Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals

Protocol Number: H-27393
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Section Aa: Title & PI
A1. Protocol Title

BIOSPECIMEN COLLECTION AND ANALYSIS IN THE NATIONAL CHILDRENS STUDY: METAGENOMIC ASSESSMENT OF THE MICROBIOME

A2. Principal Investigator

Name: KJERSTI MARIE AAGAARD-TILLERY Phone: 713-798-8467
Id: 159224 Fax: 713-798-4216
Department: OB-GYN: ADMINISTRATIVE Email: aagaardt@bcm.tmc.edu
Center: Mail Stn: BCM610

A3. Administrative Contact

Name: PATRICK B MALONE Phone: 713-798-1044
Id: 043231 Fax: 713-798-5074
Email: patrickm@bcm.tmc.edu
Mail Stn: BCM610

A3a. Financial Conflict of Interest
Does the investigator have a financial interest in any non-Baylor sponsor or funding source for this research?
No

A3b. Cooperative Agreement
Is this a cooperative agreement protocol?
No

Which institution is the IRB of record?
BCM: Baylor College of Medicine

Section Ab: General Information
A4. Co-Investigators

Name: CLAUDIA A KOZINETZ Phone: 832-824-3730
Id: 023875 Fax: 832-825-3697
Department: PEDIATRICS: ACADEMIC GENERAL Email: kozinetz@bcm.tmc.edu
Center: Mail Stn: BCM315

Name: JAMES VERSALOVIC Phone: 832-824-2213
Id: 026810 Fax: 832-825-0164
Department: PATHOLOGY Email: jamesv@bcm.tmc.edu
Center: Mail Stn: BCM315

A5. Funding Source:

Organization: NATIONAL INSTITUTES OF HEALTH (NIH)

A6a. Institutions where work will be performed:

BCM: Baylor College of Medicine
St. Luke's Episcopal Hospital

A6b. Research will be conducted outside of the United States:

Country:
Facility/Institution:
Contact/Investigator:
Phone Number:

If documentation of assurances has not been sent to the Office of Research, please explain:

A7. Research Category:

Section B: Exempt Request
B. Exempt From IRB Review

Not Applicable

Section C: Background

This study serves as one of the initial "Formative Research Initiatives" in the National Children's Study (NCS). This protocol and associated funded research serves as a meaningful modification of approved and related (but non-overlapping) protocols (H-25849, and H-26589).

Why study the human microbiome? Human-associated microorganisms are present in numbers exceeding the quantities of human cells by at least 10-fold beginning in the neonatal period, and the collective genome (the "microbiome") exceeds our human genome in terms of gene content by more than 100-fold. We have recently come to appreciate that the microbiota are a metabolically and antigenically vibrant diverse community which may function as mutualists (symbiotically beneficial), commensals (of neither harm nor benefit), or pathogens (of host detriment). While current major national research efforts (i.e., the NIH Road Map initiative known as the Human Microbiome Project (HMP)) will enable sequence-based comprehensive characterization of the adult human microbiota, how and when these diverse microbiota communities take up residence in the host are unexplored at a population-wide level. In other words, while we may soon know what constitutes the adult human microbial guild, we will not know how it is established nor will we know the impact of the microbiome on the development of common pediatric and adult diseases, including asthma and obesity.

By characterizing the pregnancy, neonatal, and early childhood microbiomes, we can begin to address a number of crucial gaps in our understanding. For example, what is the role of vertical (from mother to child) versus horizontal (across a community or household) transmission in establishing the core microbiome? How does this core microbiome change during an individual's lifetime? What is the impact of the environment on the microbiome, and what is the impact of the intestinal environment established by the microbiome on the health status of the individual? Can we bank microbial specimens acquired longitudinally from a human population base in order to analyze microbiomes with improved and efficient methodologies in the future?

There are two compelling reasons why the human microbiome should be included in this NCS formative study:

(1) Leveraging of existing research infrastructure: Given the early success of the HMP and rapid progress within the International Human Microbiome Consortium, we are uniquely poised to extend its available massive metagenomic sequencing and analysis infrastructure on other prospectively acquired datasets with linked exposure and outcomes data (i.e., NCS-acquired samples). The recent release of the first metagenomics or human microbiome gene catalogue (Nature, 2010) and anticipated completion of primary goals of the HMP within the next two years highlights the importance of the timing of such a new initiative. It is clearly advantageous to consider upcoming future initiatives in the context of sequencing and informatics pipelines and research cost extensions. In sum, it is arguably more cost effective to leverage continued utilization of our functional HMP metagenomics infrastructure at this time with NCS-acquired samples. This proposal does not constitute a redundant effort, as there is no pregnancy, neonatal, nor pediatric component to the Human Microbiome Project JumpStart initiative.

