

Study Protocol

Verifying the validity of urinary kryptopyrrole (UKP) testing in an adult population: Protocol for a multi-stage research project

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ABSTRACT

Introduction: Urinary kryptopyrroles (UKP) are described as pyrrole compounds excreted in urine and have been associated with a broad range of signs and symptoms. However, causality between elevated UKP and the described symptoms of pyrrole disorder have not been established. A four stage project has been developed to verify the validity of a urinary kryptopyrroles test and attempt to establish a reference range for a healthy population.

Methods: Stage one will consist of a survey to clinicians who are currently using UKP testing in their practice to understand their perceptions of the clinical value and applicability of the test in addition to identifying the condition for stage three. Stage two consists of a clinical trial to establish a normal reference range for UKP in a healthy population. Stage three is a clinical trial exploring the correlation, if any, between UKP in a diagnosed health condition to the healthy population group. Stage four is a pragmatic trial assessing UKP levels in patients as used by clinicians as part of routine clinical practice.

Recruitment: Recruitment for stage one will be via email invitation to SAFE Analytical laboratories Pty Ltd customer database. Stage two and three will be targeting the wider community in the greater Brisbane area in Queensland and aims to recruit 120 participants per stage. Recruitment for stage four will be conducted through community based clinical practices.

Conclusion: At the conclusion of all stage, the validation of the UKP test from SAFE Analytical laboratories will be analysed. Formulation of a reference range for a healthy population range will be explored and compared to a diagnosed condition and other conditions as tested by clinicians in practice.

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1. Introduction

The following protocol paper has been written in accordance with the SPIRIT guidelines [1].

1.1. Background and rationale

The clinical manifestation of elevated urinary kryptopyrroles (UKP) – defined as pyrrole compounds excreted in urine – is referred to as pyrrole disorder (PD) [2]. PD is reportedly associated with a broad range of signs and symptoms, notably psychological phenomena including extreme mood swings, sensitivity to light and noise, poor stress control, severe anxiety, explosive anger, and

poor dream recall [2]. However, causality between elevated UKP and the described symptoms of PD have not been established.

In 1958, the Hoffer group detected a compound in the urine of psychiatric patients that would stain purple on paper chromatograms (dubbed “the mauve factor”, or simply “Mauve”) [3]. Investigations lead to the classification of this compound within the pyrrole class. The research group reported an observed correlation between the severity of certain signs and symptoms and the presence of the pyrrole compound in patients. Further investigation by this group resulted in identification of the first pyrrole compound, named *kryptopyrrole* (KPL; CAS number 517-22-6). However, further investigations later re-identified this compound as *hydroxyhemopyrrolin-2-one* (HPL; CAS numbers 41254-89-1 and 53573-42-5, exact stereoisomer not defined) [3]. (Both structures are pictured in Fig. 1).

Despite UKP being identified over 40 years ago there is still significant ambiguity and controversy regarding its clinical value. An unpublished literature review conducted by the research team,

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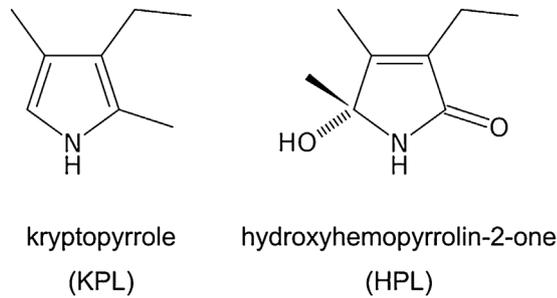


Fig. 1. Chemical structure of kryptopyrrole and hydroxyhemopyrrolin-2-one.

for example, has found substantial conflict and gaps in the empirical research regarding the PD and the Mauve Factor including no information about a normal reference range for health adults [1]. Despite these gaps, researchers have employed urinary pyrroles as an endpoint biological test in Australian clinical research [2], and as a primary outcome measure for research in both Poland [3] and Australia [4]. However, as of 2018, no UKP test has been registered for therapeutic use in Australia [5,6]. In Australia, laboratory tests to manage human patients are classified as in vitro diagnostic medical devices (IVDs), governed by the Therapeutic Goods Administration (TGA) [4]. However, prior to recent changes to TGA requirements, UKP testing was used by integrative medicine health professionals as part of their diagnostic toolkit. In light of the gaps in empirical evidence and the interest in UKP testing among both clinician and researcher communities, further research to establish the clinical significance of UKP testing is needed.

