MANDARIN study

North Cumbria Integrated Care NHS Foundation Trust

Comparison of devices for the detection of diabetic neuropathy; an evaluative diagnostic study.

('MANDARIN' , Medipin Assessment for Neuropathy in Diabetes, A Real-world INvestigation)

V1, dd 20 April 2023

Chief Investigator's Statement of Ownership and Content.

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

Name (PRINT):_____Dr Stacey Fisher_____

Signature:_____

Date: _____26 April 2023_____

RESEARCH PROTOCOL SUMMARY

TITLE:	**Comparison of devices for the detection of diabetic neuropathy; an evaluative diagnostic study.**
Short title:	'MANDARIN' , Medipin Assessment for Neuropathy in Diabetes, A Real-world INvestigation
IRAS number	325532
Device description	CE-marked neuropathy test device: Medipin (MHRA No. 1321) Medipin is a single-use precision instrument designed to optimise cutaneous pinprick perception. Medipin's protected point is designed to significantly enhance pinprick acuity to achieve useful stimulation and reduce risk of damaging delicate skin. The protective annulus inhibits depth of penetration and protects against self-inflicted "needle stick" injury.
Study design	Prospective, single-centre, controlled, non-randomised, evaluative diagnostic study
Primary objective	Respective detection rate of diabetic neuropathy determined by use of Medipin and standard care device (10g monofilament). Pragmatic real-world approach using only devices utilised in standard primary care based clinics.
Secondary objectives	Evaluation of feasibility that a 10-point scale can be applied for Medipin in relation to degree of neuropathy, as opposed to standard yes/no quantification. Concordance between Medipin and 10g monofilament test results. Relative performance/concordance achieved with 10g monofilament depending on number of tests and locations included in the assessment.
Inclusion & Exclusion criteria	Inclusion criteria: - Adult patients aged ≥ 18 years - Patients with type I or type II diabetes mellitus Exclusion criteria: - Aged < 18 years - Any reasons for the patient being unable to follow the protocol, including lack of mental capacity to consent to taking part in the study (examples include dementia, severe learning disability). - The patient has concurrent (medical) conditions that in the opinion of the investigator may compromise patient safety or study objectives (examples include receiving palliative care, active cancer treatment)

	- Amputation of a lower limb - Confirmed and ongoing foot wound / ulcer
Sample size	Sample size determined for prevalence study, with predetermined criteria of 10% prevalence (can be either based on Medipin or monofilament testing), a population of 160,000, 95% confidence interval, and precision of 5%. The required initial sample size is 139 patients. Potential to expand to 552 patients if 2.5% precision applied.
Manufacturer & provider of material	Medipin Ltd, Barry Jacobs 24 Chiltern Ave, Bushey WD23 4QB clinical@medipin.net
Chief Investigator	Dr Stacey Fisher, Research GP, North Cumbria Integrated Care NHS Foundation Trust, stacey.fisher@ncic.nhs.uk
Co-investigators	Dr Leon Jonker PhD, Science & Innovation Manager, North Cumbria Integrated Care NHS Foundation Trust Leon.jonker@ncic.nhs.uk
Sponsor and organisation where research will take place	North Cumbria Integrated Care NHS Foundation Trust R&D department Ann Burrow Thomas Centre Workington, CA14 2ED
Planned timeline	Recruitment start date (first patient, one visit): 1 May 2023, Recruitment end date (last patient, one visit): 31 Jan 2024 Study end date: 30 Mar 2024
Protocol version, date	Version 1.1, dd 20 April 2023

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Lay Summary

Nerve damage and loss of protective sensation (LOPS) is a complication of diabetes. This diabetic neuropathy (DN) can subsequently lead to further complications such as diabetic foot ulcers and even amputation of toes and lower limbs. It is therefore essential to monitor for the development of DN in diabetic patients. In standard clinical practice DN is checked for using a monofilament, a piece of nylon on a stick that is pushed onto the patient's foot; LOPS is the sign of DN having developed. Monofilament testing checks for damage to large nerve fibres. There is however evidence that small nerve fibres are damaged before the large nerves are affected. Using a practical, reliable, and simple tool to check for small nerve damage in a clinic setting may aid in detecting LOPS/DN earlier and optimising patient management. Medipin is a hygienic single-use device designed to check for small nerve fibre damage in feet. The main objective of this study is to determine how many patients have LOPS/DN when tested with the monofilament and Medipin device respectively and to what degree there is an overlap between the two tests. For this purpose a total of 139 patients will be assessed at a single clinic visit.

1 BACKGROUND AND RATIONALE

Diabetes is a chronic condition that can lead to multiple complications affecting various organs, including of the lower limbs. Initial nerve damage in the patient's foot results in loss of protective sensation, diagnosed as diabetic neuropathy (DN). Due to reduced sensation in the feet, patients may walk on a stone stuck in their shoe or not realise foot skin has been damaged; this can then result in ulceration. Poor healing associated with diabetes – because of vascular damage – can then lead to infection and amputation.

Diabetic foot ulcers are the most common type of foot ulcer and are associated with significant health and cost implications. Whereas costs for foot ulcer care were £300m in 2005-06, this had risen to ~£900m to care diabetes related ulceration and amputation care in 2014–15 (Posnett & Franks 2008; Kerr et al, 2019). In the United States, neuropathy is implicated in up to 80% of >50,000 amputations (Smieja et al, 1999). Large studies conducted in Europe in the mid-90s estimated a prevalence of diabetic neuropathy of circa 25% (Young et al, 1993; Tesfaye et al 1996; Cabezas-Cerrato et al 1998). In a more recent study where 10g monofilament testing was utilised, 11.7% of women with normal fasting glucose, 14.4% of women with impaired fasting glucose (IFG), and 18.3% of women with diabetes had LOPS (Ylitalo et al, 2013). It is imperative to ensure DN is diagnosed early in diabetes patients and that they are educated to look after their feet, thereby minimising the risk of further costly and serious complications.

