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.

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).

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

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

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 eV1.0isam4(er)eV1232>> BDC BT/F9 11.04 Tf1 0 0 1

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 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:

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 [redacted] 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, [redacted]

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, [redacted]

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

Birth sample collection:

- Samples from mother and baby retained on ward

- Consent form completed by woman
- Form checked by midwife. One copy to woman, one enclosed in notes, one to study team.
- Midwife checklist and data collection form en

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).