PROTOCOL FOR THE MANAGEMENT OF THE BABY BIO BANK (BBB)

VERSION 7.0

25th May 2016

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PROTOCOL FOR THE MANAGEMENT OF THE BABY BIO BANK (BBB)

1. What is a biobank

There is a growing demand for biological samples with well characterized histories for translational research. As a consequence, tissue banks have arisen as repositories for such material. The collection, processing, storage, maintenance and distribution of material and data are the key function of a tissue bank. That the samples are collected ethically, thereby safeguarding the donor, is of paramount importance and is also undertaken by the tissue bank.

2. The aim of the Baby Bio Bank (BBB)

2a. To generate a database of phenotypes and biological resources from four major cohorts plus controls for complications of pregnancy: recurrent miscarriage, intrauterine growth restriction (IUGR), pre-eclampsia and prematurity. Access to this database will be made available to national and international research groups to assess candidate genes and for parallel, matched RNA expression and protein analysis.

2b. The resource of biological material will include the collection of whole bloods from “families” (proband, maternal, paternal) for DNA extraction and storage of separate serum and plasma samples, and urine from mother. In addition placental membranes, placenta and cord will be collected into RNA later for RNA expression and protein analyses. Detailed clinical phenotypes will also be recorded. For the samples from pregnancies complicated by recurrent miscarriage fetal blood samples will not be available and DNA from the proband will be prepared from the collected fetal products of conception.

2c. The resource will be used to define genes and proteins that play a biological role in normal pregnancy and to identify DNA sequence variants and alterations in expression in these four complications of pregnancy. In the long-term the results of these studies will have both diagnostic and therapeutic benefits.

3. Primary Sites of the BBB

3a. Institute if Child Health (ICH)

3b. St Mary's Hospital (SMH)

4. Participating Sites of the BBB

4a. St Mary's Hospital (SMH)

4b. King's College Hospital (KCH)

4c. Queen Charlotte's and Chelsea Hospital (QCCH)

4d. University College Hospital London (UCLH)

4e. Chelsea and Westminster Hospital (CWH)

5. Personnel Structure of BBB

5a. BBB Principal Applicants: Professor Gudrun Moore and Professor Lesley Regan

5b. BBB Research Manager: Dr Nita Solanky/ Dr Sayeda Abu-Amero

5c. BBB Principal Collaborators: Professor Mark Johnson (CWH), Professor Kypros Nicolaides (KCH), Professor Catherine Williamson (QCCH), Professor Donald Peebles (UCLH), Professor John Whittaker (London School of Hygiene and Tropical Medicine (LSHTM)

5d. Head Research Nurse Ms Katherine Rogers

5e. Research Associates Dr Nita Solanky (CWH), Dr Ana Maria Miranda Perez (QCCH), Dr Anna Thomas (ICH) and Dr Shawnelle White (SMH)

6. The Resource Management Board (RMB)

6a. Structure of the RMB

The composition of the Resource Management Board is as follows:

Fiona Leishman, Chair and Chief Executive of Wellbeing of Women
Professor Lesley Regan, PI of Baby Bio Bank
Professor Gudrun Moore, PI of Baby Bio Bank
Ms Lynn Hiestand, Trustee of Wellbeing of Women

Research Scientific Advisory Committee
Dr Eamonn Sheridan, Senior Lecturer in Genetics, University of Leeds
Dr Paul Taylor, Senior Lecturer in Developmental Programming at King's College London
Professor Eric Steegers, Professor in Obstetrics and Prenatal Medicine, Erasmus MC

The Baby Bio Bank Manager will attend meetings of the Resource Management Board.

All Appointments to the Board will be subject to approval by the Wellbeing of Women Trustee Board

Wellbeing of Women Research Manager will act as the Clerk to the Board

The Chair will call the meetings and be responsible for the agenda, and ensure accuracy of record keeping. The board will use e-mail to communicate in the interim periods.

Meetings will be called at a minimum of two calendar months' notice.

The Chair will be able to call Extraordinary Meetings of the Board outside of the above arrangements if deemed necessary.

The management board has approved access to the collection for academic and commercial research purposes. Collaborators would only have access to samples on the basis that these would not be made available to a third party.

Distribution of samples will be undertaken through the central distribution centre at the ICH. Future support for the distribution of the resource will be found from budgeted research costs within grants from the interested research groups.

6b. Function of the RMB

The RMB will have the following responsibilities

1. To maintain and demonstrate confidentiality of the data and sample resource.

2. To maintain and demonstrate quality control for data entry and sample collection/storage and dispatch.

3. To maintain and demonstrate audit of the use of the resource collection.

4. To assess applications for access and provision of samples from the resource centre.

5. To hold responsibility for communication of decisions regarding sample use.

7. Ethical Issues

7a. Informed Consent

One of the fundamental steps in sample collection is informed and voluntary consent from the donor. Informed consent entails verbal and written information pertaining to the purpose, methods, demands, risks, inconveniences, discomforts and putative outcome of the study in a language and at a level that the donor understands. Of utmost importance is that the donor understands that participation is voluntary and that refusal to take part does not alter their medical care.

