PROTOCOL

CODIFI2: Randomised controlled trial of swab versus tissue sampling for infected diabetic foot ulcers, and comparison of culture versus molecular processing techniques

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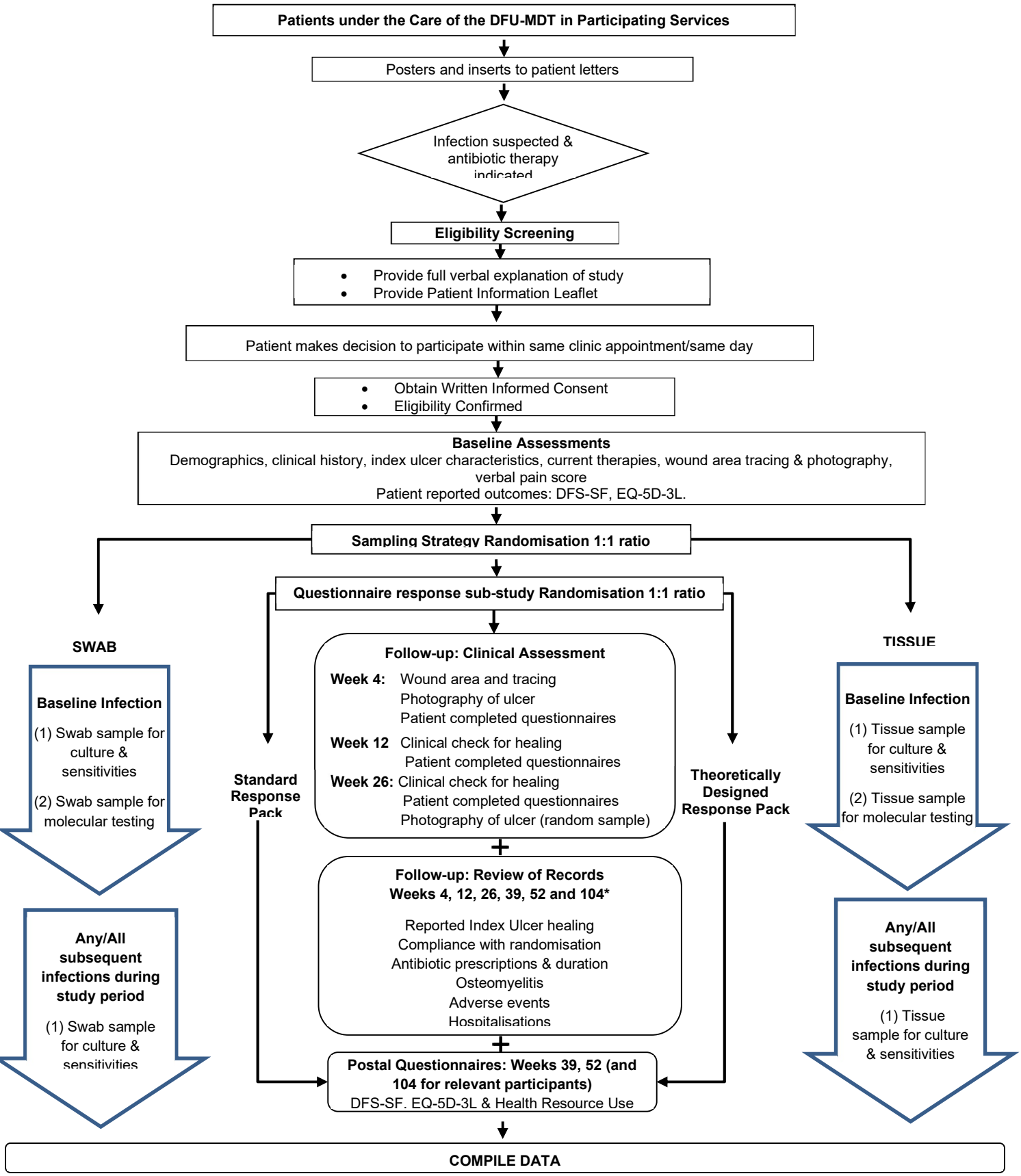

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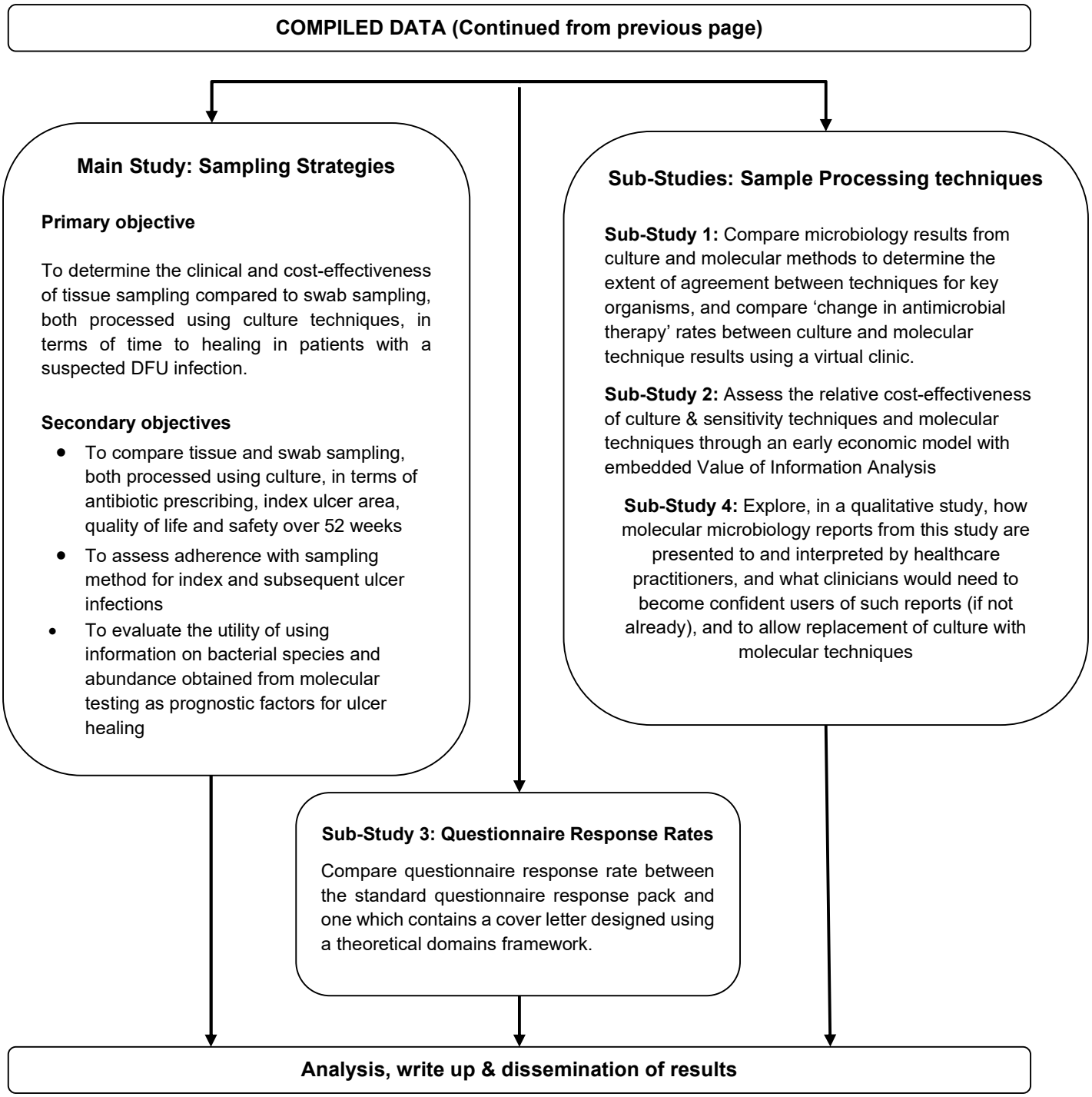
COMPILED DATA (Continued from previous page)
Main Study: Sampling Strategies
Primary objective
To determine the clinical and cost-effectiveness of tissue sampling compared to swab sampling, both processed using culture techniques, in terms of time to healing in patients with a suspected DFU infection.
Secondary objectives
To compare tissue and swab sampling, both processed using culture, in terms of antibiotic prescribing, index ulcer area, quality of life and safety over 52 weeks
To assess adherence with sampling method for index and subsequent ulcer infections
To evaluate the utility of using information on bacterial species and abundance obtained from molecular testing as prognostic factors for ulcer healing
Sub-Studies: Sample Processing techniques
Sub-Study 1: Compare microbiology results from culture and molecular methods to determine the extent of agreement between techniques for key organisms, and compare 'change in antimicrobial therapy' rates between culture and molecular technique results using a virtual clinic.
Sub-Study 2: Assess the relative cost-effectiveness of culture & sensitivity techniques and molecular techniques through an early economic model with embedded Value of Information Analysis
Sub-Study 4: Explore, in a qualitative study, how molecular microbiology reports from this study are presented to and interpreted by healthcare practitioners, and what clinicians would need to become confident users of such reports (if not already), and to allow replacement of culture with molecular techniques
Sub-Study 3: Questionnaire Response Rates
Compare questionnaire response rate between the standard questionnaire response pack and one which contains a cover letter designed using a theoretical domains framework.
Analysis, write up & dissemination of results

4 GLOSSARY OF TERMS/DEFINITIONS

AE	Adverse Event
CI	Chief Investigator
CRF	Case Report Form
CRN	Clinical Research Nurse
CTRU	Clinical Trials Research Unit
DFU	Diabetic Foot Ulcer
DNA	Deoxyribonucleic Acid
GCP	Good Clinical Practice
HTA	Health Technology Assessment
ISF	Investigator Site File
ITT	Intention To Treat
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
REC	Research Ethics Committee
RU SAE	Related Unexpected Serious Adverse Event
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SMG	Study Management Group
SSC	Study Steering Committee
SWAT	Study Within A Trial

5 BACKGROUND AND INTRODUCTION

5.1 DIABETIC FOOT ULCER INFECTION

People with diabetes can have up to a 25% lifetime risk of developing a diabetic foot ulcer (DFU) [1], largely due to diabetes related peripheral neuropathy, arterial disease, or both [2]. These risk factors may cause injuries to go undetected and restrict oxygen supply to the tissues ultimately leading to tissue damage, delayed healing and ulceration [3-5]. Any break in the skin, such as ulceration, is vulnerable to contamination by microorganisms from the surrounding skin and the external environment, commonly resulting in a complex poly-microbial wound that harbours a range of bacterial organisms. Infection is said to occur if these microorganisms begin to invade tissues, causing damage and evoking a host immune response. The longer the wound is unhealed and exposed the easier it is for bacteria to progress from colonisation to infection [6-8]. Infection is a frequent and costly complication of DFUs and represents a major cause of morbidity and mortality. The anatomical feature of the foot such as the tendons, muscle sheaths and fascia all linked together by several inter-connecting compartments, enable localised infections of DFU to travel along tendons, through the tissues and between compartments of the foot. This can result in infections of deeper tissues and bone, thrombosis (blood clots) in end-arteries and arterioles restricting blood flow to the tissues. Bone and tissue become devitalised, leading to the possibly the loss of toes, or more of the foot from gangrene or surgical intervention to prevent the spread of infection, which if disseminated through the body via the circulation, can lead to life-threatening conditions [9, 10].