In regular clinical practice, the use of a 10g monofilament is the mainstay for DN diagnosis. The 10g relates to the amount of g-force applied to the skin with the nylon strand attached to a handheld stick. More sensitive tests are available, including nerve conduction tests and biopsy of skin tissue, but monofilaments are used since they are relatively effective in detecting DN and because the test is affordable, plus straightforward to apply and interpret. Two types of nerves are present in the skin, and diabetes related damage can lead to a) large fibre neuropathy: manifests with the loss of joint position and vibration sense and sensory ataxia, and b) small fibre neuropathy: manifests with the impairment of pain, temperature and autonomic functions. Monofilament application, akin of stroking the skin, tests predominantly for large fibre DN. This means that small fibre damage is usually not specifically tested for. For example, Ylitalo et al (2013) only considered small fibre damage with the Michigan Neuropathy

Screening Instrument (MNSI) symptom questionnaire, not through a physical assessment as done for large fibres with the monofilament device. Of interest is that there were no statistically significant differences in the prevalence of monofilament insensitivity between non-diabetics and diabetics, also observed by Gregg et al (2007), but the prevalence of peripheral neuropathy symptoms (which includes questions regarding small fibre damage) did increase significantly with fasting glucose categories. There is evidence that small fibre damage develops before large fibre damage manifests, which may be of value if early intervention and prevention for DN is the goal (Malik et al, 2011; Ponirakis et al, 2014; Breiner et al, 2014). Recently, Burgess et al (2021) stated "DN is diagnosed at a late, often pre-ulcerative stage due to a lack of early systematic screening and the endorsement of monofilament testing which identifies advanced neuropathy only".

Apart from the issue of which nerve fibre type response to test for, another issue in DN diagnostics is the variability in approach with the 10g monofilament. Dros et al (2009) highlight this: 'Another problem is the lack of standardization of the monofilament test methods. Different methods are described varying from 1 testing site to 10 testing sites on 1 foot, and there is no evidence or consensus about the most appropriate threshold'. National clinical guidelines unfortunately do not settle the matter either. NICE gives no detailed guidance on how to assess for diabetic neuropathy. In their NICE guideline (26 August 2015, www.nice.org.uk/guidance/ng19) they only mention the following:

Assessing the risk of developing a diabetic foot problem. 1.3.4 When examining the feet of a person with diabetes, remove their shoes, socks, bandages and dressings, and examine both feet for evidence of the following risk factors: neuropathy (use a 10 g monofilament as part of a foot sensory examination)

The end result is that different NHS Trusts use different methods to determine if a patient has DN. Appendix 1 gives an overview with some examples. A review by Feng et al (2009) concluded: "To maximize the diagnostic value of SWME (Semmes Weinstein monofilament examination), a three site test involving the plantar aspects of the great toe, the third metatarsal, and the fifth metatarsals should be used. In the group with history of ulceration as the reference test, the four studies that used only one site had a sensitivity of around 50%, considerably lower than other studies that tested more than one site. Testing more sites allowed the SWME to be more sensitive in identifying patients with Diabetic Peripheral Neuropathy". Unfortunately, Feng and colleagues did not specify what total score of such an investigation would be deemed presence of DN. Furthermore, the references they cited for the rationale for the three anatomical sites did not actually use that exact same methodology (they measured either eight different sites, or six with one of them being the heel rather than the great toe). It is also recognized that monofilament diagnostics can be influenced by a number of variables, including the operator, how often the monofilament has been used previously, and even weather conditions (Haloua et al, 2011).

The use of a pinprick has been advocated in the past and utilized in research studies (Abbott et al,2002; Boulton et al 2008). In those studies, it was shown that inability to perceive the pinprick challenge is significantly associated with a risk of developing ulcers. However, the test does not tend to feature in current NHS guidance on screening for DN. One reason may be that in the past very rudimentary or home-made pinprick device were utilized in the absence of a fit-for-purpose device. Unlike with testing for large fibre nerve damage using the reusable monofilament device, testing for small fibre damage requires more force and therefore should always be conducted using a single use disposable device. Devices like Medipin and also Neurotips (the latter being twice the price per unit compared to the former) meet those criteria. To illustrate past suboptimal practice, in one paper by Smieja et al (1999) the following approach was taken: "Pinprick sensation was tested with a sterile or unused safety pin over the plantar aspect of the distal first, third, and fifth toe of each foot with the stimulus applied once per site.

Patients were asked to identify when they felt a sensation, and whether it was sharp or dull. Findings were scored as sharp, dull, or absent for each site".

Taken together, there is a lack of evidence concerning the use of affordable and easy-to-apply devices to diagnose small fibre DN in a real-world community setting. This study will determine the prevalence of small nerve damage using the Medipin device, and compare this to large fibre nerve damage as measured with a 10g monofilament device. Two approaches for monofilament testing will be taken, to take into account a) the variability in standard NHS practice and literature around number and locations tested b) the dorsal approach taken with the Medipin device.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVE AND OUTCOME MEASURES

Respective detection rate of diabetic neuropathy determined by use of Medipin and standard care device (10g monofilament). Pragmatic real-world approach using only devices utilised in standard community based clinics.

