The Leeds Teaching Hospitals NHS
NHS Trust

Study Title: Reference Range Project

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Sponsor:	Leeds Teaching Hospitals NHS Trust

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1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1	06/12/2010	A Luvai	Initial draft
2	2	15/03/2011	A Luvai	REC recommendations
3	3	20/06/2011	A Luvai	Alignment to C-RIDL

2. SYNOPSIS

Study Title	Reference Range Project
Study Design	Observational
Participants	Healthy volunteers among staff and visitors to LTH
Planned Sample Size	300
Study Period	01/07/2011 – 30/062/2012
Primary Objective	To determine the reference intervals of the most frequently requested analytes using all available methods and to assess the interchangeability of these reference intervals across the Yorkshire laboratories.
Secondary Objective	To keep a panel of well characterised samples that can be used for evaluation of analytes that may be employed in the future.

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
GP	General Practitioner
ICF	Informed Consent Form
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SOP	Standard Operating Procedure

4. BACKGROUND AND RATIONALE

The pathways that patients travel to diagnosis and management used to be relatively straightforward. Patients would be seen and investigated by their GP and referred, as required, to the local hospital for further management. During this process, laboratory investigations would be performed for all the involved clinical teams in a consistent manner by the local laboratory. The creation of a wider spectrum of providers and the development of clinical care networks (e.g. cancer, cardiology, renal et cetera) has introduced a new issue of data sharing and this will be particularly problematic for the laboratory investigations on which many decisions affecting outcomes and safety are based.

The main problem of data sharing is of clinical governance [1]. Laboratories use a variety of different analytical methods which are not always comparable with other laboratories; they use different units for measurement and they provide different reference ranges; all of which lead to potential problems in data interpretation which will be amplified by attempts to merge data sets. The route to raise patient safety is through standardization.

One of the key processes in laboratories that need standardisation is the provision of normal values (reference values or intervals) that are used for data interpretation. They have been routinely provided for many years as guidance but despite much exhortation to use laboratory specific values, many clinicians use values obtained from textbooks and journals. This is quite inappropriate because reference values vary in relation to age, gender, race, analytical method etc, and further confusion is caused by the use of different units (SI and arbitrary international units). All the available guidance suggests that each laboratory should investigate its own reference values but since this requires collection of at least 120 analyses from well characterised subjects, it is out of the scope of most laboratories and many use the values provided by the diagnostics industry. This has resulted in a plethora of different ranges across the country.

The potential for clinical risk caused by this variability is being exposed by distributed clinical networks which are neither geographically congruous with other specialties nor the nascent pathology networks. Furthermore, the IT networks may encompass several adjacent networks and can create further potential distortion. The only way that laboratory data can be used in a transparent manner for continuity of care will be through rationalized laboratory data. The first step of this process has been developed with the creation of a uniform messaging language, we now need to standardise the information carried.

There are models in other countries which have been developed to standardise laboratory reference values. These were pioneered in France [2] and more recently the Nordic Countries have conducted the Nordic Reference Interval Project (NORIP) with wide laboratory participation which has resulted in the creation of resultant population specific ranges across the majority of commonly used tests [3]. The Catalan Association for Clinical Laboratory Sciences [4] and the College of American Pathologists have both expressed a desire to provide such a model for their countries.

The model developed by NORIP relied on each participating laboratory collecting blood from volunteers, measuring those samples and submitting the data for central analysis of reference ranges. This permitted an overall assessment of reference ranges across the Nordic countries. We are proposing a different model in which a central library of samples would be collected and a common set of aliquots will be sent to each participating laboratory. These laboratories would analyse the samples and submit the data for central analysis. This project would be done within our geographical region but since all the common methods are represented in Yorkshire; the results would be generalisable across England and the UK.

Laboratory investigations are playing an increasingly important role in the diagnosis and management of patients in the NHS. They are being used for shared care between primary, secondary and tertiary care; as a tool for comparative clinical audit; and in target setting by the DH. However, the present lack of standardization of both clinical and laboratory systems prevents the realization of the full potential of national standards. The establishment of a means of comparing between laboratories is a requirement for data interchange. It will bring benefits for those patients and doctors who move from one hospital to another; and for the translation of research in clinical diagnostics into practice.

The aim of this study will be to collect a library of well characterised blood samples which will be analysed for all routinely measured laboratory variables (haematology & biochemistry) using all available methods across Yorkshire. This will facilitate patient pathways for the clinical networks in Yorkshire and improve patient safety.

5. OBJECTIVES

5.1 Primary Objective

To determine the reference intervals of the most frequently requested analytes using all available methods and to assess the interchangeability of these reference intervals in the Yorkshire laboratories. A parallel study will be conducted in Northern Ireland.

5.2 Secondary Objectives

To keep a panel of well characterised samples that can be used for evaluation of analytes that may be employed in the future.

6. ELIGIBILITY

6.1 Overall Description of Study Participants

Participants will be healthy volunteers recruited from among members of staff and visitors to the LTHT.

6.1.1 Inclusion Criteria

The reference individual should:

1. be feeling subjectively well.
2. be over 18 years. There is no upper age limit.
3. not be pregnant or breast-feeding for a period of 12 months.
4. not have been a hospital in-patient nor been subjectively seriously ill during the previous 4 weeks.
5. not have had any alcohol in the previous 24 hours.
6. not given blood as a donor in the previous 3 months.
7. ideally not be taking any medication but if they are taking medications, these should be recorded (medication, dose & frequency). Vitamin supplements should be included as per medications. The following medications are permitted: the contraceptive-pill or oestrogens (HRT) and thyroxine if the subject is well replaced (TSH < 6 mIU/L).
8. not smoked in the hour prior to blood sampling.

9. may have undiagnosed diabetes or diet managed diabetes - their data will be analysed separately.

6.1.2 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

1. Known diabetes on oral therapy or insulin (diet alone is acceptable).
2. Have results from their blood samples that clearly point to a disease (exclusion means that results of all analytical components are to be excluded).
3. Unusual or strenuous exercise during the previous 3 days.
4. Female participants who are pregnant or lactating.
5. History of chronic liver or kidney disease.
6. Any other significant disease or disorder which, in the opinion of the investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participants' ability to participate in the study.
7. Participants who have participated in another research study involving an investigational product in the past 12 weeks.
8. Any subject found to have a significant disease as a result of testing their blood will be excluded. This will include subjects with significant renal (eGFR <15mL/min/1.73m^2 (eGFR formula to be agreed)) or hepatic (ALT >3ULN, ALP >2ULN) impairment (values based on local laboratory limits). Other diseases resulting in exclusion will be at the discretion of the local investigator and consensus by international group.

7. ENROLMENT

7.1 Description of Enrolment Process

Subjects will be recruited by advertisements on wards and out-patient areas as well as electronic invitations to staff within the trust. Participants will be sent an information sheet and invited to attend our testing centres at either the Leeds General Infirmary or St James University Hospital. The study will be discussed and participants allowed at least 24 hours to think about participation. At a second visit which will take about thirty to forty five minutes, participants will be asked to sign an informed consent form saying that they agree to take part. A copy will be given to them for their own records.

7.2 Informed Consent

The participant will personally sign and date the approved version of the informed consent form before any study specific procedures are performed. Written and verbal information will be presented to the participants detailing the exact nature of the study and any risks involved in taking part. (Appendix E) It will be clearly stated that the participant is free to withdraw from the study at any time for any reason with no obligation to give the reason for withdrawal. The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent.

In all cases consent will be obtained by a suitably qualified health professional e.g. a research nurse or medical specialty registrar. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site.

7.3 Health Interview

Providing that they are happy to proceed, they will be asked to complete a short health interview/questionnaire about their general health. (Appendix D) It will include:

Demographics
The date of birth, gender, LMP, race, smoking pattern (0, 1-5 or more than 5 cigarettes/cigars/pipes per day) and habitual alcohol consumption of 0, 1-21 or >21 units per week (females) or 0, 1-28 and >28 units (males) will be recorded.

Medical History
Details of any history of disease in any systems will be recorded.
The total number of lifetime blood donations.

Concomitant Medication
All over-the-counter or prescription medication, iron tablets, vitamins, oral contraceptives and/or herbal supplements will be recorded.

Physical Examination
Height, weight, resting pulse and blood pressure (BP) measurements will be taken after the participant has sat for at least five minutes.

7.4 Sample Collection

The exact day and time of sampling as well as the number of hours following the preceding meal will be recorded. Subjects should rest in a seated position for 30 minutes prior to venepuncture. During this time, the subjects may complete the questionnaire or be interviewed by research staff. Locally, a minimum volume of 2.5 mL of serum and 7.5mL of EDTA blood is required for the listed analytes. We estimate that serum aliquots of 4 mL will be adequate for each participating laboratory. For the sample library a further 15mL of serum and 2.5mL EDTA plasma per volunteer will be required. The following blood samples should be taken using the preferred collection system at the participating hospital.