In addition, the donor needs to be aware that he/she can withdraw consent for the use of his/her sample and/or clinical data at any point of the study without compromising their medical care. The donor also needs to be assured that both the sample and clinical data are semi-anonymised for the purpose of the study.

7b. Obtaining Consent

Potential donors will be identified through antenatal clinics by the recruiting staff as cohorts with the following diagnosed pregnancy outcomes:

1. Intra-Uterine Growth Restriction (IUGR): IUGR can be identified by ultrasound and are noted to have slow growth.
2. Pre-eclampsia: This is usually identified by maternal hypertension and proteinuria, and possibly hepatic impairment. This is preceded by placental ischaemia and abnormal placentation.
3. Prematurity: pre-term births account for 6-8% of live births in the UK and are those occurring before 37 weeks gestation. There is often a history of prematurity, including the mother herself, although various aetiologies have been described in the literature.
4. Recurrent miscarriage: Recurrent miscarriage is described as the loss of three or more consecutive pregnancies. Various aetiologies are known but the majority of cases are idiopathic. St Mary’s Hospital run an internationally

recognised centre for mothers' suffering from recurrent miscarriage and this will be the centre targeted for this cohort.

There is overlap among the four cohorts above and patients will only be recruited once per pregnancy even if they fall into multiple cohorts.

5. Normal: in order to understand the genetic causes of the complications of pregnancies detailed above, comparisons need to be made to a reference normal cohort without any of the above or other complications.

The potential donors will then be recruited by highly motivated research nurses/midwives/associates. These nurses/midwives/associates will approach the potential donors and provide written information on the study which will be verbally explained in a language suitable for the donor. The research nurses/midwives/associates will answer all queries regarding the purpose, methods, demands, risks, inconveniences, discomforts and putative outcome of the study. The research nurses/midwives/associates will also point out contact details if the donor has any further queries. They will allow the donor to make an independent decision regarding their participation in the study or not, and assure the potential donor that their medical care is not governed by their decision to participate or not.

If the donor decides to participate, then they will be asked to sign a consent form stating that they have read the information sheet which they have understood and that they have voluntarily agreed to participate. The consent form also states that their clinical data will be used and that they are free to withdraw at any time during the study. The donor will receive a copy of the signed consent, and a copy will be held by the BBB and in the participants' hospital notes.

7c. Public Access to Information

The data generated from research projects which will use the BBB resources have enormous potential for therapeutic considerations for the future. However, as data will probably not be generated through diagnostic criteria the information cannot be used for treatment of the donor for the current pregnancy. It may be of use for the donors' future pregnancy.

Research data is usually published in peer reviewed journals which can be accessed by the public. Often, such findings are picked up by the media and receive coverage and may be used in television documentaries. All media coverage for the BBB will be handled by WoW press office.

The BBB website and newsletters will detail the progress of the sample collection. It will also detail any research programmes which are funded to study complications of pregnancies using the collected samples and clinical data. Peer reviewed publications will also be made available through this site in a timely manner. The public will be able to contact the BBB through the website with queries and comments.

8. Collection of Biological Specimens

It will be the responsibility of the research nurses/midwives/associates to collect the biological specimen and ensure that the sample is semi-anonymised by assigning a unique identifier. This unique identifier can then be used to identify clinical data. The samples will be stored at the site of collection on a daily basis and twice monthly transported to SMH (see section 13). 4ºC, -20ºC and -80ºC facilities will be available at all participating sites for appropriate storage of biological specimens.

8a. Labeling of Samples

Blood samples, placental and cord samples and products of conception will be labeled immediately on procurement using a barcode system, which will permit

the sample to be scanned and associated with clinical data, without the need for personal identifiers such as donor name, the traditionally used date of birth (DOB) or donor hospital number written on the sample container. The label will be resistant to extremes of cold storage temperatures (-80ºC) and will survive repeated freeze-thawing of the sample.

The barcode label will associate multiple samples of the same source from the same donor; multiple samples from different sources from the same donor and it will also associate the unique trio identifier code i.e. maternal, paternal and fetal samples from one family. Clinical data for each individual in the trio will be encoded into the unique trio identifier code.

All biological samples must be treated as potentially infectious and staff handling blood need to ensure that their Hepatitis B titre is acceptable every two years.

8b. Collection of Blood for DNA

20 ml of blood should be collected from donors at the time clinical samples are being taken in order not to inconvenience the donor. Bloods should ideally be refrigerated and processed within 30mins (see section 13). Mixed venous cord blood at the time of birth will be collected if possible.

DNA is to be extracted from blood collected in EDTA (purple top) tubes, these are multipurpose and are widely used.

8c. Collection of Blood for Plasma and Serum

Sodium citrate (light blue top) should be used for plasma and clotted tube (red top) used for collection of serum. Plasma can be used for bioassays, proteomic analysis and biomarker discovery and serum can be used for bioassays and proteomic analysis.

