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

VERSION 4.0

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CONTENTS **1**

1. What is a biobank **3**

2. The aim of the Baby Bio Bank (BBB) **3**

3. Primary Sites of the BBB **4**

4. Participating Sites of the BBB **4**

5. Personnel Structure of BBB **4**

6. The Resource Management Board (RMB) **5**
6a. Structure of the RMB **5**

6b. Function of the RMB **6**

7. Ethical Issues **7**
7a. Informed Consent 7
7b. Obtaining Consent 7
7c. Public Access to Information 9

8. Collection of Biological Specimens **10**
8a. Labelling of Samples 10
8b. Collection of Blood for DNA 10
8c. Collection of Blood for RNA 11
8d. Collection of Blood for Serum and Protein 11
8e. Collection of Urine 11
8f. Collection of Placental Tissue and Fetal Membrane for DNA 11
8g. Collection of Placental Tissue and Fetal Membrane for RNA 12
8h. Collection of Placental Tissue and Fetal Membrane for Histology and Proteomics 12
8i. Collection of Products of Conception 12
8j**.** Collection of Buccal Swab 13
8k. Collection of Clinical Data 13

9. Processing of Biological Specimens **14**
9a. Processing of Blood for DNA 14
9b. Processing of Blood for Plasma 14
9c. Processing of Blood for Serum 14

9d. Processing of Blood for RNA 14
9e. Processing of Urine for Biomarkers 15
9f. Processing Tissue for DNA 15
9g. Processing Tissue for RNA 16
9h. Processing of Buccal Swab for DNA 16
9i. Processing Tissues for Histology 17
9j. Processing of Tissues for Proteomics 17

10. Quantification of Biological Specimens 18
10a. Quantification of DNA 18
10b. Quantification of RNA 19
10c. Quantification of Proteins 19

11. Quality Control 19

12. Database Requirements 21

13. Storage of Specimens 22
13a. Long Term Storage of Blood 23
13b. Long Term Storage of Tissues 23
13c. Long Term Storage of Plasma and Serum 23
13d. Long Term Storage of Urine 23
13e. Long Term Storage of DNA 23
13f. Long Term Storage of RNA 23

14. Transfer of Biological Specimens from Different Sites 23

15. Transfer of Data 24

16. Conditions for Use and Access to BBB Biological Specimens 24

17. Equipment and Reagents 26
17a. Reagents 26
17b. Equipment **26**

APPENDIX 1 27
APPENDIX 2 28

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 snap frozen placental membranes, placenta and cord will be collected for RNA expression and protein analyses. Separate samples of the placenta and placental membranes will be fixed for histology. 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. Gross chromosomal abnormalities in the pregnancy complications groups will be tested by routine cytogenetic analysis.

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)

We also propose to involve other London Hospitals as the project progresses. Their details and involvement will be added as amendments.

5. Personnel Structure of BBB

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

5b. BBB Research Manager: 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 (other clinical staff to be appointed when funding available.

5e. Research Associates Dr Anna Thomas (ICH) and Dr Shawnelle White (SMH)

6. The Resource Management Board (RMB)

6a. Structure of the RMB

Resource Management:

The composition of the Resource Management Board will be as follows:

Ten investigators to this application;

Professor Gudrun Moore (ICH)

Professor Lesley Regan (SMH)

Professor Catherine Williamson (QCCH)

Professor Mark Johnson (CWH)

Professor Kypros Nicolaides (KCH)

Professor Donald Peebles (UCLH)

Ms Lorna Phelan (SMH, Consultant Obstetrics and Gynecology)

Dr Neil Sebire (Great Ormond Street Hospital (GOSH), Consultant Pediatric Pathologist)

Dr Janet Rennie (UCLH, neonatologist)

Ms Lynne Pacanowski (Guy's and St Thomas' Trust)

Two highly experienced research active bio-medical scientists not directly involved in the two management centres. Professor David Dunger, (Cambridge) and Professor Kay Davies (Oxford). Professor David Dunger has particular expertise in the analysis of quantitative genetic traits especially with respect to childhood growth and diabetes. Professor Kay Davies is internationally renowned for her work on neuromuscular disorders and has specifically been asked to be involved for her knowledge of molecular and functional genetics.

Two Emeritus Professors: Professor Philip Steer (O&G, CWH, Imperial College London) Professor Michael Preece (Paediatrics, ICH, University College London)

Two informed lay representatives: Clare Odgers (History and Science Documentary Producer) and Kate Davies (CEO Notting Hill Housing Association)

Two Representatives from the Royal College of Obstetrics and Gynaecology WellBeing of Women (to be named)

One Law and Medical Ethics Representative Professor Rosemary Scott, Professor of Medical Ethics (Kings College London).

The **R.M.B**. will be required to meet once a year and will use e-mail to communicate in the interim periods. Chairmanship of the Board will rotate annually.

Access to the collection will be for academic research purposes only. The resource itself will not be made available for commercial usage. If commercial usage were to be considered to be of potential benefit in the future, this would only be possible with the approval of the Management Board. Academic 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 anonymised for the purpose of the study.

7b. Obtaining Consent

Potential donors will be identified through the five clinics by the respective consultants 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 recognized 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 but the individual patient will be diagnosed by the consultant.

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.

The BBB will eventually launch a website, in the form of a newsletter, which 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. This website with associated facilities will be launched later on after the onset of the collection of the BBB.

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 immediately semi-anonymised by assigning a barcode generated 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 a week transported to either ICH (UCLH or KCH) or SMH (SMH, CWH or QCCH) (see section 13). 4ºC, -20ºC and -80ºC facilities and portable liquid nitrogen dewars will be available at all participating sites for appropriate storage of biological specimens if necessary.

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 trio i.e. maternal, paternal and fetal samples from one family. Clinical data for each individual in the trio will be encoded into the barcode.

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

10 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 48 hours but can be kept at -20ºC or -80ºC for long term storage unless otherwise stated (see section 13).

If DNA is to be extracted from blood then EDTA (purple top) or ACD tubes are best. EDTA are multipurpose and are widely used. ACD tubes are particularly suitable for white blood cells and permit extraction of good quality DNA up to 10 days after collection.

8c. Collection of Blood for RNA

Paxgene™ blood RNA tubes (PreAnalytiX) can be used for collection of blood intended for RNA extraction according to the manufacturer's instructions. These tubes stabilize intracellular RNA at room temperature for three days or from 2-8ºC for five days.

RNA can also be extracted from fresh whole blood using the Invitrogen iPrep™ Purification System (IS-10000; Invitrogen).

8d. 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.

8e. 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.

8f. Collection of Placental Tissue and Fetal Membrane for DNA

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 eight sites surrounding the umbilical cord will be taken and rinsed three times in room temperature 1 x PBS (Invitrogen) to remove as much maternal contamination as possible. The sample is then placed in a labeled 5 ml container and snap frozen in a portable dewar of liquid nitrogen for convenience. These will then be stored at -80ºC for long term storage.

8g. Collection of Placental Tissue and Fetal Membrane for RNA

The option of RNALater® (Invitrogen) is also available, although it is more costly. 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. Samples are excised and rinsed in 1 x PBS as for 8f and immersed in 5 volumes of RNALater®.

8h. Collection of Placental Tissue and Fetal Membrane for Histology and Proteomics

Tissues to be used for histological and proteomics investigations will be fixed in formalin and are best processed immediately on collection. Tissue specimens should not be larger than 1.5 cm x 1 cm x 0.5 cm. Specimens will be fixed in 10% neutral buffered formalin for 4 – 48 hours before embedding in paraffin using a Tissue Tek VIP Processor following conventional techniques.

Formalin-fixed, paraffin-embedded (FFPE) tissues will be stored at room temperature and are stable indefinitely for DNA, RNA and protein extraction.

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. Samples will still be processed as for tissue in 8f.

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 Clinical Data

Clinical data will be amassed by the research nurses/midwives/associates and will include information on maternal and paternal medical history and demographics and will detail fetal outcome such as birth weight, placental weight, head circumference, gender etc. (see appendix 1). The data will be encoded and relate to the barcoded biological sample and any resulting extracted sample. 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. In addition to Sodium citrate vacutainers, whole blood collected in EDTA/Lithium heparin/ACD tubes is suitable for plasma isolation. The vacutainer containing the blood sample will be spun at 815 g for 10 minutes at 4ºC in a Sorval® Legend RT centrifuge. The plasma is the upper layer and is transferred to a clean 15 ml tube without disturbing the white cells in the buffy coat (the tube can be retained for DNA extraction from the white blood cells). The transferred plasma is spun at 3200 g for 10 minutes at 4ºC and then aliquoted into 1 ml labeled cryovials before snap freezing in liquid nitrogen. Aliquots must 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 10 minutes at 1200 g in a Sorval® Legend RT centrifuge and then aliquoted into 1 ml labeled cryovials before snap freezing in liquid nitrogen. Aliquots must be stored at -80ºC.

9d. Processing of Blood for RNA

RNA can be extracted from a PAXgene™ Blood RNA tube using the PAXgene™ Blood RNA Kit according to the manufacturer's instructions. This option will be

utilized if extraction using the Invitrogen iPrep™ Purification system is not immediately possible as RNA is stable using this system at room temperature for three days or at 2-8C for five days.

RNA can be extracted from fresh blood using the Invitrogen iPrep™ Purification system in conjunction with the iPrep™ PureLink™ Total RNA kit. 5 ml of RBC (red blood cell) Lysis Buffer (supplied) will be added to 1 ml fresh whole blood and incubated on ice for 10 minutes with vortexing every three minutes during the incubation period. The sample will be centrifuged for 10 minutes at 4º C at 400 g in a Sorval® Legend RT centrifuge. The supernatant will be discarded and the white blood cell pellet resuspended in 2 ml RBC Lysis Buffer (supplied) and mixed well by vortexing before centrifuging as above. After removal of the supernatant, the pellet will be resuspended in 600 µl Lysis Buffer (supplied) and the cells homogenized. This will then be centrifuged in a benchtop centrifuge at 2600 g for 5 minutes at room temperature. The resulting lysate will be transferred to an iPrep™ Sample tube and RNA isolated according to the manufacturer's instructions. RNA is eluted in a volume of 100 µl Elution Buffer (supplied).

9e. 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.

9f. 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.

9g. 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.

9h. 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 37C. 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.

9i. Processing Tissues for Histology

Tissues for histological examination using a light microscope will be sliced (10 µm thickness) using a glass knife mounted in a microtome and the tissue slice mounted on glass microscope slides. Cellular components can be examined by using a variety of stains such as Hematoxylin and Eosin (H&E).

9j. Processing of Tissues for Proteomics

Proteins can be extracted from FFPE tissues for downstream proteomic applications such as Western blotting, protein arrays and Liquid Chromatograph Mass Spectrometry (LC-MS) by using the Qproteome FFPE Tissue Kit (Qiagen).

Tissue from FFPE blocks or unstained FFPE tissue from glass slides can be used. In both cases the sample will be deparaffinised using xylene. For FFPE slides, the slide will be serially immersed three times in fresh xylene for 10 minutes each time at room temperature. The slide will then be immersed twice in fresh 100% ethanol for 10 minutes each time at room temperature, followed by two similar washes in 96% and 70% fresh ethanol. The slide will then be transferred to a dish with fresh double distilled water for 30 seconds and then drained before excising the area of interest with a needle and transferring to a 1.5 ml collection tube (supplied). Extraction of proteins will be completed following the manufacturer's instructions for the Qproteome FFPE Tissue Kit (Qiagen).

For FFPE tissue blocks, sections approximately 10 – 15 µm will be cut and placed in a 1.5 ml collection tube (supplied) and 1 ml fresh xylene added directly. The tube will be vortexed vigorously for 10 seconds, incubated at room temperature for 10 minutes before centrifuging at 13000 g for 2 minutes. The supernatant will be pipetted off and fresh xylene wash steps

repeated twice. 1 ml of 100% fresh ethanol will then be added to the tissue pellet, the tube vortexed and incubated at room temperature for 10 minutes before centrifuging for 2 minutes at 13000 g in a benchtop centrifuge. The supernatant will be removed and the ethanol wash steps repeated twice. The washes will be repeated twice each with 96% and 70% fresh ethanol. The pellet is ready for protein extraction and will be completed following the manufacturer's instructions for the Qproteome FFPE Tissue Kit (Qiagen).

10. Quantification of Biological Specimens

Quantification of all samples will be done with 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).

11. Quality Control

11. Quality Control: taken from the BBB procedures booklet Version 4.0

Quality control encompasses:

Equipment maintenance and repair

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

Staff training records

- All BBB staff employed by the two universities requires 10 days of new skills training annually (website to programs available at Imperial and UCL). Training includes course such as: mentorship; dealing with bullying; 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.

Data management

- Data on the biological samples (type, volume, freezer location etc.) and clinical data (maternal/paternal/fetal data) are stored on the BBB database managed remotely by a secure server and is password protected.
- Trios are given a unique number and samples are barcoded.
- Data is backed up every 24 hours.
- No personal identifiers are recorded on the database rendering it semi-anonymous.
- Recruiting staff are able to identify participants only through the consultants at the hospitals to ensure that the integrity of the trio is maintained with no sample mix ups.
- Freezer maps of barcoded samples are kept in accordance with HTA requirements.
- Each month 20 samples are randomly checked for accuracy.

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 Maria Bitner-Glindzicz (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), reverse transcription PCR (RNA), and histopathology (Tissue and protein integrity)

12. Database Requirements

The BBB database will be developed with the aims to:

1. Provide 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. Quality control results will be entered into the database.

8. Access to data will be through a tiered level to restrict access to the minimum number of users.

9. The database will be used to provide reports and audit information.
10. The database will be held on the two primary sites, ICH and SMH, by means of networked laptops. This ensures that data is not stored on hard drives but rather on a secure network. Both sites will receive biological specimens for processing: ICH will receive samples from UCHL and KCH, and SMH will receive biological specimens from SMH, CWH and QCCH. The associated clinical data for each sample will be entered either at ICH or SMH by the respective research midwife with appropriate access. The data will be simultaneously updated on the sister database.

11. Processed sample information and quality control data will be entered into the database by the research technicians with appropriate access. The data will be simultaneously updated on the sister database.

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 scannable 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

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

Formalin fixed tissue will be stored in paraffin wax at room temperature.

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 -20ºC.

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 UCLH and KCH to ICH, and SMH, QCCH and CWH to SMH, the research midwives/nurses/associates will use dry ice in polystyrene 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.

In the case of FFPE material, samples will be shipped at room temperature in insulated protective wrapping.

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.

15. Transfer of Data

Research midwives/nurses/associates will collect clinical data from donors and enter the information into an Access spreadsheet on dedicated laptops with secure passwords. Data entry at this point will already be by a unique identifier. The information can then be uploaded onto the main ICH and SMH computers.

For transfer of data to prospective research users, the access database can generate reports according to the researchers' requirements which can be converted into pdfs before providing to the researchers.

ALL DATA WILL BE ANONYMISED.

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°C, -20°C and 4°C facilities for storage

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

Tissue Tek VIP Processor

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.

DONOR	INFORMATION
MATERNAL	AGE HEIGHT PRE-PREGNANCY WEIGHT PARITY DIABETES (YES/NO) HYPERTENSION(YES/NO) OTHER (YES-DETAILS/NO) SMOKING MEDICATION ALCOHOL DIET OCCUPATION PREVIOUS PREGNANCIES-DETAILS CURRENT PREGNANCY-DETAILS AGE AT MENARCHY CONSANGUINITY
PATERNAL	AGE HEIGHT DIABETES (YES/NO) HYPERTENSION(YES/NO) OTHER (YES-DETAILS/NO) 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 RESOURCES version 1.0

APPLICATION REFERENCE NUMBER:……

NAME OF RESEARCHER	
ADDRESS	
EMAIL ADDRESS	
TELEPHONE NUMBER	
FAX NUMBER	
PROJECT APPROVAL NUMBER	
APPROVING BODY	
TITLE OF PROJECT	
COLLABORATORS AND THEIR AFFILIATIONS	
500 WORD SUMMARY OF PROJECT	
TYPE OF SPECIMENS REQUESTED	
AMOUNT OF SPECIMEN REQUESTED	
NUMBER OF SPECIMENS REQUIRED	

PLEASE ATTACH ONE COPY OF THE APPROVED PROJECT. THANK YOU.