1. EDTA tube 4 x 2.5 mL
2. Plain tubes sufficient to collect 35 mL serum

One EDTA sample will be used to measure FBC (Full Blood Count) within 6 hours of venepuncture. The second EDTA sample will be analysed for ABO and Rhesus blood groups. The third EDTA sample will be analysed for glycated haemoglobin. The fourth EDTA sample will be separated and the plasma frozen as below for PTH analysis.

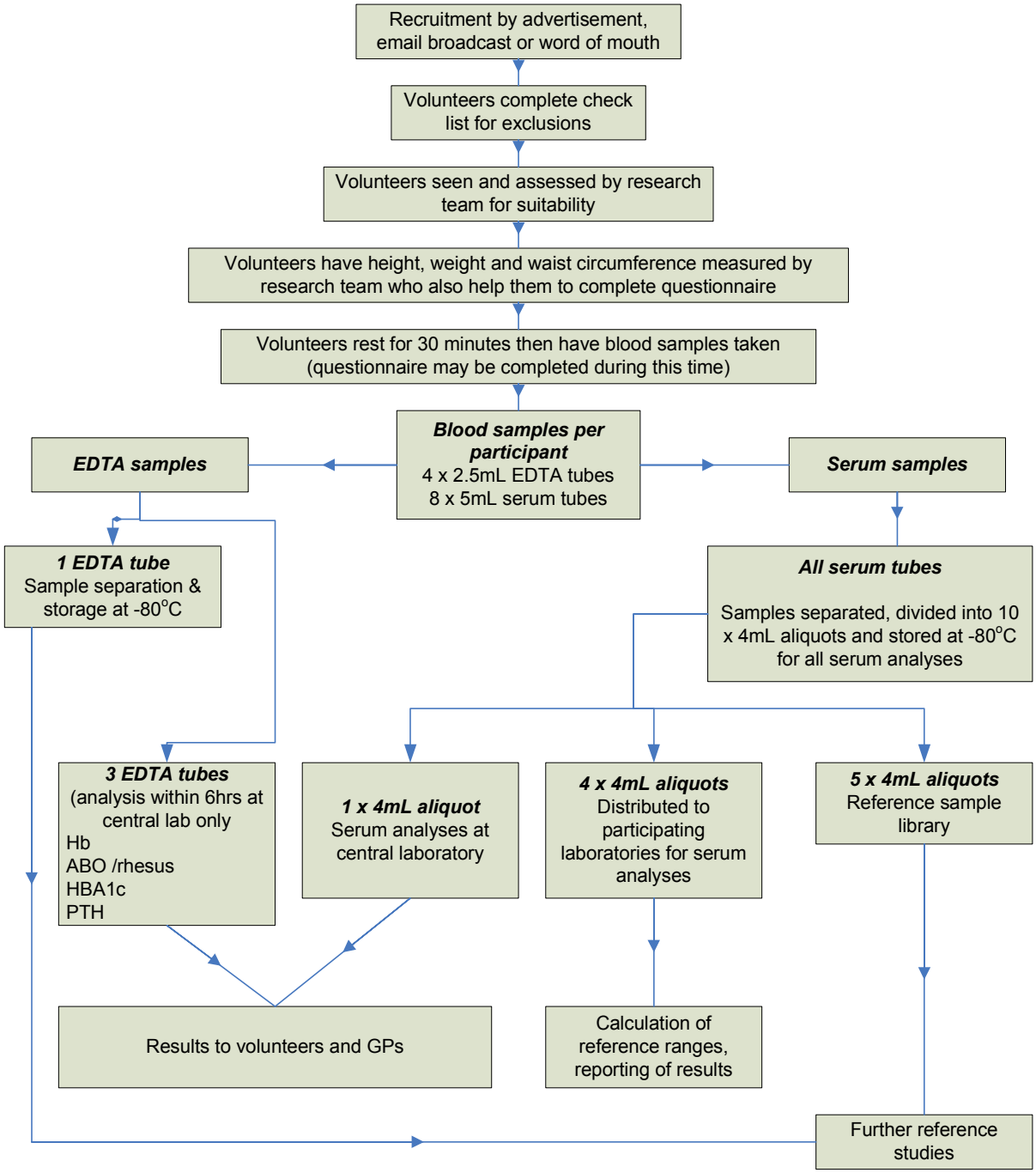
The whole blood will be allowed to clot at room temperature, then separated by centrifugation within 6 hours of venepuncture and the serum divided into ten 4 mL aliquots which will be stored at -80°C until analysis.

The following analyses will be undertaken.

FBC	Hb, WBC, Platelet count, red cell indices, blood group
U&E profiles	Sodium, potassium, bicarbonate, chloride, urea, creatinine
LFT profiles	AST, ALT, total bilirubin, albumin
Calcium profiles	Alkaline phosphatase, calcium, phosphate, magnesium
Enzymes	Amylase, creatinine kinase, gamma-glutamyltransferase, LDH
Lipids	Cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides
Other metabolites	Urate, total protein
Haematinics	Ferritin, folate, vitamin B12
Thyroid tests	TSH, fT4, TT4, fT3, TT3 (as many as are available at the site)
Other	HbA1c, Troponin, PSA, CA125
Calcium 2	PTH, Vitamin D

After venepuncture participants will be assessed to ensure that they are fit to leave the premises. A copy of their results will be sent to each participant and to their GP unless they specifically instruct otherwise. If the study uncovers seriously abnormal results, the research team will override this request and inform the GP about them. At the end of the clinic session, all samples will be conveyed to the laboratory by the research nurse/specialty registrar for processing.

7.5 Project flow chart



7.6 Target recruitment

A library of 300 samples will be collected from staff and visitors at the LTH.

7.7 Laboratory Analysis

Upon receipt in the central laboratory, the blood samples will be separated, aliquoted and stored at -20^{o} C prior to analysis. Full blood count analysis will be undertaken at the central laboratory on the day of collection. When the library is complete, serum samples and QC will then be distributed to selected laboratories in Yorkshire ensuring that all major method platforms are included i.e. Siemens, Olympus, Beckmann, Abbott and Roche. Participating laboratories will be selected from the following laboratories: Airedale, Barnsley, Bradford, Dewsbury, Halifax, Harrogate, Huddersfield, Hull, Leeds (LGI), Pinderfields, Pontefract, Rotherham, Scarborough, Sheffield and York. Each laboratory will be asked to complete an accompanying questionnaire regarding the analytical methods employed at each site. The analytical results will also be returned via an electronic format (ideally an excel spreadsheet).

8. DATA ANALYSIS

8.1 Demographic Data

Analytical results will take demographic statistics into account.

8.2 Laboratory Methods

Each laboratory will return a completed questionnaire about the analytical methods employed at each site accompanied by relevant information regarding performance. We will determine the bias that exists between laboratories using IFCC approved methods.

8.3 Analytical Results

The analytical results will be returned to the Principal Investigator in electronic format (ideally an excel spreadsheet). The services of a statistician will be employed. Data will be entered into a bespoke Access database. Reference range analysis will be performed using Analyse-it add-in package for Microsoft Excel. Reference values will be calculated using approved IFCC protocols.

8.4 Spurious Data

Extreme values will be accounted for using IFCC approved procedure.

9. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, relevant regulations and procedures. All sample analysis will be undertaken in CPA accredited laboratories in the Yorkshire region (Airedale, Barnsley, Bradford, Dewsbury, Halifax, Harrogate, Huddersfield, Hull, Leeds (LGI), Pinderfields, Pontefract, Rotherham, Scarborough, Sheffield and York). Dedicated storage and transport arrangements to the various laboratories will aim to maintain sample quality prior to analysis.

10. ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be restricted to the investigating team at the host institution.

11. ETHICS

11.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (October 2008).

11.2 Ethical Approval

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to the Yorkshire Research Ethics Committee (REC) for written approval. The Investigator will submit and obtain approval for all substantial amendments to the original approved documents.

11.3 Participant Confidentiality

The investigating team will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on any electronic database. All documents will be stored securely and only accessible by the investigating team. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

12. DATA HANDLING AND RECORD KEEPING

All study data will be entered into a secure LGI pathology results server. The participants will be identified by a study specific participants number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file after the participants have received a copy of their test results.

13. FUNDING

There is no external funding for this project. Analytical costs will be borne by LGI Blood Sciences and the collaborating laboratories.

14. PUBLICATION POLICY

The investigating team will seek to publish their findings in relevant peer reviewed journals in accordance with the LTHT publication policy.

15. REFERENCES

1 Elder NC, Hickner J. Missing clinical information: the system is down. JAMA. 2005; 293:617-619.

2 Siest G, Henny J. Commission on "Reference Values". Production of reference values in healthy subjects (document G, stage 3, version 1). Ann Biol Clin (Paris) 1981; 39:235-44.

3 Nordic Reference Interval Project. http://www.furst.no/norip/ accessed 10th August 2009

4 Fuentes-Arderiu X, Mas-Serra R, Aluma-Trullas A, Marti-Marcet MI, Dot-Bach D. Guideline for the production of multicentre physiological reference values. A proposal of the Catalan Association for Clinical Laboratory Sciences. Clin Chem Lab Med 2004; 42:778-782