Engaging Adolescent and Young Adults in Microbiome Sample Self-Collection: Strategies for Success

Chen X. Chen, Janet S. Carpenter, Tabitha Murphy, Patricia Brooks, J. Dennis Fortenberry

Abstract

Human microbiome research provides rich opportunities to elucidate factors influencing health, uncover novel biomarkers, and expand disease treatment options. A well-conducted microbiome study depends not only on a rigorous design but also on successfully engaging participants in collecting quality samples. In this paper, we aim to describe (1) strategies our team used to engage adolescents and young adults in vaginal and gut microbiome sample self-collection and (2) their effectiveness. In our prospective, longitudinal, feasibility study of 20 female adolescents and young adults, research participants self-collected vaginal and gut microbiome samples at home. Using a participatory and iterative process, we developed strategies to engage participants in sample self-collection, including (1) providing clear instructions to ensure comprehension and buy-in, (2) providing a user-friendly take-home package, (3) minimizing disgust/embarrassment associated with sample collection, and (4) follow-up communications to facilitate sample collections and return. With these strategies, we achieved 100% participant retention and 100% sample return rates. All samples (n=80, 100%) were usable for downstream 16s rRNA gene sequencing and analysis. All participants rated the study procedures as acceptable, and qualitative data showed that strategies were well received by participants. This study suggests that carefully planning and implementing strategies to engage participants in sample self-collection can result in high degrees of participant compliance, sample quality, and participant satisfaction in microbiome research.

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Research involving omics has become a priority in nursing science to unveil mechanisms of symptoms and to inform precision treatment approaches. Based on the National Institutes of Health's Symptom Science Model, using emerging omics methods can illuminate potential biomarkers and intervention targets (Cashion et al., 2016). According to Cashion et al. (2016), incorporating phenotypic and omics data is critical to developing precision-based treatments to enhance individuals' health. Among the omics methods, the study of microbiome (bacteria, fungi, and viruses, etc.) has provided insight into understanding individual differences in health and illnesses.

Human microbiome research has increased significantly in recent years (Gilbert et al., 2018). An increasing amount of evidence suggests that the human microbiome plays an important role in health and disease (Gilbert et al., 2018). For example, research suggests that the gut microbiota (microorganisms in the gut) are essential in human metabolism, immune response, and neurological health (Cryan et al., 2019). The gut microbiota communicate with the brain by regulating neurotransmitters, generating microbial-derived metabolites, and modulating inflammatory responses (Cryan et al., 2019). Research also suggests that the vaginal microbiota are important in female reproductive health and birth outcomes (Ma et al., 2012). Vaginal *Lactobacillus spp*. have found to produce anti-microbial compounds that inhibit the growth of potentially pathogenic organisms. Women who delivered preterm were reported to have lower vaginal levels of *Lactobacillus spp* and higher vaginal levels of potentially proinflammatory bacteria (Fettweis et al., 2019). Microbiome research has provided valuable information for nursing and other fields related to physiologic and pathophysiologic processes as well as prevention and treatment of disease.

A well-conducted microbiome study depends not only on rigorous design but also on successfully engaging participants to collect quality samples (Maki et al., 2019). Participants' compliance with sample self-collection protocols can affect sample quality, usability, and downstream assays and analysis. Some microbiome studies involve longitudinal sample collection. For longitudinal studies, ensuring participants feel respected, comfortable, and engaged is also important to ensure high participant retention (Abshire et al., 2017). An increasing number of resources have become available to guide the design of human microbiome research (for example, https://www.hmpdacc.org/hmp/resources/, (Maki et al., 2019); however, few publications are available to provide practical strategies on how to engage research participants to self-collect quality samples, ensure compliance, and achieve high participant satisfaction, especially among the adolescent and young adult population. In other biospecimen collections, adolescents have been cited as having lower compliance with the study protocol (Halpern et al., 2012).

In our prospective, longitudinal, pilot study of female adolescents and young adults, research participants self-collected vaginal and gut microbiome samples. The purposes of this paper are to describe (1) strategies our team used to engage adolescents and young adults in vaginal and gut microbiome sample self-collection and (2) their effectiveness. This paper is intended to provide practical suggestions for researchers and clinicians who collect human microbiome samples in their own work.