2.2 SECONDARY OBJECTIVES AND OUTCOME MEASURES

- Concordance level between Medipin and standard care device (10g monofilament) for real-world diagnosis of diabetic neuropathy will be determined. However, this is not a true 'quest' for concordance since the two devices are not designed to detect the same underlying nerve damage pathologies. Table 2 below outlines the different outcomes that may be had with the two diagnostic devices.

Table 2, Overview of comparison between two neuropathy tests

Test result	Medipin: patient reaction present	Medipin: patient reaction absent
10g monofilament*: patient reaction present	No sign of neuropathy	Only small fibre neuropathy
10g monofilament*: patient reaction absent	Only large fibre neuropathy	Both small and large fibre neuropathy

*Either dorsal test result or plantar test result, and scored accordingly since sum of multiple applications.

- Evaluation of feasibility that a 10-point/cm visual analogue scale (as instructed by manufacturer) can be applied for Medipin in relation to degree of neuropathy, as opposed to standard yes/no quantification. A three-option scoring system (sharp sensation, dull sensation, absence of sensation) applied earlier with pinprick testing will also be recorded (McNeely et al, 1995).

- Relative performance/concordance achieved with 10g monofilament depending on number of tests and locations included in the assessment. This will be application of the monofilament 4x on the dorsal part of the hallux versus application once on five locations on the plantar side of the foot.

3 INVESTIGATIONAL PLAN

3.1 TRIAL DESIGN AND TIMELINE

Prospective, single-centre, controlled, non-randomised, prospective evaluative diagnostic study.

Table 3 shows the anticipated timeline for the study. For this study, appropriately trained staff will conduct the Medipin and monofilament tests.

During the study period, all patients can continue to be managed and receive their standard treatment regime by their usual clinical team. Therefore, management of participants' diabetes is not affected by participating in this present study. If signs of diabetic neuropathy are identified then the patient's GP will be notified by means of a GP notification letter. The regular GP and/or diabetes practice nurse can then decide the next course of action in relation to those findings, should they deem this necessary.

Table 3, Anticipated study timeline

Month	Setup	Cohort	Analysis
Mar 2023	Submission to NRES and HRA		
	NIHR portfolio adoption		
May 2023	HRA and Trust approval	Start recruitment	
Jan 2024		Finish all recruitment	
Mar 2024			Finalise analysis & report

4 PARTICIPANTS

4.1 TRIAL PARTICIPANTS & LOCATIONS

Patients will be recruited from the adult population managed in one of 13 different GP practices, see Table 4. The GP practices will act as Patient Identification Centres, and the study activities will take place in the clinic spaces of North Cumbria Integrated Care NHS.

Table 4, List of participating GP practices

ODS CODE	name	POSTCODE	patient list (Jan2023)
A82016	Carlisle Healthcare	CA1 1DG	37516
A82047	James Street Group Practice	CA14 2DL	32999
A82044	Fellview Healthcare	CA28 7QE	24021
A82020	Eden Medical Group	CA2 7AJ	16330
A82041	Lowther Medical Centre	CA28 7RG	9971
A82045	Wigton Group Medical Practice	CA7 9QD	8980
A82654	Warwick Square Group Practice	CA1 1LB	7777
A82055	Aspatria Medical Group	CA7 3HH	6869

A82024	Seascale Health Centre	CA20 1PN	5878
A82038	Temple Sowerby Medical Practice	CA10 1RW	4882
A82058	Queen Street Medical Practice	CA28 7BA	4372
A82037	Silloth Group Medical Practice	CA7 4AH	4328
A82631	Court Thorn Surgery	CA4 0HP	3690

Identification of potentially eligible patients will be done by the Investigator/GP. Therefore, the patient will be approached initially by the clinical team caring for them. An invite letter and patient information sheet is sent out to the eligible patients and they then return a reply slip via Freepost if they are interested in participating. The patient is then contacted – they will have indicated how they prefer to be contacted on the reply slip – and they will attend a one-off study visit. The researchers will obtain written informed consent from the patients after talking them through the patient information sheet. Then the tests for diabetic neuropathy and questionnaires will be conducted.

4.2 INCLUSION & EXCLUSION CRITERIA

Inclusion criteria:

- Adult patients aged ≥ 18 years
- Patients with type I or type II diabetes mellitus

Exclusion criteria:

- Aged < 18 years
- Any reasons for the patient being unable to follow the protocol, including lack of mental capacity to consent to taking part in the study (examples include dementia, severe learning disability). Pure language barriers, eg lack of spoken English or eg deafness/blindness, are not an exclusion criterion.
- The patient has concurrent (medical) conditions that in the opinion of the investigator may compromise patient safety or study objectives (examples include receiving palliative care, active cancer treatment, patient immobile)
- Amputation of a lower limb
- Confirmed and ongoing wound / ulcer located on the foot

5 STUDY PROCEDURES

5.1 INFORMED CONSENT

Before being recruited to the clinical evaluation, the patient must have consented to participate, after the nature, scope and possible consequences of the evaluation have been explained in an understandable form. An initial invite letter and patient information leaflet will be provided to the patient via post. If patients are interested, they then return a reply slip and during the study visit the patient will provide written informed consent. Consent to take part in this research is obtained from adult patients, where they possess mental capacity.

During the consent procedure the following information will be outlined in writing, which will also be relayed verbally: a) The evaluation involves research, a description of the aims of the evaluation and how

it will be organised and the expected duration of the patient's participation; b) Any potential risks and benefits of taking part; c) The freedom to ask for further information, and to withdraw from the study, at any time; d) The extent to which confidentiality of records identifying the patients will be maintained and that the Regulatory Authorities may inspect the records.

