

The Baby Biome Study

STUDY PROTOCOL

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Study Group

Core group:

Prof Peter Brocklehurst
(PI)
Prof Carol Dezateux
Dr Nigel Field
Dr Claire Townsend
Dr Alison Rodger
Dr Trevor Lawley
Prof Eleanor Riley
Sarah Bailey

Coapplicants:

Prof Stephan Beck
Prof Paul Elliot
Prof Elaine Holmes
Prof Paul Kellam
Prof Simon Murch
Prof Deenan Pillay
Prof Neil Sebire

Collaborators:

Prof Judy Breuer
Prof Dame Anne Johnson
Dr Xiayi Ke
Dr Mahdad Noursedeghi
Prof Rosalind Smyth
Prof David Spratt
Dr Claire Thorne
Prof Sir Andrew McMichael
Dr Richard Pebody

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I SUMMARY

Study Rationale

The Wellcome Trust made a Strategic Award (2013-15) to fund piloting and development work to establish the feasibility and acceptability of sample collection from Life Study participants around the time of birth and in the first year of life, with a focus on infection and immunity. This pilot study, called the Infection and Immunity (I&I) Enhancement, aimed to pilot the collection of biological samples which will be used to understand how interactions between microorganisms, both pathogenic and the colonising microbiota, the immune system and clinical, social and behavioural factors during pregnancy, birth, infancy and childhood influence health throughout life. The I&I pilot also aimed to address two hypothesis-driven research questions, analysing mother and baby stool samples and cord blood. Due to lower than expected recruitment through Life Study, a costed extension was subsequently granted by the Trust until the end of October 2016. **This has been further extended to the end of February 2017.**

A new phase of the I&I pilot, the Baby Biosample Study (BBS), was implemented at Barking, Havering, and Redbridge NHS University Trust (BHRUT) in September 2015 to augment recruitment. The protocol for the collection, analysis and storage of cord blood, maternal and baby stool as part of BBS was previously approved by the London City and East REC (21/07/2015, 12/LO/1492). **In light of the closure of Life Study, the I&I Enhancement has adopted the BBS recruiting and other methodologies. The Life Study enhancement and BBS are now known as the Baby Biome Study. This protocol describes (1) the process of contacting women eligible for the Baby Biome Study during the third trimester of their pregnancy, (2) the collection of samples from mothers and babies (3) follow up of women and their babies at 4-10 and 9-15months for collection of baby stool samples and completion of a questionnaire, and (4) requesting consent for future record linkage around the time of birth.**

Objectives

Our objectives, using samples previously collected from the Life Study I&I pilot study and BBS as well as those collected prospectively through the Baby Biome Study are to:

- 1) Collect biological samples by recruiting women who arrive at the labour ward to give birth to allow acceptability and feasibility of sample collection to be tested and our scientific objectives for our pilot study to be met within the time frame of the Wellcome Trust Strategic Award.
- 2) Characterise and compare colonisation patterns in the first few weeks of life in infants born vaginally or by caesarean section using sequentially collected stool samples from babies.
- 3) Characterise and compare baby gut microbial colonisation patterns in those born vaginally or by caesarean section with (1) no exposure to antibiotics during pregnancy, (2) exposure to intra-partum antibiotics, or (3) exposure to antepartum antibiotics during the month prior to birth.
- 4) Test the association between the cord blood immune cell transcriptome profile in cases with prolonged rupture of the membranes (PROM) compared to controls without.

Participant involvement

All pregnant women booked to deliver at each participating hospital's labour ward will be given a **flyer describing the study in handheld notes at 12 weeks, and** PIS at around 36 weeks' gestation. Some women may immediately decide that they do not wish to take part and they will be able to opt out of the study by notifying their community midwife. For all other women, the PIS will be placed into the maternity notes to indicate to labour ward staff that these women have been notified about the study. All pregnant women who

arrive at the labour ward to give birth and do not already have a PIS in their notes, including women in labour before 36 weeks' gestation, and have not already declined to participate, will be provided with the PIS and asked to participate. Pre-term babies are a group of specific interest and there are no exclusion criteria based on gestational age. Women can participate even if they do not provide all of the requested samples.

Wherever possible, women will have a maternal stool sample collected, and a sample of umbilical cord blood. All women will be asked to self-collect a vaginal swab before birth. Women will also be asked to collect baby stool 7 days after birth. A subgroup of approximately 200 mothers will additionally be asked to return baby stool at four days and 21 days after birth. We will also approach women post-natally who we may have missed before birth and ask them for consent to collect baby stool, even if we have not collected other samples, this poo may be collected by a midwife once the baby has gone home.

Women will be invited to consent to donation of their samples after delivery, although they will be able to consent prior to delivery if they wish to do so. We will also ask women to consent to record linkage, future contact about further study activities, and retrieval of leftover routine antenatal and neonatal samples. Women will be able to decline this invitation or withdraw their consent at any time, including after they have left the hospital.