Materials and Methods

Design of the Parent Study

The parent study was a prospective, longitudinal, descriptive, pilot study of 20 female adolescents and young adults. The study was approved by the institutional review board. Detailed methods of the parent study are described elsewhere (Chen et al., under review). Briefly, the parent study focused on female menstrual pain. Previous research has shown that

the gut microbiota play an important role in regulating visceral pain (O' Mahony et al., 2017), while the vaginal microbiome play an important role in modulating reproductive tract inflammation (Fettweis et al., 2019; Ma et al., 2012). Participants were recruited through a clinical study registry and study flyers. Potential participants were screened over the phone and consented during an in-person enrollment visit. Participants younger than 18 years old assented, and their parents/guardians consented. Microbiome sample collection kits (OMNIgene®•GUT, OMNIgene®•VAGINAL) were provided during the study visits for participants to take home. Participants were followed for one menstrual cycle. Participants self-collected stool samples and vaginal swab samples twice (on menses and off menses) at home and returned the samples during follow-up study visits. In addition, participants provided self-reported data through online surveys at the initial visit/study enrollment, at the time of sample collection, and at the conclusion of the study.

Engagement Strategies for Microbiome Sample Self-Collection

We developed our strategies to engage participants in microbiome sample self-collection using a participatory and iterative process. The process involved testing and refining the study protocol prior to launching the study, soliciting formative and informal feedback from each research participant, and continuously adapting the engagement strategies based on feedback from research participants and staff.

Our research team has extensive experience, including investigators with over 30 years of experience engaging longitudinal cohorts of research participants, investigators familiar with biological sample collection protocols, and staff with hands-on experiences teaching participants how to self-collect biological samples (including microbiome samples). Prior to launching the study, we tested the protocols with team members. The principal investigator (a PhD-prepared researcher), project manager (a Certified Clinical Research Professional (CCRP®)), and research assistant (a RN/BSN-prepared graduate student) were all involved in designing and compiling the take-home packages, instructing research participants, and having follow-up

communications with participants. Research team members met regularly to discuss what had been working well and what could be improved. During the course of the study, we regularly sought (1) informal feedback from participants at study visits and during follow-up phone calls and (2) formal feedback from participants when they finished the study. We used this feedback to improve our strategies for at-home microbiome sample self-collection iteratively. Key strategies are described below and summarized in Table 1.

Provide Clear Instructions to Ensure Comprehension and Buy-in

To teach participants how to self-collect samples, we used a variety of techniques, as described below. The goals for these techniques were to ensure participants' comprehension and to safeguard sample quality and usability.

Provide Multiple Learning Formats. Our team prepared participant training materials in multiple formats, including instruction sheets with pictures and a rundown of the necessary steps (from the sample kit manufacturer), an instruction video (from the sample kit manufacturer, publicly available on YouTube), demonstration kits, and instruction scripts. These different formats accommodated different learning styles and varying literacy levels. During the initial visit, we verbally explained and demonstrated sample collection procedures. After the initial visit, participants were able to access video instructions (through a QR code or a shortened URL link) and instruction sheet at home.

Use Teach-back Techniques. As learning about self-collect microbiome samples involves cognitive and psychomotor skills, we used teach-back and show-back techniques. Specifically, after demonstrating and explaining the sample collection procedures to a study participant, the research assistant asked the study participant to repeat the instructions and physically demonstrate their understanding.

Avoid Technical Words. For instructional scripts, we avoided technical words. However, we did include details regarding sample collection timing, storage, and sample return to minimize variability in how instructions were followed. The scripts had a Flesch readability

level of 7th grade. During instruction, research staff used informal language when explaining the detailed procedures. While the research assistant demonstrated the collection steps using a demonstration kit, participants were encouraged to ask questions.

Provide Rational for Quality Sample Collection. Our research staff was explicit about our research questions, describing how the study aimed to advance science and women's health. We explained to participants why we collected these samples and emphasized the importance of collecting quality samples to answer our research questions.

As contamination during sample collection could confound downstream sequencing results, we introduced tips to prevent sample contamination by skin, urine, or toilet water. These included washing hands prior to sample collection, not putting the swab tip on other surfaces, using the toilet accessories correctly, and urinating before collecting the stool samples.

Provide a Take-home Package to Make Sample Collection User-friendly

Provide Tools to Ease Sample Collection. We provided each participant with a sample collection package with components to ease sample collection. For stool samples, we provided toilet accessories made of flushable paper (purchased from the sample kit manufacturer). The toilet accessories can be adhered to the back of the toilet seat to ease sample collection. A backup toilet accessory was included in case it did not properly adhere to the toilet seat or it broke on the first attempt. The commercial kit also came with a spatula to ease sample transfer to the tube. For vaginal swab samples, we provided a backup swab in case a participant was unable to collect the sample on the first attempt (e.g., accidentally drop the first swab).

Include Bathroom Reminder. Inside the package, we included a tent sign to remind participants about the timing of sample collection. The reminder was laminated and waterproof, and therefore, was suitable for the bathroom. In addition, we included the team's contact information on the reminder in case participants had questions about the sample collections.

Label Clearly. We packaged the kit to make it easier to find the instructions and sample collection devices. We labeled components of the kits to help participants identify kit components. We also used bright-colored labels on the biohazard bags for participants to note sample collection dates, time, and issues with sample collection.

Minimize disgust/embarrassment

Discussing stool and vaginal samples and collecting them can be potentially embarrassing and awkward for participants. We used several approaches to minimize embarrassment and awkwardness.

Relate to Participants as Individuals. Research staff used empathy, reassurance, and humor when appropriate. For example, when teaching participants to collect stool samples, a staff member commonly said, “Collecting stool samples sounds gross, I know, but it is not as bad as you think.” When teaching participants to collect vaginal swabs, a staff member told participants that “if you cross your eyes, that means your swab has gone too far.” We also emphasized to participants the benefits of self-collection by pointing out that they could do this in the privacy of their own homes.

Provide Trainings in a Private Room. Research staff conducted sample collection training in a private room. For participants younger than 18 years old, unless participants preferred otherwise, parents could stay in the room. Participants were encouraged to ask questions, and we emphasized that no question was dumb.

Emphasize the User-friendliness of the Kits. We showed participants the actual sample collection kits and emphasized their user-friendliness. For stool samples, participants became aware that only a small amount of sample was needed. We also emphasized to participants that they did not need to touch their stool samples with the toilet accessory and spatula. For vaginal swabs, participants became aware that the vaginal swab was much thinner than a tampon. We also emphasized that as there is a stabilizer in the kit, they did not need to store their stool and vaginal swab samples in a home freezer.

Provide an Opaque Bag. We provided participants with an opaque tote to carry their samples. This is because, based on previous research (Lecky et al., 2014) and our own experience, some people felt embarrassed by the idea of other people knowing they carried their own stool in a container.

Follow-up Communications to Facilitate Samples Collections and Return

During the training session, our research staff went over the sample collection schedule with each participant using a visual aid. We asked participants their preferred method of communication for follow-up reminders.

Using their preferred method, we provided a reminder before each scheduled sample collection to ensure specimens were appropriately collected, labeled, and returned on time. We also reminded participants about how to locate instruction materials if needed. We conducted troubleshooting with participants if they ran into any problems with the sample collection at home. For sample drop-off, we tried our best to accommodate research participants' schedules.

Methods to Evaluate the Effectiveness of the Engagement Strategies

To evaluate the feasibility of the sample collection protocol and the effectiveness of our participant engagement strategies, we conducted a post-study online survey and in-person interviews. On the post-study survey, we included several closed-ended questions. Participants were asked if the overall study procedures were acceptable to them (with two response options: yes/no). In addition to this general question, we inquired about specific sample collection procedures. Participants were asked, "How easy was it to obtain the stool samples at home?" and "How easy was it to collect vaginal swabs at home?" Both questions were rated on a scale with five response options: "very easy," "quite a bit easy," "somewhat easy," "a little bit easy," and "not at all easy." In addition, participants were asked how willing they would be to self-collect microbiome samples at additional time points with three response options: "very willing," "maybe," and "never again."

We also asked open-ended questions in the survey and during the interviews. Answers were recorded with pen and paper by research staff during the interview. Participants were asked about what they liked and what they found challenging about the study procedures. Participants also were asked to provide any other comments and suggestions about the study.

For quantitative data, we summarized them descriptively using counts, frequencies, means, and standard deviations with IBM SPSS Statistics 27. For qualitative data (participants' responses to open-ended survey questions and staff notes taken during the interviews), we conducted content analysis (Miles, Huberman, & Saldaña, 2013) to summarize themes of feedback. Two research team members reviewed the open-ended responses and staff notes repeatedly to obtain a sense of the breadth and depth of the data. Data were further coded based on its relevance to specific participant engagement strategies. Discrepancies were resolved by discussion. A third team member validated their conclusions by reexamining the data. Narrative descriptions of the qualitative findings also were reviewed and edited by the other two authors.

Results

All twenty participants were female, and the mean age was 20.9 ±3.2 years (Range: 15-24 years). The majority of participants (60%) self-identified as White, 35% as Black/African American, 5% as Asian, and none as Hispanic/Latino.

Of 20 participants who consented to be in the study, none dropped out (i.e., 100% participant retention rate). All participants managed to self-collect stool samples and vaginal microbiome samples longitudinally at both time points during the study. All microbiome samples (n=80, 40 stool samples and 40 vaginal swabs) were returned within three days of sample self-collection. All the returned samples were usable for downstream DNA extraction, PCR, and 16S rRNA gene sequencing. No sample was discarded due to low biomass or low read counts. (Chen et al., under review).

Among participants, 100% rated the study procedures acceptable. For vaginal swab collections, 75% of participants found it very easy, 5% found it quite a bit easy, 15% found it somewhat easy, 5% found it a little bit easy. None reported it as “not at all easy.” For stool sample collections, 25% of participants found it very easy, 25% found it quite a bit easy, 40% found it somewhat easy, 10% found it a little bit easy, 5% found it not at all easy. Among all participants, 75% expressed that they were “very willing,” and 25% expressed that they “may be willing” to provide samples at additional time points.

Among participants, 100% completed post-study surveys and interviews. Several participants described the study procedures as “well organized” and “enjoyable.”

For sample collection instructions, several participants commented that research staff were “knowledgeable” and “explained things well.” They expressed appreciation for the “straightforward” and “easy-to-understand” instructions provided by the research staff. They also commented that instructions were easy to follow.

For the take-home package, some participants found having back-up toilet accessories and sample collection kits very helpful. A few participants were unsuccessful at their first sample collection attempt (e.g., not attaching accessory appropriately, dropping a vaginal swab). They were able to successfully re-collect the samples using the back-up kits provided by the research team. Two participants noted that collecting stool samples was challenging because the toilet accessory broke on their first attempt. Two other participants noted that the toilet accessories did not attach well to their toilets. Despite these problems, they were able to re-collect the samples using the back-up toilet accessory. Their constructive feedback prompted the team to emphasize the importance of ensuring proper attachment and what to do in the event of breakage.

For minimizing disgust and embarrassment, participants noted that research staff made them feel comfortable. They described the research staff as “kind”, “considerate”, “and “enjoyable to work with.” A large majority of participants commented that the sample collections

were “easy,” “non-invasive,” or “not uncomfortable.” A few participants noted that having an opaque bag to carry the sample kits and collected samples was helpful.

For follow-up communication, participants noted that research staff “communicated effectively” and kept them “up to date.” Some participants found the telephone, email, or text reminders from research staff very helpful. They also expressed appreciation for accommodating their schedules for sample drop-off times.

Discussion

In this paper, we described effective strategies our research team utilized to engage adolescents and young adults in vaginal and gut microbiome sample self-collection. Specifically, we provided practical suggestions for researchers and clinicians whose work involves asking research participants or patients to self-collect microbiome samples. Strategies included providing sample collection instructions in ways that accommodated different learning styles and levels of health literacy, providing a clearly labeled take-home package with user-friendly tools, intentionally minimizing disgust/embarrassment associated with sample collection, and providing follow-up communications to ensure samples were being collected and returned on time. We found that iterative quality improvement procedures and consistent communication with participants and team members were critical to refining participant engagement strategies. Based on the quantitative and qualitative data, our strategies were well received by the research participants.

This paper contributes to the limited literature on engaging participants in microbiome sample self-collection. Sample self-collection at home allows for flexibility, accessibility, and privacy. However, there are challenges in microbiome sample self-collection related to participant compliance and sample quality (Debelius et al., 2016). As Debelius et al. (2016) described, people are often unable to follow directions, which may be even more true for adolescents and young adults. Only a few researchers have published some strategies to solve these challenges. For gut microbiome sample collections, Debelius et al. (2016) suggested that

succinct, clear, and engaging instructions are critical to minimize variability in how instructions are followed. We expanded their approach by having a detailed description of what to include in self-collection instructions, what to include in the take-home package, and how to reduce awkwardness related to sample collection. Our approaches are relevant not only for stool samples but also vaginal swab samples.

Even though our study had a small sample size, the strategies we used are likely to be appropriate for research with larger samples. These strategies require budgeting for additional costs (back-up kits, bags, etc.) and staff time (preparing package, communicating with research participants, etc.). However, such additional costs are relatively low in comparison to the costs of participant attrition due to frustration at collecting biospecimens, costs of lost or unusable samples; both of which reduce statistical power and statistical conclusion validity. In our study, these strategies ensured high participant retention and high sample quality. Therefore, these strategies should be evaluated for use and cost-effectiveness in larger studies as well.

Outside of the context of microbiome research, there is more guidance on engaging research participants in longitudinal studies. Researchers have suggested that corresponding with participants via their preferred mode of contact, providing reminders, and having research staff with good interpersonal skills are important to successful retention in longitudinal studies (M. Abshire et al., 2017; Hanna et al., 2014). Our strategies aligned with these recommendations.

We recognized the limitations of our study. First, our pilot study was limited by a small sample size and a shorter follow-up period. Additional problems may arise in a larger sample and with a longer follow up period. Second, we included relatively healthy female adolescents and young adults; therefore, some of our successful strategies may not be generalizable to other populations (e.g., younger children, people with serious or debilitating conditions). Third, participants only collected stool samples and vaginal swabs. Other types of samples (e.g., urinary samples, skin samples) may require other special considerations. Fourth, we did not

notice any pattern of differences across racial and age groups in terms of procedure acceptability, challenges encountered, or participant satisfaction. This is likely due to our small sample size. Researchers conducting large-scale projects can further investigate if participant engagement strategies need to be tailored based on demographic and social cultural factors.

In conclusion, we found that it was feasible to engage adolescents and young adults in longitudinal research involving microbiome sample self-collection. The strategies discussed require careful planning and thoughtful implementation. In terms of the outcomes of our study, these strategies were effective in achieving high participant compliance, sample quality, and participant satisfaction in microbiome research.

Table 1. Summary of Participant Engagement Strategies

Strategies	Specific Techniques
Provide clear instructions to ensure comprehension and buy-in.	• Provide multiple learning formats (video, pamphlet, verbal scripts, and demonstration kits). • Use teach-back and show-back techniques. • Avoid technical words. • Provide rational for quality sample collection and tips to prevent sample contamination.
Provide a take-home package to make sample collection user-friendly	• Provide toilet accessories and other tools to ease sample collection; provide back-up kits. • Include a bathroom reminder with timing for sample collection and team's contact information. • Label package components clearly.
Minimize disgust/embarrassment	• Relate to participants as individuals and use empathy, reassurance, and humor when appropriate. • Provide trainings in a private room. • Provide an opaque bag for sample carrying.
Follow-up communications to facilitate sample collection and return	• Use participants' preferred methods of communication. • Provide reminders before each scheduled sample collection. • Offer flexibility when scheduling sample drop-off.

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