The staff will be trained in obtaining informed consent as part of professional development, members of staff involved in the consent process will also have current ICH Good Clinical Practice training (even though this officially does not fall under the remit of Good Clinical Practice requirements).

5.2 STUDY PROCEDURES

After completing informed consent, information on relevant clinical parameters and demographics will be collected. See Table 5 for an overview of the activities conducted at the single research visit. The Medipin assessment is an added investigation, whereas the other activities are all part of a diabetes (foot) review.

Table 5, Overview of study activities for study participants at single study visit.

Activity (in this order)	Reference for rationale
Diabetes status and history	
Patient demographics	
General quality of life questionnaire (EQ-5D-5L)	Herdman et al, 2011
Michigan Neuropathy Screening Instrument symptom questionnaire	Moghtaderi et al, 2006
10g Monofilament test plantar side, five plantar locations on both left and right foot	GP notebook online resource
10g Monofilament test: four applications on dorsal side of hallux, proximal to toenail, on both left and right foot	Canadian Journal Diabetes, 2018
Medipin test: one application on dorsal side of hallux, proximal to toenail, on both left and right foot	Boulton et al, 2008

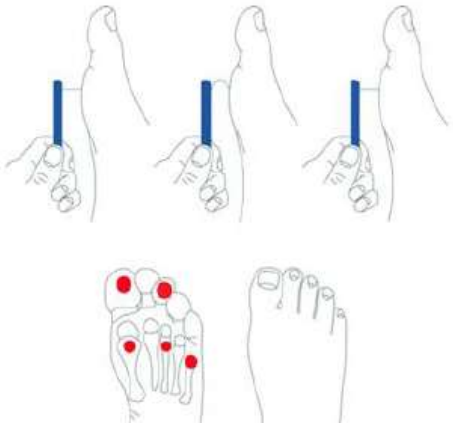
5.3 DESCRIPTION OF TESTS FOR DN

10g Monofilament test plantar side, multiple locations

https://gpnotebook.com/simplepage.cfm?ID=x20200630104981911 28 . Scoring system based on five sites on each foot = 10 sites; if score of 8 or less then indicative of neuropathy (Baker 2011; Boulton et al 2008)

- Apply the filament to a sensitive area of skin (e.g. the forearm) so that the patient is aware of the sensation they are supposed to feel.
- Test 5 sites* on both feet:
  - ✔ Plantar surface of the hallux and 3rd toe
  - ✔ 1st, 3rd and 5th metatarsal heads
  - *If callus is present at any of the sites then test at the nearest non-calloused area.
- Ask the patient to close their eyes and say 'yes' every time that they feel you touch the skin on the foot
- Place the monofilament at 90° to the skin surface
- Slowly push the monofilament until it has bent ~ 1cm (don't jab)
- Hold the monofilament in this position for 1-2 seconds, then slowly release the pressure until the monofilament is straight
- Remove contact from the skin
- If the patient does not respond, repeat the test at the site twice. If there is still no response, record as a negative response

- **Maximum score 10. A score of 8 or less indicates neuropathy**
- **Replace monofilament after 500 uses (approximately 6 monthly frequent testing, yearly infrequent testing)**



10g Monofilament test: four applications on dorsal side of hallux, proximal to toenail, on both left and right foot

A reference from Canadian Diabetes is used for this purpose,
https://www.canadianjournalofdiabetes.com/article/S1499-2671(17)30866-3/fulltext

1. Touch patient with monofilament on the forearm to establish that sensation is understood
2. Instruct patient to say 'yes' every time stimulus is felt. Answer can be 'yes, less than forearm' or 'yes, same or more as forearm'.
3. With patient's eyes closed, apply the monofilament to the dorsal area of the great proximal to the nail bed. Apply monofilament for one second each time, bending the filament. Ask patient where possible to identify and grade the quantity of deficit between them and the 'control' area as per point 2.
4. Perform the stimulus a total of 4 times, each time having the patient score the test (if sensation felt)
5. Total of 8 applications, and score can be 0 if stimulus not perceived, 0.5 if perceived substantially less than on forearm and score of 1 if perceive same or more as on forearm. Score below 3.5 means DN present, score of 3.5 to 5 means risk of DN within next four years, and score 5.5 or higher means low risk of neuropathy.

Figure 1. Application of monofilament on dorsal side of hallux

Medipin test: one application on dorsal side of hallux, proximal to toenail, on both left and right foot

1. Break tab to expose point - avoid contact with fingers.

2. Grasp device between thumb and index finger lightly enough to permit slight axial slippage if required - utilize textured surface to facilitate control.

3. Apply to skin at a perpendicular to standardize point pressure for improved test consistency and optimize annular contact to generate a 'centre surround' field of enhanced acuity. Establish a control area in an unaffected region (forearm) with an 'average' stimulation level by making several quick applications around the same locality for about 5-10 seconds. Press firmly but carefully using a repetitive, percussive contact.

Avoid high amplitude or 'stabbing' actions - penetration is checked by the annulus but never assumed 'impossible'. Instruct your patient this "normal" area represents a 'sharp' sensation and that this equates to a score of 5 out of 10 (on a 10cm scale).

4. With patient's eyes closed, apply the Medipin once to the dorsal area of the great proximal to the nail bed. Ask patient where possible to identify and grade the quantity of deficit between them and the 'control' area. Answer can be no sensation, 'dull' sensation, and 'sharp' sensation. Patient will also score the sensation on a 10cm visual analogue scale.

