Study Title: Evaluation of laboratory methods for measuring the composition of breastmilk

Internal Reference Number / Short title: The NECTAR study

Ethics Ref: 19/WS/0174

IRAS Project ID: 272907

Date and Version No: Version 1.1 dated 10th October 2019

Chief Investigator:

Dr Fadil Hannan
Director of the Oxford Centre for the Endocrinology of Human Lactation
Nuffield Department of Women's and Reproductive Health
Level 3, Women's Centre, John Radcliffe Hospital
Headington, Oxford, OX3 9DU
01865 222937
fadil.hannan@wrh.ox.ac.uk

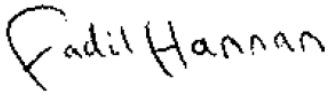
Investigators:

Dr Karl Morten
Laboratory Manager
Nuffield Department of Women's and Reproductive Health
Level 3, Women's Centre, John Radcliffe Hospital
Headington, Oxford, OX3 9DU
01865 221032
karl.morten@wrh.ox.ac.uk

Ms Helen Price
Research Midwife
Oxford Centre for the Endocrinology of Human Lactation
Nuffield Department of Women's and Reproductive Health
Level 3, Women's Centre, John Radcliffe Hospital
Headington, Oxford, OX3 9DU
01865 221074
Helen.price@wrh.ox.ac.uk

Dr Frances O'Brien
Consultant Neonatologist
Oxford Newborn Care Unit
Level 2, Women's Centre, John Radcliffe Hospital
Headington, Oxford, OX3 9DU
01865 221355
Frances.O'Brien@ouh.nhs.uk

Sponsor:	University of Oxford
Funder:	The Family Larsson-Rosenquist Foundation

Chief Investigator Signature: 

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. KEY CONTACTS

Chief Investigator	Dr Fadil Hannan Director of the Oxford Centre for the Endocrinology of Human Lactation Nuffield Department of Women's and Reproductive Health Level 3, Women's Centre, John Radcliffe Hospital Headington, Oxford, OX3 9DU 01865 222937 fadil.hannan@wrh.ox.ac.uk
Sponsor	University of Oxford Clinical Trials and Research Governance Joint Research Office 1st floor, Boundary Brook House Churchill Drive, Headington Oxford OX3 7GB
Funder(s)	Family Larsson-Rosenquist Foundation Rheinstrasse 1 8500 Frauenfeld Switzerland T +41 41 510 05 10 info@larsson-rosenquist.org www.larsson-rosenquist.org
Clinical Trials Unit	Not applicable

2. LAY SUMMARY

Aim:. The objective of this study is to establish suitable laboratory methods for measuring milk hormones, cells, antibodies and metabolites. These methods will be utilised in future clinical studies to investigate the biological mechanisms by which lactation influences the health and wellbeing of the breastfeeding infant

Outline of research: This study involves the collection of 5 – 10mls of breast milk from up to 30 women who are already expressing milk for their babies being cared for in the Oxford Newborn Care Unit, Women's Centre, John Radcliffe Hospital, Oxford. The donated milk samples will be used to evaluate the suitability of a range of laboratory tests for measuring hormones, cells and metabolites in human milk. These laboratory tests will be used in future clinical studies to establish reference standards for key breast milk constituents, and to gain a greater understanding of how human milk influences the health and wellbeing of the breastfeeding infant.

Justification: Human breast milk is a nutrient rich fluid, which additionally contains many factors with the potential to influence neonatal development, metabolism and health outcomes such as diabetes, obesity and neurodevelopment. However, little is known about the concentrations of these factors in human milk, and robust laboratory tests for measuring milk hormones have not been established.

3. SYNOPSIS

Study Title	**Evaluation of laboratory methods for measuring the composition of breast milk**		
Internal ref. no. / short title	The NECTAR study		
Study registration	The study will be registered with the clinicaltrials.gov		
Sponsor	University of Oxford Clinical Trials and Research Governance Joint Research Office 1st floor, Boundary Brook House Churchill Drive, Headington Oxford OX3 7GB		
Funder	Family Larsson-Rosenquist Foundation Rheinstrasse 1 8500 Frauenfeld Switzerland T +41 41 510 05 10 info@larsson-rosenquist.org www.larsson-rosenquist.org		
Study Design	Method Evaluation Study		
Study Participants	Healthy volunteers, who are mothers providing breast milk for their babies in the Oxford Newborn Care Unit, Women's Centre, John Radcliffe Hospital.		
Sample Size	30 participants		
Planned Study Period	24 months		
Planned Recruitment period	01/12/2019 to 30/11/2021		
	Objectives	Outcome Measures	Timepoint(s)
Primary	Evaluate suitability of laboratory tests for measuring components of human milk	Suitable assays established for the analysis of milk hormones, cells, antibodies and metabolites	Samples analysed within 3 months of collection
Secondary	Evaluate sample requirements, and conditions for processing and storage	1. Sample requirements established such as volume of milk needed per assay 2. Conditions for sample processing and storage established	Samples analysed within 3 months of collection

4. ABBREVIATIONS

CI	Chief Investigator
CRF	Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
EBM	Expressed Breast Milk
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
IFT	Infant Feeding Team
JR	John Radcliffe Hospital
NCU	Newborn Care Unit
NDWRH	Nuffield Department of Women's and Reproductive Health
NHS	National Health Service
RES	Research Ethics Service
OUHFT	Oxford University Hospitals NHS Foundation Trust
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SOP	Standard Operating Procedure

5. BACKGROUND AND RATIONALE

Human milk is a nutrient rich fluid (1,2), which additionally contains hormones (3,4), cells (5), antibodies (6), and metabolites (7,8) that are transmitted to the breastfeeding infant. These milk components have the potential to influence neonatal development, metabolism and health outcomes such as diabetes, obesity and neurodevelopment (9,10). However, little is known about the overall composition of these factors within human milk. The purpose of this study is to establish suitable laboratory methods for measuring milk hormones, cells and metabolites.

Description of the study: Laboratory method evaluation

Population to be studied: Healthy volunteers, who are mothers in established lactation and expressing milk for their babies in the Oxford Newborn Care Unit. The participants will be asked to provide 1-2 teaspoons (5-10 ml) of milk for this study.

Summary of risks: This is a non-interventional study. None of the study participants will be undergoing any invasive or potentially harmful procedures.

Summary of benefits: The main benefit of this study is the establishment of laboratory methods, which can be used in future clinical studies to investigate the biological mechanisms by which lactation influences the health and wellbeing of the breastfeeding infant.

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	**Outcome Measures**	**Timepoint(s) of evaluation of this outcome measure (if applicable)**
Primary Objectives Evaluate suitability of laboratory tests for measuring components of human milk	Suitable assays established for the analysis of milk hormones, cells, antibodies and metabolites	Samples analysed within 3 months of collection
Secondary Objectives Evaluate sample requirements, and conditions for processing and storage	1. Sample requirements established such as volume of milk needed per assay 2. Conditions for sample processing and storage established	Samples analysed within 3 months of collection

7. STUDY DESIGN

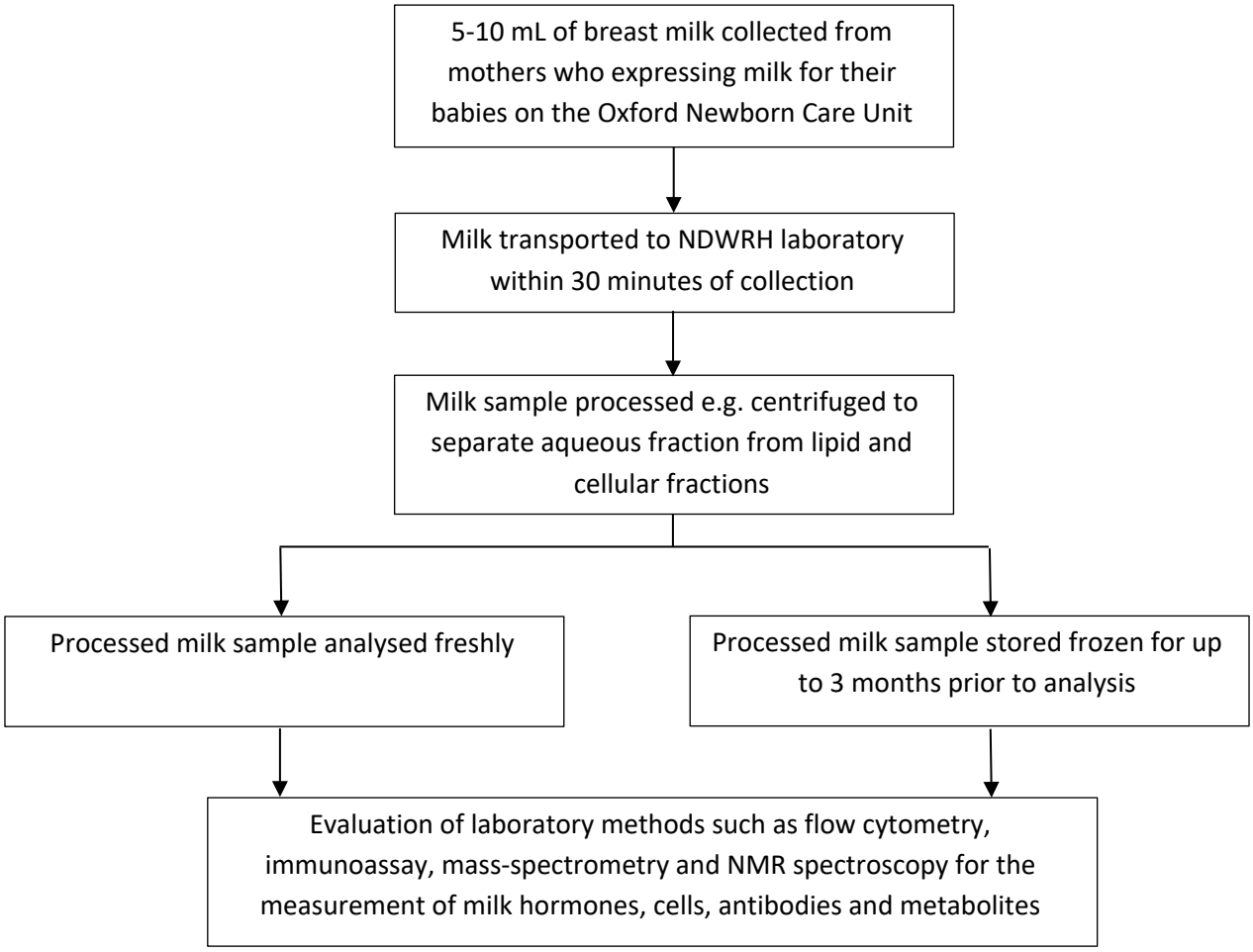
Overall study design: Laboratory method evaluation

Study setting: Samples collected in the Oxford Newborn Care Unit, The John Radcliffe Hospital.

Participants: Healthy volunteers that are in established lactation and attending the Oxford Newborn Care Unit by virtue of their babies being NHS inpatients.

Study procedures:
1. Participant approached, study discussed, and written, informed consent received
1. Lactating mother to express 5-10 mL of breast milk into a sterile container
2. Milk sample transported to NDWRH laboratory for processing e.g. centrifugation
3. Processed milk sample analysed freshly or stored frozen for up to 3 months prior to analysis
4. Samples analysed in laboratories located in the University of Oxford or in OUHFT

Study flowchart:



Participant follow-up: Most participants will produce a breast milk sample on a single occasion. However, if they are producing a substantial amount of breast milk, then they may be asked to provide further samples on subsequent visits to the Oxford Newborn Care Unit.

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants

Participants are healthy volunteers aged 18-45 years and are >1 week post-partum and in established lactation. The participants will be recruited in the Oxford Newborn Care Unit at the Oxford University Hospitals NHS Trust, where they are visiting and expressing breast milk for their babies.

8.2. Inclusion Criteria

- Female, aged 18 to 45 years in established lactation and are >1 week post-partum
- Able to express breast milk by hand or using a pump device
- Participant is willing and able to give informed consent for this study
- Able to understand and speak English

8.3. Exclusion Criteria

- The participant may not enter the study if they have limited milk supply or are not lactating for any reason.

9. PROTOCOL PROCEDURES

9.1. Recruitment

The Neonatal Research Team will identify potential participants i.e. mothers who are visiting the Oxford Newborn Care Unit and expressing milk for their babies and make the initial approach. The study Research Midwife or Neonatal Research Team will discuss the study in detail with the potential participants.

Potential participants will also be recruited using leaflets and posters displayed in the Oxford Newborn Care Unit, or through information displayed on the NDWRH and Oxford Safer Pregnancy Alliance (OSPREA) websites.

9.2. Screening and Eligibility Assessment

All breastfeeding women aged 18-45 years are eligible regardless of demographics or medical history, if they are >1 week post-partum and currently expressing a substantial amount of breastmilk and are comfortable expressing either via hand or using a pump device. Women must be available to express milk in The Women's Centre, John Radcliffe as the milk samples will typically need to be transported to the laboratory and processed within 45 min of collection. Women will not be approached if they do not understand the English language or lack capacity to consent.

Participants will be screened by the Neonatal Research Nurses during normal working hours (Monday to Friday, 8am to 4pm) in line with the working hours of the study research team and availability of the NDWRH laboratory services.

9.3. Informed Consent

The Neonatal Research Nurses or Research Midwife will seek Informed Consent at a time that is convenient to the participant. The process of Informed Consent for this study is outlined below:

1. Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the study; what it involves for the participant; the implications and constraints of the protocol; and any risks involved in taking part.

2. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to their baby's future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

3. 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.

4. 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. The recruiting team member who obtained the consent will be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator. They will be GCP trained and will have signed the delegation log.

5. A copy of the signed Informed Consent will be given to the participant. The consent forms will be scanned and stored on the University of Oxford High Compliance Servers. The original signed form will be retained at the study site and will be transferred to a locked filing cabinet in a key coded locked office of the Lead Research Midwife. The Neonatal Research Nurses will keep a screening log of all women approached to participate. This will be stored in a locked filing cabinet in their key coded locked office.

6. The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

9.4. Enrolment

Recruited individuals will be given a unique participant number at enrolment, which will be recorded in the screening log (Appendix A). The screening log will contain the participant name and number, date of enrolment, and confirmation of consent or decline. In addition, the screening log will document whether the participant is willing to provide multiple samples. The unique participant number together with enrolment date and name will also be recorded in the case report form and the trial master sheet (Appendix A). This document will be stored on the University of Oxford High Compliance Servers. Each sample will be given a unique sample number, which will be recorded in a case report form, sample log, and also in the trial master sheet (Appendix A). If the participant provides multiple samples, then each sample will be given a separate sample number and date. Verbal consent will be taken each time a new sample is donated and recorded on the screening log.

9.5. Blinding and code-breaking

Not applicable.

9.6. Description of study intervention(s), comparators and study procedures (clinical)

This is a laboratory method evaluation study and does not involve an intervention or comparator.

9.6.1. Description of study procedure(s)

1. Participants provide up to 10mls of breast milk. This will be collected in the same setting where they normally express milk for their baby e.g. in the Oxford Newborn Care Unit, Women's Centre, and during a time when they are normally expressing milk for their baby.

2. Milk samples will be collected using either an electric or manual breast pump, or by hand expression into sterile containers.

3. The milk sample will be anonymised (i.e. given a sample number) by a Research Midwife/Nurse and transferred to the NDWRH laboratory within 30 minutes of donation, for processing e.g. centrifuged to separate the aqueous fraction from the lipid and cellular fractions.

4. The processed milk sample will either be analysed freshly or stored frozen for up to 3 months prior to analysis.

5. The processed milk sample will be used to evaluate laboratory methods such as flow cytometry, immunoassay, mass-spectrometry and NMR spectroscopy for the measurement of milk hormones, cells and metabolites.

9.7. Study visit

Participants will be asked to provide a breast milk sample on a single occasion. However, if they are producing a substantial amount of breast milk, and are agreeable, they may be asked to provide samples on subsequent visits to the Oxford Newborn Care Unit.

9.8. Subsequent Visits

The procedure for milk collection on subsequent visits the Oxford Newborn Care Unit will be the same as that described in section 9.6.1.

9.9. Sample Handling

1. Each breast milk sample will be given a unique sample number, and the date it was collected will be recorded. Samples will be anonymised (i.e. participants cannot be identified by any member of the research team other than the CI and study Research Midwife).

2. Breast milk samples will be transported to the NDWRH laboratory on ice within 30 min of collection.

3. Samples will be processed e.g. centrifuged at 4°C to separate the aqueous fraction from the lipid and cellular fractions. The different fractions will then be aliquoted. Preservatives such as protease inhibitors or anti-microbial compounds may be added to the aliquots. Sample aliquots will either be analysed freshly or stored frozen (i.e. at -20 °C or -80 °C) for up to 3 months prior to analysis.

4. The cellular fraction will be used to evaluate whether techniques such as flow cytometry can measure and quantify the cellular components of breastmilk. Whilst the aqueous and lipid fractions will be used to assess the suitability of laboratory methods such as immunoassay, mass spectrometry and NMR spectroscopy for measuring milk hormones, antibodies and metabolites. These laboratory method

evaluation studies will be undertaken at the University of Oxford or within the Oxford University Hospitals NHS Foundation Trust.

5. Any residual sample aliquots will be disposed of following analysis. The samples will be disposed of on the day of analysis in accordance with University guidelines and the Human Tissue Authority's Code of Practice. A disposal log will be used to record the sample ID of the disposed samples.

9.10. Early Discontinuation/Withdrawal of Participants

During the course of this method evaluation study, a participant can choose to withdraw her donated breast milk samples from the study at any time for any reason without prejudice to her babies' future care. and with no obligation to give the reason for withdrawal. If the participant withdraws from the study, then any stored samples would be destroyed with immediate effect. Withdrawn participants will be replaced so that the target sample size of 30 can be achieved.

9.11. Definition of End of Study

The end of study is the point at which all the study data has been collected and analysed, and the database is locked.

10. SAFETY REPORTING

Adverse events are not expected as part of this study. It is highly unlikely there will be any injury or harm to study participants as this is a non-invasive and non-interventional study. Moreover, the participants are providing a sample of breast milk during their routine nursing session at the Oxford Newborn Care Unit, where they are collecting breast milk for their own baby. Thus, this study does not involve any additional procedures.

11. STATISTICS AND ANALYSIS

11.1. Statistical Analysis Plan (SAP)

The data obtained in this study will be used to determine assay precision, accuracy, sensitivity, and also analytical linearity and measurement range.

11.2. Description of the Statistical Methods

This laboratory method evaluation study involves the use of descriptive statistics (i.e. determination of mean, standard deviation and % coefficient of variation (%CV)). Moreover, within-run and between-run assay precision will be assessed. Bias plots and correlation coefficients will be used to evaluate assay accuracy and linearity, respectively.

11.3. Sample Size Determination

We plan to collect up to 30 breast milk samples in order to provide sufficient quantities of milk to evaluate a range of laboratory methods (e.g. immunoassay, mass-spectrometry, flow cytometry and NMR spectroscopy).

11.4. Analysis populations

Not applicable for this laboratory method evaluation study.

11.5. Decision points

Not applicable for this laboratory method evaluation study.

11.6. Stopping rules

If it is not possible to collect any breast milk samples, then the study will close. The decision to terminate the trial would be made by Dr Fadil Hannan, PI.

11.7. The Level of Statistical Significance

Not applicable for this laboratory method evaluation study.

11.8. Procedure for Accounting for Missing, Unused, and Spurious Data.

Not applicable for this laboratory method evaluation study.

11.9. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Not applicable for this laboratory method evaluation study.

11.10. Health Economics Analysis

Not applicable for this laboratory method evaluation study.

12. DATA MANAGEMENT

The plan for the data management of the study are outlined below. There is not a separate Data Management document in use for the study.

12.1. Source Data

All study documentation will be stored in a locked filing cabinet in a locked office only accessible to the research team. There are no other source documents other than the consent form, as the participants are healthy volunteers and are recruited by virtue of their babies being NHS Inpatients.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

Personal details will be stored as follows:

1. Participant details will initially be recorded on a paper case report forms, and then transposed onto the trial master sheet. Following this, the paper case report forms will be shredded. The trial master sheet, which holds the participant identifiers, the unique participant study number and the sample number will be held on the University of Oxford High Compliance Server.

2. Screening log, which contains names (and unique study number if participating) of all individuals approached for this study, will be kept in a locked filing cabinet in a key coded office.

3. The Informed Consent forms will be scanned and stored together with the trial master sheet on the University of Oxford High Compliance Server. Hard copies of the consent forms will be transferred to a locked filing cabinet in the key coded locked office of the Lead Research Midwife.

Study documentation will be stored for 5 years in an offsite archiving facility. The study will comply with the General Data Protection Regulation (GDPR), which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number or a sample number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

13. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

13.1. Risk assessment

This is a non-interventional and non-invasive study, which involves the collection of a small amount of breastmilk from women who are already expressing milk for their babies on the Oxford Newborn Care Unit. Therefore no formal risk assessment will be undertaken.

13.2. Study monitoring

Data will be evaluated for compliance with the protocol and accuracy. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13.3. Study Committees

As this is a feasibility method evaluation study, no subcommittees will be required.

14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

15. SERIOUS BREACHES

A "serious breach" is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice. All members of the research team who receive written informed consent will have up to date GCP training.

16.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Other Ethical Considerations

The breast milk samples will be obtained during the participants routine expressing session and will not involve any additional procedures or interventions. The samples will solely be used for laboratory method evaluation and will not generate any clinically relevant data. Thus, no results will be available to the clinicians or the participants. There are no further ethical considerations.

16.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

16.6. Transparency in Research

The study will be registered with clinicaltrials.gov

16.7. Participant Confidentiality

The study team will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant study number on all study documents and any electronic database the sample number will be used. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the General Data Protection Regulation (GDPR), which require data to be de-identified as soon as it is practical to do so.

16.8. Expenses and Benefits

A £10 gift voucher will be offered to each participant in recognition of the time they have given to provide the study sample.

17. FINANCE AND INSURANCE

17.1. Funding

This study is funded by the Family Larsson-Rosenquist Foundation (FLRF), which is an independent charitable organization supporting research into breast milk and breastfeeding.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the Family Larsson-Rosenquist Foundation. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

19. ARCHIVING

Study documentation will be archived for a 5 year period in an off-site facility.

20. REFERENCES

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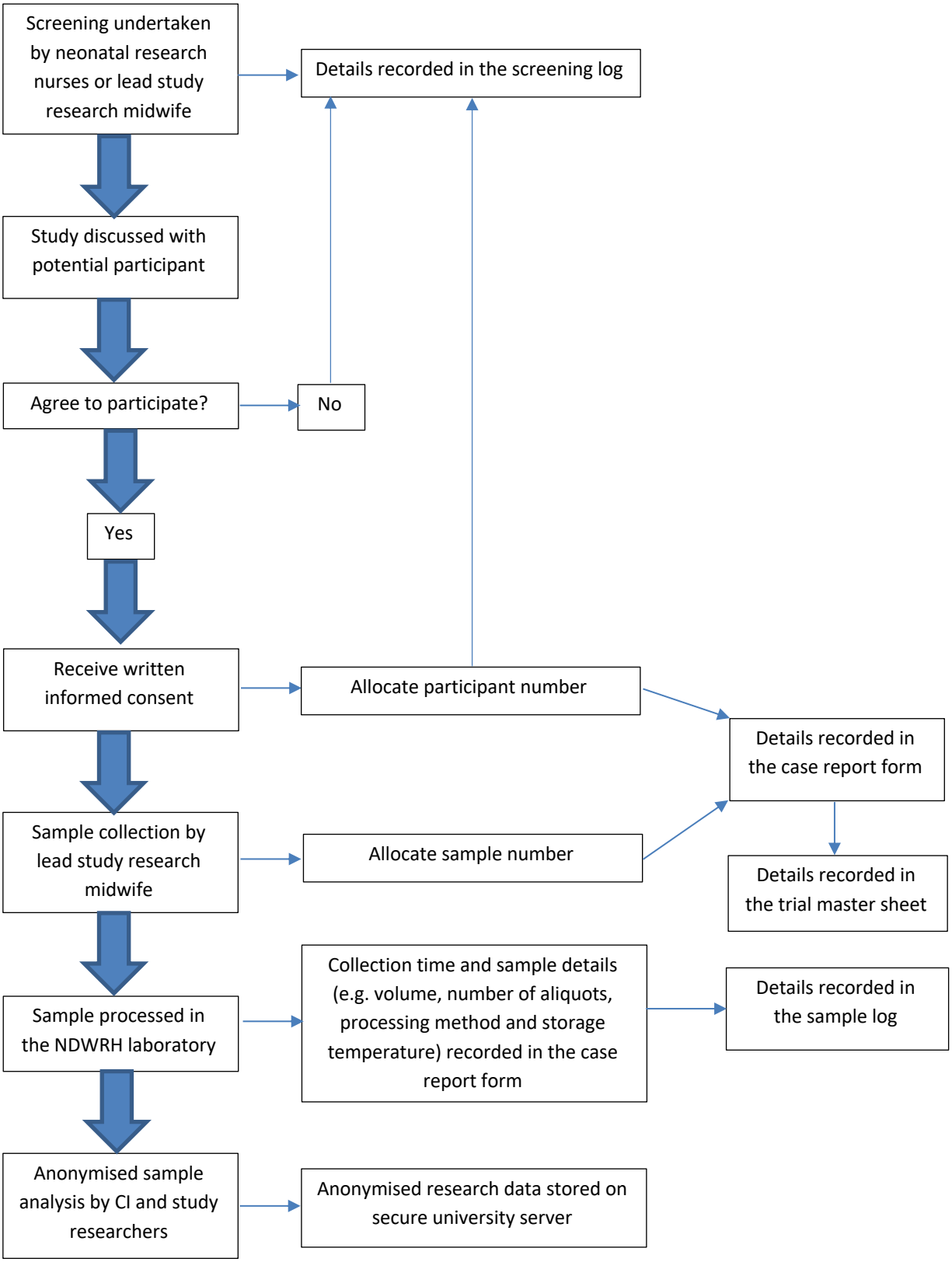
21. APPENDIX A: STUDY PROCESS FLOW CHART



22. APPENDIX: SCHEDULE OF STUDY PROCEDURES

	Date and time			
Procedures	**Screening**	**Study visit 1**	**Study visit 2**	**Study visit 3**
Eligibility assessment				
Informed consent				
Sample collection				

23. APPENDIX C: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made