(2) Strong scientific rationale: How the core microbiome is established is not understood, but multiple lines of evidence suggest influence by virtue of vertical (maternal) factors, mode of delivery, and diet and exposures through infancy. With respect to our current understanding of potential symbiont vertical transmission in pregnancy, it is evident that the microbiota of the genital tract can be altered in pregnancy and that such alterations can be pathogenic with respect to maternal and perinatal morbidity and mortality (i.e., group A streptococcus and puerperal sepsis). Conversely, we have also learned that treating common vaginal pathogens will not necessarily improve perinatal morbidity and mortality. For example, often dominant microbiota such as Trichomonas vaginalis (TV) and conditions such as bacterial vaginosis (BV) have been long-standing in their association with preterm labor and preterm birth. However, meta-analyses and randomized, double-blinded, placebo control trials have actually demonstrated that treating such “infections” actually increase the rate of preterm birth. Along these same lines, it bears mention that several species of metalloproteinase-producing bacteria, including Group B streptococcus and Prevotella, can impair amniotic membrane strength via the disruption of the matrix metalloproteinases. Such mechanisms may serve as a pathogenic nidus for the clinical condition of preterm premature rupture of the membranes.

Turning to the neonatal interval, by the end of the first week of life neonates typically acquire complex microbial communities which fluctuate in terms of relative proportions during the first year but by the end of infancy, the intestinal bacterial populations have reached an adult-like state of complexity and a relative equilibrium. Notably, populations in the neonatal period and infancy are affected by mode of delivery and early treatment with antimicrobial agents. Indeed, several lines of evidence suggest that members of the microbial community can interfere with or enhance each other’s transmission probabilities, or that some microbes can be acquired both from the in utero environment (maternal vertical transmission), or the immediate neonatal environment. For example, when delivery occurs via cesarean, infants have higher rates of colonization by horizontally transmitted, environmentally acquired microbes such as Clostridum difficile, Klebsiella, and Enterobacter species, suggesting the possibility that the presence of vertically transmitted symbionts may meaningfully alter the transmission of these taxa during vaginal birth. Similarly, in a small study of fecal samples of infants at 1 month of age, dominant determinants of gut microbial composition were mode of delivery (cesarean versus vaginal), type of feeding (breast milk versus formula), gestational age, hospitalization, and antibiotic use (Penders, 2006). Interestingly, these authors found that the most “beneficial” gut microbiota profiles (dominance of bifidobacteria over C. difficile and E. coli) were among term infants delivered at home and breast fed.

Finally, the impact of both host phenotype and the environment on microbial community establishment, notably in childhood, is poorly understood. One notable exception is the impact of obesity on the gut microbiome. In twin studies of adult female monozygotic and dizygotic twin pairs concordant for leanness or obesity and their mothers, it has been demonstrated that obesity is associated with phylum-level changes in the microbiota and reduced bacterial diversity. Broad environmental and exposure measures, such as those acquired in the full scale NCS study, will further allow us to understand broad phenotypic and environmental influences on concomitantly derived microbial profiles in humans. For the purposes of this NCS demonstration project, “exposure” will be defined by known proximal influences including mode of delivery, breast milk versus use of formula, and other limited measures (i.e., antibiotic exposure, gestational age, and length of hospital stay). In addition, these known influences on microbial diversity serve as our rationale for the number of subjects necessary to determine feasibility.

Section D: Purpose and Objectives

In this NCS Formative Research Study our broad objective is to lay the essential groundwork establishing the feasiblity of a full-scale microbiome research effort in the National Children's Study.

By way of overview, construction of a reference sequence data set for the Human Microbiome Project (HMP) was undertaken in 2007 as an NIH Road Map Initiative with the aim to fully characterize the microbiome, and is an ongoing effort at multiple centers notably including Baylor College of Medicine (JumpStart initiative, Versalovic and Aagaard co-Investigators). In our current expanded proposal with the NCS, we collectively propose to: (1) jointly leverage our microbiome genomic sciences infrastructure developed for HMP with the clinical and longitudinal data and sample collection infrastructure of the NCS, then (2) refine, optimize and ultimately establish protocols to bank NCS-acquired microbial specimens for future metagenomics analyses on the human microbiome. When applied to the full scale NCS study, our collaborative formative approach will enable a comprehensive interrogation of the human gut microbiome from establishment, to birth, and into early adulthood. Successful accomplishments of the following goals will enable achievement of our overall study aims:

(Primary Objective 1) To establish proven currently utilized methodologies for sample collection and concomitant DNA extraction for metagenomic sequencing from the HMP JumpStart (Versalovic and Aagaard) and UH2 Demonstration Projects (Versalovic) with already scheduled NCS visits and self-sampling. *Sample the vaginal microbiome at the scheduled third trimester NCS clinical visit *Sample the placental and fetal (first meconium) microbiomes at the time of delivery *Self-sample stool microbiomes at scheduled intervals of one month and 6 months

(Primary Objective 2) To develop and refine protocols to enable banking of acquired specimens prior to microbial DNA extraction in an effort to establish feasibility of deferring metagenomic sequencing and analysis of some of the samples collected through NCS, and to enhance ease of collection of high quality specimens in the context of a large multicenter study that can be used for both DNA and RNA extraction and metagenomic/metatranscriptomic analysis.

With our formative approach executed as a one year demonstration project executed jointly by investigators at the Waukesha County Vanguard Center (WCVC) and at Baylor College of Medicine (BCM) employing samples collected in conjuncture with a modified Vanguard protocol, we will demonstrate not only feasibility with easily expanded NCS protocols and HMP metagenomics infrastructure, but the ability to yield unparalleled insight into functional variation of the microbiome across diverse racial and socioeconomic populations with developmental exposures. Of note, it is important to comment that our proposed protocol reflects the ultimate intent by the investigators on this LOI with respect to what will define a “full scale” NCS initiative on microbiome research.