5. To prevent re-use destroy point by compression against a hard surface and/or dispose of in a biohazard container.

Figure 2. Medipin device.

5.4 DEFINITION OF END OF STUDY

For participants the study involves only one clinic visit, and once complete then all study involvement is complete. The study itself therefore ends once the last participant has been seen for the single study visit.

5.5 DISCONTINUATION OR WITHDRAWAL OF PARTICIPANTS

Each participant has the right to withdraw from the study at any time. Since this concerns a single visit study, data already collated as part of the study will be retained if a subject withdraws from the study. Participants do not need to give a reason for study withdrawal and their normal clinical care will not be affected should they decide to discontinue participating in the study.

5.6 SOURCE DATA

Source data will include patient's GP records and the Case Record Form for the results of the neuropathy detection test results. Medipin Ltd will have no access to patient data other than pseudo-anonymised data for the test results.

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

6 EVALUATION PRODUCT

6.1 DESCRIPTION OF MEDIPIN MEDICAL DEVICE

CE-marked neuropathy test device: Medipin (MHRA No. 1321). Medipin is a single-use precision instrument designed to optimize cutaneous pinprick perception. Medipin's protected point is designed to significantly enhance pinprick acuity to achieve useful stimulation and reduce risk of damaging delicate skin. The protective annulus inhibits depth of penetration and protects against self-inflicted "needle stick" injury.

6.2 DISTRIBUTION & ACCOUNTABILITY

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

7 SAFETY

7.1 SAFETY DEFINITIONS

Table 5, Description of different adverse event reporting definitions.

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

7.2 PROCEDURES FOR RECORDING ADVERSE EVENTS

All SAEs need to be reported to the sponsor/host Trust R&D immediately and within no more than 24 hours of the investigator team becoming aware of them.

The only devices to be in contact with the patient is the Medipin device and also the 10g monofilament device. These are both minimally-invasive devices, the patient's skin is not pierced through or damaged in proceedings.

7.3 CAUSALITY

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

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

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

8 Statistical consideration and data analysis plan

8.1 GENERAL AND BASELINE CHARACTERISTICS

The numbers of patients entering the study will be recorded, as will be number of any study withdrawals. Adverse events will also be recorded – adverse events are only included if the event occurs during the actual study clinic visit. Any preceding or subsequent events will not be considered an adverse event.

In order to describe the sample and facilitate analysis of objectives, the following characteristics and parameters will be collated, either from the patient or the patients' records (using EMIS patient clinical record system), see also Appendix 1:

- Patient demographics, including age, sex and body mass index
- Pre-existing co-morbidities, including peripheral arterial disease, medical treatment for high blood pressure, and foot/toe malformations.
- Use of diabetes related medication

8.2 SAMPLE SIZE CALCULATION

Since Medipin and monofilament do not assess the same nerve types, a true concordance study cannot be conducted. Hence a sample size is calculated for the prevalence of diabetic neuropathy being present. For the sake of a sample size calculation the assumption is that both tests will detect diabetic neuropathy in a conservative 10% of the sampled population. In the literature a ~20% prevalence of diabetic neuropathy in diabetics is reported but many of these reports are from nearly 30 years ago and therefore a more modest percentage is assumed here. Since it is not expected that the concordance will be high due to the different fibres being tested, calculation of a sample size with sufficient power based on a hypothetical concordance rate is not included here.

Using an online calculator, https://sampsize.sourceforge.net/iface/ , with the predetermined criteria of a population of 160,000, 95% confidence interval, and precision of 5%, the required sample size is 139 patients.

Subject to submission of an amendment and appraisal of how patient recruitment into the initial cohort fares, we would like to extend the study to detect 2.5% precision. Again assuming 10% prevalence (which will be reviewed once initial 139 patients have been recruited), 95% confidence interval, and the stricter 2.5% precision, a revised new total of 552 patients will be required.

8.3 PRIMARY OUTCOME STATISTICS

The Medipin result and monofilament result (either dorsal or plantar outcome) will be presented with descriptive statistics to show percentage of patients with LOPS / DN.

Table 6, Definition and score cut-offs for different DN tests (based on published papers).

Test	DN Absent	DN Present
10g Monofilament test plantar side, five plantar locations on both left and right foot	Score of 9 or 10 (out of 10)	Score of 8 or lower
10g Monofilament test: four applications on dorsal side of hallux, proximal to toenail, on both left and right foot	Score of 3.5 or higher (out of 8). Recognising that score 3.5 to 5 considered elevated risk of DN in original reference article.	Score of 3 or lower
Medipin test: one application on dorsal side of hallux, proximal to toenail, on both left and right foot	Sensation in both feet (either 'dull' or 'sharp' sensation, and score of above 0 out of 10)	No sensation in one or both feet

8.4 SECONDARY OUTCOME STATISTICS

Evaluation of feasibility that a (Likert) scale can be applied for Medipin in relation to degree of neuropathy, as opposed to standard yes/no quantification and no sensation/dull sensation/sharp sensation distribution. For this, descriptive statistics will be used to present how results are distributed amongst these three outcome modalities. Mann-Whitney U-test will be used to compare median 10-point score between those with present and absent DN respectively (ie yes/no groups), to determine if the median value differs. Similarly, Kruskall-Wallis test will be used to compare median score between the three groups of no sensation/dull sensation/sharp sensation.

Concordance between Medipin and 10g monofilament test results. The binary outcome , DN present or absent, will be used to conduct concordance and distribution comparisons. Cohen's Kappa value will be calculated to assess level of concordance. McNemar's test for paired data will be used to compare distribution of outcomes between Medipin and each 10g monofilament procedure outcome.

Relative performance/concordance achieved with 10g monofilament depending on number of tests and locations included in the assessment. As above for concordance measurement between Medipin outcome and respective 10g monofilament outcomes, the same test is conducted to compare the two different 10g monofilament diagnostic test procedures.

The patient's age, years of diabetes, smoking status, blood pressure medication and diabetes medication, foot malformations, general quality of life (EQ-5Q-DL) , and MNSI symptom questionnaire score are primarily used to define the cohort on which the DN screening has been conducted. Parameters collated at the clinic visit will be recorded and presented in a tabulated format. No identifiable data will be presented, only averages and totals.

However, binary logistic regression may be applied to evaluate if any parameters are associated with Medipin positive tests, with the the Medipin result being the dependent variable. Likewise, this regression analysis can also be used with either of the monofilament test outcomes as the dependent. This will then

allow comparison of variables associated with small fibre and large fibre screening. This type of analysis may only be feasible with the potential larger sample size outlined in Section 8.2 of this protocol. Odds ratios (logistic) will be expressed as Beta with 95% confidence intervals.

Analysis will be performed on a per protocol basis since there will be no loss to follow-up, and inferential statistics will be performed on pooled data. Data will first be collated in Microsoft Excel, followed by analyses performed using SPSS v24.

9 Data handling and Monitoring

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

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

All paper data will be held in secure locked environments in the office of the Research & Development department in Cumberland Infirmary, Carlisle, North Cumbria Integrated Care NHS Foundation Trust. Electronic data will be saved on the patient management system such as EMIS, and also a password protected research database. Data released (e.g. by publication) will contain no information that could lead to the identification of an individual participant. Upon completion of the study the site files will be archived for a period of 10 years in line with local archiving policy and procedures.

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

Final data, will be shared with Medipin Ltd company in pseudo-anonymised form.

10 Governance of study

10.1 APPROVALS

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

10.2 SPONSOR & INDEMNITY

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

Patients will not be given financial incentives for taking part in the study. Travel expenses are not offered in this study since patients are ideally seen when they attend their regular clinical appointment.

11 Publication and data-sharing policy

The results of this study will potentially be disseminated through:

- Peer-reviewed scientific journal
- Internal report

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

12 References and Further Reading

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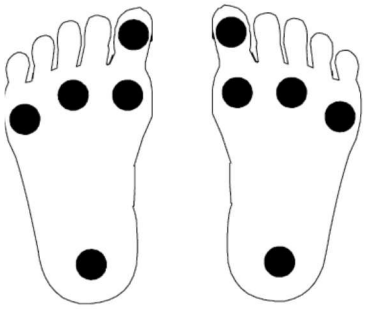
Appendix 1, Examples of Monofilament practice variation

Sussex Community NHS:

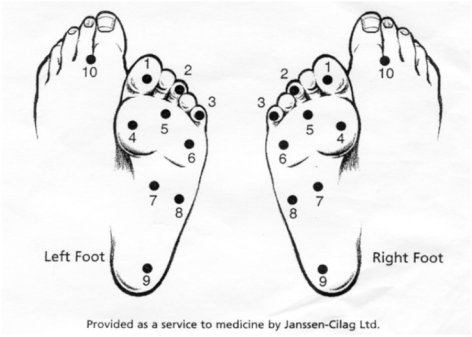
Test for neuropathy with a 10g monofilament (neuropathy is present if sensation is lost at any site)



1. Demonstrate on the patient's forearm, so they know what the sensation feels like.
2. Place the tip of the monofilament on the skin and apply pressure until it bows.
3. Use the monofilament to examine all the test sites on the feet – ask the patient to close their eyes and say "yes" when they feel the filament.
4. Do not test over callus – sensation will be reduced
5. Neuropathy is present if sensation is lost at any site.

Northern Devon and Leicestershire NHS:

The 10 sites to be tested (see figure). Loss of protective sensation = No feeling in less than 8 sites



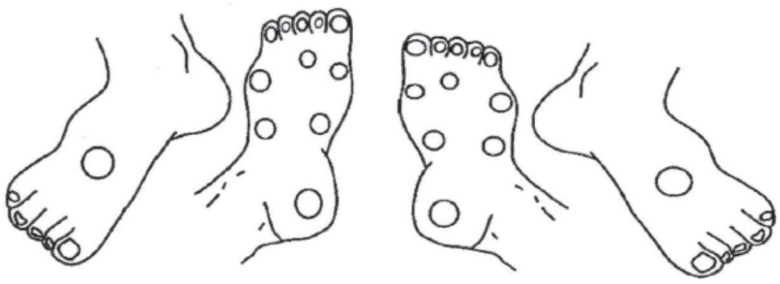
NHS Borders podiatry service:

Monofilament testing is the best current method of predicting ulceration due to loss of protective sensation. Test on all five sites in each foot (as per the chart). If the patient feels less than eight applications (in total) of the Monofilament they are considered to have loss of protective sensation.

Loss of protective sensation is a major factor in the aetiology of diabetic foot ulcers.

East Lancashire NHS:

Use 10g monofilament and use on the marked sites (apex all toes, 1/3/5 met heads, medial and lateral arch and heel). No problems is 0, any loss of sensation is 1.

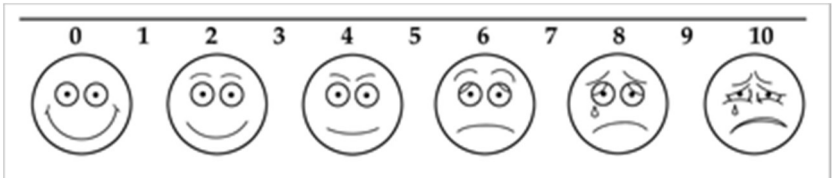
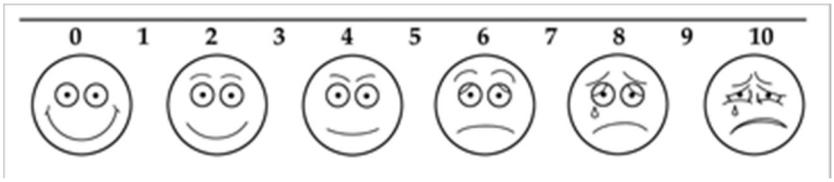


Appendix 1, Case Report Form

Patient Code: MANDARIN-…….

Researcher completing form: …….

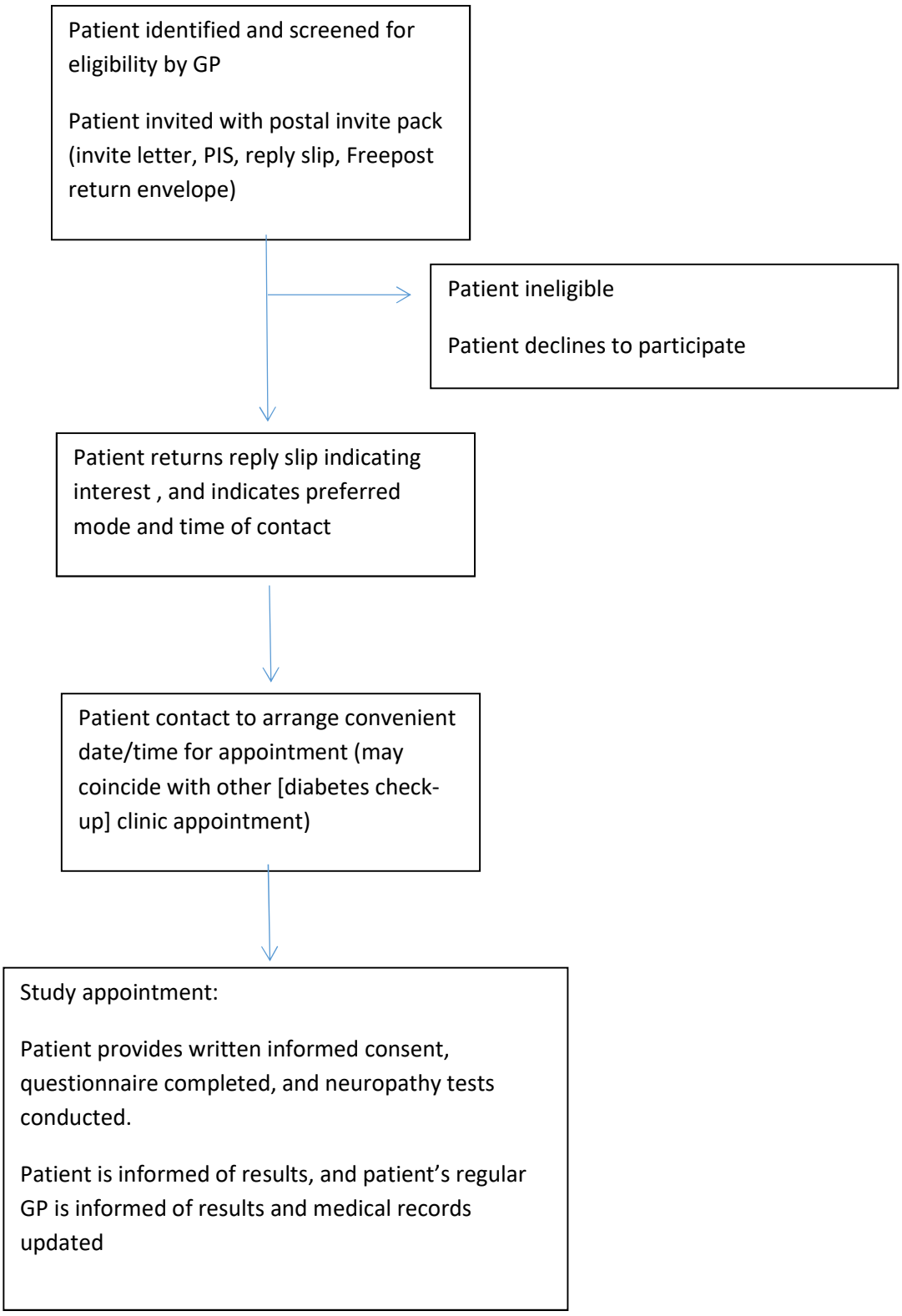
item	outcome
Patient visit date	
Patient age	Years
Patient height / weight / BMI	Height (m) Weight (kg) BMI
Year type II diabetes diagnosed	
Smoking status	Never Ex-smoker Current smoker
Peripheral Arterial Disease (PAD) recorded in notes	No Yes
Patient on antihypertensive medication?	No Yes (if yes, which medication?)
Foot and/or toe malformation recorded in notes	No Yes If yes: - Toes - Foot - Both toes and foot
Current medication for diabetes	Biguanides (Metformin) Alpha-glucosidase inhibitors (eg acarbose miglitol (Glyset)) Dipeptidyl peptidase-4 (DPP-4) inhibitors (eg alogliptin (Nesina), linagliptin (Tradjenta), saxagliptin (Onglyza), sitagliptin (Januvia) Glucagon-like peptide-1 receptor agonists (GLP-1 receptor agonists) (eg dulaglutide (Trulicity), exenatide (Byetta), liraglutide (Saxenda, Victoza) lixisenatide (Adylyxin), semaglutide (Ozempic), tirzepatide (Mounjaro) Meglitinides (eg nateglinide (Starlix), repaglinide (Prandin)) Sodium-glucose transporter (SGLT) 2 inhibitors (eg canagliflozin (Invokana), dapagliflozin (Farxiga), empagliflozin (Jardiance), ertugliflozin (Steglatro) Sulfonylureas (eg glimepiride (Amaryl), gliclazide Glipizide, glyburide (Glynase), glyburide-metformin)