About 40% of recent onset DFUs are considered clinically infected at presentation [11]; unless quickly diagnosed and treated, these infections can lead to substantial morbidity, including amputation. DFUs present for more than 30 days have up to a 5-fold increased risk of infection compared to those that are less chronic. Patients with a DFU that is infected at presentation have a significantly reduced likelihood of being alive and ulcer free at 12 weeks (OR 0.76) [11] and increased risk of minor amputation (OR 1.96) [11] and, in the presence of peripheral arterial disease, major amputation [12]. Thus, any patient with a DFU should undergo assessment for symptoms or signs of infection at initial presentation and as well as at each subsequent visit. This assessment should include a thorough history and physical examination to detect (or monitor) pain, tenderness, fever, purulent exudate, redness, warmth, induration, foul odour and visible or palpable bone [13].

Infection of a DFU is identified through a clinical assessment of the individual, the limb and the wound, where signs and symptoms such as fever, exudate, redness, inflammation, pus, odour and bleeding could indicate an infection. In some DFUs however, some signs and symptoms may be suppressed, for example in cases of arterial insufficiency where there is poor perfusion of the tissues, signs of inflammation and redness may be subdued or concealed, making identification of infection challenging [14]. As the risks associated with DFU infections can be substantial, empiric antibiotic therapy is usually initiated immediately with the antimicrobial selected in accordance with departmental guidelines, probable causative organisms and local susceptibility patterns. Wound samples for microbiology analysis are obtained to aid the identification of the causative organisms, but perhaps more importantly to facilitate the judicious use of antibiotics by guiding subsequent modifications of the empiric antibiotic therapy [15]. Due to the poly-microbial colonisation of DFU wounds, culture samples should only be taken once a clinical diagnosis of infection has been made, with the resultant microbiological results interpreted in relation to clinical circumstances [10, 14, 16].

When infection of a DFU is suspected, current practice involves 3 steps: 1) obtain specimens from the ulcer for microbiological tests; 2) initiate protocol-based (often broad-spectrum) empiric antibiotic therapy; and, 3) reassess the antibiotic regimen, and change if necessary, when the culture and sensitivity results are available [17, 18].

5.2 DFU MICROBIOLOGY SAMPLING METHODS

Most clinicians obtain ulcer specimens with a cotton swab, as the equipment is readily available, sampling is quick, easy to perform, non-invasive, inexpensive and can be assigned to a non-physician or a non-registered practitioner. Clinical practice guidelines, however, recommend obtaining a tissue sample from the wound bed, as these samples are more likely to detect more pathogens, less likely to harbour colonising bacteria and better support growth of anaerobic or fastidious organisms [13, 19, 20]. Several studies including CODIFI confirm that cultures of tissue samples identify bacteria from DFU more often than swab samples, and they find more isolates per sample [21-23]. Detecting more isolates from tissue samples may be beneficial if it represents a higher yield of pathogens, i.e. have greater sensitivity. Conversely, this greater sensitivity may detect more clinically non-pathogenic (colonising)

bacteria, at the cost of reduced specificity. If finding more pathogens leads to better tailoring of antibiotic therapy to pathogens, then tissue sampling could lead to improved prescribing. This, in turn, may increase the likelihood and rapidity of resolution of infection, and perhaps quicker healing. Importantly, this could potentially reduce the use of broad-spectrum antibiotic agents, thereby reducing the likelihood of development of antibiotic resistance, an emerging crisis for healthcare [24, 25]. Conversely, if tissue sampling detects more colonising organisms due to lower specificity, then this may increase inappropriate prescribing, particularly with an overly broad-spectrum regimen, leading to increased costs and antibiotic resistance. The clinical relevance of the higher number of isolates reported from tissue rather than swab samples has been previously assessed in the original CODIFI in a 'virtual clinic' where clinicians blinded to the sample source (swab or tissue) were asked whether they would change the prescribed antibiotic regimen based on the culture results (each report was presented to each clinician to allow comparison) [21]. Clinicians provided with the tissue result indicated that they were more likely to want to change antibiotic treatment (44.5%) than when they were provided the corresponding swab result (35.6%) [21]. What remains unknown is whether using tissue rather than swab sampling would lead to real changes in prescribing behaviour in a true (rather than virtual) clinical setting. Nor is it known if any changes in prescribing behaviour would lead to improved clinical outcomes.

5.3 DFU MICROBIOLOGY SAMPLE PROCESSING

Once a sample is obtained the current standard for microbiological processing of wound specimens is to culture any isolated bacteria in a laboratory, identify organisms that have grown using additional tests and determine their susceptibility to selected antibiotics by assessing their ability to grow in the presence of an antibiotic. Whilst traditional microbiology culture methods require at least 2 to 3 days to provide results, as molecular methods work by identifying the presence of bacteria through the detection of bacterial DNA they have the potential to be faster than culture methods, with some taking <1 hour. For a patient with a DFU infection, a faster result might allow clinicians to prescribe tailored antibiotic therapy at the initial patient visit or the next day, potentially omitting a stage of empiric treatment with a broad-spectrum regimen while awaiting culture results. In practice, up to 72% [26] of patients presenting to secondary care have already received a course of antibiotic therapy, which can increase the likelihood of false negative routine microbiology cultures. In this scenario molecular microbiological techniques, which do not rely on the presence of viable bacteria,

may more accurately detect the bacterial species present. For DFU infections any microbiological method used must be able to identify the commonest DFU pathogens listed in the UK standards for microbiology investigations of skin and superficial soft tissue infections [10]. Currently available molecular microbiology techniques have been shown to be generally reliable and valid, but none are routinely used for DFU infection or validated specifically for specimens isolated from such infected tissue.

5.4 WHY IS THIS RESEARCH IMPORTANT?

Diabetic foot ulcers have a substantial effect on health-related quality of life (HRQoL), with physical, social, psychological, and financial effects [27, 28]. Mortality rates after diabetic foot ulceration and related amputation are high, with 5 year death rates of 43-55% after developing a diabetic foot ulcer and up to 70% after amputation, related to the presence of systemic cardiovascular disease, including heart disease. Optimally treating DFU infection by avoiding both under-treatment (failing to cover pathogens) and overtreatment (by using an unnecessarily wide-spectrum regimen), is necessary to avoid on one hand worsening infection and potentially serious sepsis for the patient, but, on the other the wider societal effects related to antibiotic resistance. The key issues in assessing the causative pathogen(s) in a wound are firstly, collecting an appropriate sample; and secondly processing the specimen to identify all relevant pathogenic organisms. The aim of the sampling is to detect all potentially pathogenic microorganisms in the wound whilst minimising the collection of non-pathogenic colonising flora (which do not require treatment). These two aspects are related as implementing modern processing techniques on inappropriate samples or those collected sub-optimally may waste resources.

5.5 WHY IS IT IMPORTANT TO DO THIS RESEARCH NOW?

It is anticipated that molecular techniques will become more widely available, especially as the number of targets which they can detect grows. Clinicians are not yet familiar with these techniques, nor the reports produced from molecular diagnostic techniques, which may identify many more species than culture, leading to uncertainties in how clinicians will interpret these reports and how they might use them to inform prescribing practice. If molecular techniques are quick, safe, well accepted and effectively replace traditional culture methods, then there is the potential that DFU care can be tailored more quickly, at potentially

reduced cost, and with minimal delay. There is a risk, however, that the growing availability of molecular testing in clinical settings may lead to clinicians replacing culture methods before we know if this approach is safe. Alternatively, clinicians may add molecular techniques to conventional microbiology tests (thus increasing both costs and complexity). There is a urgent need to avoid over-prescribing antibiotics as this can lead to antibiotic resistance, (an emerging crisis for healthcare [24, 25]), therefore choosing the most appropriate diagnostic methods to mitigate this is important.

5.6 OVERVIEW OF PROGRAMME OF WORK

This programme of work sets out to address two important, related aspects of the assessment of infected diabetic foot ulcers through a series of studies. The key clinical questions relate to strategies for collecting a sample from an infected diabetic foot ulcer (tissue or swab), and how to process this (culture or molecular technique). A number of other sub-studies provide additional information on the potential challenges and value of molecular methods of sample analysis, and the value of further research. A 'study within a trial' also seeks to contribute to the evidence base around questionnaire response rates in research.

The main study is a 2-arm randomised controlled trial comparing clinical, cost-effectiveness and process outcomes associated with strategies for sampling infected foot ulcers. This contains an embedded cross-sectional study (**sub-study 1**) with the first sample sent for culture and sensitivity immediately (as per regular NHS practice), with the results available to the clinicians to inform care. A second sample will be processed according to local procedures and batch-processed using molecular techniques. Hence for each participant we will have paired samples of culture results for comparison with molecular results, in a cross-sectional study. This will allow us to determine the extent of agreement (or not) between culture and molecular techniques for key organisms. A virtual clinic will present un-paired results to clinicians to investigate whether a change in antimicrobial therapy would be recommended (using vignettes for each participant). This will allow us to understand whether 'different' information from culture or molecular techniques would potentially be associated with clinical differences in management.

We will also undertake a value of information analysis, in **sub-study 2**, to inform the potential value of future research about the choice of sample processing method.

In **sub-study 3** we will assess whether questionnaire response rates can be improved in trials by randomising to standard vs theoretically designed cover letter to participants (the details of this element are in Appendix I).

Finally, we are also undertaking **sub-study 4** to evaluate clinician perspectives on sample processing, because the adoption of a novel sample processing technique depends upon clinicians being able to interpret the results from a molecular sample processing technique. We will determine willingness to *replace* culture and sensitivity with molecular techniques, ease of interpretation of molecular microbiology reports, and what clinicians would need to become confident users of such reports (if not already). These elements will be investigated using qualitative methods, with a detailed separate protocol which will be submitted for separate ethics and HRA approval.

In summary, funding has been provided for the main RCT and 4 sub-studies. This protocol includes details relating to the main study; sub-study 1; sub-study 2 and; sub-study 3 (see Appendix 1). Data collected in the main study will be utilised in sub-study 4 but methods and analysis will be detailed in a separate protocol.

6 AIMS AND OBJECTIVES

6.1 PRIMARY OBJECTIVE

To determine the clinical effectiveness of tissue sampling compared to swab sampling, both processed using culture and susceptibility (C&S) methods, in terms of time to healing in patients with a suspected DFU infection.

