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Maternal–Child Microbiome: Specimen Collection, Storage and Implications for Research and Practice

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Abstract

Background—The maternal microbiome is a key contributor to the development and outcomes of pregnancy and the health status of both mother and infant. Significant advances are occurring in the science of the maternal and child microbiome and hold promise in improving outcomes related to pregnancy complications, child development, and chronic health conditions of mother and child.

Objectives—The purpose of the paper is to review site-specific considerations in the collection and storage of maternal and child microbiome samples and its implications for nursing research and practice.

Approach—Microbiome sampling protocols were reviewed and synthesized. Precautions across sampling protocols were also noted.

Results—Oral, vaginal, gut, placental, and breastmilk are viable sources for sampling the maternal and/or child microbiome. Prior to sampling special considerations need to be addressed

related to various factors including current medications, health status, and hygiene practices. Proper storage of samples will avoid degradation of cellular and DNA structures vital for analysis.

Discussion—Changes in the microbiome throughout the perinatal, postpartum and childhood periods are dramatic and significant to outcomes of the pregnancy and the long-term health of mother and child. Proper sampling techniques are required to produce reliable results from which evidence-based practice recommendations will be built. Ethical and practical issues surrounding study design and protocol development must also be considered when researching vulnerable groups such as pregnant women and infants. Nurses hold the responsibility to both perform the research and to translate findings from microbiome investigations for clinical use.

Keywords

mothers; microbiome; newborns; pregnancy; specimen handling; specimen collection

Characterization of the human microbiome—the ecological community of commensal, symbiotic, and pathogenic microorganisms that inhabit the human body—has become a topic of increased scientific study within the context of human health and disease (Peterson et al., 2009). As such, there is a growing interest in understanding the role of the human microbiome in maternal child health (MCH), as dysbiotic microbial communities have been implicated in a variety of adverse MCH outcomes including preterm birth, cardiometabolic complications of pregnancy and childhood, along with neonatal complications including necrotizing enterocolitis (Dunlop et al., 2015; Koren et al., 2012; Peterson et al., 2009). Furthermore, the maternal microbiome is a key contributor in the initial microbial colonization and development of the infant microbiome which may have long-term implications for child physical and neurocognitive development (Dominguez-Bello, 2010; Yang et al., 2016). These factors highlight the importance of improving our understanding of the human microbiome and its role in an individual's health and in adverse outcomes among maternal–child populations.

Traditionally, human microbiological studies focused on the identification of pathogenic microbes and their association with particular diseases, with little consideration of symbiotic microbes and their health implications (Peterson et al., 2009). Much of what is known about microbes and their phylogenetic relationships to one another has been obtained via culture-based methods, which rely on the observation of phenotypic and genetic traits of bacteria grown on culture media (Staley & Konopka, 1985). Recently, newer culture-independent methods and next generation sequencing strategies targeting the identification of the 16S ribosomal gene of bacteria has resulted in the detection of new microbial species and has allowed for detailed DNA-based comparisons to be made between microbial species (Griffen et al., 2012). Identification of the 16S ribosomal gene was first identified in 1985 via culture-independent polymerase chain reaction (PCR) methods (Lane et al., 1985), a method which allowed for the amplification of the 16S ribosomal region of DNA that is unique to bacteria. This method has since been further streamlined as multiple amplicons of several bacterial genes can be completed simultaneously, thereby allowing for the identification of all bacterial species present in a sample. The process involves the use of DNA primers that anneal to select regions of the DNA and amplify the target region; the amplified genes are then sequenced and classified (Shendure & Ji, 2008). As a result, new

species of bacteria have been identified and previously known species of bacteria are better characterized, based on specific and detailed DNA analysis. Advances in these methodologies have resulted in increased scientific inquiry regarding the microbial diversity, and metabolic and genetic potential of the human microbiome among select populations.

A key step in accurately assessing the human microbiome involves the appropriate selection of collection methods and protocols to ensure accurate sampling and handling of biological samples to prevent biased results. In most cases, biologic samples are obtained relatively easily from patients as most microbiome collection protocols are simple for patients to follow; however, appropriate collection techniques and storage methods specific to the body site of interest are needed to prevent postcollection microbial growth or contamination (Kumar et al., 2014). Additionally, some study designs require the collection and transport of viable microbes calling for accurate methods to ensure that the integrity of the samples is maintained.

Purpose

The purpose of this manuscript is to review collection methods and considerations for the acquisition of oral, gut, vaginal, placental, and breastmilk microbiome samples in maternal child health populations. Implications for nursing research and practice are discussed.

Oral Microbiome

The oral cavity represents the second most complex bacterial community in the human body, next to the colon (Human Microbiome Project Consortium, 2012). The oral cavity microbiome consists of over 1,000 different species on a variety of habitats including the teeth, the gingival sulcus, tongue, cheeks, hard and soft palates, tonsils and saliva (Dewhirst et al., 2010). Several of these species are associated with common oral diseases such as dental caries and periodontal disease (Dewhirst et al., 2010). Additionally, evidence exists for strong associations between oral bacterial organisms and a number of adverse maternal and child health outcomes including preterm birth, cardiovascular disease, stroke, diabetes, and ventilator associated pneumonia in preterm infants (Dewhirst et al., 2010; Sohn, Kalanetra, Mills, & Underwood, 2016).

Oral Screening Considerations

Oral microbiome research requires specific methodological considerations for collection. Methods for appropriate specimen collection from each habitat are described based on sampling procedures in the Manual of Procedures for Human Microbiome Project (McInnes & Cutting, 2010). In children and adults, tooth brushing, flossing, and mouthwashes should be avoided 12 hours before sample collection; eating, drinking, smoking, and chewing of flavored gum should be avoided a minimum of 30 minutes prior to saliva collection, although some protocols recommend 60 minutes (Kumar et al., 2014; Salimetrics, 2015). Tongue piercings should be removed prior to specimen collection, and noted (McInnes & Cutting, 2010). Additionally, participants should be advised to avoid oral sex 48 hours prior to sample collection (McInnes & Cutting, 2010). Caregivers of infants and young children should be advised to limit or withhold pacifier use, pre-chewing (adult pre-chews solid food

then feeds to infant or child), and bottle-feeding or breastfeeding for 30 to 60 minutes (Kumar et al., 2014; Salimetrics, 2015). The presence of an oral candida infection (thrush) should be noted as a potential confounder to results. For infants and young children, safety is paramount as collection devices represent a choking hazard to young children; special swabs (Table 1) that remain in the collectors hands are available that minimize the risk of choking (Salimetrics, 2015).

When sampling multiple oral sites from the same participant the following order should be adhered to saliva, soft tissue sites, hard tissues sites, then throat (McInnes & Cutting, 2010). The throat should be the last specimen collected due to the gag reflex which may cause considerable participant discomfort (McInnes & Cutting, 2010). Different oral cavity sites host distinct communities therefore care must be taken to avoid cross-contamination when sampling (Dewhirst et al., 2010). Samples from any of the oral microbiome sites should be frozen and stored at -80°C until analysis is conducted (McInnes & Cutting, 2010).

Saliva Sample Collection

Saliva, while part of the oral cavity provides a distinctive medium for the microbiome. In older children and adults, instruct subjects to let saliva collect in the mouths for one minute then drool into a collection tube. This may be repeated several times to collect the required volume; generally 1-2 ml is sufficient for most tests. Collection devices that allow for cleaner collection process and/or collection vessels with lysis buffers that preserve the bacterial microbiome at the time of sampling are also available (Table 1). For infants and young children, oral swabs are the most common method used to collect saliva. Care to avoid soft tissue contamination can be a challenge in this age group. Passive drool has been used to collect saliva in children as young as three years (Rice et al., 2016).

Soft tissue Site Sample Collection

Soft tissue sites consist of tongue, hard palate, buccal mucosa, keratinized gingiva, tonsils, and throat (McInnes & Cutting, 2010). All soft tissue sites should be swabbed with sterile cotton swabs that are placed in collection vials and swirled in buffer solution.

Hard Tissue Site Sample Collection

Hard tissue sites consist of supra- and subgingival plaque. Collection sites are prepared by isolating with cotton rolls and drying with a stream of air from an air-water syringe. Supragingival plaque is removed from the mesial surface using a dental scaler. The plaque is then released into the buffer solution of the collection tube by swirling for four to five seconds. The site may need to be sampled again or plaque from other sites may be pooled into one vial in to obtain an adequate amount of plaque (McInnes & Cutting, 2010).

Subgingival sites are prepared in the same manner, with the additional step of wiping off supragingival plaque to avoid contamination (McInnes & Cutting, 2010). Subgingival plaque is collected by inserting a scaler into the gingival sulcus at the mesio-buccal surface of the selected teeth (McInnes & Cutting, 2010). Alternatively, studies have used endodontic paper points, inserted into the sulcus, left for 10 seconds, then removed and placed into the buffer solution (Bizzarro, Loos, Laine, Crielaard, & Zaura, 2013; Griffen et al., 2012).

Gut Microbiome

The human intestinal microbiome is the most commonly investigated and the most complex of all human microbiome sites. It is comprised roughly 500–1,000 unique species that have key roles in food digestion, energy and nutrient extraction, drug metabolism, regulation of host metabolism, and modifying immune response (Tuddenham & Sears, 2015). The composition and function of the gut microbiota have been implicated in a wide range of health outcomes, including obesity, atherosclerosis, diabetes, gastrointestinal disorders, and developmental and mental health (Manges, Steiner, & Wright, 2016; Rogers et al., 2016; Tuddenham & Sears, 2015). Specifically in maternal–child health, the gut microbiota composition and function develop throughout the first two years of life and then shift during pregnancy and appear to differ in women with pregnancy-associated complications such as gestational diabetes (Koren et al., 2012).

Given its role in multiple health outcomes and its ability to be modified by dietary intake and environmental exposures, the gut microbiota may soon prove to be an effective target for therapeutic interventions across many health outcomes. A promising field of microbiome research involving fecal transplant has proven successful in treating severe gastrointestinal (GI) infection (*Clostridium difficile*) and may be beneficial in treating other inflammatory GI disorders such as Crohn's disease (Manges, Steiner, & Wright, 2016).

Gut Screening Considerations

Any investigation of the gut microbiome must consider multiple variables that impact microbiota composition: host, environment, and habitual dietary intake. Depending on the focus of the study, an adequate GI-focused health history should be noted, including a history of major GI surgeries and GI disorders (i.e., inflammatory bowel disease, irritable bowel disease, colitis, gastroesophageal reflux, or cancer). Additionally, bowel history, including recent diarrhea, constipation, anal sex or treatments/procedures such as enema or colonoscopy preparation can also significantly impact gut microbiome. A detailed account of dietary intake should be documented when possible, such as with a food frequency questionnaire or dietary recall. Medication history, including over the counter and homeopathic remedies, in particular current and recent intake of antibiotic therapy, should be noted (Aagaard et al., 2014; McInnes & Cutting, 2010).

Gut Sample Collection

As with any sample collected for assessing the microbiome, the method of gut microbiome collection should maximize the recovery of fecal microbial DNA while minimizing sampling bias and contamination (Robinson et al., 2016). A self-collected fecal sample is the most common form of specimen collection and either involves a full fecal sample or some variation of swabbing (e.g., swabbing the rectum, swabbing the fecal sample, swiping feces onto a collection card). It is recommended that any new swab sampling method be validated in pilot studies before being employed in larger scale studies (Robinson et al., 2016). Participants should be provided the method-specific collection tools (Table 1) and instructed on the self-collection procedure: either self-swab of the rectum or stool collection using disposable trays, ensuring no urine is collected with the stool sample (McInnes & Cutting,

2010). For any self-swabbing procedure, participants must be instructed such that contamination from the skin around the rectal area, specimen handling, and sample collection equipment. Provider-collected rectal swabs are another form of specimen collection when self-collection is not feasible (Kabayiza et al., 2013). For infants, swabbing of fecal samples from the diaper is the common collection method; however, rectal swab collection has also been used successfully (Dominguez-Bello, 2010; Yatsunenko, 2012).

Supplies for the collection of gut microbiome are dependent upon the method of specimen collection. Simplified instructions with pictures of each step are essential in self-collection protocols. Plastic bags for storage, ice packs (only required with some kits) and biospecimen approved shipping containers for transport or shipping may be required (McInnes & Cutting, 2010). The packet should include a return mailer box or envelope with postage prepaid if patients are not at the clinical setting.

Although there is no standard protocol for sample storage and handling of gut microbiome samples, multiple studies have investigated the effect of time and temperature on microbial composition and DNA integrity. Ideally, it is recommended that gut microbiome samples be frozen at -80°C as quickly as possible after collection. Storage at temperatures other than -80°C , as well as variations in preservative buffers, have significant effect on microbiota composition and DNA integrity (Choo, Leong, & Rogers, 2015). However, previous studies have demonstrated that samples stored at room temperature for 24 hours or less and in cold storage (Cardona et al., 2012; Wu et al., 2010) remain stable. A more recent study concluded that samples stored at -20°C for one week, at $+4^{\circ}\text{C}$ for 24 hours, and at room temperature for 24 hours, remain stable (Tedjo, 2015). This has been applied to the storage protocols of other microbiome studies as well (Choo, Leong, & Rogers, 2015).

Vaginal Microbiome

The composition of the vaginal microbiome is instrumental in the development of several reproductive tract infections including bacterial vaginosis, yeast infections, urinary tract infections, and sexually transmitted infections (Haahr, 2016; Jayaprakash, Schellenberg, & Hill, 2012). General consensus attributes this to a dynamic equilibrium amongst bacterial communities in which indigenous microbes prevent the overgrowth of potentially pathogenic bacteria (Ravel et al., 2011). The *Lactobacillus* species is the primary protective vaginal bacterial species identified in the majority of women and traditionally associated with a healthy vaginal microbiome (Thomas, 1928; Ravel et al., 2011). *Lactobacillus* spp. are considered protective through its ability to produce lactic acid resulting in a low and protective pH and production of various bacteriostatic and bacteriocidal compounds (Voravuthikunchai, Bilasoi, & Supamala, 2006). *Lactobacillus* spp. also protect the vaginal environment through competitive exclusion of harmful, typically anaerobic, bacterial strains (Voravuthikunchai et al., 2006). Recent findings suggest that there may be other undiscovered protective microbial species as observations of healthy vaginal microenvironment have been observed in otherwise healthy women that lack *Lactobacillus* species (Jayaprakash, Schellenberg, & Hill, 2012; Ravel et al., 2011).

Improved DNA sequencing strategies have allowed researchers to better characterize the vaginal microflora which, thus far, seems to have a smaller amount of biodiversity compared to the gut or oral microbiomes (Human Microbiome Project Consortium, 2012). Differences in both stability and diversity of species have been observed in association with varying times during the menstrual cycle, sexual activity, race/ethnicity, menopausal status, and nongravid versus gravid status (Brotman et al., 2014; Human Microbiome Project Consortium, 2012; Ravel et al., 2011; Romero et al., 2014).

Vaginal Screening Considerations

Women should be carefully screened and given clear instructions before vaginal microbiome specimens are collected. Screening should be scheduled 48 hours after the cessation of menstruation. Additionally vaginal sexual activity, vaginal medications or suppositories, diaphragms, cervical caps, feminine sprays, and genital wipes should be avoided for 48 hours prior to collection of specimens (Aagaard et al., 2013; McInnes & Cutting, 2010; Ravel et al., 2011). Specifically, vaginal antifungal agents and antibiotics should not be used in the prior seven days (McInnes & Cutting, 2010). These guidelines are applicable to both pregnant and non-pregnant women; however, clinical judgment should also be employed to avoid any additional risk to the pregnancy or in the immediate postpartum period when vaginal sampling may be contraindicated. Research questions, study design, and clinical guidelines should drive final decisions related to appropriate timing and frequency of sampling during these time periods. Exclusion criteria that should be considered include women who utilize a combination hormone vaginal ring contraceptive device and those whose vaginal pH is < 4.5 during screening (McInnes & Cutting, 2010). Hygiene and health history should be included in screening, depending on the focus of the research, and may include: menstrual cycle, sexual activity, medications, and sanitary product use.

Vaginal Sample Collection

Provider versus self-collection of vaginal microbiome specimens has produced comparable results (Forney et al., 2010; Nelson, Bellamy, Gray, & Nachamkin, 2003). Provider collected samples allow for the visualization of the vaginal introitus, posterior fornix, and midpoint of the vagina for selective sampling at these sites if desired (Aagaard et al., 2013; McInnes & Cutting, 2010; Romero et al., 2014). For examination, providers should use a sterile speculum without lubrication or one that has been moistened with sterile saline or water. Other lubricants can alter the microbiome sample and are to be avoided during sample collection (Aagaard et al., 2013; Forney et al., 2010; McInnes & Cutting, 2010). During collection, the swab (Table 1) should be rotated five times against the vaginal wall to ensure swab saturation (Aagaard et al., 2013; McInnes & Cutting, 2010). Sampling in the vaginal areas that has been in contact with the speculum should be avoided, therefore careful progressive sampling into the vaginal canal is required (McInnes & Cutting, 2010).

For self-collected samples women should be instructed to insert the vaginal swab 1-2 inches into the vagina to access the vaginal midpoint (Forney et al., 2010; Nelson, Bellamy, Gray, & Nachamkin, 2003; Ravel et al., 2011). After insertion the patient should gently twist the swab in order to expose all sides of the cotton tip to secretions. Other collection methods instruct women to purposefully wipe the swab against the vaginal wall as well as hold the

swab in the vagina for 20 seconds (Brotman et al., 2014; Forney et al., 2010). Either method is acceptable, but should be consistent with all participants to ensure study design rigor. Studies point out that women are typically willing to self-collect and may even prefer it to a pelvic exam (Huynh, Howard, & Lytwyn, 2010). This method may also be financially feasible and prove more accessible for use in nonclinical settings (Forney et al., 2010; Nelson, Bellamy, Gray, & Nachamkin, 2003).

Regardless of who collects the sample, care should be taken to avoid contamination as the swab is withdrawn from the vagina. Contact with outside skin surfaces, such as the labia and inner thigh, should be avoided. To transfer the collected specimen, all swabs should be immediately introduced into individual sterilized medium and placed on ice for transport if required, then stored at -80°C within four hours until analysis (McInnes & Cutting, 2010).

Placental Microbiome

Traditionally, the contents of the pregnant uterus including the placenta, membranes, and amniotic fluid were thought to be sterile; however, recent findings suggest that the placenta contains a metabolically rich microbiome that closely resembles the human oral microbiome (Aagaard et al., 2014). In a cross-sectional study of placentas, gram-positive and gram-negative intracellular bacteria were discovered at the basal plate (the layer of tissue directly at or below the maternal-fetal interface) which was traditionally thought to be sterile, leading researchers to believe the placenta has its own microbiome (Stout et al., 2013). The transfer of oral microbes to the placenta is thought to occur when select oral microbes alter the structure of blood vessels, pass into the blood, and seed the placenta. The transfer of microbes into the intrauterine environment may potentially activate immune mechanisms and inflammation thereby increasing the risk for perinatal complications (Aagaard et al., 2014).

Placental Screening Considerations

General microbiome screening considerations include the evaluation of factors related to diet and exposure to antibiotics, although, studies of the placental microenvironment should also consider factors related to pregnancy including history of intrauterine fetal demise, severe or fatal fetal congenital and/or chromosomal anomalies, or infections such as intrapartum chorioamnionitis, *Streptococcus agalactiae* (GBS) colonization, and HIV (Aagaard et al., 2014; Amarasekara, Jayasekara, Senanayake, & Dissanayake, 2015). Other elements to consider include mode of delivery, rupture of membranes during labor, gestational age at delivery, and maternal complications that may influence placental health such as preeclampsia (Aagaard et al., 2014; Amarasekara et al., 2015; Stout et al., 2013). Further investigations are warranted to evaluate the influence of other clinical procedures on the placental microbiome including chorionic villus sampling, percutaneous villus sampling, and amniocentesis.

Placental Sample Collection

Sampling the placental microbiome requires that placentas be transferred to a sterile container immediately following delivery and strict procedures for specimen extraction be

followed per identified protocol (Aagaard et al., 2014; Amarasekara et al., 2015; Stout et al., 2013). The premier researcher for placental microbiome describes the following protocol for tissue extraction and sampling: under clean and sterile conditions cuboidal sections are circumferentially cut from different areas of the placenta that are 4 cm from the cord insertion site (Aagaard et al., 2014). Placental sampling is specifically performed only by trained personnel using sterile equipment and wearing sterile face masks and gloves (Aagaard et al., 2014; Amarasekara et al., 2015; Stout et al., 2013). After tissue extraction, samples should be transported on ice in sterile containers to the appropriate laboratory, and stored at -80°C until DNA extraction (Aagaard et al., 2014).

Breast Milk Microbiome

The microbial composition of breastmilk is unique from that of other mucosal sites of the body such as the vagina, gut, skin, and mouth (Cabrera-Rubio et al., 2012). Human breast milk is highly variable in microbial content but accumulating evidence shows a dominance of *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* (Cabrera-Rubio et al., 2012; Soto et al., 2014). Breast milk provides the infant with a foundation for a diverse microbial gut community, essential nourishment, and immunological compounds. It also supplies specific bacteria and probiotic compounds which encourage the colonization of beneficial gut bacteria in the newborn (Yang et al., 2016). Anaerobic gut bacteria such as *Bifidobacterium* confer protective mechanisms which allow the infant to combat infection and mount an immune response (Soto et al., 2014). Thus, a more complete understanding of the breast milk microbiome enables researchers, nurses, and concerned mothers to encourage behaviors which lead to a more diverse, healthy microbial community within breast milk, ultimately encouraging development of a healthy infant gut microbiome.

Breast Milk Screening Considerations

Screening criteria include any condition that might contaminate the breast milk sample, e.g., mammary abscesses (Arroyo et al., 2010). Other considerations include pregnancy status, current medications and supplements, especially hormonal birth control, length of time breastfeeding, and feeding mode, i.e., exclusive breastfeeding versus exclusive pumping (Arroyo et al., 2010; Cabrera-Rubio et al., 2012). Infant factors that have been noted to impact breast milk microbiome include gestational age of the infant and the infant microbiota (Gomez-Gallego et al., 2016). Questionnaires serve as a valuable tool to document additional circumstances such as maternal and/or child infections and health status, frequency and duration of feedings, lactation stage, or drastic changes in maternal diet that could affect the breast milk microbial community during the study.

Breast Milk Sample Collection

While breast milk collection timing and frequency should be based on study design, collection times should be standardized across participants to maximize comparability. Samples of colostrum should be collected within five days of birth and careful thought given toward collection of foremilk, hind milk, or both. Factors to consider when determining collection timing and frequency are synchronization with collection of other data sources, lactation phase, patient burden, and rationale for collecting from one or both breasts. Often,

only one breast is used for milk collection throughout the study period (Cabrera-Rubio et al., 2012). It is important to remember new mothers are often under significant stress and may not realistically be able to adhere to frequent data collection. Therefore, study designs that allow for flexibility in timing of sample collection are favorable. In order to ensure adequate sampling volume, mothers should wait two hours after feeding before collecting a breast milk sample for the study (Hunt et al., 2011).

Prior to collection, the breast should be cleaned with soap and water or other suitable alternatives, such as iodine, to minimize contamination from skin or exogenous bacteria on the nipple (Table 1) (Cabrera-Rubio et al., 2012). If substances like iodine or chlorhexidine are used, care should be taken to remove these substances from the nipple prior to the infant's next feeding. The first drops of milk (~500 µL) should be discarded and subsequent milk can be expressed either manually or with an electric pump into a sterile collection vial (Cabrera-Rubio et al., 2012; Hunt et al., 2011). The required sample volume is determined by subsequent analysis techniques. If collected at home, milk should be immediately refrigerated, then placed on ice (if collection kit requires) for transfer to the laboratory (Cabrera-Rubio et al., 2012; Hunt et al., 2011). If collected in the clinical setting samples can be immediately analyzed otherwise, the same guidelines as home collection should be followed. Previous studies have found storage of milk samples at -20°C and -80°C to be adequate for subsequent DNA sequencing (Cabrera-Rubio et al., 2012; Hunt et al., 2011).

Beyond the considerations noted above, researchers have an ethical obligation to prioritize the nutritional health of the nursing infant above data collection. All efforts should be made to protect the development of the mother's milk supply and not take more than absolutely necessary for the analysis of the research study.

Importance for Nursing Research and Practice

The unique sources of microbiome samples from perinatal women, neonates, and children require special consideration. Shifts in the microbiome throughout the perinatal period for the woman and her neonate are dramatic and persist throughout early childhood. Although this is a new frontier of scientific exploration, we are now able to formulate relevant questions that surround complex health concerns for the human maternal-child population. Given the multiple steps described in the collection, storage and analysis of human microbiome samples there are many ways that bias can be introduced into a study. Such bias becomes more significant at lower taxonomic levels and when the taxa examined are less abundant (Freitas, Li, Scholz, & Chain, 2015). Further standardization of collection and storage methods will occur as larger, more complex studies are conducted and sources of variation are more readily determined.

A fall in the price of sequencing technology, coupled with greater bioinformatics capabilities, has led to the application of findings in research and in the clinical setting. Nursing plays a vital role in the exploration of the microbiome by not only formulating innovative hypotheses but also by developing and refining protocols and study designs that are patient-centered yet maintain research rigor. Given the complexity and limits of the science in this burgeoning area, care providers must use caution when counseling research

participants and educating patients to ensure their understanding. Nurses will remain at the forefront of translating relevant findings to maternal–child health care providers and patients to improve health outcomes.

The Precision Medicine Initiative announced by President Obama in January 2015, now titled *All of Us*, was created to support the development of new approaches for detecting, measuring, and analyzing a wide range of biomedical information (The White House, 2015). The advancement of this initiative will depend, in part, on human microbiome research and the application of this research on individualized care for health promotion, disease prevention, and treatment. The National Microbiome Initiative, announced May 2016, set forth the goal of studying microbiomes across different ecosystems (The White House, 2016). These recent initiatives underscore the high level prioritization and importance placed on advancing human microbiome research.

Much remains to be uncovered about the maternal-child microbiome; however, what appears to have been established—that the foundation of an individual's microbiome is set in the first two years of life—suggests that the optimization of maternal child health during this time is critical (Yang et al., 2016). Human microbiome research findings may aid in this effort. Following tested collection and storage methods will contribute to valid and reliable outcomes and ultimately inform evidenced based practice.

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TABLE 1

Site Specific Collection of Microbiome Specimens

Habitat	Supplies	Vendor	Website
Oral cavity (soft and hard tissue) Gut Vagina	Catch-All™ swabs – soft, foam swabs with plastic handles for general collection in adults and children	Epicentre®	http://www.epibio.com/applications/nucleic-acid-purification-extractionkits/buccal-swabs-and-dnaextraction/buccal-swabs-and-bru
	PowerBead collection tube – collection tube to help isolate RNA from samples	MoBio	https://mobio.com/powermicrobiometm-rna-isolation-kit-1188.html
Saliva	Falcon (50 ml) – basic collection tube for saliva	Fisher Scientific	https://www.fishersci.com/shop/products/falcon-50ml-conical-centrifuge-tubes-2/p-193321
	SalivaBio Passive Drool Method – allows for the direct funneling of saliva into the tube	Salimetrics	https://www.salimetrics.com/collection-system/passive-drool
	SalivaBio Children's Swab (SCS) Method – helps to eliminate choking hazard	Salimetrics	https://www.salimetrics.com/collection-system/childrens-swab
	SalivaBio Infant's Swab (SIS) Method – for use in children age less than 6 months	Salimetrics	https://www.salimetrics.com/collection-system/infants-swab
	OMNIgene Oral (OM-505) – self-collection kit that provides stable storage at	DNA Genotek	http://www.dnagenotek.com/US/products/OM505.html

Habitat	Supplies	Vendor	Website
Gut	room temperature for three weeks		
	FecalSwab™ – liquid based collection and preservation system	ThermoFisher	https://www.thermofisher.com/order/catalog/product/R723487
	OMNIgene-GUT (OMR-200) kit – self-collection kit that provides room temperature storage for sixty days	DNAgenotek	http://www.dnagenotek.com/US/products/OMR200.html
Breast	Skin preparation kit – for use before milk collection	BD	http://www.sklarcorp.com/sterilekits/general-purpose/basic-skin-prepray.html
	HygieniKit® – milk collection system with protective barrier	Am da	http://www.amedia.com/accessories/milk-collection-storage/hygienikit-milkcollection-system