Section E: Protocol Risks/Subjects
E1. Risk Category

(45 CFR 46.404) Category 1: Research not involving greater than minimum risk.

E2. Subjects

Gender:
Both

Age:
Adolescent (13-17 yrs), Adult (18-64 yrs), Infant/Toddler (0-36 mos), Premature Infant (<37 weeks gestational age)

Ethnicity:
All Ethnicities

Primary Language:
English

Groups to be recruited will include:
Both patients and healthy, non-patient, normals

Vulnerable populations to be recruited as subjects:
Children, Emancipated minors, Pregnant women

Vulnerable populations require special protections. How will you obtain informed consent, protect subject confidentiality, and prevent undue coercion?

Informed consent will be obtained according to federal regulations and BCM and its affiliated hospital policies. Pregnant women will be informed of the objectives of this study by their obstetrician, primary nurse or study personnel at the time of the office visit during the third trimester of pregnancy.

The procedures involved will be described and the subjects will be asked if they would like to participate. The subjects will be made aware that there is no compensation for participation, and their care will not be altered as a result of their participation. No access to care will be withheld if subjects decline participation. Full disclosure of the nature of participating in the study will be made. The consent form will include a statement regarding long term storage of the samples. Subjects will be made aware their participation involves the analysis of genetic material, including the potential for genome wide association studies wherein the results of their DNA code will become part of public archives.

Subjects will further be made aware that confidentiality will be protected within the limits allowed by law. This will include using unique study identifier codes such that samples and data (including genetic data) cannot be linked to a subject or their child.

E3. Pregnant woman/fetus
Will pregnant women be enrolled in the research?

Yes

E4. Neonates
Will neonates be enrolled in the research?
Yes

E5. Children
Will children be enrolled in the research?
Yes

Section F: Design/Procedure
F1. Design
Select one category that most adequately describes your research:
c) Pilot

Discuss the research design including but not limited to such issues as: probability of group assignment, potential for subject to be randomized to placebo group, use of control subjects, etc.

This study is designed to collect a total of three sets of samples from women and their offspring in a feasibility study for formative research with the National Children's Study. Of note, all investigator obtained sample collection points correspond with existing visits within the currently approved NCS protocol (Kozinetz, PI).

Briefly, samples for this formative project collected will include: 1. A vaginal specimen from pregnant women during their third trimester visit (existing time frame with current NCS protocol). 2. At the time of delivery, placental tissue and the infant's first meconium will be collected (existing time frame with current NCS protocol). 3. Subjects will be asked to submit a stool sample from their baby 1 month and 6 months after delivery.

In accordance with the NCS Protocol, subjects will be screened using a modified general health and pregnancy questionnaire and will be examined by an obstetrician-gynecologist at the time of sampling to verify eligibility. Subjects will be pregnant females between the ages of 18 and 40. Subjects who are 16 years old and meet the definition of an emancipated minor in the state of Texas will also be eligible for participation.

Eligible and consented subjects will be enrolled and the vaginal specimen will be collected at the time of the scheduled NCS protocol office visit during the third trimester. Each subject (defined as the gravidae) will provide a total of three sets of specimens: (1) vaginal swab for determination of the vaginal microbiome, taken at a single time point of 28 weeks gestation, (2) placental and fetal (meconium) swab, taken at the time of delivery (or shortly thereafter, dependent upon passage of first meconium sample), for determination of the placental and fetal microbiome. The infant stool samples at the one month and six month reference point will be collected at home using a self-collection kit and then sent to the investigator at Baylor College of Medicine.

Specific subject information will be collected via review of the medical record(s), with information being recorded on health history forms and study specific case report form (so-called NCS minimal data forms, see attachments). After specimen collection and coding, microbial DNA will be isolated from the microbiome specimens. The DNA will then be used to produce DNA sequence information (metagenomics) in accordance with fully functional and existing protocols through the Human Microbiome Project (H-25849, H-22895) and the Burroughs Welcome Fund Preterm Birth Initiative (H-26589).

Selection of the study population in this feasibility study for formative research will occur in alliance with current recruitment methodology for the NCS (Kozinetz, PI). The target for this multi-center study here at Baylor are 30 gravidae between the ages of 16 and 40. While there are two enrolling sites (Baylor College of Medicine and Waukesha County Vanguard Center (WCVC)), the second enrolling site will operate under their own IRB protocol as mandated by the National Children's Study.

The requisite gestational age range at the time of enrollment will be 28 weeks. A goal for minority participation will be at least 20% of the total number of subjects.

All samples will be sent to and processed by study personnel at Baylor College of Medicine (Aagaard lab, Versalovic lab, Petrosino lab).

For this protocol there is no group assignment, randomization or placebo group.

Inclusion Criteria:

Gravidae 28 weeks of gestation anticipated to undergo delivery at St. Luke's Episcopal Hospital. This will focus our recruitment efforts to subjects under care with Baylor College of Medicine Obstetrics and Gynecology.

Exclusion Criteria:

Gravidae with a non-viable fetus.

