SUPPLEMENTAL MATERIAL

Sampling procedures:

Blood transcriptome samples

Cellular genetic information is *transcribed* from DNA into complementary messenger RNA (mRNA), a sequence of nucleotides that is later partially translated into a polymer of amino acids (i.e. proteins). The aggregate of all transcribed genes is referred to as ‘transcriptome’.\[1\] While the genome (the entirety of the genetic material of an organism) is marked by its steadiness, the abundance of transcribed mRNA is of dynamic nature as gene transcription frequency changes in different conditions. Assessing perturbations in whole blood transcriptome abundance have been successfully used in health and disease.\[2-4\] Following a modular data-mining strategy,\[5\] we will measure the abundance of up to 272 transcripts that have been selected based on analyses of an extensive collection of whole genome blood transcriptional profiles generated with microarrays. RNA quality, integrity and overall gene expression data obtained via capillary blood sampling (e.g. 50 microliter), is appropriate for serial monitoring and field based application.\[6\] To evaluate these transcriptome signatures, we will collect 50 microliter of capillary blood using RAM Scientific: SAFE-T-FILL® Capillary Blood Collection Tubes (Kabe Labortechnik, Nümbrecht, Germany (Product number GK100, [http://www.kabe-labortechnik.de/download/kapillarblut_en.pdf](http://www.kabe-labortechnik.de/download/kapillarblut_en.pdf)). Before blood collection, Tempus™ RNA stabilization and isolation reagent (ThermoFischer Scientific, Waltham, MA, USA; Product number 4342792; [https://www.thermofisher.com/order/catalog/product/4342792](https://www.thermofisher.com/order/catalog/product/4342792)) will be added to the tubes. Once the sample is transferred into the microtube it will be shaken vigorously and stored at -20°C. The recommended ratio of whole blood to RNA stabilizing reagent is 1 volume of blood to 2 volumes of Tempus™ solution; hence 50 μl of blood will be added to 100 μl of RNA stabilizing reagent in this project. Detailed standard operating procedures for finger prick sampling have been made available elsewhere [7] and a demonstration video is available here: [https://www.youtube.com/watch?v=xnrXidwg83I](https://www.youtube.com/watch?v=xnrXidwg83I).

Stool sampling

OMNigene®•GUT Collection and Stabilization kits (DNA Genotek Inc, Ottawa, Ontario, Canada; [http://www.dnagenotek.com/ROW/products/OM-200.html](http://www.dnagenotek.com/ROW/products/OM-200.html)) will
be used to collect stool samples. OMNiGene®•GUT is an all in one system for the easy self-collection and stabilization of microbial DNA from faeces/stool for gut microbiome profiling. After collection, the OMNiGene®•GUT stabilizes and stores the DNA at room temperature for up to 14 days and ensures the microbiota profiling at the in vivo state. The OMNiGene®•GUT tubes will be temporary stored at -20°C to avoid cracking of the tube and then transferred into -80°C for long-term storage.

Vaginal swab sampling

At each of the 6 sampling time-points (once in each trimester, delivery and on two occasions post-partum) a midwife will take 4 vaginal swabs from the posterior fornix of the vagina under direct visualization by using a Copan ESwab™(Copan Diagnostics Inc, Murrieta, CA, USA; Cataloge Number 480C; http://www.copanusa.com/products/collection-transport/eswab/). Each tube contains 1mL of Liquid Amies, which is a liquid-based multipurpose collection, and transport system that maintains viability of aerobic, anaerobic and fastidious bacteria for up to 48 hours at room and refrigerator temperature. After collection, the swabs will be placed back in the tube that contains different storage media for targeted analysis. Table S1 summarises the purpose and the storage media preparation of the swabs.