6.2 SECONDARY OBJECTIVES

a. To compare tissue and swab sampling, both processed using culture and susceptibility methods, in terms of:
    I. healing status of the index ulcer at 12, 26, 39, 52 (104 weeks in relevant participants) post-randomisation
    II. antibiotic prescribing over 52 weeks (104 weeks in relevant participants) post-randomisation
    III. reduction in index ulcer area at 4 weeks post-randomisation
    IV. quality of life using DFS-DF and EQ-5D-3L over 52 weeks (104 weeks in relevant participants) post randomisation
    V. incidence of adverse events relating to sampling, to the DFU (including the development of osteomyelitis, amputation (major and minor), hospital admission) and antibiotics-related adverse events (such as incidence of *Clostridium Difficile* and mortality) over 52 weeks (to a maximum of 104 weeks) post randomisation
    VI. cost-effectiveness over 52 weeks (104 weeks in relevant participants)

b. To assess protocol adherence with randomised sampling strategy for index and subsequent ulcer infections
c. To evaluate the utility of using information on bacterial species and abundance obtained from molecular testing (when compared with culture) as prognostic factors for ulcer healing
d. To assess the agreement between C&S and molecular processing methods separately for each index ulcer sampling technique (swab and tissue) (Sub-study 1)

e. To assess the potential impact of information obtained via molecular techniques upon the clinical approach to antibiotic treatment plan at review (stop/ amend / continue), presented in a vignette to clinicians via a virtual clinic (Sub-Study 1).
f. To assess the relative cost-effectiveness of culture and sensitivity techniques and molecular techniques through an early economic model with embedded Value of Information Analysis (VOIA). In particular we are interested in the value of further research aimed at reducing the likely uncertainty surrounding the economic model's parameters associated with molecular techniques (Sub-study 2)
g. To evaluate the impact of a theoretically informed cover-letter on questionnaire response rates (Sub-study 3, Appendix 1).

7 DESIGN

The trial is a multi-centre, Phase III, open, prospective, parallel group, randomised controlled trial (RCT) comparing two sample collection techniques in clinically infected diabetic foot ulcer (DFU) patients, with blinded outcome assessment. The embedded sampling processing sub-study (Sub-study 1) is a cross-sectional study comparing the agreement in reporting of the presence of pathogens using molecular or culture techniques.

A total of 730 eligible and consenting participants will be randomised in a 1:1 allocation ratio to either swab or tissue sampling from randomisation to the end of the interventional period (defined as randomisation to a minimum of week 52 post randomisation for all participants or up to week 104 post randomisation for relevant participants). All participants will have two samples taken at baseline, one for standard C&S and one for molecular processing for central batching and processing using molecular techniques, and will attend study visits at 4,12 and 26 weeks. There will also be further data extracted from the healthcare records at weeks 4, 12, 26, 39 and 52 (and 104 for relevant participants) e.g., incidence of osteomyelitis, antibiotic prescriptions and duration. Participants will also be asked to complete Quality of Life questionnaires, a Health Resource Use questionnaire and an antibiotic diary throughout the study.

The trial will include a 12-month internal pilot phase to the evaluate feasibility of recruitment and therefore the delivery of the trial (see section 19.3).

7.1 BLINDING

As both swab and tissue sample methods are distinct requiring different equipment and approaches it is not possible to blind a participant or the treating clinician to sampling strategy. Outcome assessments will be conducted by an independent clinical research nurse/assessor, who will have no previous involvement with, or knowledge of the sampling methods used, and as such will be blind to the randomised sampling strategy (see Section 12). The blinded assessor can be a clinician, research nurse or registered healthcare professional who is suitably trained in the assessment of wound healing. To mitigate the risk of assessment bias the blinded assessor will not be informed as to the randomised group and have no access to the trial Case Report Forms (CRFs) prior to or during the blinded assessment visit. In order to minimise bias, tracings and photographs at baseline and week 4, and confirmation of the index ulcer healing assessments will be returned to the CTRU separate to the main trial CRFs.

7.1.1 Blinding at Week 4 Assessment

Outcome assessors at each site will complete an acetate tracing and take a digital photograph of the index ulcer at week 4, which will be submitted to the CTRU. Area measurements will be obtained from the index ulcer tracing using 'Image J' software by a member of the CTRU team independent of the research team at each site and blind to randomised sampling strategy.

A photograph of the index ulcer will be taken as a back-up in the event that a tracing cannot be taken or the tracing is of insufficient quality to determine the index ulcer outline. Photographs will be transferred electronically to CTRU. Where required an independent registered healthcare professional will delineate the index ulcer margin on the photograph and use Image J software to calculate the index ulcer area. The clinician will be independent of the research team at each site and blind to sampling strategy and other outcomes. The measurements obtained using photographs will be used to inform imputed values for missing index ulcer area measurements when tracings are not available.

7.1.2 Blinding at the Post Healing Assessment

Following notification to the research team by the attending clinical team or the patient, that the index DFU has healed a blinded assessment visit will be arranged for assessment and

photography of the index ulcer by the blinded assessor. The visit will be undertaken within 3 days of healing being reported and may be at the participant's routine clinic assessment or at home. The photograph will undergo blinded central review by the clinical members of the Trial Management Group, who will not be aware of the participant's identity or the randomised sampling strategy.

7.1.3 Other Photographic Assessments for Blind Review

In order to assess the risk of under-reporting of healing, a random sample of participants will have their index ulcer photographed by the local principal investigator (or delegate) at their week 26 visit. These photographs will be included with the photographs of healed ulcers and will be reviewed by the panel in a blinded fashion to ensure they are unaware of whether the photo is for confirmation of healing or an assessment of under-reporting.

All photographs will be submitted to CTRU. Photographs taken at first follow-up visit and confirmation of healing visits, and of unhealed index ulcers for randomly selected participants at baseline and week 26 visits will be centrally reviewed at the CTRU by blinded Trial Management Group clinical members.

7.2 SUB-STUDY 1 (CROSS-SECTIONAL COMPARISON OF PAIRED CULTURE RESULTS OBTAINED BY C&S OR MOLECULAR TECHNIQUES)

In the cross sectional sub-study we will report the extent of agreement between molecular techniques and culture for the presence of key organisms. In addition, the utility of rapid molecular microbiology in antibiotic prescribing decisions will be explored by a 'virtual clinic' sub-study. This will comprise of a panel of clinicians from study sites, both medical and non-medical prescribers. Panel members will be presented with unpaired culture and molecular microbiology reports and will be asked based on the report; to review the 'coverage' of the prescribed antibiotic/s against the stated organisms and to report if they would continue, amend or stop antimicrobial therapy.

7.3 SUB-STUDY 2 (VALUE OF INFORMATION ANALYSIS (VOIA))

A Markov model with VOIA from the perspective of the UK NHS and Personal Social Services will be developed to assess the relative cost-effectiveness of culture and sensitivity techniques and molecular techniques. The VOIA will assess the value of undertaking further research to reduce decision uncertainty in the model.

7.4 SUB_STUDY 3 (STUDY WITHIN A TRIAL (EVALUATION OF THE IMPACT OF A THEORETICALLY INFORMED COVER-LETTER ON QUESTIONNAIRE RESPONSE RATES)

For the embedded study within a trial (SWAT) we have provided the protocol in APPENDIX 1 – Sub-Study 3: STUDY WITHIN A TRIAL (evaluation of the impact of a theoretically informed cover-letter on questionnaire response rates), page 63.

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.

***Waivers to the eligibility criteria are NOT permitted.**

8.1 INCLUSION CRITERIA

- 18 years of age or older at the time of signing the consent form
- Diagnosis of diabetes mellitus (according to WHO criteria [29])
- Presence of a DFU with a suspected mild to moderate soft tissue infection (as per IDSA guidelines [17])
- Able and willing to provide informed consent for participation in the study
- Consent for foot photography

8.2 EXCLUSION CRITERIA

- Index ulcer present for >2 years

- Presence of suspected osteomyelitis of the index limb
- Previous participation in the trial
- Not expected to comply with the sampling strategies (i.e., has a preference)
- Not expected to comply with the follow-up schedule
- In the opinion of the local investigator the participant's foot infection is too severe to include them in the study

9 RECRUITMENT

9.1 RECRUITMENT SETTING

Recruitment will take place in secondary care and community clinics that provide an MDT-DFU service. Research centres will be required to have obtained local confirmation of capability and capacity, undertaken a site initiation meeting with the Clinical Trials Research Unit (CTRU) and received a Sponsor green light prior to the start of recruitment into the study.

9.2 RECRUITMENT PROCESS

All patients under the care of the DFU-MDT in participating services will be considered as potentially eligible for this study if they have a current DFU.

We will alert patients attending clinics that this study is happening and should the attending clinical team suspect the patient has a DFU infection, then we will ask them to consider taking part in the study.

To alert patients to the study and provide preliminary general information about the study we will provide patients with an information leaflet on arrival at clinic visits, insert a patient introductory information letter into clinic appointment letters for existing and newly referred patients, and provide posters in clinic. Members of the attending clinical team will also alert patients to the study during foot ulcer clinic visits and will provide further information as required and the opportunity for patients to ask questions, whilst not alarming them in relation to the risk of infection.

Where subsequent infection is suspected by the attending clinical team, assenting patients will receive a full verbal explanation of the study and a Participant Information Leaflet (PIL) will be provided by either the attending clinical team or the CODIFI2 CRN for the patient to consider. This will include detailed information about the rationale, design and personal implications of the study. Ideally samples need to be taken prior to newly initiated antibiotics or a change in antibiotic treatment, but the antibiotics cannot be delayed. The interval between determining suspected infection and the start time for the antibiotic treatment is the same day, and so patients will consider study participation within the period of the clinic visit and if wishing to participate will be required to provide consent at that visit.

10 ELIGIBILITY 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

10.1 INFORMED CONSENT AND ELIGIBILITY

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 participant will be free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment/care.

Participants will also be asked to provide consent to receive periodic text messages and/or e-mails about the study e.g., reminders shortly prior to their questionnaire being sent to remind them to let us know of any address changes or approximately two weeks after their questionnaire has been sent to remind them to complete it where it has not yet been returned to CTRU.

Consent will be sought as part of the main CODIFI 2 trial to allow the provision for further long term follow up, subject to obtaining further funding; information gathered from CRFs and participant questionnaires will be linked to electronic health records (EHRs) other central NHS UK bodies, such as the Office for National Statistics, Hospital Episodes Statistics, Primary Care databases and NHS Summary Care Records.

Identifiable information will be held within a dedicated safe haven at the CTRU.

A record of the consent process detailing the date of consent and all those present will be kept in the participant'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 participant and a copy will be returned to the CTRU, at the University of Leeds.

11 RANDOMISATION

Participants who have formal confirmation of eligibility for randomisation and have provided written informed consent will be randomised into the trial by an authorised member of staff at the research site. Randomisation will be performed centrally using the CTRU automated, secure, 24-hour randomisation service which can be accessed via the web or telephone. For the telephone randomisation, the site code, authorisation code and Personal Identification Number (PIN) will be required to access this system. For the web randomisation, a site staff email address, site code and PIN will be required. Authorisation codes and PINs will be provided by the CTRU to access the randomisation service. These codes will only be issued

once a site has been fully approved and all the necessary documentation has been received at CTRU.

Baseline questionnaires must be completed prior to randomisation.