Laboratory procedures

Stool samples will be used for phenotypic analyses and DNA will be extracted for sequencing using MiSeq. Initial PCR experiments will be performed in Dr Mahdad Noursadeghi's laboratory at University College London (UCL) or in the study laboratory at BHRUT. Sequencing experiments will be performed by Dr Trevor Lawley's group at the Wellcome Trust Sanger Institute in Cambridge, or in Dr Noursadeghi's laboratory at UCL. RNA will be extracted from cord blood samples and cDNA microarrays performed in Dr Mahdad Noursadeghi's laboratory at UCL. We may send samples to the laboratory at BHRUT for initial processing and DNA extraction prior to analysis.

Study management

Overall study management will be overseen by Professor Peter Brocklehurst (Principal Investigator), with Nigel Field as the Scientific Lead. Laboratory and sample management will be overseen by Dr Trevor Lawley (stool samples and vaginal swabs), Dr Mahdad Noursadeghi (cord blood samples).

Ethical implications

We will initially approach women during pregnancy to provide information about the study in advance of giving birth, **with a flyer in booking notes at around 12 weeks' gestation, and a PIS at around 36 weeks gestation.** We believe that this will improve recruitment by giving women more time to consider what the study involves and means that most eligible women should be aware of the Baby Biome Study before they give birth. However, there may be some women who do not receive this information and they will be given a PIS when they arrive at the labour ward in labour.

Women will be asked to consent to participation after birth, on the labour ward, usually by their attending midwife who has cared for them during the birth or by one of our study midwives. Some women, for example those who have received an epidural or who are booked for an elective caesarean, and those who have received the PIS during pregnancy, may feel able to consent prior to giving birth. Women who need more time to decide, for example medical emergencies or women in advanced labour, will be asked to provide written consent on the postnatal ward. It will be made very clear that women can refuse to take part immediately or

withdraw. Women will be able to withdraw at any time, including after discharge from the hospital, in which case all samples and associated data collected will be destroyed.

Overview of outcome

Through the collection of samples from women and babies around the time of birth, we aim to answer central questions about the role of infection and immunity in later health outcomes by undertaking experimental studies to answer questions about the early gut microbiota and developing immune system.

II BACKGROUND AND RATIONALE

The Baby Biome Study

The Baby Biosample Study (BBS) was implemented at Barking, Havering, and Redbridge NHS University Trust (BHRUT) in September 2015 to augment recruitment into the pilot for the Infection and Immunity Enhancement to Life Study. In light of the closure of Life Study, we extended BBS to additional hospital sites (including University Hospitals Leicester (UHL), University College London Hospital (UCLH)) and modified the study so that we can obtain follow up information on women and their babies. This includes asking women to consent to future record linkage and contacting women taking part to complete a questionnaire between 6 weeks and 6 months after birth and to donate baby stool samples both between 6 weeks and 6 months and 9-12 months after birth. We have also changed the study name from the I&I Enhancement and BBS to the Baby Biome Study to reflect the change from our study being a Life Study Enhancement to a standalone study. We will retain samples already collected as part of Life Study and BBS for analysis as part of the Baby Biome Study.

III AIM AND OBJECTIVES

Aim

The Baby Biome Study aims to explore causal pathways linking early infectious and immunological events with subsequent health outcomes. The overall aim of sample collection at the time of birth is, therefore, to recruit women to donate samples for use in our pilot phase before the end of our grant **(current end date: February 2017)** to enable us to answer the primary scientific objectives for our pilot study, listed below.

Objectives

Our primary scientific objectives for the Baby Biome Study are to:

- 1) Characterise and compare colonisation patterns in the first few weeks of life in those born vaginally or by caesarean section using sequentially collected stool samples from babies.
- 2) Characterise and compare baby gut microbial colonisation patterns in those born vaginally or by caesarean section with (a) no exposure to antibiotics during pregnancy, (b) exposure to intra-partum antibiotics, or (c) exposure to antepartum antibiotics during the month prior to birth.
- 3) Test the association between the baby peripheral blood immune cell transcriptome profile in babies who are exposed to, and those who are not exposed to, prolonged rupture of the membranes (PROM), as evidence for ascending placental infection.

IV METHODS

A. Setting

Sample collection will take place at BHRUT, UHL and UCLH. In the future, we plan to expand the Baby Biome Study to other hospital sites and will seek local permissions in each instance as appropriate. The Baby Biome Study will recruit pregnant women who arrive at each hospital to give birth before the end of **February 2017**. Women will be approached and recruited sequentially in order to collect sufficient samples to test the feasibility of sample collection as well as to answer the scientific objectives of the Baby Biome Study.

B. Recruitment

The recruitment process is described in Appendix 1. We will collect a focused set of samples as well as clinical data such as mode of delivery, gestational age at delivery and perinatal and intrapartum antibiotic use.

Women will first be made directly aware of the opportunity to take part in the Baby Biome Study during pregnancy, at around 12 weeks' gestation at booking, a flyer (BBS A5 FLYER V1.0) will be placed in notes.

Women will be provided with a PIS during pregnancy by their community midwife which will explain the study and advises women that they may be approached to take part when they come to the labour ward to give birth. This PIS will, [version 2.0], include details about self-collection of a vaginal swab, retrieval of leftover routine antenatal and neonatal samples, record linkage and future contact.

Some women may immediately decide that they do not wish to take part and they will be able to opt out of the study by notifying their community midwife who will place a sticker indicating that they have declined participation on the front of their maternity notes. These women should not be approached again. For all other women, community midwives will be asked to place the PIS into the maternity notes to indicate to labour ward staff that these women have been notified about the study.