Table S1. Purposes and storage media preparation of the vaginal swabs

<table>
<thead>
<tr>
<th>Intended use</th>
<th>Transport media preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>First swab</td>
<td>Extracting genomic DNA</td>
</tr>
<tr>
<td>Second swab</td>
<td>Gram stain smear</td>
</tr>
<tr>
<td>Third swab</td>
<td>Metatranscriptomics analyses</td>
</tr>
<tr>
<td>Fourth swab</td>
<td>Analysis of vaginal cytokines</td>
</tr>
</tbody>
</table>

*PhosSTOP™ Phosphatase Inhibitor Tablets, Roche, Basel, Switzerland

*Complete™, EDTA-free Protease Inhibitor Cocktail Tablets, Roche, Basel, Switzerland

The second swab will be used to prepare a smear that will later be Gram stained, and scored using Nugent criteria at the University of Alabama at Birmingham.[8] Nugent scores are composite scores based on the cellular morphologies of the bacteria present in a sample. A score of 0-3 is designated normal, 4-6 as intermediate and 7-10 is considered to be abnormal and indicative of bacterial vaginosis. After collection, all samples will be stored in -80°C.
Saliva Sampling

Saliva samples will be collected at 2 time points: at 24-26 weeks of gestation and at delivery. The samples will be taken at least 30 minutes after the last food intake. Prior to sample collection the participant has to rinse her mouth with clean water for at least 30 seconds. Thereafter 3 ml of saliva will be spat into a sterile falcon tube. Two aliquots of 0.5 ml will be transferred into sterile Eppendorf tubes and stored unprocessed and two 0.5 ml aliquots will be transferred into sterile Eppendorf tubes and mixed with 0.5 ml RNAlater, an RNA stabilizing and protecting solution (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/7020M.pdf). All saliva samples will be stored at -80°C before analysis.

Placental Tissue Samples

All placenta samples will be taken and processed within 30 minutes of placental expulsion. Sterile technique will be applied to harvest placental tissue. Narrative of the procedure: Healthy placenta tissue, 3 cm from the edge of the placenta will be identified. A rectangle from the maternal surface that is 0.5 cm across and 3 cm in length and about 1 – 1.5 cm deep will be cut out, while avoiding cutting through the membranes covering the fetal side. Thereafter 0.25-0.5 cm of the maternal surface of the placenta will be removed and 12 cubes (each 0.5 x 0.5 x 0.5 cm) will be excised. Six cubes are intended for assessment of microbial colonization and 6 cubes for genome wide regulation of gene expression. All samples will be rinsed in sterile PBS and then transferred into cryovials. Nine cubes will be flash frozen unprocessed in liquid nitrogen while the remaining 3 samples will be mixed with RNAlater, an RNA stabilizing and protecting solution (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/7020M.pdf) and stored at -80°C.

Cord Blood Collection

Umbilical cord blood will be taken within 30 minutes of the expulsion of the placenta or preferably whilst the placenta is still in utero. Seven ml of cord blood will be taken and dispensed into a tube containing a clot activator. By inverting the tubes gently 8-10 times the blood-clot activator compound will be mixed. The tube will then be kept in room temperature for a minimum of 30 minutes to allow the
clot to form. Thereafter the tube will be centrifuged at 1,000 x g for 5 minutes at room temperature (20°C) and 2 ml of serum will be collected and stored at -20°C. By applying the same collection procedure, 6 ml of umbilical cord blood will be dispensed in an EDTA tube and stored at -80°C. This sample will be used to evaluate DNA.

Oral Glucose Tolerance Test (OGTT)
All study participants will have a 75g oral glucose tolerance test (OGTT) to screen for GDM at 24-26 weeks of gestation or earlier if indicated. Diagnosis of GDM will be based on cut-off values according to the HAPO trial: an OGTT is considered positive when any of the following three thresholds for capillary blood glucose are met or exceeded: fasting 92 mg/dL, 180 mg/dL and 153 mg/dL at one and 2 hours after the 75g OGTT respectively.[9]

Fever screening battery
In the event of a febrile episode a capillary blood sample, a stool sample (including 24-hour food recall) and a set of vaginal swab samples will be taken to evaluate perturbations of whole blood transcriptome and microbiome respectively. Additionally an attempt to diagnose the underlying cause for the fever will be made by taking the following samples.

- 50µL blood (malaria smear)
- 5 ml blood (acute serology)
- 30 µg Stool (wet smear)
- Nasopharyngeal aspirate

If indicated: - Cerebrospinal fluid (cell count and culture)
- Sputum (acid-fast-stain, GeneXpert® and culture)

10-30 days after the event, 5 ml blood will be taken for convalescent serology. Samples will be stored appropriately for later analysis.
Routine ANC procedures and neonatal anthropometry at SMRU

» Baseline antenatal care/assessment:
• Demographics: age, ethnic group, marriage status
• Medical History: disease or surgical interventions
• Obstetric history: complications, type of delivery, outcome
• Concomitant medication and toxins: smoking and areca nut consumption
• Vitamin supplements: including the start date and dosing
• Vital signs: auricular temperature, resting pulse and blood pressure, performed after the participant has sat for at least twenty minutes
• Physical exam: including height, weight, and mid-upper arm circumference
• Obstetric examination: fundal height and fetal heart beat
• Obstetric ultrasound: crown rump length measurement and fetal heartbeat according to the methodology of the INTERGROWTH-21st Project [10]
  • Laboratory tests: CBC, Blood group, Malaria smear or RDT
  HCT, Rhesus-factor, HIV
  Hb-typing, Fresh stool smear, Hepatitis B
  G6PD-status, Urine culture, Syphilis
  CRP and serum ferritin (in the event of anemia)

» Routine follow-up procedures:
• Vital signs
• Physical examination
• Obstetric examination: fundal height, fetal heartbeat and the fetal presentation
• Maternal growth monitoring according to standards of the Intergrowth 21st project [11]*
  Available at: https://globalhealthtrainingcentre.tghn.org
• Obstetric ultrasound (6-weekly) to monitor progress of pregnancy and fetal growth.
  According to Ultrasound methodology of the INTERGROWTH-21st Project [9]*
  Including: fetal heartbeat, fetal presentation, placental position
  Head circumference, biparietal diameter, abdominal circumference
  Femur length, cervical length, amniotic fluid index
  Umbilical Doppler pulse index

» Routine anthropometry of the neonate:
As soon as possible after birth, the infant will be assessed according to the neonatal anthropometric standards of the Intergrowth 21st project [11]* (available at: https://globalhealthtrainingcentre.tghn.org)
  Including: birth weight, body length, head circumference
  Mid-arm circumference, malformations (if present)
* All study staff will be certified and quality controlled according to the standards.

Sample collection, transport and storage
All samples will be collected according to standard operating procedures that will be drafted and reviewed prior to study commencement. Skilled health care workers in SMRU clinics alongside the Thailand-Myanmar border will be trained in sampling procedures and thereafter collect study samples. Samples will then be transferred to the SMRU central laboratory in Mae Sot, Thailand for long-term storage on a daily basis.
All samples will then be transferred to Sidra (Qatar) for further processing and analysis. For international shipping samples will be kept on dry ice in a freezer box with thick Styrofoam walls.
# Application Form for Datasets under the Custodianship of Mahidol Oxford Tropical Medicine Research Unit (MORU) Tropical Network

## 1. APPLICANT DETAILS

<table>
<thead>
<tr>
<th>Name of applicant</th>
<th>Institution name and address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phone</td>
<td>Email</td>
</tr>
<tr>
<td>Name of applicant's supervisor/ manager/ head of department (delete as appropriate)</td>
<td></td>
</tr>
<tr>
<td>Phone</td>
<td>Email</td>
</tr>
</tbody>
</table>

## 2. DETAILS OF PROJECT

<table>
<thead>
<tr>
<th>Title of Project</th>
<th>Start Date</th>
<th>End Date</th>
</tr>
</thead>
</table>

## 3. CO-APPLICANTS INCLUDING MORU COLLABORATORS (if applicable)

<table>
<thead>
<tr>
<th>Name</th>
<th>Role</th>
<th>Institution</th>
</tr>
</thead>
</table>

Anticipated users or user groups other the above individuals e.g. data management team of applicant's institution

## 4. BRIEF DESCRIPTION OF DATA REQUESTED

## 5. OBJECTIVES OF PROJECT

## 6. BRIEF DESCRIPTION OF ANALYSIS PLANNED

## 7. POTENTIAL ETHICAL ISSUES INCLUDING RISKS e.g. stigmatisation or breaches of privacy

## 8. POTENTIAL BENEFITS OF THE STUDY including to participant communities, scientific capacity building or health policy

## 9. PLANNED OUTPUTS including publications

Signature of applicant: ___________ DATE: ____
Signature of applicant's supervisor/ manager/ head of department: ___________ DATE: ____

Note: Additional information may be requested to support the application. We are not charging for data access but the applicant may be required to cover the cost of preparing the data for sharing. Primary contact: phaikyeong@tropmedres.ac
References: