence of pathogens

For each pathogen reported, a cross-tabulation on the extent of growth (none, + to ++++) will be generated for swab vs. curettage samples, by type of diabetic foot ulcer (neuropathic, ischaemic) and overall and weighted kappa will be reported for all tables. Categories + to ++++ will be combined to record any reported presence of the pathogen.

The corresponding 2 by 2 table will be created and several statistics will be reported: prevalence and bias adjusted kappa, unadjusted kappa and overall percentage agreement.

McNemar's test will be used to test for a difference between swab and curettage sampling techniques in the proportion of samples with the reported pathogen present, to further investigate the pattern of disagreement.

Summary of pathogens reported

An overall summary of pathogens reported will be generated (Slater et al. 2004). Each pair of results (swab and curettage) will be coded as follows: Swab and curettage report the same pathogens; swab reports same pathogens as curettage plus extra pathogens; curettage reports same pathogens as swab plus extra pathogens; both curettage and swab report different species (with or without overlap in species found).

Multinomial logistic regression will model the proportions in each category on type of ulcer (predominantly neuropathic or ischaemic), grade of ulcer, previous antibiotic therapy, wound duration and centre to determine whether agreement is influenced by any of the specified covariates. The reference category will be same pathogens reported by both tests; estimates of odds ratios for each covariate will be presented along with 95% confidence intervals.

Reported presence of antimicrobial resistance among likely pathogens

Methicillin-resistant *S. aureus*, Coagulase-negative *Staphylococci* and Vancomycin-resistant *Enterococcus* are the three anti-microbial resistant pathogens of most interest. For each of these resistant pathogens, 2 by 2 tables will be created (presence or absence of resistant pathogen) and the following statistics will be reported: prevalence and bias adjusted kappa, unadjusted kappa and overall percentage agreement.

McNemar's test will be used to test for a difference between swab and curettage sampling techniques in the proportion of samples in which the specified resistant pathogen is reported.

For each resistant pathogen the following codes will be created: resistant pathogen reported by swab not curettage, resistant pathogen reported by curettage not swab, swab and curettage results agree. Multinomial regression modelling will model these categories on type of ulcer (predominantly neuropathic or ischaemic), grade of ulcer, previous antibiotic therapy, wound duration and centre to determine whether agreement is influenced by any of the specified covariates

Number of species reported

Summaries (including cross-tabulations) on the number of species reported per specimen will be generated for swab vs. curettage samples. Samples will be further coded as follows: curettage had two or more extra pathogens reported, curettage had one extra pathogen reported, curettage and swab had the same number of pathogens reported, swab had one extra pathogen reported, swab had two more extra pathogens reported.

Ordinal logistic regression will model the number of species reported per specimen on type of ulcer (predominantly neuropathic or ischaemic), grade of ulcer, previous antibiotic therapy, wound duration and centre to determine whether agreement is influenced by any of the specified covariates. The reference category will be same number of pathogens reported by both tests; estimates of odds ratios for each covariate will be presented along with 95% confidence intervals.

14.3 Secondary Endpoint Analysis

Appropriateness of empirical antibiotic therapy

Summaries (including cross-tabulations) will be generated for the codings: no change to therapy required, possible change of therapy following review of patient, definite change of therapy required.

The first two categories will be combined and the resultant 2 by 2 table analysed using McNemar's test to identify if one test identifies significantly more patients requiring a definite change in treatment.

Sample pairs will be further coded: swab but not curettage indicates change in therapy, curettage but not swab indicates a change in therapy, swab and curettage in agreement on change in therapy. Multinomial regression modelling will model these categories on type of ulcer (predominantly neuropathic or ischaemic), grade of ulcer, previous antibiotic therapy, wound duration and centre to determine whether agreement is influenced by any of the specified covariates.

Number and presence of pathogens reported using molecular or culture techniques

An overall summary of pathogens reported using culture and molecular techniques will be generated. Each pair of results (molecular and cultured) will be coded as follows: molecular and culture report the same pathogens; molecular reports same pathogens as culture plus extra pathogens; culture reports same pathogens as molecular plus extra pathogens; both culture and molecular report different species (with or without overlap in species found).

Summaries (including cross-tabulations) on the number of pathogens reported will be generated.

Adverse Events

Safety analyses will summarise all AEs, SAEs, and RU SAEs. The number of events and number of patients with events will be summarised.

Sampling Costs

Sampling costs will be summarised for each technique.

15. DATA MONITORING

Data will be monitored for quality and completeness by the CTRU. Missing data will be chased until it is received, confirmed as not available or the study is at analysis. The CTRU/Sponsor will reserve the right to intermittently conduct source data verification exercises on a sample of patients, which will be carried out by staff from the CTRU/Sponsor. Source data verification will involve direct access to patient notes at the participating sites and the collection of copies of consent forms and other relevant

investigation reports. A Study Monitoring Plan will be developed and a Meeting Group Monitoring Schedule including primary endpoint and safety data will be defined and agreed by the Study Management Group (SMG) if necessary.

15.1 Clinical Governance Issues

To ensure responsibility and accountability for the overall quality of care received by patients during the study period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the SSC and, where applicable, to the participating NHS Trusts.

16. QUALITY ASSURANCE AND ETHICAL CONSIDERATIONS

16.1 Quality Assurance

The study will be conducted in accordance with the principles of GCP, the NHS Research Governance Framework and through adherence to CTRU SOPs.

16.2 Ethical Considerations

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 52nd World Medical Association General Assembly, Edinburgh, Scotland, October 2000. Informed written consent will be obtained from the patients prior to registration into the study. The right of a patient to refuse participation without giving reasons must be respected. The patient must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment. The study will be submitted to and approved by a Main REC prior to entering patients into the study. The CTRU will provide the Main REC with a copy of the final protocol, patient information sheets, consent forms and all other relevant study documentation.

17. CONFIDENTIALITY

All information collected during the course of the study will be kept strictly confidential. Information will be held securely on paper and electronically at the CTRU. The CTRU will comply with all aspects of the 1998 Data Protection Act and operationally this will include:

- Consent from patients to record personal details including name, date of birth, NHS ID and hospital number.

- Appropriate storage, restricted access and disposal arrangements for patient personal and clinical details.
- Consent from patients for access to their healthcare records by responsible individuals from the research staff or from regulatory authorities, where it is relevant to study participation.
- Consent from patients for the data collected for the study to be used to evaluate safety and develop new research.
- Patient name will be collected when a patient is registered into the study but all other data collection forms that are transferred to or from the CTRU will be coded with a study number and will include two patient identifiers, usually the patient's initials and date of birth.
- Where central monitoring of source documents by CTRU (or copies of source documents) is required, the patient's name must be obliterated by site before sending.
- Where anonymisation of documentation is required, sites are responsible for ensuring only the instructed identifiers are present before sending to CTRU.

If a patient withdraws consent from further research and / or further collection of data, all collected data will be included in the final study analysis.

17.1 Archiving

At the end of the study, data will be securely archived in line with the Sponsor's procedures for a minimum of 5 years. Data held by the CTRU will be archived in the Leeds Sponsor archive facility and site data and documents will be archived at site. Following authorisation from the Sponsor, arrangements for confidential destruction will then be made.

18. STATEMENT OF INDEMNITY

This study is sponsored by The University of Leeds and The University of Leeds will be liable for negligent harm caused by the design of the study. The NHS has a duty of care to patients treated, whether or not the patient is taking part in a clinical study, and the NHS remains liable for clinical negligence and other negligent harm to patients under this duty of care.

As this is a clinician-led study there are no arrangements for no-fault compensation.

19. STUDY ORGANISATIONAL STRUCTURE

19.1 Responsibilities

Chief Investigator - The Chief Investigator, as defined by the NHS Research Governance Framework, is responsible for the design, management and reporting of the study.

CTRU – The CTRU will have responsibility for conduct of the study in accordance with the Research Governance Framework and CTRU SOPs.

19.2 Operational Structure

Chief Investigator – The Chief Investigator is involved in the design, conduct, co-ordination and management of the study.

Study Management Group (SMG) - The SMG, comprising the Chief Investigator, CTRU team and co-investigators will be assigned responsibility for the clinical set-up, on-going management, promotion of the study, and for the interpretation of results. Specifically the SMG will be responsible for (i) protocol completion, (ii) CRF development, (iii) obtaining approval from the main REC and supporting application for Site Specific Assessment, (iv) completing cost estimates and project initiation, (v) reporting of related unexpected serious adverse events, (vi) monitoring of screening, recruitment, treatment and follow-up procedures, (vii) monitoring consent procedures, data collection, and database development.

Clinical Trials Research Unit (CTRU) - The CTRU will provide set-up and monitoring of study conduct to CTRU SOPs including registration, database development and provision, protocol development, CRF design, study design, source data verification, monitoring schedule and statistical analysis for the study. In addition the CTRU will support main REC, Site Specific Assessment and R&D submissions and clinical set-up, ongoing management including training, monitoring reports and promotion of the study. The CTRU will be responsible for the day-to-day running of the study including study administration, database administration functions, data management, safety reporting and all statistical analyses.

Study Steering Committee (SSC) - The SSC, with an independent Chair, will provide overall supervision of the study, in particular study progress, adherence to protocol, patient safety and consideration of new information. It will include an Independent Chair, not less than two other

independent members and a consumer representative. The Chief Investigator and other members of the SMG may attend the SSC meetings and present and report progress. The Committee will meet annually as a minimum.

Data Monitoring and Ethics Committee (DMEC): The DMEC will comprise a sub-group of the main SSC and will review the safety and ethics of the trial by reviewing interim data during recruitment. The committee will meet prior to scheduled SSC meetings as deemed appropriate.

Clinical Co-ordinator - The clinical co-ordinator will be responsible for clinical set-up, clinical co-ordination and the overall day-to-day clinical management of the study, including R&D submissions, liaison with local collaborators, the local research networks and liaison relating to the performance and conduct of the clinical research team and promotion of the study. They will also co-ordinate site initiations and local collaborator and clinical research team training in research procedures. The clinical co-ordinator will support the CTRU in collation of essential documentation, CRF design, database development, safety reporting and the monitoring schedule.

19.3 Funding

This study is funded by the Health Technology Assessment (HTA) as part of the National Institute for Health Research (NIHR) (09/75/01): *Concordance in Diabetic Foot Infection*.

20. PUBLICATION POLICY

20.1 Authorship and Acknowledgment

The success of the study depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the study, through authorship and by contribution. Uniform requirements for authorship for manuscripts submitted to medical journals will

guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- conception and design, or acquisition of data, or analysis and interpretation of data
- drafting the article or revising it critically for important intellectual content
- final approval of the version to be published
- and that all these conditions must be met (www.icmje.org).

In light of this, the Chief Investigator, Co-Applicants and senior CTRU staff will be named as authors in any publication. In addition, all collaborators will be listed as contributors for the main study publication, giving details of their roles in planning, conducting and reporting the study.

The Chair and Independent members of the SSC will be acknowledged, but will not qualify for full authorship, in order to maintain their independence.

20.2 Data Release

To maintain the scientific integrity of the study, data will not be released prior to the first publication of the analysis of the primary endpoints, either for study publication or oral presentation purposes, without the permission of the SSC.

The SSC will agree a publication plan and must be consulted prior to release or publication of any study data.

Individual collaborators must not publish data concerning their patients which is directly relevant to the questions posed in the study until the main results of the study have been published. Local collaborators may not have access to study data until after publication of the main study results.

21. REFERENCES

Armstrong D.G., Lipsky B.A. (2004) Diabetic foot infections: stepwise medical and surgical management. *Int. Wound J.* 1:123 - 132

Bill T.J. *et al*. (2001) Quantitative swab culture versus tissue biopsy: a comparison in chronic wounds. *Ostom. Wound Man.* 47:34 - 7

Cuny C., Witte W. (2005) PCR for the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains using a single primer pair specific for SCCmec elements and the neighbouring chromosome-borne orfX. *Clin. Microbiol. Infect.* 11:834 - 837

Dowd S.E., et al. (2005) Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded flx amplicon pyrosequencing (bTEFAP). *PLoS ONE*; 3(10): e3326. doi:10.1371/journal.pone.0003326)

Frykberg, RG. (2003) Diabetic Foot Ulcers: Pathogenesis and Management'. *American Family Physician*; 66; 9

Gardner SE, et al (2001) A tool to assess clinical signs and symptoms of localized infection in chronic wounds: development and reliability. *Ostomy Wound Manage.*;47(1):40-7

Health Protection Agency 2009a. Investigation of skin, superficial and non-surgical wound swabs. www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop11.pdf

Health Protection Agency 2009b. Investigation of abscesses and deep seated infections. www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop14.pdf

International Working Group on the Diabetic Foot. International consensus on the diabetic foot [CDROM]. Amsterdam, The Netherlands: International Diabetes Federation, 2003.

Knapp P. et al (2009) Performance-based readability testing of participant materials for a phase I trial: TGN1412. *J. Med. Ethics* 35(9):573 - 578.

Landis J.R., Koch G. (1977) The measurement of observer agreement for categorical data. *Biometrics* 33:159 - 174

Iversen M.M., *et al.* (2009) History of foot ulcer increases mortality among individuals with diabetes ten-year follow-up of the Nord-Trøndelag Health Study, Norway. *Diabetes Care* 32(12):2193 - 2199.

Narayan K.M. *et al.* (2003) Lifetime risk for diabetes mellitus in the United States. *JAMA.* 290(14):1884 – 90.

O'Meara S.M., *et al*. (2006) A series of systematic reviews to inform a decision analysis for sampling and treating infected diabetic foot ulcers. *Health Technology Assessment* 10(12)

Pellizzer G. *et al*. (2001) Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb-threatening diabetic foot infection. *Diabetic Medicine* 18:822 - 827

Redkar R., *et al*. (2000) Identification of bacteria from a non-healing diabetic foot wound by 16 S rDNA sequencing. *Mol Cell Probes* 14:163 - 9.

Relman D.A., *et al*. (2002) Identification of the uncultured bacillus of Whipple's disease. *New Engl. J. Med.* 327(5):293 - 301.

Schabereiter-Gurtner C., *et al*. (2001) 16S rDNA-Based identification of bacteria from conjunctival swabs by PCR and DGGE Fingerprinting. *Investigative Ophthalmo.l Visual Sci.* 42(6):1164 - 2883.

Schaper N.C.. (2004) Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab. Res. Rev.* 20 (Suppl 1):S90 – S95.

Senior C. (2000) Assessment of infection in diabetic foot ulcers. *J. Wound Care* 9:313 – 317.

Singh N., Armstrong D.G., Lipsky B.A. (2005) Preventing foot ulcers in patients with diabetes. *JAMA* 293:217 - 228

Slater R.A. *et al*. (2004) Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. *Diabetic Medicine* 21(7): 705 – 709

Wagner FW Jr. The diabetic foot. Orthopedics 1987;10:163-72. (scale as adapted by R G. Frykberg, Diabetic Foot Ulcers: Pathogenesis and Management. *American Family Physician* 2003; 66; 9)

Weisburg W.G., Barns S.M., Pelletier D.A. and Lane D.J. (1991) 16s Ribosomal DNA amplification for phylogenetic study. *J Bacteriol.* 173(2):697 - 703.

Wild S., et al (2004) Global Prevalence of Diabetes. *Diabetes Care* 27(5):1047 - 1053.

Williams R., Airey M. The size of the problem: epidemiological and economic aspects of foot problems in diabetes. In Boulton A, Connor H, Cavanagh P. *The Foot in Diabetes.* Chichester: Wiley 2000. Doi 10.1002/0470846399.ch2

22. APPENDICES

22.1 Appendix 1: Methods and Materials for molecular analysis

a) Extraction of nucleic acids from samples

Bacterial DNA will be released from swabs using a protocol adapted from Schabereiter-Gurtner et al, 2001. Swabs are to be processed in batches that include negative and positive swabs to control for

contamination and extraction efficacy (see 'Quality control' below). Swabs are broken off into labelled 2ml sample tubes containing 500µl of XB buffer (150mM Na2EDTA, 225mM NaCl, pH8.5), 60µl lysozyme (100mg/ml) and 25µl lysostaphin (1mg/ml). The swabs are then incubated at 37□C for 0.5h and then subjected to 3 cycles of freezing (-70^{o}C) and thawing (72^{o}C).

220µl of each bacterial suspension will be removed into a fresh tube and processed for nucleic acid extraction using the QIAamp DNA Blood Biorobot MDx kit combined with the Qiagen MDx Biorobot so that nucleic acids from 200uL of sample are eluted into 200µl TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The remainder of each sample containing the original swab will be stored at -70 ^{o}C.

b) Bacterial 16s rRNA gene detection and quantitation (bacterial load PCR)

Nucleic acids from each swab will be subjected to real-time quantitative PCR using the following universal 16S rRNA gene primers: forward, 5'-AGA GTT TGA TCA TGG CTC AG-3' (Weisburg *et al*, 1991), reverse, 5'-ACC GCG GCT GCT GGC AC-3' (Relman *et al*, 1992). The reaction mixture consists of ReadyMix Taq PCR reaction mix (12µl, Sigma-Aldrich, Poole, UK), combined primers (1µl, 5µM), MgCl2 (1.5µl, 25mM), Evagreen fluorescent dye (1µl, Biotium, Hayward, USA) and template (10µl, extracted nucleic acids). Realtime PCR will be performed in a Rotorgene 3000 (Corbett Research UK Ltd, St Neots, UK) under the following parameters: 95 ^{o}C for 40s, followed by 30 cycles of 97 ^{o}C for 20s, 55 ^{o}C for 20s, and 72 ^{o}C for 20s (acquiring to Sybr channel).

A standard curve will be generated for each assay using 10-fold dilutions of DNA extracted from a culture of *Staphylococcus aureus* that has been quantitated by viable and particle counting methods. Unknowns will be calculated by interpolation and results expressed as 16s rRNA gene copies per ml. The lowest limit of detection of the assay will be 103 copies/ml.

c) Quality Control

Positive and negative extraction controls are included in each batch of swabs processed. Positive controls are prepared by absorbing 500µl of a 4h broth culture of S. aureus onto sterile swabs, stored at -70 ^{o}C until required. Negative controls were sterile swabs only. Extraction controls are processed using the same protocols as live samples and the extracted NA analysed as described in (b) above. Positive extraction control swabs are expected to provide bacterial load results of at least 1x104 S. aureus genome equivalents/ml and negative control swabs are expected to have a bacterial load of less than 1x103 S. aureus genome equivalents for results from that batch of swabs to be accepted.

Each quantitative PCR assay also includes a quality control sample of extracted nucleic acids of a known bacterial load (internal quality control, IQC). This IQC sample has to provide a result varying no more than 0.5 of a log from the predicted value for the results of that PCR assay to be accepted.

d) MRSA assay

Methicillin-resistant *S. aureus* will be detected using a single round PCR assay specific for the 3‘ terminal region of the Staphylococcal Chromosomal Cassette containing the mecA gene responsible for methicillin resistance (SCCmec) when specifically inserted into the Staphylococcus aureus genome at *orfX*.

Nucleic acids will be obtained from swabs as described above and subjected to single round PCR using the following primers: forward rjmec 5‘-TAT GAT ATG CTT CTC C-3‘ and reverse orfX 5‘-AAC GTT TAG GCC CAT ACA CCA-3‘ (Cuny and Witte, 2005). The reaction mixture consists of PCR buffer (5µl, Labmaster, Sevenoaks, UK), dNTP's (5µl, Web Scientific, Crewe, UK), combined primers (1.5uL, 5µM), water (18.5µl, Sigma-Aldrich, Poole, UK), MgCl2 (1µl, 25mM, Sigma-Aldrich, Poole, UK), and Immolase DNA polymerase (0.5µl, Bioline, London, UK). The cycling parameters are 95 ^{o}C for 20s, followed by 45 cycles of 97 ^{o}C for 20s, 57 ^{o}C for 20s, and 72oC for 20s. PCR products are detected by ethidium bromide and agarose gel electrophoresis. Products are referenced to a positive control (MRSA, provided by Prof. Dowson, University of Warwick).

22.2 Appendix 2: Baseline and Sampling Assessments

22.2.1 PEDIS Grading System

<u>Perfusion Classification</u>

GRADE 1

No symptoms or signs of peripheral arterial disease (PAD) in the affected foot, in combination with

- palpable dorsal pedal and posterior tibial artery **or**
- ankle-brachial index 0.9 to 1.10 **or**
- toe-brachial index >0.6 **or**
- transcutaneous oxygen pressure ($tcpO_2$) >60 mm Hg

GRADE 2

Symptoms or signs of PAD, but not of critical limb ischemia (CLI):

- Presence of intermittent claudication **or**
- Ankle-brachial index < 0.9, but with ankle pressure >50 mm Hg **or**
- Toe-brachial index < 0.6, but systolic toe blood pressure >30 mm Hg **or**
- $tcpO_2$ 30 to 60 mm Hg

GRADE 3

CLI, as defined by

- systolic ankle blood pressure <50 mm Hg **or**
- systolic toe blood pressure <30 mm Hg **or**
- $tcpO_2$ < 30 mm Hg

Extent/Size

Wound area, obtained by tracing with a clear acetate film. If this is not possible, then the length and width of the ulcer should be recorded instead.

Depth/Tissue Loss Classification

GRADE 1

- Superficial full-thickness ulcer, not penetrating any structure deeper than the dermis.

GRADE 2

- Deep ulcer, penetrating below the dermis to subcutaneous structures, involving fascia, muscle or tendon.

GRADE 3

- All subsequent layers of the foot involved, including bone and/or joint (exposed bone, probing to bone).

Infection Classification

GRADE 1

No symptoms or signs of infection.

GRADE 2

Infection involving the skin and the subcutaneous tissue only (without involvement of deeper tissues and without systemic signs, as described below). At least two of the following items are present:

- Local swelling or induration
- Erythema >0.5 to 2 cm around the ulcer
- Local tenderness or pain
- Local warmth
- Purulent discharge (thick, opaque to white or sanguineous secretion).

Other causes of an inflammatory response of the skin should be excluded (e.g. trauma, gout, acute Charcot neuro-arthropathy, fracture, thrombosis, venous stasis).

GRADE 3

- Erythema > 2 cm around the ulcer

And one of the following items

- Local swelling or induration
- Local tenderness or pain
- Local warmth
- Purulent discharge (thick, opaque to white or sanguineous secretion).

OR

Infection involving structures deeper than skin and subcutaneous tissues

- Abscess
- Osteomyelitis
- Septic arthritis
- Fasciitis

GRADE 4

Any foot infection with the following signs of a systemic inflammatory response syndrome. This response is manifested by two or more of the following conditions:

- Temperature >38 or <36 Â°C

- Heart rate >90 beats/min
- Respiratory rate >20 breaths/min
- PaCO2 <32-mm Hg
- White blood cell count >12.000 or <4.000/cu mm
- 10% immature (band) forms

Sensation

GRADE 1

No loss of protective sensation on the affected foot detected, defined as the presence of sensory modalities described below.

GRADE 2

Loss of protective sensation on the affected foot is defined as the absence of perception of the one of the following tests in the affected foot:

- Absent pressure sensation, OR
- Absent vibration sensation,

Schaper NC (2004) Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabet Metab Res Rev:* Volume 20 S1: S90-95

22.2.2 Clinical Signs and Symptoms Checklist

The following will be collected on the CRFs:

Foul wound odour

Pocketing in the wound

Discoloured granulation tissue

Friable granulation tissue

Recent increase, or decrease in pain

Recent increase in size of wound

Breakdown of newly formed epithelium

Gardner SE, et al (2001) A tool to assess clinical signs and symptoms of localized infection in chronic wounds: development and reliability. *Ostomy Wound Manage:* 47(1):40-7

22.2.3 Wagner Scale (Wagner Ulcer Classification System)

Grade	Lesion
0	No open lesions; may have deformity or cellulitis

1	Superficial diabetic ulcer (partial or full thickness)
2	Ulcer extension to ligament, tendon, joint capsule, or deep fascia without abscess or osteomyelitis
3	Deep ulcer with abscess, osteomyelitis, or joint sepsis
4	Gangrene localized to portion of forefoot or heel
5	Extensive gangrenous involvement of the entire foot

From Frykberg RG (2003) Diabetic foot ulcers: pathogenesis and management. *American Family Physician:* 66-9