8d. Collection of Urine

Urine will be collected in standard specimen containers by the donor and will be aliquoted within 1 hour for long-term storage at -80ºC. Urine can be used for proteome mapping and biomarker discovery.

8e. Collection of Placental Tissue for DNA and RNA

It is best to collect and process tissues within an hour of excision to minimize degradation of RNA and minimize the effect of hypoxia on gene expression. As excised tissues stored for extraction of DNA will be the same source for RNA extraction, the same care will be taken for both end points.

For fetal DNA and RNA, 1 cm x 1 cm x 1 cm excisions from four sites surrounding the umbilical cord on the fetal side will be taken and rinsed in room temperature 1 x PBS (Invitrogen) to remove as much maternal contamination as possible. The sample will then be placed in 5 x volume RNALater® (Invitrogen). Samples will be stored overnight at 4ºC before transfer to -20ºC or -80ºC for long term storage

Although it is more costly than other methods the advantage of RNALater® is that samples can be stored at room temperature for five days or a month at 4ºC in the eventuality that liquid nitrogen is not immediately available.

8f. Collection of Placental Villous Tissue for DNA and RNA

0.5cm^2 placental villous tissue will be taken from 4 sites on the chorionic (maternal) side of the placenta. These will be rinsed in room temperature 1 x PBS (Invitrogen) then placed in 5 x volume RNALater® (Invitrogen). Samples will be stored overnight at 4ºC before transfer to -20ºC or -80ºC for long term storage.

8g. Collection of Umbilical Cord for DNA and RNA

2x 2cm lengths of umbilical cord adjacent to the placental insertion site will be collected. These will be rinsed in room temperature 1 x PBS (Invitrogen) then

placed in 5 x volume RNALater® (Invitrogen). Samples will be stored overnight at 4ºC before transfer to -20ºC or -80ºC for long term storage

8h. Collection of Fetal Membrane for DNA and RNA

The chorionic and amninonic membranes will be separated and ~5cm^2 of each will be rinsed in room temperature 1 x PBS (Invitrogen) then placed in 5 x volume RNALater® (Invitrogen). Samples will be stored overnight at 4ºC before transfer to -20ºC or -80ºC for long term storage

8i. Collection of Products of Conception

Products of conception will be obtained from ERPC (Evacuation of Retained Products of Conception) surgery in a sterile container. Due to the nature of products of conception it is unlikely that RNA will remain undegraded or that protein structure will remain intact-therefore only DNA will be extracted.

8j. Collection of Baby Buccal Swab

Occasionally the placenta, cord and cord blood may not be available (e.g. baby delivered at home, in an emergency or another hospital). In such instances a buccal swab from the baby may be requested. The SK-2 Isohelix Swab (SK-2S; Isohelix, Cell Projects) are in sterile packaging which need to be removed and the swab stick removed from the clear plastic tube. The swab is then rubbed against the inside of the cheek for a minimum of 20 seconds and up to one minute. The swab stick is then placed back into the plastic tube, the stick removed so that only the swab containing the buccal cells is in the plastic tube, permitting easier manipulation for DNA extraction.

8k. Collection of Paternal Saliva

Occasionally blood collection may not be available (e.g. father is unable or uncomfortable to give a blood sample). In such instances a sample of saliva or a buccal swab will be requested either in person, via the mother or via the post.

The SK-2 Isohelix Swab (SK-2S; Isohelix, Cell Projects) will be used for collection of a buccal sample (see 8j above).
Saliva samples will be collected using the Oragene DNA saliva collection kit (DNA Genetek Inc), The ability to send the product to participants through the mail increases participant compliance and provides high quality DNA.

Alternatively 5mL of saliva will be collected in a sterile bijou and transfered to -20ºC or -80ºC for long term storage upon receipt.

8l. Collection of Clinical Data

Maternal clinical data will be extracted from the Hospital CMiS database by HD Clinical. Data transfer to and from HD Clinical will be through password protected Excel documents via nhs.net.
Research nurses/midwives/associates will collect paternal data via verbal qestionaire and will include information on paternal medical history and demographics. Data will also be collected on pregnancy and fetal outcome such as birth weight, head circumference, gender etc which will be used to verify data obtained from electronic NHS database downloads. Placental weight (trimmed and untrimmed will be measured by the Research nurses/midwives/associates prior to research sample collection (see appendix 1).

The data will be encoded and related to the barcoded biological sample and any resulting extracted sample by the BBB database manager. This will also be linked to the type, number of aliquots and physical location of the biological and extracted samples.

When biological samples are to be re-extracted it will be noted in the database in order to track availability of samples for future projects.

9. Processing of Biological Specimens

9a. Processing of Blood for DNA

DNA from blood will be extracted using the Invitrogen iPrep™ Purification system in conjunction with the iPrep™ Card: gDNA Blood and iPrep™ PureLink™ gDNA Blood Kit. A total volume of 350 µl of fresh or frozen blood is used for extraction with expected yields of approximately 6 µg and 10 µg respectively in a final volume of 200 µl Elution Buffer (supplied). Samples are processed according to the manufacturer's instructions.