1.2. Objectives

The major objective of this study is to verify the validity of the use of UKP testing. To achieve this, several aspects of UKP testing must be examined: (1) establishing a normal reference range for UKP; (2) determine if a correlation exists between UKP levels and a diagnosed health condition; and (3) describe the clinical value of UKP.

2. Trial design

The study employs a multi-phase observational design. No interventions are administered as part of the study. The four stages are as follows:

2.1. Stage 1: clinician survey

Stage 1 surveys clinicians previously using UKP testing within their practice to understand their perceptions of the clinical value and applicability of the test. The data from this stage will be used to identify the condition most commonly associated with elevated UKP levels, which will inform the sample population chosen for Stage 3 of this study. An overview of Stage 1 is shown in Fig. 2.

2.2. Stage 2: UKP levels in a healthy adult population

Stage 2 seeks to establish a normal reference range for UKP in a healthy adult population. An overview of Stage 2 is seen in Fig. 3.

2.3. Stage 3: UKP levels in an adult population with a diagnosed health condition

Stage 3 explores the correlation, if any, between UKP and a diagnosed health condition. An overview of Stage 3 is seen in Fig. 3.

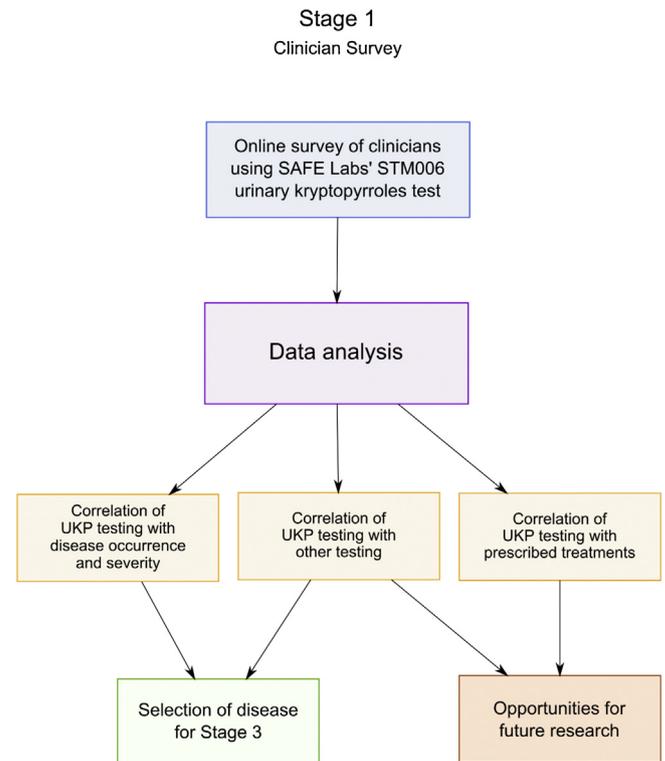


Fig. 2. Timeline of Stage 1 and integration of survey results with Stage 3.

2.4. Stage 4: case control study

Stage 4 describes UKP levels in patients as used by clinicians as part of routine clinical practice. An overview of Stage 4 is seen in Fig. 4.

3. Methods: participants, interventions, UKP test, and outcomes

3.1. Study setting

All data for this study will be collected in Australia. Stage 1 is an online survey of clinicians. Stages 2 and 3 are to be conducted in a clinical environment at the Endeavour College of Natural Health Brisbane (Fortitude Valley) campus. Stage 4 is to be conducted through treatment rooms of individual clinicians in clinics around Australia.

3.2. Eligibility criteria

3.2.1. Stage 1

Clinicians who identify as having used UKP tests as part of their clinical management of patients prior to the changes to TGA legislation affecting access to UKP tests are able to participate in the survey.

3.2.2. Stage 2

Healthy adults aged 18 years or more who have no currently active diagnosed health condition. Exclusion criteria include use of any medication or other drugs, or women who are pregnant or breastfeeding.