Upon arrival at the hospital, labour ward midwives will provide women who do not already have a PIS in their maternity notes, including women who go into labour before term, with the study PIS to read. Midwives will speak further to women after delivery of their baby, once clinically stable, and before discharge from the labour ward, which is usually around 6 hours after delivery. Women will have had a chance to review the PIS and midwives will offer to answer questions and to go through the PIS with them if they wish.

C. Sample collection

Samples

1. **Stool** will be obtained from the mother during labour where women are happy to do this, during delivery, or in the postnatal period before they leave hospital.
2. Women will be asked to self-collect a **vaginal swab** before delivery.
3. A **cord blood** sample will be taken as soon as is feasible after delivery without causing any delay or disruption to the birth or clinical processes after birth.
4. Approximately seven days after birth, a sample of stool will be collected from the nappies of babies by mothers. In most cases, this will be at home and the sample will be posted to the hospital pathology laboratory. For babies remaining in hospital, attending midwives will be responsible for collecting the sample.

We will also ask a subsample of approximately 200 women to collect **stool** from their babies four and 21 days after birth to allow sequential colonisation of the microbiota to be investigated. We will ask women whether they are happy to provide these samples until we have asked sufficient numbers to obtain these additional samples from around 200 babies. These samples will also be posted back to the hospital pathology laboratory. For babies remaining in hospital, attending midwives will be responsible for collecting these samples.

- 5. Participants will be contacted (BBS Request for sample letter_V1.0 27/09/2016) when their baby is six months old to request a baby poo sample with a short form detailing date and time of collection (BBS 6 Month stool form V1.0 27/09/2016). Following this contact, participants will be contacted and asked to complete a questionnaire (BBS 6M Questionnaire cover letter_V1.0 27.09.2016 and BBS 6M Questionnaire_V1.0 27/09/2016)**

When women give birth, the biological samples listed above will be collected either by midwives on the labour ward or self-collected by women, with the sampling process described in detail in Section VI, part D. We also plan to retrieve leftover routine antenatal maternal blood samples and dried blood spots from infant heel prick tests to investigate the potential effects of infection during pregnancy on babies' immune systems at birth. The only blood samples collected around the time of birth by the Baby Biome Study will therefore be cord blood; no other blood samples will be taken from mother or baby specifically for this study around the time of birth.

Biological samples will be tracked using the labelling system which was established for Life Study. Systems appropriate to local hospitals will be put in place. Further details are provided in Section V, part A (page 14).

D. Sampling methods

Stool sample from mothers

Women will be asked to provide a stool sample during labour. Many women not passing stool before birth will pass stool during a normal vaginal delivery. In this case, the maternity unit midwife will place any stool passed into a stool specimen collection pot. **This kit also includes a stool specimen frame that sits within the toilet and supports the collection container. Women may also self-collect stool using the kits provided.** Women delivering by elective caesarean section will be asked to provide a stool sample before (or after) delivery, during their hospital stay. Samples will be stored in the study fridge until processing.

Vaginal swab

This sample will be self-collected by women before delivery, with a kit comprising a swab and collection tube along with collection instructions. If women prefer, and are happy to donate this sample, they may ask their midwife to collect the sample. Samples will be stored in the study fridge until processing.

Umbilical cord blood

The umbilical cord will be doubly clamped and transected after delivery. Once the placenta and cord are delivered they will be placed on an inco sheet in the delivery room and umbilical cord blood will be extracted from the lowest point in the cord using a butterfly needle. During caesarean sections, after the birth of the baby, cord blood will be collected from the removed placenta outside the operating theatre. Efforts will be made to obtain maximal volumes from each collection. The delivery of the newborn will not be influenced in any way by the study and, in particular, the clamping of the cord will not be accelerated, nor the delivery of the placenta delayed. Samples will be stored at room temperature until processing.

Stool sample from baby

Mothers will be given a collection kit for baby stool when they leave the labour ward. They will be asked to collect a sample of their baby's stool seven days after birth by collecting a small amount of stool from the nappy using a scoop (if possible, avoiding any mixing with urine) and place it into a tube. In most cases, this will be at home and the sample will be sent to the pathology laboratory at BHRUT by First Class post. For babies remaining in hospital, attending midwives will be responsible for collecting the sample. A subsample of approximately 200 women will also be asked to collect stool from their babies at four and 21 days after birth, again sending the samples to the pathology laboratory by First Class post. **All participants will be approached 4-10 months after the birth of their baby to request a sample of their baby's poo, which they will be asked to return by post in a prepaid envelope with a form detailing date and time of sample collection (BBS 6 Month stool form V1.0 27/09/2016).**

E. Research Question 1: Microbiota

Inclusion criteria

We will use stool samples **and vaginal swabs** from women and their babies who have samples collected as part of the Baby Biome Study. Women will be selected according to whether they fall into one of the following groups:

1. Vaginal deliveries with antibiotic exposure in the month prior to birth;
2. Vaginal deliveries with intrapartum antibiotic exposure;
3. Caesarean deliveries with antibiotic exposure, or;
4. Vaginal deliveries with no antibiotic exposure.