9b. Processing of Blood for Plasma

It is advisable to process blood samples for plasma to be used in downstream proteomics applications within an hour of collection. Sodium citrate vacutainers containing the blood sample will be spun at 1200 g for 15 minutes at 4ºC in a Sorval® Legend RT centrifuge. The plasma is the upper layer is then aliquoted into 2 ml labeled cryovials without disturbing the white cells in the buffy coat which is retained for DNA extraction from the white blood cells. Aliquots will be stored at -80ºC.

9c. Processing of Blood for Serum

It is advisable to process blood samples for serum to be used in downstream proteomics applications within an hour of collection. The appropriate vacutainers containing the blood samples will be spun for 15 minutes at 1200 g in a Sorval® Legend RT centrifuge and then aliquoted into 2 ml labeled cryovials. Aliquots will be stored at -80ºC.

9d. Processing of Urine for Biomarkers

A variety of methods are available for proteomic analysis of urine, generally relying on a separation step, followed by ionization and subsequently mass spectrometry. The methodologies are beyond the scope of this protocol booklet for the time being and will be added as appendices as and when appropriate.

9e. Processing Tissue for DNA

DNA from tissue will be extracted using the Invitrogen iPrep™ Purification system in conjunction with the iPrep™ Card:gDNA Tissue and iPrep™ ChargeSwitch® gDNA Tissue Kit. Up to 10 mg of frozen tissue is homogenized with 1 ml Lysis Buffer (supplied). 10 µl of *RNase* A (5 mg/ml) (supplied) is added to the homogenate, vortexed and the samples incubated for 10 minutes at room temperature. 20 µl Proteinase K (20 mg/ml) (supplied) will be added, the samples mixed by vortexing and incubated for 1-3 hours at 55ºC. If the homogenate is not clear the Proteinase K incubation can be extended overnight. The treated samples will be processed according to the manufacturer's instructions. Approximately 12 µg of DNA in 200 µl Elution Buffer (supplied) is expected from 10 mg of tissue.

9f. Processing Tissue for RNA

RNA from tissue will be extracted using the Invitrogen iPrep™ Purification system in conjunction with the iPrep™ Card:Total RNA and iPrep™ PureLink™ Total RNA kit or the iPrep™ Trizol® Plus RNA kit. 50 mg of frozen tissue will be transferred to a sterile tube on ice and completely immersed in 1 ml Trizol® Reagent (supplied) before homogenizing well and incubating at room temperature for 5 minutes. 0.2 ml chloroform is added and the sample mixed by inversion for 15 seconds and then centrifuged at 12 000 g for 15 minutes at 4ºC in a benchtop centrifuge. After centrifugation, approximately 500 µl of inorganic upper phase is transferred to a clean iPrep™ Sample tube and RNA extracted using the iPrep™ PureLink™ Total RNA kit according to the manufacturer's instructions. RNA is eluted in a volume of 100 µl Elution Buffer (supplied).

If a *DNase* I (supplied) step is required, it can be added to the cartridge and the step will be automated according to the manufacturer's instructions.

9g. Processing of Buccal Swab for DNA

DNA from buccal swabs will be extracted using the Invitrogen iPrep™ ChargeSwitch® Buccal Cell Kit in conjunction with the iPrep™ Forensic Card. 1 ml ChargeSwitch® Lysis Buffer and 10 ul Proteinase K are added together to make the Lysis Mix and this added to the buccal swab in a sterile 1.5 ml microcentrifuge tube. The buccal swab is completely immersed in the Lysis Mix and vortexed for 10-15 seconds to facilitate mixing and then incubated for 20 minutes at 37^{o}C. The sample is then transferred to the iPrep Sample and Elution Tube and DNA extracted using the Invitrogen iPrep™ ChargeSwitch® Buccal Cell Kit according to the manufacturer's instructions. DNA is eluted in a volume of 75 -150 µl Elution Buffer (supplied) with an expected yield around 1 - 3 ug.

10. Quantification of Biological Specimens

All nucleic acid extraction will be quantified using the Nanodrop® ND 1000 (Nanodrop Technologies Inc.). Although the sample size is not critical, it is essential that a liquid column be formed so that the gap between the upper and lower measurement pedestal is bridged by the sample.

On start up, the pedestal surfaces will be thoroughly wiped with water and a clean water sample loaded to initialize the instrument. The Nanodrop® ND 1000 will then prompt to blank the instrument which should be the buffer used to resuspend or elute the samples. The Nanodrop® ND 1000 is ready for use.

10a. Quantification of DNA

The minimum amount of DNA for accurate quantification using the Nanodrop® ND 1000 is 1 µl. Select the Nucleic Acid option and from the drop down menu select dsDNA (double stranded DNA). Enter the identification of the sample and quantify according to the on-screen instructions (see manufacturer's instructions for details).