3.2.3. Stage 3

Adults aged 18 years or more who have a current diagnosis of a predetermined health condition (informed by the findings of Stage

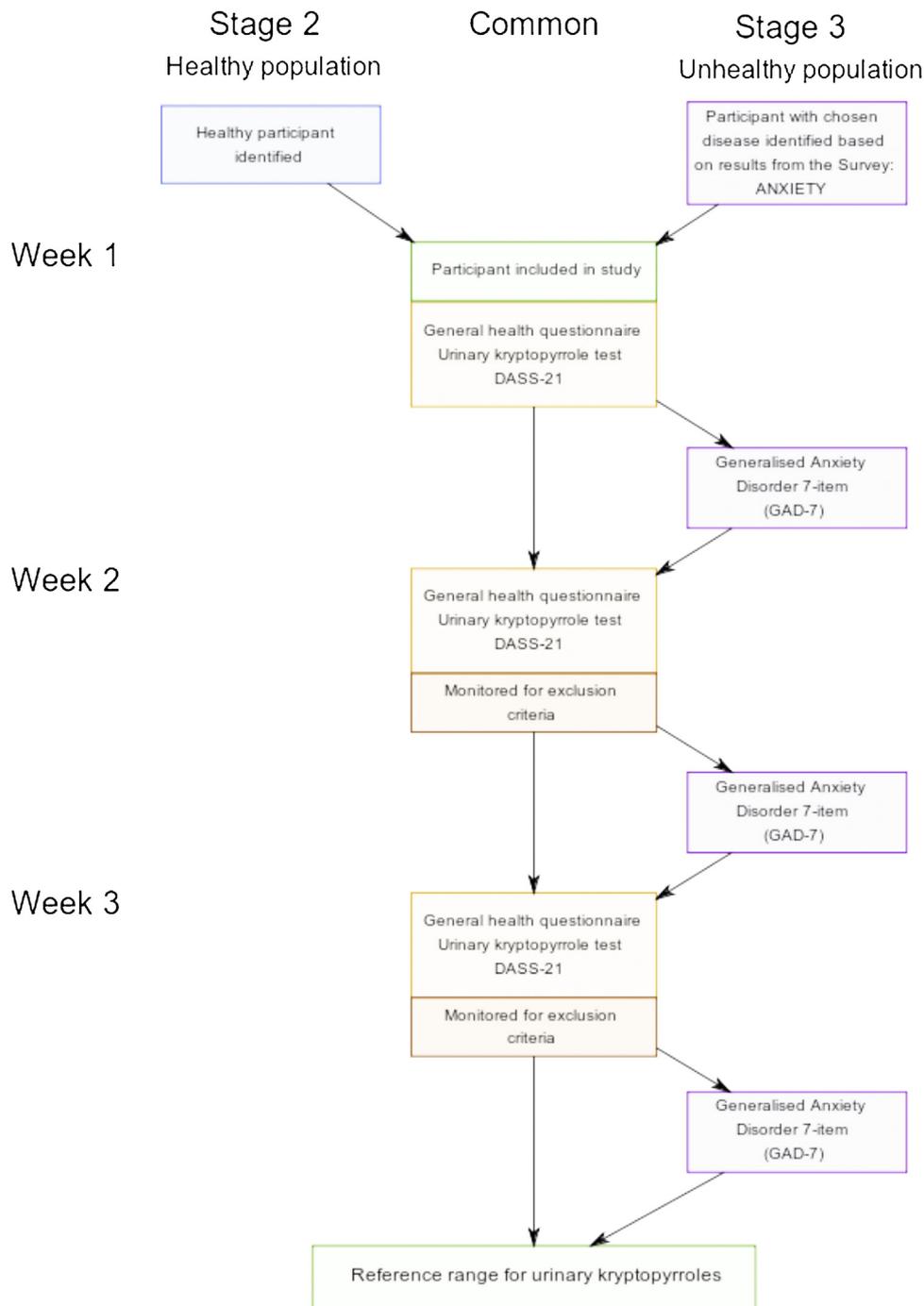


Fig. 3. Timeline for Stages 2 and 3.

1). Exclusion criteria include women who are pregnant or breastfeeding.

3.2.4. Stage 4

Patients aged 18 years or more who are identified by a clinician as potentially benefiting from UKP testing to inform clinical care. Patients are considered to potentially benefit if clinicians would have considered UKP testing to inform clinical care prior to changes to TGA legislation. Exclusion criteria include women who are pregnant or breastfeeding.

3.3. Interventions

This is an observational study, there are no interventions.

3.4. The UKP test

Urinary kryptopyrrole testing in Stages 2, 3 and 4 will be carried out by SAFE Analytical Laboratories Pty Ltd using a proprietary test method (STM006) based upon methodology described previously by A Sohler, E Holsztyimska and C Pfeiffer [5]. The test is a colorimetric assay using Ehrlich's reagent. Due to the fragile nature of HPL and KP, measures are taken to protect samples from heat, light, and oxidation, including immediate freezing of collected samples, transport on dry ice, not re-freezing samples, and using control samples preserved with L-ascorbic acid. Urine specific gravity is used to control for hydration status. Collection time of all samples is taken in to account. The analytical method being

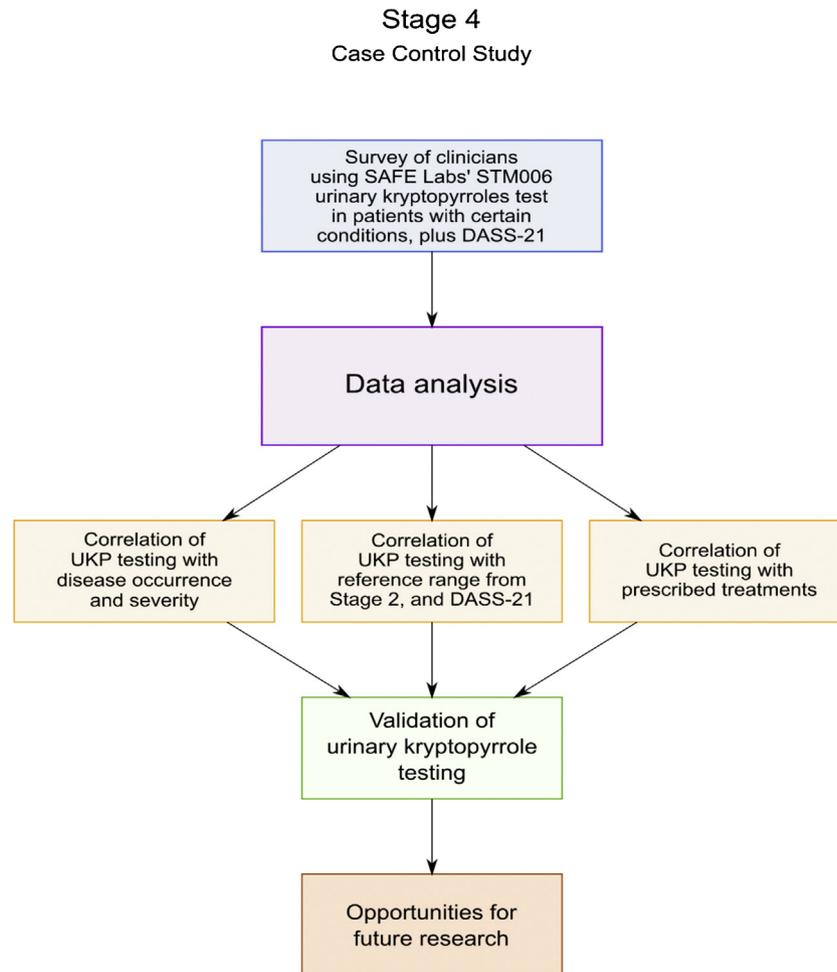


Fig. 4. Timeline for Stages 2 and 3.

studied does not control for elevated levels of urobilinogen, a tetrapyrrolic, Ehrlich-reactive substance present in urine [6]. Due to the analytes in question (HPL and KP) also being Ehrlich-reactive, the presence of significantly elevated urobilinogen may lead to positively biased results. Further research would be required to determine the impact of urobilinogen in samples on the results reported. This limitation will be reported in any publications arising from Stages 2, 3 or 4 of this study.

3.5. Outcomes

3.5.1. Stage 1

The primary outcome for the survey is the health condition most frequently identified as correlated with elevated UKP as described by clinicians. The survey will also be used to determine the clinical treatments and adjunct tests reported as useful alongside UKP testing.

3.5.2. Stage 2 and 3

The primary outcome is the level of UKP as provided by SAFE Analytical Laboratories Pty Ltd (test code STM006) for a healthy population range and for a population of people diagnosed with anxiety.

3.5.3. Stage 4

The primary outcome is the correlation of UKP levels (SAFE Analytical Laboratories Pty Ltd [test code STM006]) of different

diseases from practitioners around Australia to the reference level found in Stage 2 of healthy adults.

3.6. Participant timeline

The Stage 1 survey will be open for four months for practitioners to answer the online survey (see Fig. 2). Stage 2 and 3 follows participants for three visits, each one week apart (see Fig. 3). Stage 4 follows patients between 1 visit to 2 or more depending on the practitioner and how many times they test the patient using the UKP test from SAFE Analytical Laboratories Pty Ltd (test code STM006) (see Fig. 4).

3.7. Sample size

Population samples will be obtained via convenience and simple random sampling.

Stage 1: 50 or more (convenience sampling).

Stage 2: 120 (simple random sampling).

Stage 3: 120 (simple random sampling).

Stage 4: 50 or more (convenience sampling).

3.7.1. Sample size calculation: stage 2 and 3

The sample size calculation for references ranges from a pathology test is different to interventional clinical trials. As reference ranges are values used to describe the dispersion of variables in healthy individuals, it is based on population-based

reference intervals comprising of 95% of the health population. The international standard for nonparametric determination for reference ranges is 120 reference individuals in a trial [7]. To confirm the number of people required for each stage, previous studies examining reference intervals were examined and calculated for sample sizes [8,9].

3.7.2. Sample size calculation: stage 4

No sample size calculation was conducted for stage 4 with only a minimum number given for convenience sampling. Numbers will depend on the number of clinicians who agree to participate and assist in recruitment and the number of participants tested by each clinician.

3.8. Recruitment

3.8.1. Stage 1

Recruitment for Stage 1 will occur via email invitation to SAFE Analytical Laboratories Pty Ltd's customer database and targeted social media groups comprising clinicians known or likely to have used UKP testing prior to changes to TGA legislation.

3.8.2. Stages 2 and 3

Recruitment for Stages 2 and 3 will occur in only the greater Brisbane area in Queensland, Australia. The advertising of the trial will occur through online social media platforms, flyers in local businesses, advertising at the Endeavour College of Natural Health both in Brisbane and the Gold Coast and radio and public forums.

3.8.3. Stage 4

Recruitment for Stage 4 is conducted through community-based clinical practices. Clinician's assisting with the study will invite any patients for whom they would usually undertake UKP testing to participate in the study. Clinicians will be invited to assist with the study via SAFE Analytical Laboratories Pty Ltd whereby an email invitation as sent to all Australian clinicians registered on their customer database as having used the SAFE Analytical Laboratories Pty Ltd's STM006 test prior to changes to the TGA legislation.

3.9. Data collection methods

Data collection in the various stages of this study are as follows:

3.9.1. Stage 1

A survey administered via the online platform SurveyGizmo. The survey will include questions related to practice characteristics, clinical observations between UKP and diagnosed health conditions, clinical management informed by UKP testing (e.g. adjunctive testing, treatment choices), and observed associations between elevated UKP and potential causative factors or clinical outcomes.

3.9.2. Stage 2 and 3

Urinary kryptopyrrole (UKP) samples will be collected in a registered biological specimens collection centre. Other relevant participant information will be collected on paper by the research team. Instruments to be used are the Depression, Anxiety Stress Scales (DASS-21) [10] and an additional validated instrument relevant to the selected health condition (Stage 3 only). Participant socio-demographics, health behaviours and medication use will be collected via a participant case report.

3.9.3. Stage 4

The mode of data collection in Stage 4 will include a case report form that includes basic participant socio-demographics, medical

history, current medication and complementary medicine supplementation, other pathological tests being conducted, the treatment the clinician prescribed from the test and the DASS-21.

3.10. Data management

3.10.1. Stage 1

Data will be stored on secure, password protected hard drive. All results will be anonymous.

3.10.2. Stage 2 and 3

Data will be stored in both hard copy and digital formats, with identifying data being stored in password protected files, and physically locked cabinets only accessed by the research team. The test results are de-identified via the participant code and are stored by their codes. As SAFE Analytical Laboratories Pty Ltd are conducting the tests, the results will be directly sent to Endeavour all under the de-identified participant number.

3.10.3. Stage 4

The patient name and clinician name will be on the results as the pathology result will be sent to the clinician as well as the researchers. Once the researchers have received the results from clinicians in Stage 4, the data manager will decode the patient's results by the clinician's initials and participant number. A password-protected spreadsheet will be used to store participant codes linked to participant names. All remaining electronic copies of the results that can identify the patient will be deleted so no patient can be identified.

3.11. Statistical methods

3.11.1. Stage 1

Descriptive frequencies and percentages for all survey items will be reported. Basic inferential statistics (i.e. chi square test) will be used to explore relationships between variables.

3.11.2. Stage 2

For the analysis of the primary outcome, the reference range will be determined by non-parametric analysis of urinary kryptopyrrole data. Secondary analysis of descriptive statistics, logistic regression and chi square analysis will be conducted to ascertain any relationships that can be identified using all information from the case report including the DASS-21, UKP results and descriptive data. Any anomalies identified will be assessed to ascertain any relationship to tests results and medication, mental state, or lifestyle events.

3.11.3. Stage 3

For the analysis of the primary data, the reference range for this specific condition group will be determined by non-parametric analysis of urinary kryptopyrrole data. Unpaired T-tests and percentage proportions will be completed to compare difference in ranges to Stage 2. Descriptive statistics for DASS-21 and GAD-7 [11] will be conducted with chi square analysis and logistic regression to determine disease severity and any other relationship. Any anomalies identified will be assessed to ascertain any relationship to tests results and medication, mental state, or lifestyle events.

3.11.4. Stage 4

Unpaired T-tests and percentage proportions will be used to compare difference in ranges to Stage 2. Descriptive statistics for DASS-21 will be conducted with chi square tests and logistic regression to determine disease severity and any other relationships. Any anomalies identified will be assessed to ascertain any

relationship to tests results and medication, mental state, or lifestyle events.

4. Ethics and dissemination

4.1. Research ethics approval

Ethical approval for the study has been granted by the Endeavour HREC (NHMRC registration code EC00358), approval number #20171111, in November 2017.

4.2. Consent or assent

4.2.1. Stage 1

Participants provide consent via an online consent form prior to accessing the survey.

4.2.2. Stages 2 and 3

The research team will be obtaining informed consent directly from participants.

4.2.3. Stage 4

Individual clinicians will be obtaining informed consent from participants.

4.3. Confidentiality

Information about the trial participants will be kept confidential and managed according to the requirements of the Australian Standards of Good Clinical Practice. The regulations require a signed participant authorisation informing the participant of the following:

In the event that a participant revokes authorisation to collect or use patient health information (PHI), the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorisation. For participants that have revoked authorisation to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

All data that can identify a participant will be kept in a password protected file on a password protected software and computer. All other data will be de-identified. For Stage 4, the patient name and clinician name will be on the results as the pathology result will be sent to the clinician as well as the researchers. Once the researchers have received the results from clinicians in Stage 4, the data manager will decode the patient's results by the clinician's initials and participant number. A password-protected spreadsheet will be used to link participant code and participant name. All remaining electronic copies of the results that can identify the patient will be deleted so no patient can be identified.

Declaration of interests

This project is funded by SAFE Analytical Laboratories Pty Ltd. The research design, implementation and analysis have been conducted by the research team independent of SAFE Analytical Laboratories Pty Ltd.

Access to data

The sponsor has access to UKP test data. The research team has access to all data. Final data will be in its de-identified, and analysed. The research team will have the only access to all data and the analysis and results will be published.

Ancillary and post-trial care

As this is an observational study, no post-study care is expected to be necessary. However, any participants experiencing adverse events will be referred for appropriate medical care.

Dissemination policy

A publishing embargo exists for 12 months after completion of the study. Results will be published in peer-reviewed journals as open-access where possible.

Trial registration

The trial is registered on the Australian New Zealand Clinical Trials Registry (ANZCTR), registration number ACTRN12618000032257.

Author Statement

The study was designed by AS and JS. All authors contributed to the drafting of this manuscript.

Ethical statement

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