F2. Procedure

Vaginal sample collection: Two vaginal swabs in triplicate will be acquired by clinical providers at the time of the scheduled 3rd trimester clinical visit from the vaginal introitus and posterior fornix. As part of eligibility, the gestational age will be determined by the first trimester sonogram and compliance with the third trimester visit. A CatchAll® swab will be employed under direct visualization to sample the vaginal introitus and posterior fornix. The nucleic acid will be stored in SCF-1 buffer media, and transferred to the laboratory for immediate extraction. In the “banking approach”, samples will be placed into either (1) RNAlater or (2) SCF-1 media, and thereafter placed on dry ice and stored at -80oC. At approximately 60 days from sample acquisition, nucleic acid extraction will be performed on both banking protocol samples in a small replicate cohort.

Placental sample collection: At the time of delivery, a CatchAll® swab will be employed under direct visualization to sample in triplicate the placenta on the fetal side in a uniform fashion (4 cm medial to the cord insertion). In the scientific protocol, nucleic acid is again stored in SCF-1 buffer media and transported to the laboratory for immediate extraction. In the “banking approach”, samples will be placed into either (1) RNAlater

or (2) SCF-1 media, and thereafter placed on dry ice and stored at -80oC. At approximately 60 days from sample acquisition, nucleic acid extraction will be performed on both banking protocol samples in a replicate cohort (10%).

Meconium and stool sample collection: First meconium samples will be collected in the diaper. For longitudinal stool sample collection, subjects will be instructed to perform self-collection of fecal specimens at home. In addition, a portion of the fecal specimen will be preserved in RNAlater as part of the specimen banking protocol. Briefly, in this demonstration project children and their or guardians will receive at least two sterile plastic collection containers with plastic stool collection frames. They will be instructed to place stool collection frames on the back of the toilet bowl prior to securing the stool container. After defecation, a portion of the specimen will be placed into RNAlater for preservation and banking protocol, and then the stool container will be closed firmly with a plastic lid and placed into a Styrofoam box with cold gel packs. Eight to ten polar gel packs per fecal specimen will be stored for a maximum of 12 hours in a freezer (at subject's home) prior to stool collection. Gel packs will be placed below and around (completely surrounding) stool container within the Styrofoam box. This box will be delivered to the laboratory for immediate nucleic acid extraction.The RNAlater sample will be stored at -80oC. At approximately 60 days from sample acquisition, nucleic acid extraction will be performed on both banking protocol.

DNA extraction: For fresh extraction, samples will arrive in PowerBead tubes provided. For banked approach samples, initial thawing on ice with subsequent sample addition to PowerBead tubes. Nucleic acid is freed with extensive vortexing, and subsequent transfer of supernatant to collection tubes for extraction using filter-based centrifugation in a modified MoBio protocol (HMP MOP, available upon request).

RNA extraction: In order to assess potential feasibility of employing concomitant transcriptomics analysis in banked specimens, RNeasy or NucleospinII will be employed to extract RNA from the "banked approach" specimens. Given the timeline and expense associated with transcriptomics, demonstrative analysis will be deferred. Alternately, RNA yield and integrity will be assessed by standard Sybr and Nanodrop methodologies.

Metagenomic sequencing and analysis: All samples, whether collected and extracted at the BCM site or the WCVC site, will be shipped to BCM for metagenomic sequencing and analysis.

Sequencing overview: The predominant metagenomics sequencing strategy will focus on 16S rDNA 454 sequencing. This takes advantage of existing and fully functional technology and pipelines with proven reproducibility and quality reads in a self-sampled pediatric population. In addition to bacteria, archaea are surveyed by the addition of different primer sets to amplify archaeal 16S rDNA sequences, and fungi are identified by 18S rRNA gene/ITS-based sequencing. Sequencing of the bacterial 16S rRNA gene targets will be performed in collaboration with the existing HMP Genome Sequencing Centers (Baylor College of Medicine, Washington University Genomics Center, and the Broad Institute) by 454 GS Titanium sequencing. A detailed presentation of metagenomics DNA sequencing, informatics/biostatistics, and DNA sequencing approaches, is outside the scope of this protocol, but suffice it to say we are employing our present and proven methodology for sequencing, informatics pipelining, and analysis. All data are maintained on secure servers, in a coded (deidentified) fashion.

Bioinformatics overview: Read data will be filtered and analyzed so that accurate taxonomic assignments may be made while the HMP is completing its initial JumpStart phase, next-generation sequencing and analysis

approaches have been optimized, including our first analysis of 18 pregnant reference subjects (H-25849), and therefore further methodologic details are not included in this protocol.

Section G: Sample Size/Data Analysis

G1. Sample Size

How many subjects (or specimens, or charts) will be used in this study?

Local: 30 Worldwide: 120

Please indicate why you chose the sample size proposed:

This formative study seeks to evaluate feasibility of incorporating routine microbiome sampling as part of the National Children's Study (NCS).

The majority of sample collection (90 of 120 subjects) will take place at WCVC, where participant enrollment began in 2008 and averages 10 to 12 NCS births per month with anticipated increasing accrual. A smaller cohort of participants (30 of 120 subjects) will be enrolled at BCM.

BCM, although not currently enrolling in the Vanguard pilot, has established and optimized approaches for microbial sampling of vagina, placenta, meconium, and feces, through participation in the HMP JumpStart and HMP Clinical Demonstration Project (UH2) initiatives and will thus serve as a measured control against any methodological concerns related to microbial sampling and nucleic acid extraction (see prior referenced IRB approved protocols).

The combined involvement of these sites will leverage the NCS experience and extensive environmental data collection of WCVC with the HMP metagenomics infrastructure at BCM, and allow us to evaluate the feasibility of adding microbiome sampling and metagenomic analysis to the NCS.

G2. Data Analysis

Provide a description of your plan for data analysis. State the types of comparisons you plan (e.g. comparison of means, comparison of proportions, regressions, analysis of variance). Which is the PRIMARY comparison/analysis? How will the analyses proposed relate to the primary purposes of your study?

Outline of goals and strategies for the collection, analysis, and characterization of metagenomics data from NCS acquired specimens employing established methodology from the HMP JumpStart initiative have been previously outlined.

Briefly, our collaborative proposal will use a two layered analysis strategy.

We will collect microbial specimens (n=120 gravidae, 90 from WCVC and 30 from BCM) at NCS specified timepoints, and aliquot specimens for both fresh extraction and banked specimen protocols. We will extract nucleic acids from at the time of specimen collection and subsequently subject such immediate extractions to flash freezing for later batched-run metagenomic sequencing and analysis. In the second-layer strategy, we will collect parallel samples from each subject for immediate banking (using 1 of 2 “banking” protocols) rather than already proven immediate nucleic acid extraction. A randomly-selected cohort comprising 20% (10% of each of

the two banking protocols) of these “banked” specimens will be run in batched parallel samples in order to establish and refine banking methodology.

We will thereafter compare the methodologic approaches with respect to: 1. Length and quality of sequencing reads 2. Interpretation of reads by virtue of taxonomy (phyla, taxa, genus, species) and OTU binning 3. Variation in read quality, length and interpretation by virtue of known clinical factors (mode of delivery, BMI, method of feeding as breast or bottle with formula).

In such a manner we will dually assess and demonstrate not only feasibility and methodology as outlined above, but establish banking protocols for the full scale NCS.

Section H: Potential Risks/Discomforts

H1. Potential Risks/Discomforts

Describe and assess any potential risks/discomforts and assess the likelihood and seriousness of such risks:

The potential risks/discomforts are minimal. Slight irritation at the sampling site (vagina) may occur and some subjects may experience embarrassment at having the procedure. The placental, meconium and fecal sampling pose no additional risks to the subjects since no invasive procedures are used to collect this material.

H2. Data and safety monitoring plan

Do the study activities impart greater than minimal risk to subjects?

No

H3. Coordination of information among sites for multi-site research

Is the BCM Principal Investigator acting as the SPONSOR-INVESTIGATOR for this multi-site research?

Yes

Is BCM the COORDINATING CENTER for this multi-site research?

Yes

If the answer to EITHER of the questions above is "Yes", please complete the following questions:

If this is a multicenter study and the BCM PI is an INVESTIGATOR with responsibilities of SPONSOR or if BCM is the COORDINATING CENTER, describe the management of information among the sites related to participant protections. Your description should include reporting of unanticipated problems, protocol modifications, IRB and/or institutional approvals, and interim results among the sites.

The laboratories of Drs. Aagaard, Versalovic, and Petrosino at Baylor College of Medicine will process all samples obtained from this formative project on the human microbiome within the NCS. Metagenomic sequencing will be run in the Human Genome Sequencing Center and data will be downloaded onto secure

servers in a manner consistent with aforementioned protocols presently employed for the Human Microbiome Project.

Because this is a feasibility study where the results will not be compared until completion of sequencing of all eligible subject samples, not protocol modifications will be instituted. Once IRB approval is obtained here at BCM, the approved IRB will be sent to the University of Wisconsin for institutional approval. In this feasibility study of minimal risk, no interim analysis is planned.

When research is conducted in collaboration with outside entities or organizations, the PI must obtain the necessary approvals from those entities. The BCM IRB may request documentation that such approvals have been obtained. Please list and describe the planned sites for this multi-site research for which the BCM PI is either Sponsor-Investigator and/or Coordinating Center. Sites that do not meet the requirements for inclusion in section A6a of the protocol summary and BCM informed consent documents should be listed here.

Collaborating Site: Medical College of Wisconsin Waukesha County Vanguard Center (WCVC)

Section I: Potential Benefits

Describe potential benefits to be gained by the individual subject as a result of participating in the planned work.

Subjects will not derive direct benefit nor treatment for participation.

Describe potential benefits to society of the planned work.

The benefits to society are reflective of the general benefits in acquiring knowledge regarding human health and disease.

In this feasibility study, subjects will not benefit personally from giving the specimens because this research will take a long time to produce medically useful results. However, subjects will be part of a study to help researchers determine feasibility so that they may ultimately gain knowledge and understand more about the human microbiome and how it relates to pregnancy health and disease.

Do anticipated benefits outweigh potential risks? Discuss the risk-to-benefit ratio.

The results of research cannot be foreseen so it is possible that new risks may arise in the future that cannot be predicted now. However, it is believed that the benefits of learning more about the human microbiome and how it relates to health and disease outweigh the current and potential future minimal risks. Therefore, the risk-to-benefit ratio is favorable.

Section J: Consent Procedures

J1. Waiver of Consent

Will this research require a waiver of consent and authorization?

No

Will additional pertinent information be provided to subjects after participation?
No

Explain why providing subjects additional pertinent information after participation is not appropriate.
All information obtained from sample collection will be de-identified to protect the subjects' identities.

J1a. Waiver of requirement for written documentation of Consent
Is this research subject to FDA regulations?
No

Explain how the research involves no more than minimal risk to the participants, and the specifics demonstrating that the research does not involve procedures for which written consent is normally required outside of the research context.

Explain how the only record linking the participant and the research would be the consent document, and how the principal risk would be potential harm resulting from a breach of confidentiality, and how each participant will be asked whether he or she wants documentation linking the participant with the research and their wishes will govern.

J2. Consent Procedures
Who will recruit subjects for this study?
PI
PI's staff

Describe how research population will be identified, recruitment procedures, and consent procedures in detail.
The target for this multi-center study is 120 healthy gravidae between the ages of 18 and 40, 30 from Baylor College of Medicine and 90 through the WCVC Vangard Site. The requisite gestational age range will be at approximately 28 weeks gestational age. In 2007, 2988 deliveries at SLEH occurred at >28 weeks gestation, making recruitment and initial enrollment of appropriately confirmed gestational-age subjects unlikely to be problematic. A goal for minority participation will be at least 20% of the total number of subjects.

At Baylor College of Medicine (BCM), subjects will be recruited through the Baylor Clinic under the direction of Kjersti Aagaard, MD PhD. Longitudinal assessment and retention will occur with the support of the full NCS study staff, under the direction of Dr. Kozinetz and Dr. Aagaard.

With respect to consenting and executing the research, Dr. Aagaard will serve as Principal Investigator for the study and will supervise the research/nursing team trained in clinical assessment and specimen gathering

(histories/physicals and microbiome specimen gathering of placenta and meconium). Based on her experience with this line of research, Dr. Aagaard will perform all of the human vaginal sampling at the time of subject enrollment.

Three venues will be utilized to make providers (primarily Maternal-Fetal Medicine providers at SLEH) aware of the study to optimize enrollment. Venue 1: Prior address of all OB/GYN and MFM residents and faculty during Grand Rounds on March 17th, 2010 generated intial enthusiasm for the overall microbiome research intitiaves presently underway. As a reflection of this enthusiasm, we have enrolled and sampled 147/150 subjects for HMP and 24 subjects for the BWF. Venue 2. Baylor Clinic Obstetrics and Gynecology Department: As a result of Venue 1, Dr. Aagaard will continue to work with the Departmental providers to recruit subjects for this study.

Specifically with respect to recruitment of the 30 subjects here at Baylor College of Medicine, subjects receiving their prenatal care from the first trimester onward through the Baylor Clinic and with the Baylor College of Medicine obstetrics and gynecology practice group (approximately 900 potential subjects/year) will be approached by their primary providers prior to 26 weeks gestation for interest. When potential subjects display interest, research coordinators will be contacted by study personnel, consented, and thereafter enrolled. After enrollment, data will be entered into the NCS secure web-based minimal data system and subjects will be assigned a subject identification code. They will be sampled as detailed in methodology for collection of their vaginal microbiome.

Upon admission with labor, all subjects will be instructed to call the "NCS pager", which will enable NCS study personnel to be called in for both first meconium sampling as well as placental sampling. Subjects will be called by study personnel weekly from 32 weeks gestation onward to remind them of need to have the NCS study personnel paged upon admission in labor.

For neonates of uncertain viability: The legally effective informed consent will be obtained of either parent of the neonate, except that the consent of the father or his legally authorized representative need not be obtained if the pregnancy resulted from rape or incest (If neither parent is able to consent because of unavailability, incompetence, or temporary incapacity, the legally effective informed consent of either parent's legally authorized representative will be obtained, except that the consent of the father or his legally authorized representative need not be obtained if the pregnancy resulted from rape or incest) Each individual providing consent will be fully informed regarding the reasonably foreseeable impact of the research on the neonate.

For longitudinal sampling, subjects will be called and reminded, with "self" (parental) sampling as detailed previously.

Are foreign language consent forms required for this protocol?
No

J3. Privacy and Intrusiveness
Will the research involve observation or intrusion in situations where the subjects would normally have an expectation of privacy?
No

J4. Children
Will children be enrolled in the research?
Yes

J5. Neonates
Will non-viable neonates or neonates of uncertain viability be involved in research?
Yes

J6. Consent Capacity - Adults who lack capacity
Will Adult subjects who lack the capacity to give informed consent be enrolled in the research?
No

J7. Prisoners
Will Prisoners be enrolled in the research?
No

Section K: Confidentiality
Will research data include health information by which subjects can be identified?
Yes

Where will research data be kept? How will such data be secured?
The research data will be stored on a secure server at BCM. Once samples are collected identifiers are removed and only the Study ID will be retained. Subject's data is maintained on certified NCS servers, and all sequencing data is maintained in a coded (deidentified) fashion on secure servers.

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological specimens and genetic tests in addition to the clinical information relating to participating subjects. The study documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

Specifically, all data will be maintained in a deidentified fashion using unique generated study codes as previously described.

The nature of the information collected from subjects may involve risk to their privacy. This study will create a resource that will be available over the internet. The microbial DNA, which will be coded, will be deposited and be available through an open-access database. The clinical data will be available only through a NCS controlled-access database.

There is a small risk that some information could be disclosed to someone outside of the project or that, at some point, information stored in the controlled access databases could in some way be linked back to a specific subject. Despite the recent passage of a federal law that bars genetic discrimination in employment and some types of insurance, the use by others of genetic information from the eventual study of the blood specimens could conceivably impact the subject negatively in some way. For example, there is a very small risk that genetic information obtained by this study could be used by law enforcement officials to try to learn more about the subjects or their family members for the purpose of a criminal investigation. However, anyone seeking access to the information in the controlled access databases will need prior approval of site investigators.

Who, besides the PI, the study staff, the IRB and the sponsor, will have access to identifiable research data?

Access to study specific records will be limited to the PI, study personnel and appropriate regulatory agencies.

Will you obtain a Certificate of Confidentiality for this study?

No

Please further discuss any potential confidentiality issues related to this study.

Section L: Cost/Payment

Delineate clinical procedures from research procedures. Will subject's insurance (or subject) be responsible for research related costs? If so state for which items subject's insurance (or subject) will be responsible (surgery, device, drugs, etc). If appropriate, discuss the availability of financial counseling.

Patients will not be charged to participate and all ensuant costs to the research will be covered by the study grant.

If subjects will be paid (money, gift certificates, coupons, etc.) to participate in this research project, please note the total dollar amount (or dollar value amount) and distribution plan (one payment, pro-rated payment, paid upon completion, etc) of the payment.

Dollar Amount:

100

Distribution Plan:

Single payment following receipt of final specimen. In instances of fetal or neonatal or infant demise, renumeration will occur with last specimen received.

The rationale for deferring payment until the final specimen is received, rather than by study visit, is that the design of the study requires complete data sets.

Section M: Genetics
How would you classify your genetic study?

Discuss the potential for psychological, social, and/or physical harm subsequent to participation in this research. Please discuss, considering the following areas: risks to privacy, confidentiality, insurability, employability, immigration status, paternity status, educational opportunities, or social stigma.

Not applicable, as this a microbiome metagenomics study and host DNA is not analyzed.

Will subjects be offered any type of genetic education or counseling, and if so, who will provide the education or counseling and under what conditions will it be provided? If there is the possibility that a family's pedigree will be presented or published, please describe how you will protect family member's confidentiality?

Not applicable.

Section N: Sample Collection
SAMPLE: Other: Vagina
What is the purpose of the sample collection?

With respect to the vagina and upper genital tract, it is well established that among reproductive aged women the composition of the vaginal microbiota is affected by several host factors including age, menarche, sexual activity, and exogenous exposures such as the use of certain contraceptives and “feminine hygene” products. It is presumed that these alterations confer protection again the overgrowth of potentially pathogenic microbes. For example, at menarche lactobacilli are the dominant organisms but even among healthy women anaerobic bacteria and Candida species are easily detected with traditional culture methodology. Similarly, bacterial vaginosis (BV) is a common “disease” in as great as 20% of women and manifests following polymicrobial overgrowth dominated by Gardnerella and anaerobic bacteria such as Prevotella. Of interest to our proposal herein, the precise pathogenic organisms contributing to the clinical syndrome of BV remains unknown although recent advances with high throughput molecular approaches (i.e., terminal RFLP) suggests that additional phylotypes may be primary contributors.

This study involves broad determination of the microbiota found in vagina during pregnancy. This study will enroll gravidae at 28 weeks gestation. The participation of healthy individuals will create a baseline for discovery of the core microbiota in association with adverse pregnancy conditions. All subjects will be between the ages of 18 and 40. The age range of subjects has been established based on reproductive capacity and in consideration of our other studies which have provided reference data.

Specifically, swabs will be obtained from the vaginal introitus, the mid vagina, and the posterior fornix.

For blood draws, specify the amount drawn, in teaspoons, at each visit and across the course of the subjects entire participation time.
Not applicable.

Is there the possibility that cell lines will be developed with this sample?No

Sample will be obtained from:
Other: Clinic

Will the sample be stripped of identifiers?
Yes

If sample will be released outside the hospital:

Will sample be released to anyone not listed as an investigator on the protocol? Will the information be identifiable, coded or de-identified?
No

Will sample material be sold or transferred to any third parties? Will the information be de-identified?
No

If sample will be banked for future use:

Where will the sample be banked and for how long?
The sample will be in the locked laboratory and locked -80 freezer of Dr. Aagaard-Tillery or designated co-Investigator at Baylor College of Medicine indefinitely.

Does the banking institution have an approved policy for the distribution of samples?
Not applicable.

If the entire sample will NOT be used during the course of this research study:

Will the remaining tissue be discarded? If not what will be done with the remaining sample after study completion and how long will the sample be kept?

The sample will be in the locked laboratory and locked -80 freezer of Dr. Aagaard-Tillery or designated co-Investigator at Baylor College of Medicine indefinitely.

Will samples be made available to the research subject (or his/her medical doctor) for other testing?
No

If a subject withdraws from the study:

Will subject have the option to get the remaining portion of their sample back?
No

Will samples be destroyed? If not, will they be kept anonymously? What will happen to the sample if the subject revokes authorization?
Destroyed if revoked.

Will data obtained from their sample be deleted? What will happen to the sample if the subject revokes authorization?
Yes.

Will study data or test results be recorded in the subject's medical records?
No

Will results of specific tests and/or results of the overall study be revealed to the research subject and or his/her doctor?
No.

Please identify all third parties, including the subject's physician, to receive the test results.
None.

SAMPLE: Genetic Material
What is the purpose of the sample collection?
Placental swabs will be collected for microbiome profiling.

For blood draws, specify the amount drawn, in teaspoons, at each visit and across the course of the subjects entire participation time.
NA

Is there the possibility that cell lines will be developed with this sample?No

Sample will be obtained from:
Other: Labor and delivery

Will the sample be stripped of identifiers?
No

If sample will be released outside the hospital:

Will sample be released to anyone not listed as an investigator on the protocol? Will the information be identifiable, coded or de-identified?
No.

Will sample material be sold or transferred to any third parties? Will the information be de-identified?
No.

If sample will be banked for future use:

Where will the sample be banked and for how long?
The sample will be in the locked laboratory and locked -80 freezer of Dr. Aagaard-Tillery or designated co-Investigator at Baylor College of Medicine indefinitely.

Does the banking institution have an approved policy for the distribution of samples?
Not applicable.

If the entire sample will NOT be used during the course of this research study:

Will the remaining tissue be discarded? If not what will be done with the remaining sample after study completion and how long will the sample be kept?
The sample will be in the locked laboratory and locked -80 freezer of Dr. Aagaard-Tillery or designated co-Investigator at Baylor College of Medicine indefinitely.

Will samples be made available to the research subject (or his/her medical doctor) for other testing?
No

If a subject withdraws from the study:

Will subject have the option to get the remaining portion of their sample back?
No

Will samples be destroyed? If not, will they be kept anonymously? What will happen to the sample if the subject revokes authorization?
Destroyed on revoke.

Will data obtained from their sample be deleted? What will happen to the sample if the subject revokes authorization?
Destroyed on revoke.

Will study data or test results be recorded in the subject's medical records?
No

Will results of specific tests and/or results of the overall study be revealed to the research subject and or his/her doctor?
No.

Please identify all third parties, including the subject's physician, to receive the test results.
None.

SAMPLE: Tissue
What is the purpose of the sample collection?
Stool (first meconium and at 1 and 6 months of age) will be collected for determination of the fetal and infant microbiome.

For blood draws, specify the amount drawn, in teaspoons, at each visit and across the course of the subjects entire participation time.
Not applicable.

Is there the possibility that cell lines will be developed with this sample?No

Sample will be obtained from:
Other: nursery, parent

Will the sample be stripped of identifiers?
Yes

If sample will be released outside the hospital:

Will sample be released to anyone not listed as an investigator on the protocol? Will the information be identifiable, coded or de-identified?
If released to our collaborative investigators within the NCS, all information will be coded.

Will sample material be sold or transferred to any third parties? Will the information be de-identified?
No.

If sample will be banked for future use:

Where will the sample be banked and for how long?
The sample will be in the locked laboratory and locked -80 freezer of Dr. Aagaard-Tillery or designated co-Investigator at Baylor College of Medicine indefinitely.

Does the banking institution have an approved policy for the distribution of samples?
If samples are requested by other NCS investigators, an approved IRB from their institution as well as a materials transfer agreement between the institutions will first be arranged.

If the entire sample will NOT be used during the course of this research study:

Will the remaining tissue be discarded? If not what will be done with the remaining sample after study completion and how long will the sample be kept?
Yes. The sample will be in the locked laboratory and locked -80 freezer of Dr. Aagaard-Tillery or designated co-Investigator at Baylor College of Medicine indefinitely.

Will samples be made available to the research subject (or his/her medical doctor) for other testing?
No

If a subject withdraws from the study:

Will subject have the option to get the remaining portion of their sample back?
No

Will samples be destroyed? If not, will they be kept anonymously? What will happen to the sample if the subject revokes authorization?

Destroyed on revoke.

Will data obtained from their sample be deleted? What will happen to the sample if the subject revokes authorization?

Yes. Destroyed.

Will study data or test results be recorded in the subject's medical records?

No

Will results of specific tests and/or results of the overall study be revealed to the research subject and or his/her doctor?

No.

Please identify all third parties, including the subject's physician, to receive the test results.

None

Section O: Drug Studies

Is this study placebo-controlled?

No

Does the research involve a drug or biologic (including radioactive drugs) that is not approved by the FDA?

No

Will the research involve a radioactive drug that is not approved by the FDA?

No

IND Number:

Section P: Device Studies

Does this research study involve the use of ANY device?

No

Section Q: Consent Form(s)
Biospecimen Collection and Analysis in the National Children's Study: Microbiome

Biospecimen Collection and Analysis in the National Children's Study: Microbiome (Children's Stool Consent)

Section R: Advertisements
None