10b. Quantification of RNA

The minimum amount of RNA for accurate quantification using the Nanodrop® ND 1000 is 1 µl. Select the Nucelic Acid option and from the drop down menu select RNA. Enter the identification of the sample and quantify according to the on-screen instructions (see manufacturer's instructions for details).

10c. Quantification of Proteins

The minimum amount of protein for accurate quantification using the Nanodrop® ND 1000 is 2 µl. Select the Protein option and from the drop down menu select the type of protein. Enter the identification of the sample and quantify according to the on-screen instructions (see manufacturer's instructions for details).

Before dispatch a subset of both DNA and RNA samples will additionally be verified by UCL Genomics using an Agilent 2100 Bioanalyser. (https://www.ucl.ac.uk/ich/research/genetics-genomic-medicine/ucl-genomics).

Researchers are able to request bioanalyser verification and results of samples prior to dispatch at a cost.

11. Quality Control

11a. Equipment maintenance and repair

All equipment is maintained under contract with their supplier's and serviced annually.

11b. Staff training

- All BBB staff employed by the two universities requires 10 days of new skills training annually. Training includes course such as: Good Clinical Practice; research and human tissue legislation and statistical analysis.

- All recruiting staff are certified phlebotomists.

- BBB staff also attend relevant meetings and conferences related to biobanking and fetal/pregnancy complications. Usually one national per annum and several if based in London.

- Note that staff training records are part of HTA compliance.

11c Data management

- Data on the biological samples (type, volume, freezer location etc.) and clinical data (maternal/paternal/fetal data) are stored in the BBB database on a secure server hosted by AssetPerformer for which data security is ongoing and regularly conducted such as third-party penetration testing of the server, where AssetPerformer subscribe to services provided by companies who test the resilience of servers to hacking attacks. Proprietary software as an extra level security , which monitor certain areas of the server and can log and prevent any suspicious activity is also present.
- User names and passwords are held in a separate database which is not accessed by customers. The passwords are also encrypted.
- Physically the server's hosting company is ISO 27001 accredited, with the servers themselves held in secure, premises with constant CCTV surveillance.
- The database is fully backed up every 24 hours and all changes to the database are logged.
- No personal identifiers are recorded on the database rendering it semi-anonymous.
- Freezer maps of barcoded samples are kept in accordance with HTA requirements.
- Trios are given a unique number and samples are barcoded.

11d Record keeping

Biobanks are also auditable by the HTA and are inspected annually.

Standard Operating Protocols (SOPs)

SOPs are documented in the PROTOCOL FOR THE MANAGEMENT OF THE BABY BIO BANK which are approved by the ethics committee and are in compliance with the HTA. HTA require that all SOPs are reviewed annually by the HTA Designated Individual (DI) at each site - Dr Andrew Copp (UCL) and Dr Graham Taylor (Imperial).

Quality of samples

- This is a series of measures which checks the qualities and performance against standards, to verify that these standards are met.

- It is critical that the biological samples are maintained appropriately (see section 13) to ensure integrity of DNA, RNA and proteins.

- At least 15% of randomly selected samples from each complication will be tested by detailed quantification using PCR and sequencing (DNA) and reverse transcription PCR (RNA).

12. Database Requirements

The BBB database:

1. Provides a unique identifier by means of an alphanumeric barcode for each donor. This unique identifier will be assigned immediately on collection of the biological specimen and collection of clinical data to ensure semi-anonymisation of the donor at the onset. This unique identifier will be used to track the donor sample from collection, processing and distribution.

2. Each donor will provide biological sample(s) (e.g. blood, urine, placenta, cord or products of conception) which needs to be identifiable. Each biological sample

will have multiple aliquots which need to be identifiable. Each biological sample will be processed into DNA, RNA and protein which needs to be identifiable.

3. The clinical data (and research data) associated with each donor will be linked through the unique identifier.

4. All biological and processed specimens, and clinical data comprising the trio (mother, father, baby) will be linked.

5. Consent (and withdrawal of consent) will be tracked through the database.

6. The location, availability and usage of the donor sample will be tracked through the database.

7. Access to data will be user specific to restrict access to the minimum number of users.

8. The database will be used to generate reports and audit information.

9. The database will be held on a cloud based secure server. This ensures that data is not stored on hard drives but rather on a secure network and can be accessed and updated by staff on all sites.

10. Processed sample information and quality control data will be entered into the database by the research technicians with appropriate access.

12. The BBB research manager will ensure that the database is maintained efficiently and all information is current and accurate.

13. Storage of Specimens

We will have duplicated samples stored on the two primary sites, ICH and SMH, to ensure that no sample is lost due to breakdown of storage facilities.
For storage of biological specimens it is generally preferable for them to be cooled gradually to minimize formation of ice crystals but thawing should be as quick as possible. Repeat freeze thawing should be avoided by generating aliquots. Generally, the lower the temperature the longer the viable storage period.