Study population and eligibility

Stool samples from a total of 300 babies will be needed to answer this question, with approximately 75 in each group 1-4 above. Samples will be collected from the first 300 mother-infant pairs from whom it is possible to collect stool samples.

Identification of samples

A total of two aliquots will be taken from each mother and infant stool sample for the purposes of the experimental studies described here: one will comprise extracted DNA for sequencing and one will comprise raw stool for phenotypic analyses.

Overview of experiments

Both mother and baby stool samples will be processed into separate aliquots of extracted DNA and raw stool. Using DNA extracted from stool samples, amplicons will be produced for 16S rRNA sequencing using MiSeq and metagenomics. Raw stool samples will be used for phenotypic and functional analyses. The experiments will be performed by Dr Trevor Lawley's group at the Wellcome Trust Sanger Institute and in Dr Mahdad Noursedeghi's laboratory in UCL. More detail of the laboratory procedures and the outcome measures for the experiments is provided in Appendix 3.

Data collection

Data will be collected onto paper forms or electronic devices (e.g. iPads) by labour ward midwives or research staff from the Baby Biome Study after birth to collect basic clinical information about each woman and their baby around the time of birth, by reviewing their paper-based notes, including the gestational age of the baby at birth, mode of delivery, whether any antibiotics were given at the time of delivery (and if so, which antibiotic and for how long), PROM status and whether they were breast feeding at discharge. Where

required, the study midwife will fill in any missing data using hospital-based electronic records after discharge of the woman.

A questionnaire (BBS 6M Questionnaire_V1.0 27/09/2016) will be sent to participants four to ten months after the birth of their baby. The questionnaire will be used to collect information on exposures the baby has encountered in their first six months, as well as some further lifestyle information.

Paper-based medical notes of participants will be identified using a coloured sticker on the front cover. Notes will be collected by the ward clerk on the postnatal ward at the time of discharge and collected by the I&I research midwives for review. For the purposes of record linkage we will retain each woman's NHS number in their study record.

Metadata will be collected for all samples. This will include, for example, the date and time of sample collection, processing, and storage as well as information about sample volumes. The following information will also be collected for each sample:

- Date and time samples are sent to the Sanger Institute / UCL
- Date and time samples are received at the Sanger Institute / UCL

Key clinical data required to meet the objectives of this research question include:

- Antibiotic use
- Mode of delivery
- Gestational age at delivery

This list is an indicator of the type of information required and is not exhaustive.

Data analysis

Microbiota sequencing and culturing data from stool **and vaginal** samples will be used to analyse potential differences in microbiota composition in babies delivered by caesarean section versus vaginally, further stratified by whether women did or did not receive antibiotics. Sequencing and bioinformatic analysis of microbiota samples will be used to identify antimicrobial genes, virulence and beneficial genomic pathways that are present within infant's microbiota and will be associated with clinical practices and health outcomes of babies. Microbial culturing will be used to validate phenotypes. We will undertake microbiota analyses on vaginal swab samples however we may require additional funding in order to do this. These samples will therefore be stored until analysis.

Descriptive analyses of demographic and clinical characteristics will also be undertaken and these data will be linked to samples with analyses undertaken within the context of a large-scale epidemiological cohort study. As such it will be appropriate to include potential confounders in analyses and these may include: maternal ethnicity, gestational age at birth and use of antibiotics. In order to account for differences in antibiotic administration depending on whether surgery is elective or emergency, data on the timing of antibiotic administration will be collected. This will allow us, by stratification, to determine the potential effect of caesarean delivery on the microbiota independently of the effect of antibiotics and to investigate any potential dose-response effect of antibiotics on the microbiota.

Handling of leftover samples

Leftover extracted DNA and raw stool from samples collected as part of the Baby Biome Study will be stored longterm (for more than 10 years) at the Sanger Institute.

Sample size

We will collect and analyse 300 paired mother-infant stool samples. Power calculations for microbiome studies in human cohorts are extremely challenging, as they must simultaneously address multiple areas including depth of sequencing, and taxon detection. We will work with experts in the field to maximise statistical power within the constraints of time and manpower to undertake these analyses. Within the first 300 women and babies for whom paired stool samples are available, we expect to collect samples from: (1) vaginal deliveries with antibiotic exposure in the month prior to birth, (2) vaginal deliveries with intrapartum antibiotic exposure, (3) caesarean deliveries with antibiotic exposure, and (4) vaginal deliveries with no antibiotic exposure, with approximately 75 women in each group. These four groups will allow us to answer questions about the effect of mode of delivery and antibiotic exposure on microbiota colonisation patterns set out in our objectives. Based on an approximate caesarean section rate of 25% in the UK and that all women who deliver by caesarean section in the UK receive antibiotics (NICE), we estimate that 500 women in a cohort of 2,000 births will receive antibiotics prior to caesarean delivery. Studies on antibiotic use in pregnancy have reported that 5% of women received a prescription for oral antibiotics in the last month of pregnancy prior to the onset of labour. We therefore estimate that approximately 5% of women, whose babies are delivered vaginally, will also be given antibiotics in the immediate antepartum period, translating into 100 participating mothers and babies exposed to antibiotics in our cohort. Many published microbiota studies have detected differences between participants using small sample sizes, suggesting adequate power within our sample to explore differences in early gut colonisation using these groups.

F. Research Question 2: Immunology

Definitions and inclusion criteria

The exposure for this question is PROM and the outcome of interest is transcriptional immune response profiles in cord blood.

The exposed group will be defined as:

- Babies of women who deliver at term (at or after 37 completed weeks of gestation) where delivery occurred more than 24 hours after rupture of membranes.

The unexposed group will be defined as:

- Babies of women who deliver at term, within 8 hours of rupture of membranes, frequency matched to women exposed to PROM based on parity and mode of delivery.

Study population and eligibility

We will collect cord blood samples from the babies of women with PROM observed in our cohort who are participating in the Baby Biome Study. We anticipate that we will need to collect samples for between 20 and 30 women with PROM and their babies from an expected 160 PROM cases observed during the pilot study comprising approximately 2000 women, based on an 8-10% prevalence of PROM in the UK population (NICE Guideline CG70 Induction of labour: full guideline, 2008). We will also use cord blood samples from approximately the first 100 control women and their babies not meeting the definition of PROM from whom cord blood was obtained

Identification of samples

Babies exposed and not exposed to PROM will be identified by research midwives on a weekly basis by following up the Baby Biome Study participants who give birth and meet the case definition of PROM. Women who meet the case definition will have their Tempus tube of cord blood set aside for batch transportation to UCL.

Overview of experiments

Using cord blood samples collected into Tempus tubes, RNA will be extracted and cDNA microarrays performed in Dr Mahdad Noursedeghi's laboratory in UCL. More detail of the laboratory procedures and the outcome measures for the experiments is provided in Appendix 3.

Data collection

Data collection methods are the same as described in Section IV, part E (page 10).

Key clinical data required to meet the objectives of this research question include:

- PROM status of each woman
- Evidence of ascending intrauterine infection
- Gestational age at delivery

This list is an indicator of the type of information required and is not exhaustive.

Data analysis

RNA from cord blood samples will be used to generate transcriptional profiles of babies' immune systems at birth. Descriptive analyses of demographic and clinical characteristics will also be undertaken and these data will be linked to samples with analyses undertaken within the context of a large-scale epidemiological cohort study. As such it will be appropriate to include potential confounders in analyses and these may include: maternal ethnicity, gestational age at birth, presence of concurrent infection and use of antibiotics.

Handling of leftover samples

Following RNA extraction from cord blood samples, excess RNA from each anonymised the Baby Biome Study sample will be stored long-term (for more than 10 years) at the laboratory where the proposed experiments are to be carried out.

Sample size

We have statistical power (>90%) to detect as significant (at the 0.1% level) differences in the transcriptional profile of white cells isolated from peripheral blood in cord blood reflecting *in utero* exposure to pathogens as a result of PROM. In the UK, approximately 8-10% of term births can be attributed to PROM (NICE IPC guideline, 2008). We therefore conservatively estimate that, from 2,000 births we will observe an expected 160 PROM cases, from whom we will be able to compare cord blood gene expression in 20-30 samples associated with PROM and 80-100 control samples. Gene expression profiling of peripheral blood shows significant differences attributable to infectious diseases in sample sizes of 15-30. We have also used existing pilot data from patients presenting to UCLH with suspected infectious diseases in an on-line tool that combines parametric mixture modelling and bootstrapping to estimate the relationship between power and sample size (<http://www.poweratlas.org/>) in genome wide data sets. On this basis, comparing samples from 20 women with and 80 without history of PROM, gives >90% power to detect significant differences at a pvalue threshold of 0.001.

V CONDUCT OF THE RESEARCH

A. Ethical review process

Plans for ethical review

The Baby Biome Study falls under the London City and East Research Ethics Committee (REC), under the number 12/LO/1492.

Participant information

We will provide **a flyer at around 12 weeks gestation, and** an antenatal PIS to women, at around 36 weeks' gestation. This PIS will explain the Baby Biome Study and the samples that will be collected, including a vaginal swab. Although vaginal swabs, unlike other samples collected, do not represent a waste sample which would otherwise be discarded, we have found that women donate a self-collected vaginal sample more frequently than the stool sample, **with 61% of Baby Biome Study participants providing this sample, indicating good acceptability.**

Consent

Consent for the Baby Biome Study will cover the following parts of the study:

- Collection of a vaginal swab before delivery;
- Collection during labour or immediately after birth of stool from the mother and cord blood;
- Collection of stool from babies seven days after birth, **in most cases** once they have left the hospital;
- Collection of additional stool from babies at four and 21 days after birth, once they have left the hospital, for a subsample of approximately 200 women and their babies who agree to this;
- Retrieval of leftover antenatal maternal blood samples and dried blood spots from babies' heel prick tests;
- Collection of routine health-related information about women and their babies including future record linkage;
- Long-term storage of the collected samples in the laboratories where samples are analysed, where long-term means for more than 10 years;
- Future contact about further study activities.

Women will be asked to provide written consent to donating their samples and having routine health-related information collected after birth, whilst on the labour ward. Consent will be taken by an appropriately trained midwife, nurse, or research staff member. Most women will therefore be asked to consent either around the time of discharge home or when transferred to the postnatal ward. Women can consent prior to delivery if they wish to do so. Some women may require more time to read the PIS and consider whether they want to take part. If women do not appear able to consider the study, midwives, nurses and/or research staff will be asked to use their clinical and/or professional judgement to decide when (or whether) is most appropriate to discuss the study and whether a woman needs more time. All women who consent to donate samples will then also be asked to collect baby stool. We will also approach women postnatally who we may have missed before birth and ask them for consent to collect baby stool, even if we have not collected any other samples. If a woman does not give consent, then her samples will be discarded when she leaves the hospital.

Labour ward midwives will be trained by our research team in how to obtain informed consent and a record of who has been trained will be kept. Women will indicate consent by initialling each section of the consent form and signing and dating the completed form. For all women who opt to participate, it will be made clear that they may choose to withdraw at any time, as described in Section V, part I (page 17).

All women will be allocated an anonymous participant ID and the original copy of the signed consent form will be given to the woman to take home. One carbon copy of the consent form will be placed into the woman's notes. A second carbon copy of the signed consent form will be collected and retained by study staff along with the Baby Biome Study checklist and data collection form, adapted from the checklist currently used at to include collection of a vaginal swab and placenta (Appendix 4). Women will take a copy of the PIS home with them as it contains contact details for the research team and information about withdrawal.

For women who were not given a **flyer at around 12 weeks gestation, or a** PIS at around 36 weeks' gestation, the approach of allowing participants to consent to take part in the study on the same day as they are first approached, has been used successfully in other studies (e.g. Baby Biobank - <http://www.ucl.ac.uk/tapb/sample-and-data-collections-at-ucl/biobanksucl/baby-biobank/protocol-for-babybiobank-management>) and was approved by the Life Study REC for both our earlier pilot study at UCLH and for the original BBS protocol. At all stages, women will be fully informed about their rights to withdraw from the study at any time to ensure participants are empowered to take part or not, exactly as they wish.

We estimate that it will take approximately five to ten minutes to go through the PIS, and a further five minutes to read and sign the consent form. A record will be kept of the number of women who decline to take part after being given all the study information, although no personal identifying information will be collected about these women.

Confidentiality and data security

Confidentiality will be ensured throughout the study. Initially, named samples will be collected, using routinely available hospital labels, which include name, date of birth, and hospital number. This will reduce the risk of any transcription errors when labelling multiple samples from mother and baby on a busy maternity ward (which might occur if using only an anonymised study number to identify samples). This will also limit any wastage of samples due to labelling errors and will be done with the explicit consent of the participants for named sample collection (see PIS).

A Baby Biome Study-dedicated technician will be responsible for pseudonymisation of the samples by transferring the stool samples and vaginal swabs from containers with hospital labels to sample tubes pre-barcoded with an anonymous barcode number. **DNA extraction will also be performed on stool samples and aliquots of DNA for each sample will also be stored in sample tubes pre-barcoded with an anonymous barcode number. To anonymise the samples of cord blood, the sticker on the sample collection tube will be overlaid with an anonymised barcoded sticker containing a study ID and the date and time of sample collection before transportation to UCL for processing. All samples will therefore already be pseudonymised when they reach the Sanger Institute, and Dr Mahdad Noursadeghi's laboratory at UCL. Anonymised samples may also be sent to UK Biocentre for long-term storage.**

B. Study timeline

Sample collection for the Baby Biome Study will take place up until the current end date for our grant (end of February 2017) in the first instance.

Testing of samples will be batched accordingly and analysis of data will take place once adequate numbers of samples have been collected, with dissemination of results anticipated as published data in peer-reviewed journals and presented at international conferences in 2016-17 and beyond.

C. Incentives for participants

No financial incentives will be used for recruitment into the Baby Biome Study. However, baby stool samples are the most important sample for answering objectives 2 and 3 and the only sample that will be collected after women are discharged from hospital. We will send reminders to return samples by text (or phone for women who do not provide a mobile telephone number). If we find that inadequate numbers of women are returning their baby's stool sample we will pilot offering financial incentives to see whether this increases responses. These incentives will be based on the incentive vouchers already approved by the REC for Life Study, with a value of up to £20 given for the return of all three sequential baby stool samples.

D. Feedback of health-related information to participants

We do not intend to feedback any specific results from testing of samples collected in this pilot study because none of the tests performed will have clinically meaningful or diagnostic implications, i.e. there will be no results that are relevant to the participants' health. The purpose of collecting and analysing these samples is to answer hypothesis-driven research questions about the effect on microbiota of antibiotic exposure and the effect of PROM on neonatal immune responses. Furthermore, the samples are not being collected or processed according to clinical diagnostic standards, and may be tested in batches with some considerable delay between collection and data being available. Our approach of not providing feedback is described in the PIS, and participants will be asked to consent to the specific provision of named samples without the return of results. Participants will be able to access anonymised and collated information on study results and other outcomes on the study website, which will be developed.

E. Dissemination

The data generated will be used for peer-reviewed publication in relevant journals and conferences, and informing public health policy. Only anonymised, aggregate data will be presented and no individual participant will be identifiable from any of the data reported.

F. Transfer of biological samples

After sample collection on the labour ward, samples collected at hospitals other than BHRUT will be sent to the pathology laboratory at BHRUT for initial processing, aliquoting and storage, before being sent to the following sites for storage and/or analysis, labelled appropriately:

- Extracted DNA and raw stool aliquots for each paired mother infant sample will be transported to the laboratory of Dr Mahdad Noursedghi where PCR will be performed and then to the Sanger Institute in Cambridge to the laboratory of Dr Trevor Lawley for sequencing. Dr Lawley will have overall responsibility for these samples. The samples will be processed and stored within this lab.
- **Vaginal swabs will be sent to the Sanger Institute in Cambridge for storage prior to analysis.**
- Cord blood samples will be collected into a Tempus tube to adequately preserve the sample for RNA extraction then sent to the laboratory of at UCL where RNA extraction will be performed. Dr Noursedghi will have overall responsibility for these samples.

For stool, vaginal swabs and cord blood, samples will be transported in batches to the Sanger Institute and UCL Cruciform building, respectively, once a sufficient number of samples has been collected.

G. Destruction of samples

Once testing and analysis of the samples has been completed, Baby Biome Study samples will be stored at the laboratories where the tests are carried out. Women will be asked to consent for samples to be stored long term that is, for more than 10 years after collection. If, at any time, consent is withdrawn for any of the samples collected, these samples will be destroyed.

H. Data Management

Initial data collection will be onto a paper-based collection form. After collection, data will be entered into a secure database by members of the Baby Biome Study. This database will only be accessible to members of the Baby Biome Study team with a username and password.

I. Withdrawal of consent

Women will be free to withdraw consent at any time (including before delivery, at the time of delivery, or after discharge from hospital), and their samples will be discarded or destroyed. If a woman chooses to withdraw from the study after her samples have already been analysed we will destroy both the samples and associated data. These options will be described in each PIS, including information about how they can withdraw consent by telephoning or writing to the researchers, or by telling the research midwives. The research midwives' contact details will be provided to all participants before they leave hospital to ensure they understand that they can withdraw from the study after leaving hospital and how to do this. **Until end of February 2017** women will be able to withdraw by contacting the research midwives. After this date they will be advised to contact the researchers.

VI STUDY MANAGEMENT

Funding and sponsorship

The Baby Biome Study is funded by a Wellcome Trust Strategic Award (grant number WT101169AIA) to University College London (UCL). Trevor Lawley at the Sanger Institute, Cambridge, has secured funding to undertake sequencing work on around 300 paired mother infant stool samples from grant WT08851. UCL will act as sponsor for the study.

Management of the Study

The chief investigator (CI) for the Baby Biome Study, Peter Brocklehurst, has overall responsibility for this work, including day-to-day management and financial oversight. Nigel Field is the scientific lead for the study. Sequencing and phenotypic analyses of stool samples will be overseen by Trevor Lawley at the Sanger Institute, Cambridge. RNA extraction from cord blood samples and transcriptional analysis will be overseen by Mahdad Noursadeghi in the Department of Infection and Immunity, UCL

VII COMPLIANCE WITH GUIDELINES

Human Tissue Act

All samples and materials derived from these samples will be treated and used in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto.

The samples will not be transferred to any party not identified in this protocol and are not to be processed and/or transferred other than in accordance with the patients' consent. At the end of the study, all samples will be disposed of in accordance with the Human Tissue Act 2004 and any amendments thereto.

All data and samples will be collected and handled in accordance with appropriate UCL guidelines and policy:

Recommendations of the Caldicott committee and The Caldicott Guardian Manual 2010

Data Protection Act 1998 (and outstanding changes)

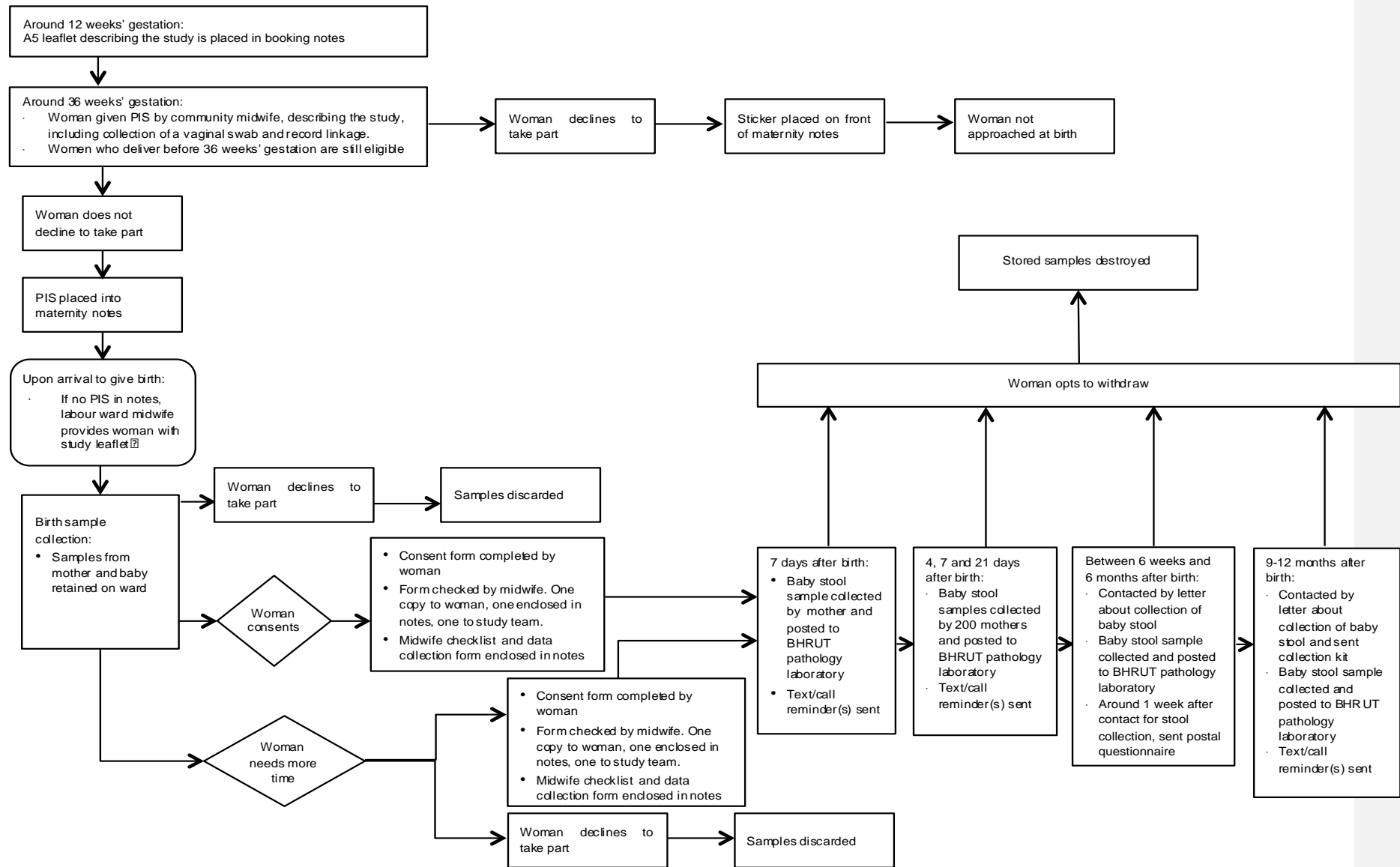
Human Rights Act 1998

Human Tissue Act 2004

Section 251 of the NHS Act 2006

Confidentiality Advisory Group (CAG) within the Health Research Authority (HR

APPENDIX 1 - Recruitment process



APPENDIX 2- Description of samples and planned assays for the Baby Biome Study

Sample	Objective	Collection	Assay
Maternal stool (around the time of birth)	Define microbes present and their phenotypes such as virulence and antimicrobial resistance	Lysing matrix E tubes	Lysis, DNA extraction and batch sequencing, using 16 rRNA gene Illumina MiSeq PCR or metagenomic sequencing, culturing of microbes to sequencing genomes and perform phenotypic analysis (i.e. antimicrobial resistance).
Baby stool (after birth)	Define microbes present and their phenotypes such as virulence and antimicrobial resistance	Lysing matrix E tubes	Lysis, DNA extraction and batch sequencing, using 16 rRNA gene Illumina MiSeq PCR or metagenomic sequencing, culturing of microbes to sequencing genomes and perform phenotypic analysis (i.e. antimicrobial resistance).
Cord blood RNA	Extract RNA	1x Tempus tubes (3 mL)	RNA extraction and integrity analysis RNA labelling QC cDNA microarray expression arrays
Vaginal swab	Define microbes present and their phenotypes such as virulence and antimicrobial resistance	Lysing matrix E tubes	Lysis, DNA extraction and batch sequencing, using 16 rRNA gene Illumina MiSeq PCR or metagenomic sequencing, culturing of microbes to sequencing genomes and perform phenotypic analysis (i.e. antimicrobial resistance).

APPENDIX 3 – Midwife checklist

Baby Biome Study checklist

To be used in conjunction with the Baby Biome Study sample log

Notes for maternity unit staff:

- Please complete full checklist before discharge, ensuring that a reason (e.g. consent declined) is provided if a sample is not given.
- Midwives should collect or assist with collection of samples if required.

Consent

	Action	Task completed?	Reason if task not completed
On labour ward			
1	Participant information sheet issued	<input type="checkbox"/>	
2	Consent form signed	<input type="checkbox"/>	

Kit checklist

	Action	Task completed?	Reason if task not completed
Kits to be issued on the labour ward			
1	Vaginal swab kit	<input type="checkbox"/>	
2	Maternal poo kit	<input type="checkbox"/>	
Kits to be issued when discharged from the labour ward			
3	Baby poo kit	<input type="checkbox"/>	

Collection checklist

	Action	Task completed?	Reason if sample not collected
Samples to be stored in the Baby Biome Study fridge			
1	Maternal poo collected	<input type="checkbox"/>	
2	Vaginal swab collected	<input type="checkbox"/>	
Samples to be stored at room temperature			
3	Cord blood collected:		
	RNA (blue cap)	<input type="checkbox"/>	
	Serum (white cap)	<input type="checkbox"/>	

Date completed _____

Signed (discharging midwife) _____

PLACE COMPLETED FORM INTO THE BABY BIOME STUDY BOX IN THE RESEARCH OFFICE