	Thiazolidinediones (eg rosiglitazone, pioglitazone-alogliptin (Oseni)) Insulin - rapid- or short-acting insulin. - mixed insulin. - long-acting insulin.
Medipin outcome (dorsal, hallux)	Left foot (patient's left foot) - Sharp sensation - Dull sensation - Absent sensation - Score if sensation felt (5 being forearm reference score):  Right foot - Sharp sensation - Dull sensation - Absent sensation - Score if sensation felt (5 being forearm reference score): 
Monofilament 10g (dorsal, hallux)	Left foot (1st application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation Left foot (2nd application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation Left foot (3rd application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation Left foot (4th application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation

	Right foot (1st application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation Right foot (2nd application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation Right foot (3rd application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation Right foot (4th application) - Present sensation (same or more as forearm) - Present sensation (substantially less than forearm) - Absent sensation
Monofilament 10g (plantar: hallux, third toe, the first metatarsal, the third metatarsal, and the fifth metatarsals)	Left foot Hallux (plantar) - Present sensation - Absent sensation - Third toe (plantar) - Present sensation - Absent sensation First metatarsal - Present sensation - Absent sensation Third metatarsal - Present sensation - Absent sensation Fifth metatarsal - Present sensation - Absent sensation Right foot Hallux (plantar) - Present sensation - Absent sensation - Third toe (plantar) - Present sensation - Absent sensation First metatarsal - Present sensation

	- Absent sensation Third metatarsal - Present sensation - Absent sensation Fifth metatarsal - Present sensation - Absent sensation
Michigan Neuropathy Screening Instrument symptom questionnaire completed (yes / no)	
QoL EQ-5D-5L completed (yes / no)	

Appendix 2, Study participant flowchart



Appendix 3, Quality of life: EQ-5D-5L

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

MOBILITY

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

SELF-CARE

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

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

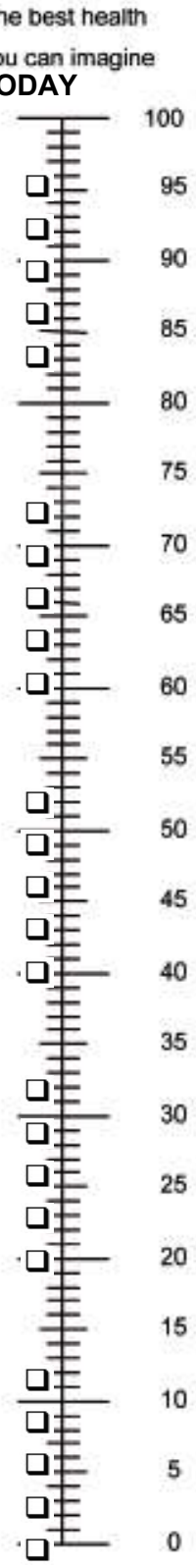
I have no problems doing my usual activities
I have slight problems doing my usual activities
I have moderate problems doing my usual activities
I have severe problems doing my usual activities
I am unable to do my usual activities

PAIN / DISCOMFORT

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

ANXIETY / DEPRESSION

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



EQ-5D

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

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

YOUR HEALTH TODAY = ☐

Appendix 4, Michigan Neuropathy Screening Instrument symptom questionnaire

MNSI symptom questionnaire

1. Are your legs and/or feet numb?	Yes / No
2. Do you ever have any burning pain in your legs and/or feet?	Yes / No
3. Are your feet too sensitive to touch?	Yes / No
4. Do you get muscle cramps in your legs and/or feet?	Yes / No
5. Do you ever have any prickling feelings in your legs or feet?	Yes / No
6. Does it hurt when the bed covers touch your skin?	Yes / No
7. When you get into the bath or shower, can you tell hot water from cold water?	Yes / No
8. Have you ever had an open sore (ulcer) on your foot?	Yes / No
9. Has your doctor ever told you that you have diabetic neuropathy?	Yes / No
10. Do you feel weak all over most of the time?	Yes / No
11. Are your symptoms worse at night?	Yes / No
12. Do your legs hurt when you walk?	Yes / No
13. Are you able to sense your feet when you walk?	Yes / No
14. Is the skin on your feet so dry that it cracks open?	Yes / No
15. Have you ever had a (toe/foot/leg) amputation?	Yes / No

Total number of reported symptoms based on composite score of MNSI symptom questionnaire "yes" responses, with questions 7 and 13 reverse-scored.

From Ylitalo et al (2013):

Questions 1, 8, 13, and 15 are considered indicators of large fibre nerve dysfunction.

Questions 2, 3, 5, 6, 7, and 14 are considered indicators of small fibre nerve dysfunction