An important consideration is the type of storage vehicle e.g. cryovials, sample containers etc which have to withstand extremes of cold (-80ºC) and sudden rises in temperature (thawing) and therefore need to be resistant. Additionally, the use of appropriate barcode labels which can remain adherent and readable is critical.

13a. Long Term Storage of Blood

Whole blood and white cell pellets will be stored at -80ºC.

13b. Long Term Storage of Tissues

Tissues in RNAlater® can be stored indefinitely at -20ºC but will be stored at -80ºC to control storage space.

13c. Long Term Storage of Plasma and Serum

Both plasma and serum will be stored at -80ºC.

13d. Long Term Storage of Urine

Urine will be stored at -80ºC.

13e. Long Term Storage of DNA

DNA is stable over prolonged period of times even at room temperature. For long term storage however, DNA will be stored at -80ºC.

13f. Long Term Storage of RNA

Extracted RNA will be stored at -80ºC.

14. Transfer of Biological Specimens from Different Sites

For the transfer of biological and processed samples from QCCH and CWH to SMH, the research midwives/nurses/associates will use dry ice in polystyrene boxes or cool bags/boxes for the safe and efficient transfer from the collection site to the deposit site. It is anticipated that movement among these sites will be an hour or less and that this method is appropriate. Individual samples will be in appropriately sealed containers and bagged to ensure spillages are minimized.

For samples to be transferred to research users, the above methodology will be used with consideration for shipping time, distance, climate, method of transport, type and number of samples and regulations of the carrier. Packages will be labeled with standardized BIOSPECIMEN HAZARD labels. Samples will not be shipped without an authorized MTA (Template academic MTA Appendix 3).

15. Transfer of Data

Research midwives/nurses/associates will collect clinical data from donors and enter the information into an Excel spreadsheet on dedicated laptops with secure passwords. Data entry at this point will already be by a unique trio identifier code. Data transfer between recruitment team will be via nhs.net.

Additional data will be uploaded from the relevant NHS databases by HD Clinical. Data transfer between BBB and HD clinical will be via nhs.net.

Clinical and sample data will be stored on a bespoke database hosted by Asset trac. This will provide security with personalised access and editing rights, 24hr complete database backup, remote (cloud based) access and tracking of all changes and amendments to the dataset. All data stored on the Asset trac database will be semi-anonymised. Individual biological samples are identified

via a unique barcode which is the database is able to link with the unique BBB trio identifier code.

16. Conditions for Use and Access to BBB Biological Specimens

See Appendix 2 for 'APPLICATION FORM FOR USE OF BBB RESOURCES version 1.0'

1. Prospective researchers will have to provide written evidence of an ethically approved project to the RMB Board prior to access of the BBB resources.

2. Researchers should request an appropriate number and amount of samples for their study.

3. Samples should only be used for the submitted project. Sample left over after the project should be returned to the BBB.

4. Samples handed over to a researcher will be for their sole use and should not be passed on to other researchers.

5. The BBB will endeavor to provide the best quality sample but cannot guarantee suitability for other researchers' downstream applications.

6. Costs for transportation of samples will be the researchers' responsibility.

7. The BBB should be acknowledged in all publications and presentations where samples have been used to obtain data. A copy of any resulting abstract or publication should be sent to the BBB.

8. Findings that may have implications for a particular donor should be highlighted to the BBB, even though these findings will not be reported to the donor.

17. Equipment and Reagents

17a. Reagents

1. Kits

The Invitrogen iPrep™ Purification System is an automated nucleic acid purification system with assorted kits containing inclusive reagents.

iPrep™ Card:gDNA Blood (IS-10012) http://www.promega.com

iPrep™ Card:gDNA Tissue (IS-10013)

iPrep™ Card:Total RNA (IS—10014)

iPrep™ Forensic Card (IS—10011)

iPrep™ PureLink™ gDNA Blood Kit (IS-10005)

iPrep™ ChargeSwitch® gDNA Tissue Kit (IS-10004)

iPrep™ PureLink™ Total RNA (IS-10006)

iPrep™ Trizol® Plus RNA kit (IS-10007)

iPrep™ ChargeSwitch® Buccal Cell Kit (IS-10003)

PAXgene Blood RNA System (PreAnaytiX)

Qproteome FFPE Tissue Kit (Qiagen) 37625

SK-2 Isohelix Swab Isohelix, Cell Projects (SK-2S) http://www.isohelix.com

2. Chemicals

RNALater® (Ambion)

17b. Equipment

-80^{o}C, -20^{o}C and 4^{o}C facilities for storage

Invitrogen iPrep™ Purification System (IS-10000; Invitrogen)

Nanodrop® ND 1000 (Nanodrop Technologies Inc.)

Sorval® Legend RT (Kendro Laboratories)

Benchtop centrifuge

Waterbath

Barcode System

Freezer Bondz II labels (for cryogenic usage) (Brady)

Vacutainers (Becton, Dickinson and Company)

APPENDIX 1
Information to be collected pertaining to each trio. Additional maternal fields from participating hospitals database will be available via HD Clinical

DONOR	INFORMATION
MATERNAL	AGE HEIGHT PRE-PREGNANCY WEIGHT PARITY DIABETES (YES/NO) HYPERTENSION(YES/NO) OTHER SMOKING MEDICATION ALCOHOL DIET OCCUPATION PREVIOUS PREGNANCIES-DETAILS CURRENT PREGNANCY-DETAILS CONSANGUINITY
PATERNAL	AGE HEIGHT DIABETES (YES/NO) HYPERTENSION(YES/NO) OTHER SMOKING MEDICATION ALCOHOL DIET OCCUPATION
FETAL	GENDER GESTATION BIRTH WEIGHT PLACENTAL WEIGHT HEAD CIRCUMFERENCE BIRTH LENGTH APGAR SCORE MODE OF DELIVERY ADDITIONAL INFORMATION

APPENDIX 2

APPLICATION FORM FOR USE OF BBB BIORESOURCES version 2.0

BBB APPLICATION REFERENCE NUMBER:……

PLEASE READ AND SIGN OUR TERMS AND CONDITIONS FOR BBB USE AND UPLOAD WITH YOUR APPLICATION AND ACCOMPANYING DOCUMENT

BY SIGNING THIS AGREEMENT YOU ARE ALSO AGREEING TO DEPOSIT YOUR DATA IN THE BBB AFTER PUBLICATIONS AND IP ACKNOWLEDGED

APPLICATIONS ARE CONSIDERED APPROXIMATELY EVERY FOUR MONTHS APRIL, JULY AND NOVEMBER. YOU WILL HEAR BACK REGARDING YOUR APPLICATION WITHIN TWO WEEKS OF THE OF THE COMMITTEE MEETING.

COMPLETE ALL FIELDS AND SUBMIT VIA THE BBB WEBSITE

IF YOU HAVE ANY QUERIES REGARDING THIS FORM PLEASE EMAIL THE BBB MANAGER s.abu-amero@ucl.ac.uk

NAME OF APPLICANT, TITLE	
ADDRESS	
EMAIL ADDRESS	
TELEPHONE NUMBER	
FAX NUMBER	
DO YOU HOLD EXISITING PATENTS OR PATENT APPLICATIONS THAT COVER THE PROJECT	
LOCAL R&D PROJECT	

APPROVAL NUMBER	
APPROVING BODY	
TITLE OF PROJECT	
COLLABORATORS AND THEIR AFFILIATIONS-ADD AS NECESSARY	
500 WORD SUMMARY OF PROJECT	
TYPE OF SPECIMENS REQUESTED-PLEASE TICK BOX *OR* SEE BBB WEBSITE FOR LIST OF TYPE OF SPECIMENS AVAILABLE	FETAL PLACENTAL TISSUE FETAL UMBILICAL CORD FETAL CORD BLOOD EDTA FETAL CORD BLOOD SERUM FETAL CORD BLOOD PLASMA PLACENTAL MEMBRANES MATERNAL BLOOD EDTA MATERNAL SERUM MATERNAL PLASMA MATERNAL WHITE BLOOD CELLS MATERNAL URINE PATERNAL BLOOD EDTA PATERNAL SERUM PATERNAL PLASMA PATERNAL WHITE BLOOD CELLS FETAL DNA FROM PLACENTAL TISSUE FETAL RNA FROM PLACENTAL TISSUE FETAL DNA FROM UMBILICAL CORD FETAL RNA FROM UMBILICAL CORD FETAL CORD BLOOD DNA MATERNAL BLOOD DNA PATERNAL BLOOD DNA

CATEGORY OF SPECIMENS	PRE-ECLAMPSIA PREMATURE RECURRENT MISCARRIAGE IUGR NORMAL
VOLUME/AMOUNT OF SPECIMEN REQUESTED	
NUMBER OF SPECIMENS REQUIRED PER CATEGORY	
FUNDING BODY DETAILS	

PLEASE PROVIDE ONE COPY OF THE APPLICANTS CV.

PLEASE ATTACH ONE COPY OF THE APPROVED PROJECT.

PLEASE ATTACH ONE COPY PROOF OF FUNDING.

THANK YOU.

SIGNATURE OF APPLICANT-DATE	SIGNATURE OF R&D OFFICE/HOST INSTITUTION-DATE

APPENDIX 3

THE BABY BIO BANK MATERIAL TRANSFER AGREEMENT

(For non-commercial research by non-profit organisations)

THIS AGREEMENT is made as of the last date of signature by the parties.

BETWEEN:

(1) University College London, incorporated by Royal Charter in the United Kingdom whose principal address is Gower Street, London WC1E 6BT, United Kingdom (the "Provider");

and

(2) [insert details of the recipient institution] (the "Recipient")

THE PARTIES AGREE as follows:

1. Within 30 days following receipt of the payment as referred to in clause 12, the Provider shall provide to the Recipient anonymised clinical data and materials in such quantities (the "Materials") as set out in the Baby Bio Bank approved application in Schedule 1 (the "Approved Application"). For the purpose of this agreement, the term "Material" shall include the material provided to the Recipient pursuant to this clause 1 and any portions, unmodified progeny and unmodified derivatives thereof and any data and information provided to the Recipient in connection therewith (the "Material"), including any of them contained or incorporated in other substances.