The person telephoning or accessing the web address to randomise the participant must have the completed Randomisation CRF available at the time of telephoning/accessing the web, as the following information will be required:

- Participant details including initials, date of birth, and NHS number
- Site code
- Confirmation of eligibility for randomisation
- Confirmation of completion of baseline questionnaires
- Confirmation of completion of baseline assessments
- Details relating to the stratification factors (stated below)

Participants will be randomised on a 1:1 basis to undergo either a swab or a tissue sampling strategy of their infected DFU, and be allocated a unique trial number. A minimisation algorithm, incorporating a random element, will be used to ensure sampling strategy groups are well-balanced for the following characteristics:

- Randomising centre
- Index ulcer area (< $1cm^2$ or ≥ $1cm^2$)
- Index ulcer duration (<6 months or >= 6 months)
- Index ulcer aetiology (neuropathic, ischaemic, or neuro-ischaemic)*
- Number of ulcers across both feet (Single or Multiple)
- Location of the index ulcer (forefoot or mid/hindfoot)

* Neuropathy will be confirmed if there is lack of sensation in 2 of 3 sites on the Diabetic Foot Screen Test sites when using a 10g microfilament

* Ischaemia will be ruled out by the presence of a palpable pedal pulse or multiphasic hand held Doppler signal

Direct line for **randomisation** +44 (0)113 343 2290

After allocation to either swab sampling or tissue sampling, a proportion of participants will be randomly selected (stratified by randomising site and study intervention arm) to have a photograph of their ulcer taken at week 26 in addition to the assessments planned at this timepoint. A randomised participant who is selected for the additional photography will have this outcome included as part of the randomisation notification provided to site. The sampling proportion will be disclosed after completion of recruitment. Participants will also be allocated to receive either a standard or an enhanced cover letter to be sent with their postal questionnaire pack at the weeks 39, 52 and 104 post-randomisation time points (where applicable) as part of Sub-study 3 (Appendix 1) although the outcome of this randomisation will not be disclosed to the participating site or the participant.

Following randomisation the research site will:

- Provide each participant with a trial information card and inform them to keep this with them at all times and present to any attending clinical team responsible for the care of their ulcer. The card will advise the attending clinical team to contact the research team should the following occur:
    - i) the index ulcer heals, in order that an appointment can be arranged with a blinded assessor
      or
    - ii) there are any further infections suspected (index ulcer or any other DFU), to ensure that compliance with sampling strategy is maintained (from 10th October 2022, any wound samples taken from ulcers requiring further sampling will be as per local guidelines and clinical judgement).
- Provide each participant with an Antibiotic Diary for them to log all antibiotics prescribed to them.
- Ensure that participants are notified of their appointment dates.
- Notify the participant's GP of participation in the trial.

Following participant randomisation, CTRU will fax or email a Participant Randomisation Notification to the member of the research team member who randomised the participant. The participant ID number will be provided during randomisation. This should be added to the consent and relevant CRFs.

Participants may only be randomised into the trial once.

12 INTERVENTION – RCT OF SAMPLING STRATEGIES

The randomised sampling strategy allocated to each participant will be adopted (where sampling is indicated by the attending clinical team) for all* of the participant's DFUs and the full duration of the intervention period (52- a maximum 104 weeks). From 10th October 2022, any wound samples taken from ulcers requiring further sampling will be as per local guidelines and clinical judgement.

The index ulcer is defined as the largest infected ulcer present (unless osteomyelitis is present).

*Whilst one 'index ulcer' will be identified which will be the subject of wound tracing, photography and healing assessment, treatment of other ulcer sites may affect the index ulcer. Therefore, if infection is suspected in a non-index ulcer site, any samples for microbiology investigations taken will use the randomised sampling strategy, where practical / clinically acceptable. In order to maintain compliance with the randomised sampling strategy, participants will be provided with a trial information card to present to any clinician with responsibility for treating their foot ulcers to ensure that compliance with the sampling strategy is maintained. From 10th October 2022, any wound samples taken from ulcers requiring further sampling will be as per local guidelines and clinical judgement.

The intervention period is defined as time from randomisation to a minimum of week 52 (or a maximum of week 104 for relevant participants recruited prior to 10th October 2022). From 10th October 2022, participants will complete up to a maximum of 52 week follow up.

The indication for sampling may include the initial suspected infection, suspected persistent infection, a new episode of infection after initial resolution, or any newly diagnosed infection in any foot ulcer.

12.1 SWAB TECHNIQUE

After wound cleansing (using sterile saline and gauze) and debridement (removal of necrotic tissue, foreign material, callus, undermining), a cotton-tipped swab, (standard/routine microbiology swab) should be rubbed over the wound surface to sample superficial wound fluid and tissue debris. The swabs will be placed with sufficient pressure on the wound bed to capture expressed wound fluid (as per Levine's technique) [30], and will be positioned deep in the ulcer to collect from likely infected areas. In line with standard NHS care swab samples will be placed in a transport tube, labelled and delivered as per local practice to the local medical microbiology laboratory as soon as possible. A national standard method will be used for collecting and processing samples [10].

12.2 TISSUE SAMPLING TECHNIQUE

After wound cleansing (using sterile saline and gauze) and debridement (removal of necrotic tissue, foreign material, callus, undermining), a small piece of wound tissue will be taken from the base of the wound by scraping or scooping using a dermal curette or sterile scalpel blade. In line with standard NHS care tissue samples will be placed in universal containers which will be labelled and delivered as per local practice to the local medical microbiology laboratory as soon as possible. A national standard method will be used for collecting and processing samples [10].

12.3 TRAINING AND AUDITING OF SWAB AND SAMPLING STRATEGIES

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.

12.4 MOLECULAR SAMPLE LABELLING AND TRANSPORT

At baseline a second sample (swab or tissue as randomised) will be obtained from each participant for molecular testing. These samples will be labelled as a research sample and delivered as per local practice to the local medical microbiology laboratory as soon as possible. Upon receipt, the research samples will be refrigerated until delivery to the Nottingham University Hospitals NHS Trust molecular research facility.

13 ASSESSMENT, SAMPLES AND DATA COLLECTION

A summary of the clinical assessments is given in Table 1.

Participants will attend up to 5 research visits (aligned with the standard clinic visits) as follows: eligibility screening/baseline/randomisation (on one day), weeks 4, 12, 26, and a post-healing report blinded assessment of healing visit (if blinded assessment not performed on the day of healing).

13.1 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. Participants will also be asked to complete questionnaires about their quality of life, health resource usage and antibiotics prescribed to them outside of the DFU-MDT either in clinic or at home. 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.

13.2 ASSESSMENTS

Data collection includes:

Clinical Assessments/Clinical Check of Healing Assessment: at baseline, weeks 4, 12 and 26, and these will be undertaken by a member of the clinical research team usually a clinical research nurse.

Baseline Sampling: as per the randomised allocation (either swab or tissue), the treating clinician (usually a podiatrist, but may be a doctor or nurse) will obtain 2 samples (i.e. 2 swabs or 2 tissue samples), one for standard laboratory processing using C&S and one for storage by the local laboratory for subsequent molecular processing.

Participant Questionnaires: will be completed in clinic at baseline, weeks 4, 12 and 26 and via post to participants at weeks 39, 52 (104 for relevant participants).

Healthcare Records: data will be extracted from the healthcare record by a member of the clinical research team at weeks 4, 12, 26, 39, 52 (104 for relevant participants).

Antibiotic Diary: participants will be asked to complete a brief diary relating to any antibiotics prescribed to them.

Healing Confirmation Visit: will be undertaken by a member of the clinical research team, blind to the randomised sampling strategy.

Table 1 provides a summary of the assessment schedule and the target time window either side of the expected visit time point.

Table 1: Summary of Clinical Assessments

		0	4w	12w	26w	39w	52w	104w (1)
	Formal eligibility & Baseline assessments **pre randomisation**	Randomisation & sampling						
Target time window			**+- 1w**	**+-1w**	**+-2w**	**+-2w**	**+-2w**	**+-4w**
Clinical Assessments & Interventions								
Confirm eligibility	X							
Informed consent	X							
Demographics	X							
Clinical history	X							
Current therapies	X							
Index ulcer characteristics	X							
Wound area and tracing	X		X					
Photography of index ulcer	X		X		X(4)			
Verbal Pain Score	X	X						
Randomisation		X						
Swab or tissue sampling C&S processing		X(2)	●	●	●	●	●	●
Swab or tissue sampling Molecular processing		X(3)						
Clinical check for healing			X	X	X			
Post-healing report blinded assessment visit (within 3 days of healing report by attending team)			●	●	●	●	●	●
Participant Questionnaires								
DFS-SF and EQ-5D-3L	X							
DFS-SF, EQ-5D-3L & Health Resource Use			X	X	X	Q	Q	Q (1)
Review of hospital records								
Change in treatment following baseline sample			X					
Reported index ulcer healing			●	●	●	●	●	●
Compliance with randomised intervention			●	●	●	●	●	●
Antibiotic prescriptions and duration			X	X	X	X	X	X
Osteomyelitis			X	X	X	X	X	X
Adverse events			X	X	X	X	X	X
Hospitalisations			X	X	X	X	X	X

Key: X = In-person clinical assessment visit. Q = Postal questionnaire. X = Blinded Assessment visits. (1) Participants recruited within an adequate timeframe prior to 10th October 2022 will be followed up for 104 weeks. (2) Samples (taken as per randomisation) will undergo routine culture and sensitivity, the results of which will determine participant management. (3) A second sample (taken as per randomisation) will be taken at time of original sample, this will be labelled as a research sample, stored at local microbiology laboratory facilities. These will be submitted centrally for batch molecular processing on an ongoing basis throughout the study. (4) Photography of index ulcer in a pre-selected random sample of participants if the index ulcer remains unhealed.

● May occur at any point during follow-up period

13.3 BASELINE ASSESSMENTS

Baseline assessment will be conducted in clinic, prior to randomisation and initiation of randomised sampling strategy (swab or tissue).

The following personal data will be collected (to be securely retained at site) in order to be able to arrange subsequent trial assessments and notify the participant's GP of their involvement in study: participant name, hospital number, participant address and telephone number, GP name and address.

The following data will also be collected on the Participant Contact Details Form to be returned to CTRU to facilitate sending the postal questionnaires and questionnaire reminders: participant name and participant address. In addition, the participant's mobile phone number and/or e-mail address will be provided to CTRU if the participant provides consent for this.

The following data will be collected at Baseline:
Demographic data: date of birth, ethnicity, gender, smoking status
Clinical history: diabetes type, duration of diabetes, number of ulcers on infected foot / both feet, ischaemic/neuropathic/neuro-ischaemic, aetiology (clinical assessment), recent HbA1C result (within 3 month) (available from clinical records as per standard care)
Current therapies: current and proposed antibiotic treatment (agent and dates), type of diabetes management (e.g. insulin, oral hypoglycaemic agents, other non-insulin injectables', diet alone), primary wound dressing
Index ulcer characteristics: first or recurrent ulcer, duration of index ulcer, site of index ulcer, depth, neuropathy by 10g monofilament, pulses and severity of infection [17]
Index Wound area: tracing and measurement
Foot photography: to identify the index ulcer
Pain Score using a Verbal Rating Scale (VRS)
Quality of Life: Diabetic Foot Ulcer Scale- Short Form (DFS-SF) and EQ-5D-3L

13.4 POST RANDOMISATION AND SAMPLING

Allocation of sampling strategy (swab vs tissue). Two samples will be taken from each wound: one for C&S processing (sent to NHS laboratory and results communicated as per standard care pathway to clinical team), and one for research purposes (for molecular techniques: sent to local NHS laboratory for batch transfer to centralised laboratory). See section 11 for randomisation and section 12 for sampling methods. The participant will be asked to verbally rate the pain in their ulcer being sampled immediately after sampling.

13.4.1 Follow-up assessments to 52 weeks post randomisation (or 104 weeks where relevant)

Participants will be routinely seen at MDT DFU clinics as per standard care. Three clinical follow-up visits for the study will occur at week 4, 12 and week 26.

- At week 4 there will be index ulcer tracing and photography to assess wound area reduction.
- At weeks 12 and 26 there will be a clinical assessment of healing but no requirement for tracings.
- At week 26 a random sample of participants, pre-selected during the randomisation process, will have a photograph taken of their ulcer if still unhealed at this point.

All further data collection will be undertaken by searching healthcare records to confirm non-healing of the index ulcer and assess compliance with randomised sample method (swab or tissue sample) and secondary outcomes including antibiotic prescribing, amputation and serious adverse events from randomisation to 52 (or 104 weeks for relevant participants).

13.4.2 Assessment of Healing

In addition to the healing assessments performed by the clinical research team, the index DFU will be assessed for healing at each standard care visit by the attending clinical team. In the event of healing whilst the participant is treated in the MDT DFU clinic or the community podiatry clinic/at home, the treating podiatrist or nurse will contact the research nurse to report the date the index ulcer was first noted as healed. The research nurse will then arrange assessment of the index ulcer by the blinded assessor to confirm healing and conduct photography for central review. If healing is reported during the routine MDT DFU clinic the assessment will ideally be performed during this visit. Where this is not possible or where healing is reported in the community podiatry clinic/at home a follow-up visit with a blinded assessor will be arranged. This must take place within 3 days of healing being reported. The

photographs will be sent to the trial team using an established secure transfer process which maintains confidentiality, for central blinded photography review by clinical members of the TMG.

13.4.3 Participant-completed QOL questionnaires

All participants will be asked to complete quality of life questionnaires at their in-person clinical assessment visits at baseline and at weeks 4, 12 and 26, and by mailed questionnaire at weeks 39, 52 (and week 104 for relevant participants). All participants will also be required to complete a health resource use questionnaire in clinic at weeks 4, 12 and 26, and by mailed questionnaire at weeks 39 and 52 (and week 104 for relevant participants). At all-time points, the following questionnaires will be administered in the form of an English language paper booklet (local hospital translators will be available for the in-person clinic visits). Where questionnaires are mailed to participants at home they will be provided with a self-addressed envelope to return questionnaires to CTRU.

13.4.3.1 EQ-5D-3L

The EQ-5D-3L questionnaire will be administered for health economic evaluation. The EQ-5D is a generic instrument for capturing patient-reported outcomes (www.euroqol.org) and forms part of the NICE reference case for undertaking a cost per Quality Adjusted Life Years (QALY) analysis [31]. The EQ-5D-3L comprises the five health domain questions (mobility, usual activities, self-care, pain/discomfort, anxiety/depression) each with three response levels (no problems, some, severe).

13.4.3.2 DFS-SF

The Diabetic Foot Ulcer Scale Short Form (DFS-SF [32]) is a 29 item questionnaire – each on a 5-point Likert scale – whose answers can produce six subscale scores (leisure, physical health, dependence/daily life, negative emotions, worried about ulcers/feet and bothered by ulcer care) ranging from 0-100, with higher scores meaning better health-related quality of life. No specific recall period is specified in this questionnaire. In the DFS-SF validation study [32] Cronbach's alpha ranged from 0.74 to 0.94, and ulcer closure was associated with average changes of between 7.5 and 14.1 points in the subscales, demonstrating sensitivity to change.

13.4.4 Health resource use

This is a bespoke questionnaire, formulated specifically for this group of patients, which will be administered for health economic evaluation. The questionnaire contains questions about

the use of primary, secondary and social care resource as a consequence of each intervention. In formulating the questionnaire, the NHS and Personal Social Service perspective will be assumed [31].

13.4.5 Antibiotic diary

All antibiotics prescribed to the participant will be captured during an antibiotic diary. This is a bespoke diary designed to capture the name and duration of any prescribed antibiotics.

14 WITHDRAWAL

In line with usual clinical care, cessation or alteration of the randomised intervention will be at the discretion of the attending clinical staff or the participants themselves. Withdrawal from the intervention or the research will be documented in the corresponding CRF.

Cessation of randomised allocation or withdrawal includes the following situations: the participant withdraws consent for microbiological sample collection; the participant withdraws consent for their samples to be used for research purposes; the participant withdraws consent from clinical follow-up; the participant withdraws consent from postal follow-up and/or; the participant withdraws consent from access to their healthcare records.

Participants who provide written/witnessed verbal informed consent who subsequently lose capacity will be withdrawn from the trial. Any data collected up to the point of withdrawal will be kept on record and used in the trial analysis.

15 DEFINITION OF END OF TRIAL

The end of trial is the date of receipt of the last data item for the last participant remaining in the study.

16 SERIOUS ADVERSE EVENTS PROCEDURES

16.1 GENERAL DEFINITIONS

An adverse event (AE) is any untoward medical occurrence in a participant 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[1]
- requires or prolongs hospitalisation
- is significantly or permanently disabling or incapacitating
- constitutes a congenital anomaly or a birth defect
- may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

A SAE occurring to a participant 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 procedure; and
- 'unexpected' – that is, the type of event is not listed in the protocol as an expected occurrence.

16.2 OPERATIONAL DEFINITIONS AND PROCEDURES

This is a randomised controlled trial using established sampling strategies with well-known safety profiles. In recognition of this, events fulfilling the definition of an adverse event or serious adverse event will not be reported in this study unless they are defined as:

1. Expected and related to DFUs and trial sampling strategies and classified as an AE (see section 16.2.2)
2. Expected and related to DFUs and trial sampling strategies and classified as an SAE (see section 15.2.2)
3. Expected but not related to DFUs or trial sampling strategies and classified as an SAE (see section 16.2.3)

[1] The term life-threatening in the definition of a SAE 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 was more severe.

4. Unexpected and related to DFU or trial sampling strategies and classified as a RUSAE (see section 16.2.44)

16.2.1 Expected AEs / SAEs – Not reportable

This study is being undertaken in a patient population with high levels of morbidity and co-morbid diseases and as such, acute illness resulting in hospitalisation, new medical problems and deterioration of existing medical problems are expected.
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 pre-specified expected events as detailed in sections 16.2.2 and 16.2.3 or 'related and unexpected' (16.2.4).

16.2.2 Expected and Related AEs/SAEs – Reportable

The following AEs/SAEs are expected within the study population and will be reported by the clinical research team using standardised Case Report Forms (CRFs):

AEs:

- Pain or bleeding relating to the swab or tissue sampling
- Development of new or recurrent DFUs on the foot of the index ulcer (including whether the DFU developed due to an offloading device or cast)
- Infection of any DFU on the foot of the index ulcer (as per IDSA guidelines [33])
- Osteomyelitis on either lower limb
- AEs related to antibiotics
- Other AEs related to trial procedures

SAEs:

- Hospital admission related to any DFU (including cause)
- Amputation (any site of either lower limb)
- Other SAEs related to trial procedures

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

16.2.3 Expected and Unrelated – standard reporting

The following unrelated SAEs are expected within the study population and will be reported by the clinical research team using standardised Case Report Forms (CRFs):

SAEs:

- Acute hospital admission any other cause (not DFU related)
- Death

As these events are expected within the study population they will bot be subjected to expedited reporting to the main REC.

16.2.4 Related and Unexpected SAEs (RUSAEs)

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 sent to the CTRU via secure file transfer 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 RUSAE 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
- seriousness criteria
- causality (i.e. relatedness to the investigation), in the opinion of the investigator
- whether the event would be considered expected or unexpected.

Any change of condition or other follow-up information should be sent to CTRU by secure file transfer as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached.

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.

16.3 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 the Trial Steering Committee (TSC), Data Monitoring and Ethics Committee 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.

16.4 RESPONSIBILITIES

Principal Investigator/Authorised individual:

- Checking for SAEs when participants attend for follow-up and via review of participants medical notes.
- Judgement in assigning:

  - Seriousness

  - Relatedness

  - Expectedness
- To ensure all RUSAEs 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 RUSAEs to local committees in line with local arrangements.

Chief Investigator (CI) or delegate:

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

CTRU:

- Expedited reporting of Related / Unexpected SAEs to the main REC and Sponsor within required timelines.
- Preparing annual safety reports to main REC and periodic safety reports to

TSC and Data Monitoring and Ethics Committee (DMEC) as appropriate.

- Notifying Investigators of Related / Unexpected SAEs which compromise participant safety.

TSC:

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data and liaising with the DMEC regarding safety issues.

DMEC:

In accordance with the Trial Terms of Reference for the DMEC, periodically reviewing unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis.

17 OUTCOME MEASURES

17.1 PRIMARY OUTCOME MEASURE

17.1.1 Time to Healing of Index Ulcer

Time from randomisation to healing of index ulcer (up to maximum follow-up of 104 weeks). Healing defined as complete epithelialisation [34], confirmed by a blinded assessor within 3 days after first report of healing.

In deriving time to healing / censoring, deaths and amputations will be treated as competing risks. Participants whose index ulcers are not healed by the end of follow-up will be censored at last follow-up, withdrawals from follow-up not considered to be competing risks will be censored.

17.2 SECONDARY OUTCOME MEASURES

17.2.1 Compliance with Randomised Sampling Method:

- ***Baseline sampling compliance***

  Whether or not all DFUs sampled in accordance with allocated sampling method at primary sampling assessment.

- ***Full sampling compliance***

  Whether or not all DFUs sampled in accordance with the allocated sampling method at all assessments requiring sampling, until week 52 / 104 (or earlier if healed, amputation, withdrawal or death).

17.2.2 Healing Status at 12, 26, 39, 52 and 104 Weeks Post-Randomisation

Healing status will be defined as for the primary endpoint at 12, 26, 39, 52 (104 weeks for relevant participants) post-randomisation.

17.2.3 Percentage Reduction in Index Ulcer Area at Week 4 Post-randomisation

Defined as the relative reduction in index ulcer area from baseline at 4 weeks post-randomisation.

17.2.4 Number of Days on Antibiotics for the Index Ulcer

Defined as number of days on antibiotics for the index ulcer over time at risk. This includes time on antibiotics beyond initial regimens, including rebound/recurrent infections and new infections in any DFU.

17.2.5 Adverse Events

All Adverse Events (including serious) relating to DFU, the sampling technique and antibiotic use (such as incidence of *Clostridium Difficile*) over maximum of 104 weeks post randomisation will be recorded from hospital notes.

17.2.6 Number of Days in Hospital

Number of days in hospital for admissions related to the diabetic foot ulcer over time at risk of being in hospital. The date and reason for all admissions to hospital will be recorded from hospital notes.

17.2.7 Number of Amputations (Major and Minor)

Time to amputation of the limb on which the index ulcer(s) is located. Details of the amputation, including the date, extent and site of amputation (whether it includes the site of the index ulcer) will be recorded.

17.2.8 Incidence of Osteomyelitis

The occurrence of new osteomyelitis [13] post randomisation within 52 / 104 weeks. Diagnosis date will be recorded from hospital notes.

17.2.9 All-Cause Mortality at 52 Weeks and 104 Weeks

Deaths from all causes over 52 weeks post randomisation (or 104 weeks for relevant participants). Date of death will be recorded.

17.2.10 Diabetic Foot Ulcer Short Form DFS-SF Scores

DFS-SF [32]) questionnaire subscale scores at 4,12, 26, 39, 52 (104 weeks for relevant participants) post-randomisation. Responses to this questionnaire – combined into the six subscales of leisure, physical health, dependence/daily life, negative emotions, worried about ulcers/feet and bothered by ulcer care as defined by the scoring instructions - at the above time points

17.2.11 EQ-5D-3L

We will derive utility using EQ-5D-3L utility [35, 36] at 4, 12, 26, 39, 52 (104 weeks for relevant participants) post-randomisation.

17.2.12 Cost-Effectiveness at 52 weeks Post-Randomisation

Trial-based economic evaluation will be undertaken at week 52 (or at week 104) of the trial. The proposed methods for the economic evaluation follow the reference case set out by NICE [31]. See section 19: Health Economics Analysis

17.3 SUB-STUDY 1: PROCESSING TECHNIQUES

17.3.1 Cross-sectional Study of Agreement

Presence or absence of key organisms including class of pathogen or 1 or more pathogens for a participant sample analysed by C&S or by molecular method.

The organisms will be derived directly from the analysis report for each analysis of each sample at the baseline assessment.

17.3.2 Appropriateness of Antimicrobial Therapy.

The outcome measure is whether or not a change in antimicrobial therapy is recommended for a participant when the molecular microbiology report is reviewed in a virtual clinic. Clinicians will be presented with actual prescribed antibiotics for each participant, a short vignette describing their ulcer history, and unpaired molecular and C&S microbiology results, and asked whether a change in therapy is indicated.

17.4 SUB-STUDY 2: VALUE OF INFORMATION ANALYSIS

Cost effectiveness analysis outcome measures

- See section 20.2

18 STATISTICAL CONSIDERATIONS

18.1 SAMPLE SIZE

The total sample size for CODIFI2 will be determined by the main study to ensure that clinically meaningful outcomes are addressed. From this fixed sample size of 730 participants, estimated effect sizes are provided for key outcome measures from sub-study 1 and prognostic modelling (secondary objective c).

Main Study: RCT of Sampling strategies

Outcome measure	Time to healing of index ulcer
Outcome measure analysis method	Time to event (Survival analysis)
Swab Group event rate	38.75% healed at 12 months (ie $S_1(12) = 61.25\%$)
Tissue Group event rate	51.25% healed at 12 months (ie $S_2(12) = 48.75\%$)
Effect size	Hazard Ratio ~= 1.466 (3dp)
Assumptions	Exponential healing rate (ie constant hazard); constant hazard ratio; non-informative censoring; fixed follow-up.
Type I error rate / alpha	5%
1 or 2-sided significance test	2-sided
Type II error rate (Power)	10% (ie 90% Power)
Computed number of events	288 healed
Required number of participants	328 per arm with complete data
Attrition / loss to follow-up allowed for	10%
Final sample size per arm	**365 per arm = 730 total**
Resource	nQuery 7.0 (Statistical Solutions Ltd, Republic of Ireland), table “Log-rank test of equality of 2 survival curves, fixed follow-up” [37]

Effect size (12.5 percent difference in 12-month healing rates) decided by consensus of co-applicants as minimum difference to change practice. Average 12 month healing rate is expected to be 45%, as reported by the CODIFI prognosis sub-study [Chapter 6 of [21]].

Sub-Study 1: Processing Techniques

With a fixed sample size of 365 swab / tissue pairs, the detectable effect size in terms of agreement is as follows:

Outcome measure	Agreement between pathogen presence between standard PC&S and molecular processing methods.
Outcome measure analysis method	Kappa
Fixed sample size	365 swab pairs and 365 tissue pairs
Type I error rate / alpha	5%
1 or 2-sided comparison	2-sided
Type II error rate (Power)	20% (ie 80% Power)
Pathogen prevalence	10%
Kappa under null hypothesis	0.6
Attrition / loss to follow-up allowed for	10% (from the main study)
Detectable Kappa	**0.803 (3dp)**
Resource	nQuery 7.0 (Statistical Solutions Ltd, Republic of Ireland), table "Agreement between two dichotomous ratings (intraclass kappa)" [38]

Null Hypothesis Kappa corresponds to "Good" agreement. Assumption of 10% pathogen prevalence based on range of prevalences of less-prevalent pathogens (Gram-positive bacilli, CNS, Cornebacterium, pseudomonas and MRSA) in CODIFI [21]. Power to detect this difference will be greater (and 80% power to detect smaller differences) as the prevalence of pathogens increase.

18.2 PLANNED RECRUITMENT RATE

It is estimated that a total of 2220 patients will need to be screened of whom 66% are expected to be eligible [21, 39] and 50% of those eligible will consent. To recruit a target of 730 participants will require an average recruitment rate of 1.1 patient per centre per month across 33 centres over a 33 month period.

19 STATISTICAL ANALYSIS

19.1 GENERAL CONSIDERATIONS

Statistical analysis is the responsibility of the CTRU Statistician. A full statistical analysis plan will be finalised and signed-off before any analyses are undertaken.

The full analysis set of all participants randomised (except those who withdrew consent for their data to be part of any efficacy analysis, or where written informed consent has not been provided) where all participants are included in their randomised group will be used for all intention to treat analyses. A per-protocol population (if required) which will include all eligible randomised participants according to the sampling actually received but will exclude major protocol violations. This population will be defined following discussion with the Trial Management Group.

19.2 FREQUENCY OF ANALYSIS

Statistical monitoring of safety data will be conducted throughout the trial and reported at agreed intervals to the DMEC. No interim efficacy analyses are planned

19.3 INTERNAL PILOT PHASE

An internal pilot phase will determine the likelihood of achieving the planned recruitment rate and of opening the required number of actively recruiting centres, and therefore confirming feasibility of trial delivery to the maximum target recruitment within the planned timelines (see section 18.2). Participant compliance with the allocated sampling method will also be reviewed. Details of the progression criteria are detailed separately in the “Internal Pilot Phase” document.

19.4 PRIMARY OUTCOME MEASURE ANALYSIS

Time to healing: Primary analysis on time to healing will be conducted on the ITT population using Zhou et al., extension [40] to Fine and Gray’s model [41] with covariates for the minimisation factors: centre (random effect), index ulcer area, duration, aetiology, presence of multiple ulcers and location; the effect of adding sampling method to this model will then be assessed using a likelihood ratio test. Death and amputations will be considered as competing risks. Surgical revascularisation will be considered as a time dependent covariate. Estimated hazard ratios and corresponding confidence intervals and p-values will be reported. An exploratory analysis using a multi-state model will be conducted to investigate disease progression [42].

19.5 SECONDARY OUTCOME MEASURE ANALYSIS

19.5.1 Compliance with the allocated sampling method:

Proportion of participants who complied with the allocated sampling method and at a) their primary sampling assessment, and b) at all assessments requiring sampling until week 52 (or earlier if healed, amputation, withdrawal or death).

19.5.2 Participant Management:

Summary statistics on the key elements of participant management which are expected to be on the causal pathway between sampling method and time to healing will be presented for those participants recruited during the internal pilot phase and for all participants recruited. The empiric antibiotic regimen, antibiotic regimen post allocated sampling results, whether there was change in regimen with reasons for change (e.g., sample result, no infection, lack of response), and time to first change in antibiotic regimen post allocated sampling result will be presented by sampling method.

19.5.3 Proportion of Index Ulcers Healed by 12, 26, 39, 52 and 104 Weeks:

Cumulative incidence of healing at 12, 26, 39 and 52 (104) weeks post randomisation by sampling method, corresponding confidence intervals and p-values will be reported (with those recruited within a timeframe to be followed up to 104 weeks). To be obtained as estimates from the primary endpoint analysis model.

19.5.4 Reduction in Index Ulcer Area at Week 4:

Linear regression with percentage reduction in index ulcer area at 4 weeks post randomisation as outcome, with covariates for the minimisation factors: centre (random effect), index ulcer area, duration, aetiology, presence of multiple ulcers and location; the effect of adding sampling method to this model will then be assessed using a likelihood ratio test. Parameter estimates and corresponding confidence intervals and p-values will be reported.

19.5.5 Number of Days on Antibiotics for the Index Ulcer:

A multivariable Poisson regression model will be fitted to whether participant is on antibiotics over time, with an offset term for time at risk of being on antibiotics, adjusting for the minimisation factors. The effect of sampling strategy group will be assessed using a likelihood ratio test. Centre and participant random effects will be explored. Parameter estimates, 95% confidence intervals and p-values will be reported.

19.5.6 Safety:

All adverse events and serious adverse events related to sampling, to the DFU and to antibiotics, including amputations and admissions to hospital, will be summarised by sampling strategy received.

19.5.7 Number of Days in Hospital:

A multivariable Poisson regression model will be fitted to whether participant is in hospital for admissions related to the DFU over time with an offset term for time at risk of being in hospital, adjusting for the minimisation factors. The effect of sampling method will be assessed using a likelihood ratio test. Centre and participant random effects will be explored. Parameter estimates, 95% confidence intervals and p-values will be reported.

19.5.8 Number of Amputations (Major and Minor):

Zhou et al's extension [40] to Fine and Gray's model [41] model will be fitted to time to amputation, adjusting for the minimisation factors. Death will be treated as a competing risk. The effect of sampling method will be assessed using a likelihood ratio test. Centre and participant random effects will be explored. Parameter estimates, 95% confidence intervals and p-values will be reported.

19.5.9 Incidence of New Osteomyelitis:

Zhou et al's extension [40] to Fine and Gray's model [41] model will be fitted to time to developing osteomyelitis with covariates for the minimisation factors; the effect of adding sampling method will be assessed using a likelihood ratio test. Death and amputations will be considered as competing risks. Cumulative incidence of developing osteomyelitis at 52 and 104 weeks in relevant participants, corresponding confidence intervals and p-values will be reported.

19.5.10 Death:

Multivariable logistic regression, with covariates for the minimisation factors; the effect of adding sampling method to this model will then be assessed using a likelihood ratio test. Estimated odds ratios and corresponding confidence intervals and p-values will be reported.

19.5.11 Quality of Life (Diabetic Foot Ulcer Scale-Short Form & EQ-5D-3L) at Weeks 4, 12, 26, 39, 52 and 104:

A comparison of sampling methods on the DFS-SF scores will be conducted using a multivariable, repeated measures, random coefficients, linear regression models fitted to DFS-SF scores over time adjusting for the minimisation factors, baseline DFS-SF score and

sampling method. Centre, participant and participant by time interaction random effects will be explored. Time, sampling method and sampling method by time interaction will be fitted as fixed effects. Contrasts for tissue compared to swab sampling at each time point will be reported in terms of the difference in means, corresponding confidence intervals and p-values.

A similar analysis will model EQ5D-3L utilities, with adjustment for baseline EQ-5D value, rather than the baseline DFS-SF value.

19.6 SUB-STUDY 1: OUTCOME MEASURE ANALYSES

19.6.1 Cross-Sectional Study

Presence or absence of key organisms (including class of pathogens, or 1 or more pathogens) for a participant sample analysed by C&S or by molecular method.
Agreement statistics (Kappa, Prevalence and bias-adjusted Kappa, intra-class correlation coefficient), differences in proportions of samples (molecular minus C&S) reporting each pathogen / class of pathogens. McNemar's Test to test for difference in proportions of samples.

19.6.2 Appropriateness of Empirical Antibiotic Therapy

Summary statistics of changes to prescribing indicated. Agreement statistics (Kappa, Prevalence and bias-adjusted Kappa, intra-class correlation coefficient), McNemar's Test to test for difference in proportions of samples with proposed change to antibiotic regimen.

19.7 PROGNOSTIC MODEL

19.7.1 Time to Healing of Index Ulcer

Clinical risk factors and the presence of particular pathogens identified from the molecular analysis will be fitted to the primary endpoint analysis model (time to healing, competing risks of amputation and death, adjusted for minimisation factors and fixed effect of the main study randomised group). These additional factors will be added individually to assess the relationship with time to healing (albeit, adjusted for the minimisation factors).

20 HEALTH ECONOMICS ANALYSIS

20.1 ECONOMIC EVALUATION IN MAIN STUDY: RCT OF SAMPLING STRATEGIES

For the main study, trial-based economic evaluation will be undertaken at week 52 (or at week 104) of the trial. The proposed methods for the economic evaluation follow the reference case set out by NICE [31]. The economic analysis will be a cost-utility analysis presenting incremental cost-effectiveness ratios (ICER) of tissue sampling versus swab sampling, with effects expressed in terms of quality-adjusted life years (QALY).

20.1.1 Perspective and time horizon

The cost utility analysis will adopt an NHS and Personal Social Services (PSS) perspective for cost and benefit evaluation. Costs and effect for each intervention will be calculated for the trial follow-up period of 52 weeks (to a maximum of 104 weeks).

20.1.2 Measures of effectiveness

Quality-Adjusted Life Years will be used as the main outcome measure. Participants' responses to the EQ-5D-3L questionnaire will be converted to health-related quality of life (HRQoL) scores using the standard UK general population time-trade off tariff values [43].

20.1.3 Measures of resource use

The health resource use questionnaire will collect information on NHS and personal social care use in line with NICE guidelines [31]. This will include primary, secondary, and community resource use. Unit cost data will be obtained from national databases such as the NHS Reference Cost and Personal Social Services Research Unit (PSSRU) Costs of Health and Social Care.

20.1.3.1 Costing the interventions

'Treatment' costs include the cost of delivering each intervention (mainly given by person-time of health-care professionals) and the cost of the necessary equipment. The scope of resources considered includes the direct healthcare cost incurred for necessary participant care and excludes resources driven by the study protocol (e.g., whilst routine clinics will be

included, the cost of photography and visit time for collecting data for study purposes will be excluded).

20.1.4 ICER and Net incremental monetary benefit (NMB)

The differences in mean costs and effects will be presented using incremental cost-effectiveness ratios, where ICER = Δ Cost / Δ effect. Net incremental monetary benefit (NMB) will also be computed. Net benefit combines cost-effectiveness and willingness to pay for health benefit. It is calculated by rearranging the ICER calculation such that: NMB=(λ * ΔQALYs) – Δcosts, where λ is typically referred to as cost-effectiveness threshold. The National Institute for Health and Care excellence considers a cost per QALY within the range of £20,000-£30,000 to be acceptable [31]. The lower limit of this threshold will be used such that, for λ=£20,000, an intervention with a positive mean incremental net monetary benefit (i.e. NMB >0) should be adopted.

20.1.5 Dealing with missing data

Our approach to missing data will follow good practice guidelines for cost effectiveness analysis alongside clinical trials [44]. Multiple imputation methods will be used to generate estimates of missing values based on the distribution of observed data. The multiple imputation approach is the recommended method of imputation for economic evaluation alongside clinical trials as it includes randomness to reflect the uncertainty inherent in missing data by using iterative multivariable regression techniques.

20.1.6 Sensitivity analyses

Alternative scenarios will be explored in the sensitivity analysis to test the robustness of the main trial analysis results. The effect of not imputing missing data will be considered with an analysis that includes only complete cases. Further sensitivity analyses may also be necessary to explore assumptions that are made during the primary analysis. ICERs from each of the scenarios will be presented and compared to the main trial results to identify areas of uncertainty.

20.1.7 Dealing with uncertainty

The level of sampling uncertainty around the ICER will be determined using a non-parametric bootstrap to generate 10,000 estimates of incremental costs and effects. Bootstrapped

estimates will be plotted on the cost-effectiveness plane to illustrate the uncertainty surrounding cost-effectiveness estimates [45].

Bootstrapped estimates of cost and effects will also be used to compute the probability that each intervention is cost-effective for a range of cost-effectiveness thresholds. The results will be presented as cost-effectiveness acceptability curves (CEAC) [46]. Whilst the decision to fund or not fund an intervention should be made on the expected NMB, the CEAC provides decision makers with useful information regarding the risk that the option with the largest expected NMB is not the best alternative.

20.2 SUB-STUDY 2: VALUE OF INFORMATION ANALYSIS (VOIA)

At the early stages of Sub-Study 1 we will develop a Markov model with Value of Information Analysis (VOIA) from the perspective of the UK NHS and Personal Social Services to assess the cost-effectiveness of culture and sensitivity techniques and molecular techniques. The VOIA will assess the value of undertaking further research to reduce decision uncertainty in the model. Variables of the model include transition probabilities, costs and quality of life associated with each state. At development stage, literature reviews will identify existing decision analytic models in this area and evidence on transition probabilities, economic costs and quality of life associated with each health state, all of which will enable us to populate the proposed model. The model will be then re-visited and updated at the end of the study when it will be populated with the trial's data. Finally, the model will be constructed and described in line with best practice in decision economic modelling [47].

20.2.1 Dealing with uncertainty

Uncertainty will be addressed by a set of sensitivity analyses. One-way sensitivity analyses will be conducted where key variables will be changed one at a time to see how these changes impact on the results. Parameter uncertainty will also be addressed using probabilistic sensitivity analysis, by fitting a probability distribution to each variable in the model, and running Monte Carlo simulations. The distribution of expected costs and effects will be provided and the probability that the intervention is cost-effective given a range of willingness to pay thresholds will be represented via cost effectiveness acceptability curves (CEACs).

20.2.2 Value of Information Analysis

We will conduct VOIA to assess the value of undertaking further research. VOIA relies on the theory that if evidence for the effectiveness or cost-effectiveness of a new intervention, in our case molecular techniques, is uncertain at the time of making the decision, there is a risk of making a wrong choice about which to adopt. Making a wrong adoption decision has consequences in terms of forgone health benefits and monetary resources. If the cost of further research is less than the cost of the consequences of the wrong decision, then further investigation is worthwhile. Finally, VOIA will tell us whether it is worth conducting future research and determine the optimal design of that research. The following statistics will be produced: 1) Expected value of perfect information (EVPI) which represents the ceiling value of future research, hence also the maximum cost the funder for health research should accept and 2) Expected value of perfect parameter information (EVPPI), which measures the value of reducing the uncertainty surrounding particular variables in the decision model.

21 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 participants, which will be carried out by staff from the CTRU/Sponsor. Source data verification will involve direct access to participant 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 Trial Management Group (TMG) if necessary.

21.1 CLINICAL GOVERNANCE ISSUES

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

22 QUALITY ASSURANCE AND ETHICAL CONSIDERATIONS

22.1 QUALITY ASSURANCE

The study will be conducted in accordance with the principles of GCP, the UK Policy Framework for Health and Social Care Research and through adherence to CTRU SOPs.

22.2 SERIOUS BREACHES

Investigators are required to promptly notify the CTRU of a serious breach (as defined in the latest version of the National Research Ethics Service (NRES) SOP). A 'serious breach' is defined as a breach of the protocol or of the conditions or principles of GCP (or equivalent standards for conduct of non-CTIMPs) which is likely to affect to a significant degree the safety or physical or mental integrity of the trial subjects, or the scientific value of the research. In the event of doubt or for further information, the Investigator should contact the Senior Trial Co-ordinator at the CTRU.

22.3 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 participants prior to registration into the study. The right of a patient to refuse participation without giving reasons must be respected. The participant 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.

23 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 2018 Data Protection Act and operationally this will include:

- Consent from participants to record personal details including name, date of birth, address, NHS number and hospital number, GP name and address (and optional consent to provide mobile telephone number and/ or e-mail address).
- Consent from participants for a letter to be sent to their GP to let them know they are taking part in the study.
- Consent from participants for the CTRU to receive a copy of their consent form (which includes their name, signature, Date of Birth and NHS number) to check they have not been previously registered and to facilitate collection of long term follow up data from electronic healthcare records, subject to further funding.
- Consent from participants to take photographs of their ulcer and for the electronic transfer of these images (with identifiers study number initials and date of birth only; the participant's name must be obliterated by site before sending).
- Consent from participants to have their name and address sent to CTRU for the purposes of sending questionnaires at week 39, 52 and 104 (where applicable).
- Appropriate storage, restricted access and disposal arrangements for patient personal and clinical details.
- Consent from participants 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 participants for the data collected for the study to be used to evaluate safety and develop new research.
- Participant name and address will be collected when they are randomised 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 participant'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 participant withdraws consent from further collection of data, all data collected to the point of withdrawal will be included in the final study analysis.

23.1 ARCHIVING

At the end of the study, data will be securely archived in line with the Sponsor's procedures for a minimum of 15 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.

24 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.

25 STUDY ORGANISATIONAL STRUCTURE

25.1 INDIVIDUALS AND INDIVIDUAL ORGANISATIONS

Chief Investigators – As defined by the NHS Research Governance Framework, the CI is responsible for the design, management and reporting of the study.

Trial Sponsor – The Sponsor is responsible for trial initiation management and financing of the trial as defined by Directive 2001/20/EC. These responsibilities are delegated to the CTRU as detailed in the trial contract.

Clinical Trials Research Unit (CTRU) – The CTRU will have responsibility for conduct of the study in accordance with the NHS UK Framework for Health and Social Care Research and CTRU SOPs. The CTRU will provide set-up and monitoring of trial conduct to CTRU SOPs and the UK Framework for Health and Social Care Research, including, randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, monitoring schedule and statistical analysis for the trial. In addition the CTRU will support main REC, Site Specific Assessment and NHS Permissions submissions and clinical set-up,

ongoing management including training, monitoring reports and promotion of the trial. The CTRU will be responsible for the day-to-day running of the trial including trial administration, database administrative functions, data management, safety reporting and all statistical analyses.

25.2 OVERSIGHT / TRIAL MONITORING GROUPS

Trial Management Group (TMG) - The TMG, comprising the Chief Investigators, CTRU team and other key external member of staff involved in the trial will be assigned responsibility for the clinical set-up, on-going management, promotion of the study, and for the interpretation of results. Specifically the TMG will be responsible for (i) protocol completion, (ii) CRF development, (iii) obtaining approval from the main REC and supporting application for Capacity and Capability review, (iv) completing cost estimates and project initiation, (v) nominating members and facilitating the TSC and DMEC, (vi) reporting of related unexpected serious adverse events, (vii) monitoring of screening, recruitment, sampling strategy and follow-up procedures, (viii) monitoring consent procedures, data collection, trial end-point validation and database development.

Trial Steering Committee (TSC) - The TSC, with an independent Chair, will provide overall supervision of the trial, in particular trial 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 Investigators and other members of the TMG may attend the TSC meetings and present and report progress. The Committee will meet annually as a minimum.

Data Monitoring and Ethics Committee (DMEC): The DMEC will include independent membership and will review the safety and ethics of the trial by reviewing interim data during recruitment. The committee will meet prior to scheduled TSC meetings as deemed appropriate and annually as a minimum.

25.3 FUNDING

This study is funded by the Health Technology Assessment (HTA) as part of the National Institute for Health Research (NIHR)

26 PUBLICATION POLICY

26.1 AUTHORSHIP AND ACKNOWLEDGMENT

The trial will be registered with an authorised registry, according to the International Committee of Medical Journal Editors (ICMJE) Guidelines, prior to the start of recruitment.

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).

For core publications, co-applicants and members of the CTRU trial team will be given the opportunity to contribute to drafting and reviewing manuscripts; those who contribute as per the ICMJE guidance will be named authors on publications. For methodology papers, authorship will be discussed with the TMG and an authorship sub-team agreed.

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

26.2 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 TSC.

The TSC 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.

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28 APPENDIX 1 – SUB-STUDY 3: STUDY WITHIN A TRIAL (EVALUATION OF THE IMPACT OF A THEORETICALLY INFORMED COVER-LETTER ON QUESTIONNAIRE RESPONSE RATES)

28.1 BACKGROUND

Many trials collect outcome data direct from participants using mailed questionnaires. A low response rate to these questionnaires puts the validity and generalisability of the trial results in jeopardy [1-5].

Triallists recognise the challenge and use many interventions to improve retention but it is generally difficult to predict their effect. The Cochrane systematic review of strategies to improve retention [6] found only a handful of interventions with high quality evidence of benefit. Given how central recruitment and retention are to all trials, it is crucial that more rigorous evaluations of retention interventions are done.

One way of doing this is to do a Study Within a Trial, or SWAT (See, for example, [7]). A SWAT provides a protocol for the evaluation of an intervention to improve some part of the trial process, such as recruitment or retention. This evaluation is then embedded within a host trial, such as the present trial, CODIFI2. Several teams can follow the same SWAT protocol, meaning the results can be combined in a meta-analysis. This coordinated and collaborative approach means triallists will have faster access to high-quality evidence to inform their trial design, conduct, analysis and reporting decisions.

Since returning the questionnaire is a behaviour, this opens up the possibility of designing a behaviour change intervention to influence the willingness of participants to do that behaviour. To that end, we would like to make use of SWAT 24 [8] in CODIFI2, which describes the use of a theory-based [9-11] cover letter to improve response rates to mailed questionnaires used to collect trial data. The theory-based letter shows promise as a way of improving retention [12] but requires evaluation in more trials before we can conclude that it is effective.

This SWAT 24 study is part of the Trial Forge initiative to improve trial efficiency [13].

28.2 AIMS AND OBJECTIVES

To estimate the effect on mailed questionnaire response rates of a covering letter designed with reference to behavioural change theory to encourage response compared to a standard cover letter merely requesting a response over 52 weeks (to a maximum of 104 weeks for relevant participants) post randomisation.

28.3 TRIAL DESIGN

28.3.1 Sub-Study 3: Trial Design

The embedded cover letter sub-study is a 2-arm parallel group randomised controlled trial comparing the response rates to postal questionnaires when accompanied by either a short standard cover letter requesting response to those accompanied by an “enhanced” cover letter, designed with reference to behavioural change theory [9-11]. The effect will be estimated at each time point where a questionnaire is mailed to the participant.
Eligible and consenting patients who are randomised within study 1 will be randomised 1:1 to either standard or enhanced cover letter. Participants’ cover letters will be according to randomised group at all mailed questionnaire timepoints: there will be no cross-over and no re-randomisation to different letters.

28.3.2 Blinding

Neither the randomising site nor the participant will be informed of their cover letter allocation at randomisation. The participant will become aware of their cover letter once they receive their first mailing. Site may become unblind to that patient’s allocation should the participant bring the entire questionnaire pack to clinic for their clinical follow-up. To mitigate possible biases, participants will be informed that the trial will look at the effect of a communication intervention on their data completeness, but they will not be informed of the exact nature of this intervention.

28.3.3 Randomistion

Immediately after randomisation in the main CODIFI2 study is complete, randomisation to the “cover letter” sub-study will occur. Participants will be allocated to receive either the standard or the “Enhanced” cover letter with their postal questionnaire. A second minimisation

algorithm, also incorporating a random element will be used to ensure that the sampling strategy groups are well balanced for the following characteristics:

- Randomising centre
- Sampling strategy allocation (Swab vs Tissue)
- Participant age (65 or younger, 66 or older)
- Gender (Male, Female)

Participants will retain the participant identifier already allocated.

28.4 INTERVENTIONS

28.4.1 Sub-study 3: Comparison of "Enhanced" Theory-Based Cover Letter Vs Standard Cover Letter

The interventions to be assessed in this sub-study are covering letters sent in addition to the mailed questionnaire booklet of quality of life questionnaires. All mailed questionnaire packages will comprise a blank questionnaire booklet for completion, a stamped addressed envelope for return and a covering letter address to the participant. The content of the cover letter will differ according to the patient's randomised group within this sub-study, and the wording will differ slightly for each follow-up time point. (eg: "please find enclosed the 39 week / 52 week / 104 week questionnaire").

28.4.2 "Enhanced" theory-based cover letter

The Theoretical Domains Framework (TDF) is a tool for identifying theoretical targets for behaviour change interventions [9-11]. The TDF and behaviour change techniques were used by the IQuaD trial team [12] to produce a template that trial teams can use to structure a theory-informed cover letter to send together with their trial questionnaires. Examples of relevant theoretical domains and behavioural change techniques include "Motivation and Goals" (providing general encouragement and information about others support and approval), "Beliefs about consequences" (Providing information on the benefits of action or costs of inaction) and "Knowledge" (Providing information on behaviour and outcomes, i.e. why are we asking these questions and what it means for the study) This template has been adapted for use in the CODIFI2 trial as the intervention cover letter in this study.

At the 39 week follow-up visit, participants randomised to the “enhanced” cover letter group will be sent cover letter A (filename xxxxxxxxxxx_letter A) in addition to the questionnaire booklet and a stamped addressed envelope. At 52 weeks, cover letter B (filename xxxxxxxxxxx_letter B) will be sent in addition to the booklet and envelope. Participants eligible for the 104 week follow-up will receive cover letter C with their final questionnaire pack. (filename xxxxxxxxxxx_letter C).

28.4.3 Standard cover letter

The standard comparator cover letter conveys the usual information provided by the CTRU to mailed questionnaire respondents: the title of the study, a reminder that they are taking part in the study, contact details for a staff member and that a questionnaire is enclosed for their completion and return. At week 39, participants randomised to the control cover letter group will receive follow-up letter I (filename xxxxxxxxxxx_letter I) with their questionnaire pack. At 52 weeks, cover letter II (filename xxxxxxxxxxx_letter II) will be sent with the pack. Participants eligible for the 104 week follow-up will receive cover letter III (filename xxxxxxxxxxx_letter III) with their pack.

28.5 OUTCOME ASSESSMENT

The return of questionnaire either by mail to the Clinical Trials Research Unit, or by hand at a clinic visit will be recorded. The date of receipt at clinic or at the Clinical Trials Unit will be recorded for determining time to response.

For the sub-study, the full analysis set is based on questionnaires issued to the participant. This population will include all participants randomised to take part in the CODIFI2 study. However, if a participant withdraws or dies before the first questionnaire is mailed, no questionnaires will be included in the analysis set for this participant.

28.5.1 Sub-study 3: outcome measures

Primary outcome measure: Return of mailed questionnaire at 39, 52 and 104 weeks.
Exploratory outcome measure: Time from mailing to return of questionnaire at the same timepoints.

28.6 SUB-STUDY 3: OUTCOME MEASURE ANALYSES

Multivariable logistic regression – accounting for repeated measures clustered within each participant – with fixed effects for randomised cover letter, age, sex, CODIFI Sampling arm, time-point and timepoint-by-cover letter interaction and random effects for randomising centre (if feasible), participant and participant -by-timepoint. The odds ratio for the overall effect of the enhanced letter on return (with 95% confidence interval) will be presented, along with the same for the effect at each time point.

An exploratory analysis taking a time-to-return approach (accounting for repeated measures clustered within patients) will be considered, if feasible. Such an analysis – multivariable time-to-event regression – will adjust for the sub-study minimisation factors, if feasible.

28.7 SAMPLE SIZE CALCULATION

Sub-Study 3: Questionnaire response rate

Outcome measure	Return of questionnaire
Outcome measure analysis method	Comparing proportions (logistic regression)
Fixed Sample Size	365 per arm
Control Group return rate	50%
Assumptions	ICC of response rates = 0.7 (Note unit of allocation=patient, unit of analysis = questionnaire. Multiple questionnaires per patient); Constant intervention effect over all follow-ups; All participants attend 2 follow-ups.
Type I error rate / alpha	5%
1 or 2-sided significance test	2-sided
Type II error rate (Power)	20% (ie 80% Power)
Resource	nTerim 3.0 (Statistical Solutions Ltd, Republic of Ireland), table “Fixed Term, Cluster randomised two proportions inequality”
Attrition / loss to follow-up allowed for	10% (from the main study 1)
Effect size (absolute difference in proportions Control - Enhanced)	**10.03%**

Expected ICC of 0.7 justified by repeated measures within patient (rather than many different patients clustered within a hospital) so expect high correlation of outcomes between patients. For example, Essers et al [61] found that in 12 month longitudinal quality of life outcomes in ankylosing spondylitis, the ICC ranged from 0.58 to 0.73. Detectable effect decreases as ICC decreases. Control group return rate 50% as worst case scenario (maximum variance occurs when both return rates are 50%)

28.8 REFERENCES

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