2. Where the Material is "Relevant Material" as defined in the Human Tissue Act 2004, custodianship of the Material shall transfer to the Recipient upon delivery to the Recipient's premises. Where the Material is not "Relevant Material" as defined in the Human Tissue Act 2004, ownership of the Material shall be retained by the Provider.

3. The Recipient shall ensure that the Material will be used solely by the lead scientist as named in the Approved Application ("Recipient Scientist") and other employees and students of the Recipient within the Recipient Scientist's laboratory or under the Recipient Scientist's supervision, and solely for the research as described in the Approved Application. The Material shall not be used for profit-making or commercial purposes, including without limitation use in screening, testing, evaluation, design and/or development of drug or other commercial products, use in commercially-sponsored research or provision of a commercial service, use on behalf of any commercial entity and use in research under which any commercial entity obtains rights to research results or any other benefit.

4. The Recipient shall not provide the Material in whole or in part to any third party or disclose any clinical data in connection therewith without the prior written consent of the Provider.

5. The Recipient shall not use the Material for administration to human subjects or human application, or for clinical or diagnostic purposes. The Recipient shall comply fully with all applicable environmental, health and safety laws, the Human Tissue Act 2004 or equivalent in the Recipient's country (where applicable) and other applicable laws, rules, regulations, codes of practice, research governance or ethical guidelines, or other requirements of any relevant

regulatory authority with respect to the use (including, but not limited to, disposal or return) of the Material.

6. The Recipient shall acknowledge the source of the Material in any publications and presentations, oral or written, generated through use of the Material, by including the following wording:

The [insert description of material] was provided by the Wellbeing of Women Baby Bio Bank (http://www.ucl.ac.uk/babybiobank) supported by University College London, Imperial College London and Wellbeing of Women.

The Recipient shall provide a copy of such publication to [insert email].

7. The Provider warrants that where required by law the Material has been obtained from humans with the appropriate consent as required by the Human Tissue Act 2004 and with ethical approval.

8. The Recipient understands that the Material may have hazardous properties, contain infectious agents or pose other health and safety risks. Subject to clause 7, the Provider and its collaborators including [University College London, UCL Business PLC, Imperial College London and Wellbeing of Women] ("Collaborators") make no representations and give no warranties either express or implied with respect to the Material, including without limitation any implied warranty of quality or fitness for any particular purpose or any warranty in relation to the identity, purity, safety, or activity of the Material. The Provider and its Collaborators will not be liable to the Recipient for any loss, claim or demand made by the Recipient, or made against the Recipient by any third party, due to or arising from the use, storage or disposal of the Material by the Recipient, except to the extent the law otherwise requires. Notwithstanding the foregoing, nothing in this Agreement shall exclude or limit liability for fraud, fraudulent misrepresentation, death or personal injury caused by negligence or any other matter for which it would be unlawful to exclude liability.

9. The Recipient shall fully and promptly inform the Provider of any discoveries or inventions generated by the Recipient relating to or through use of the Material.

10. This Agreement shall commence on the date on which it is approved by both parties and shall continue until completion of the research as described in the Approved Application, unless terminated sooner in accordance with clause 11.

11. The Provider has the right to terminate this agreement forthwith at any time by means of written notice to Recipient if the ethical approval is withdrawn or if the Recipient is in breach of this Agreement. Upon termination of this Agreement or completion of the research as described in the Approved Application, the Recipient shall immediately discontinue all use of the Material and promptly destroy (at the Recipient's own cost) all unused Material and provide the Provider with written confirmation that this has been completed. Should an individual donor or their next of kin rescind their consent, the Provider will require and the Recipient agrees to discontinue using the appropriately identified sample and return or destroy it in accordance with the Provider's instructions.

12. The Recipient shall pay the sum as set out in Schedule 2 to cover preparation, administration and other related costs that may be incurred in relation to the supply of the Material to the Recipient. Such payment shall be made within 30 days following execution of this Agreement to the following

account number 4017 8691, sort code 20 10 53, account name University College London, held with Barclays Bank plc,6 – 17 Tottenham Court Road, London, W1T 1AZ. In addition, the Recipient shall use a courier with suitable skill and experience to safely transport the Material in accordance with all applicable laws and will bear the cost of shipping and any necessary insurance. If so requested by the Provider, the Recipient shall provide it with written confirmation of the safe receipt of the Material promptly after their delivery to the Recipient's premises.

Schedule 1

[Attach Approved Application]

Schedule 2

[Insert cost]

Agreed by the Parties though their authorised signatories:

University College London

Signed:

Name:

Title:

Date:

[Insert name of the recipient institution]

Signed:

Name:

Title:

Date: