**Feasibility and Acceptability of Frequent Vaginal Self-Sampling at Home**

**by Rwandan Women at High Risk of Urogenital Tract Infections**

*(for submission as a short report to Sexually Transmitted Infections)*

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**Abstract**

**Objectives** To establish temporal links between vaginal microbiota (VMB) data and incident clinical events, frequent longitudinal vaginal sampling is required. Self-collection of swabs at the participant’s home may be useful to avoid overburdening research clinics and participants. One-off vaginal self-sampling for sexually transmitted infection or cervical cancer screening programs has been shown to be feasible and acceptable to women in multiple studies, including in sub-Saharan Africa, but the feasibility and acceptability of frequent longitudinal vaginal sampling in the context of VMB sequencing studies is unknown.

**Methods** Twelve participants of a randomised clinical trial in Kigali, Rwanda, self-collected vaginal swabs three times a week for a month. We studied feasibility by comparing DNA concentrations, proportions of samples with >1,000 16S rRNA amplicon sequencing reads, and VMB composition outcomes, of self-collected swabs with clinician-collected swabs. We evaluated the acceptability of self-collection using structured face-to-face interviews and a focus group discussion.

**Results** The participants collected vaginal swabs at 131 different time points. One woman stopped self-sampling after one try due to a social harm. All self-sampled swabs generated >1,000 rRNA amplicon sequencing reads, and the DNA concentration of self-sampled swabs and clinician-sampled swabs did not differ significantly (Kruskall Wallis p=0.484). Self-sampled and clinician-sampled swabs generated similar VMB composition data. Participants reported to feel very comfortable during self-sampling (91.7%) and that self-sampling had become easier over time (100%). They mentioned reduced travel time and travel costs as advantages of self-sampling at home.

**Conclusions** Frequent longitudinal vaginal sampling at home is feasible and acceptable to participants, even in the context of a low-resource setting, as long as adequate counselling is provided.

Keywords (maximum 5): vaginal microbiome, self-sampling, sexually transmitted infections, gynaecological examination, women.

**Introduction**

The vaginal microbiota (VMB) have been extensively characterised using molecular methods such as polymerase chain reaction (PCR) and sequencing since the turn of the century.[1] Although most VMB studies to date have been descriptive and cross-sectional, longitudinal studies that take the VMB’s temporal dynamics into account are on the increase.[2,3] Linkage of VMB composition data to incident clinical events may require frequent vaginal sample collection. To avoid overburdening health clinics and study participants, sample collection by the participant herself (i.e. self-sampling) at home may be considered. The feasibility and acceptability of vaginal self-sampling in sexually transmitted infection and cervical cancer screening programs has been evaluated, but sampling in most of these studies was infrequent.[4–9] We evaluated the feasibility and acceptability of frequent vaginal self-sampling in Rwandan women participating in a clinical trial at the request of anonymous grant reviewers who questioned the feasibility and acceptability of our proposed sampling scheme.

**Methods**

The trial was conducted in 2015-2016 in Kigali, Rwanda, and has been described elsewhere.[3] Briefly, 68 HIV-negative, non-pregnant women at high risk of HIV transmission were randomised to either no intervention or one of three interventions (intermittent use of oral metronidazole or two vaginal lactobacilli-containing probiotics) after successful oral metronidazole treatment for bacterial vaginosis and/or trichomoniasis. All participants had vaginal swabs taken by a clinician at the screening, enrolment, Day 7, Month 1, Month 2, and Month 6 visits. In addition, a sub-sample of twelve participants (three per randomisation group) were asked to self-sample three times per week between the enrolment and Month 1 visits, collecting two vaginal swabs at each sampling time point. All participants collected their first two vaginal swabs (Copan Technologies, Brescia, Italy) under supervision of a staff member, but further self-sampling occurred at home. Participants were asked to break the swab heads off the stems at the incision point, and to place each swab head in a cryovial containing 1 ml of RNALater (Thermo Fisher, Paisley, UK). They were asked to store the cryovials in a refrigerator (if available) or in a cool place away from direct sunlight. They were encouraged to continue sample collection during menses. Participants could choose to have their cryovials collected by a staff member at home, or to drop them off, at least once per week at a time and location that was acceptable to them. They were stored at -80 °C immediately upon arrival at the clinic. Swab heads collected by clinicians at study visits were stored dry at -80 °C on the collection day.

After four weeks of self-sampling, women were interviewed by a staff member using a structured questionnaire about their self-sampling experiences. These data were analysed using Stata version 13 (StataCorp, 2013. College Station, USA). Moreover, a focus-group discussion (FGD) with nine self-sampling participants was conducted to further investigate acceptability. The FGD was held in a private room in the local language (Kinyarwanda) and women used nicknames to protect confidentiality. The FGD was recorded on tape, transcribed verbatim, and translated into English. The interview guide was semi-structured, and thematic analysis was done using NVivo 10.0 software (QSR International, 2012. Melbourne, Australia).

One swab head per participant per time point (N=131) was used for DNA extraction and sequencing as previously described.[3] The DNA concentration was measured by Qubit (Invitrogen, Thermo Fisher, Paisley, UK), directly after DNA extraction (random subset, N=46) and after the two PCR rounds for 16S rRNA gene amplification and barcoding (all 131 samples). 16S rRNA gene sequencing was performed on an Illumina HiSeq instrument set in rapid mode (Illumina, San Diego, USA). Samples collected by clinicians during regular study visits underwent DNA extraction and sequencing as well (N=71). Bio-informatics methods and VMB outcome definitions are described elsewhere.[3] Feasibility was investigated by comparing DNA concentrations, the proportion of samples with >1,000 reads, and VMB composition outcomes (alpha diversity, and VMB types as described earlier [3]), between self-collected and clinician-sampled vaginal swabs. DNA concentrations and alpha diversity were compared using the Kruskall Wallis test.

All participants provided written informed consent, including separate consent for participation in the FGD. Participants were reimbursed for each study visit at the clinic but not for self-sampling. The study was sponsored by the University of Liverpool, and approved by the National Ethics Committee of Rwanda and the University of Liverpool Committee on Research Ethics.

**Results**

The median age of the 12 women was 30.5 (range 21-37). All reported having exchanged sex for money or goods in the month prior to screening, five (41.7%) reported a steady partner, and eight (66.7%) had not completed primary school. One participant was beaten by her partner when he discovered the self-sampling materials. Study staff visited this participant at her home, and after discussion, the participant decided to stop the self-sampling procedures but to continue in the trial. This participant collected only two vaginal swabs at the first time point in total. The remaining 11 participants collected swabs at 129 out of 132 possible time points (97.8%; details in table 1).

The self-sampled swabs were processed in the laboratory a median of two days (inter-quartile range zero to three days; range zero to twelve days) after sample collection. Measurable DNA was present in all self-collected samples after DNA extraction and in 98.5% of samples after the two PCR steps. DNA concentrations of the self-collected and clinician-collected samples after the two PCR steps were comparable (Kruskall Wallis p=0.484). All 131 self-collected samples had >1,000 high-quality reads after Illumina sequencing and quality control procedures. The VMB alpha diversities of paired self-sampled and clinician-collected samples that were taken within one day of one-another were comparable (Kruskall Wallis p=0.795) and VMB types were identical in 16/20 cases (table 1, supplementary figure 1). Two discrepant cases differed somewhat in BV-anaerobe relative abundance and two in pathobiont relative abundance, resulting in VMB type classification discrepancies (supplementary figure 1).

In the survey at the end of the self-sampling period, participants reported to feel very comfortable during self-sampling (91.7%) and that self-sampling had become easier over time (100%) (table 1). Of the nine participants in the FGD, three reported having been worried initially about their ability to collect samples safely and accurately. One participant said: “*My fear was, I was wondering if I insert that thing and push it deeply, it may stay inside; and* […] *hurt myself. So, I was worried.”* However, they gained confidence over time. Most FGD participants reported no practical difficulties during the self-sampling period, but one participant replaced the RNALater medium for tap water in one cryovial: “[…] *when I was taking my samples for the first time* […] *when I opened it, I forgot that there was a liquid inside and it was dropped down, then I added water inside* [laughing], *I was really confused*.” Two FGD participants explicitly stated not having told their steady partners about their study participation whereas one explicitly stated the opposite.

Most FGD participants were willing to self-sample frequently in future studies as long as they would receive counselling prior to participation and self-sampling was proven to be as accurate as clinician-sampling. They mentioned reduced travel time and travel costs as advantages of self-sampling. In the survey at the end of the self-sampling period, a quarter of the women said that they would agree to daily self-sampling in future studies, whereas all women would consent to every other day (table 1). Half of the surveyed women would choose home collection and the other half sample drop-off next time. FGD participants thought that possible obstacles for women to perform self-sampling could be side-effects and/or pain experienced during their first experience with self-sampling (n=2) or fear of pain (n=1), living together with children in a one-room house (n=2), and disapproval by loved ones (n=1).

**Discussion**

This study showed that frequent vaginal self-sampling at home was acceptable to Rwandan women as evidenced by the low drop-out and the positive responses in the survey and the FGDs. However, the social harm described in this study accentuates the need for counselling prior to home-sampling to assist participants with risk assessment and mitigation. If possible and desired by the participant, counselling of loved ones should also be considered. Frequent vaginal self-sampling was also feasible, as evidenced by the fact that almost all expected samples were collected and all samples yielded sufficient sequencing reads for in-depth VMB analyses. VMB profiles generated by self-collected swabs did not differ significantly from clinician-collected swabs, and discrepancies observed may have been related to natural day-to-day VMB variability.

This pilot study only included 12 participants, of whom 11 completed the full self-sampling period which lasted for only one month. The study also has limited generalisability: all participants were sex workers with little education and living in an urban setting. Study strengths include the fact that we used mixed methods to assess acceptability and feasibility, and included extracted DNA quantities and VMB profiles in our feasibility assessment.

We conclude that it is feasible and acceptable to include frequent vaginal self-sampling at home in study designs, even in the context of a low-resource setting, but that counselling to prepare participants should be considered.

**Competing interests** None declared.

**Author contributions** JvdW obtained the research funding and wrote the study protocol and data collection documents. SA, MMU, and JvdW collected the primary data. MMU performed the FGD. MV developed the analytical approach, performed the experiments, analysed the FGD, performed the statistical analyses, and wrote the first version of the manuscript. All authors commented on and approved the final manuscript.

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**Ethical approval** The study was sponsored by the University of Liverpool, and was approved by the National Ethics Committee of Rwanda and the University of Liverpool Committee on Research Ethics, and registered on ClinicalTrials.gov (NCT02459665). All participants provided written consent prior to enrolling in the study, including separate written consent for participation in the focus-group discussion.

**Data Availability Statement** Participants were not explicitly asked for consent related to use of their data by external parties or use that does not address the research questions described in the approved study protocol (publicly available at <https://datacat.liverpool.ac.uk/>). Data were therefore deposited in a controlled access repository at the University of Liverpool. Data can be requested by emailing the Research Data Management team (rdm@liverpool.ac.uk) and the data steward (Professor Janneke van de Wijgert; j.vandewijgert@liverpool.ac.uk). Requests will be submitted to the University of Liverpool ethics committee (ethics@liverpool.ac.uk) for approval.

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**Table 1: Feasibility and acceptability of frequent self-sampling**

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| --- | --- |
| **Feasibility of self-sampling** | **n (%) or median (IQR)** |
| Number of swabs handed in per woman (out of expected 24 swabs) | 24 (22.5 – 24) |
| Total number of self-sampled vaginal swabs that underwent DNA extraction and sequencing: N | 131\* |
| DNA concentration by Qubit of self-sampled swabs, after DNA extraction, in µg/ml† | 26.8 (18.3 – 42.9) |
| DNA concentration by Qubit of self-sampled swabs, after two PCR rounds (16S rRNA gene amplification and barcoding for multiplexing),in µg/ml‡ | 5.64 (3.58 – 8.01) |
| DNA concentration by Qubit of clinician-collected swabs, after two PCR rounds (16S rRNA gene amplification and barcoding for multiplexing),in µg/ml§ | 5.79 (4.28 – 8.23) |
| Number of self-sampled swabs with >1,000 high-quality Illumina HiSeq sequencing reads | 131/131 (100%) |
| Number of clinician-collected swabs with >1,000 high-quality Illumina HiSeq sequencing reads | 71/71 (100%) |
| Accordance in VMB types of self-sampled swabs paired with clinician-sampled swabs¶ | 16/20 (80%) |
| Alpha diversity (inverse Simpson index) of self-sampled swabs: mean (95% CI)|| | 0.50 (0.39 – 0.61) |
| Alpha diversity (inverse Simpson index) of paired clinician-sampled swabs: mean (95% CI)|| | 0.52 (0.38 – 0.66) |
| **Acceptability of self-sampling** | **n (% of 12)** |
| Preferred participant-reported choice of swab collection, prior to start frequent self-sampling:  - Home collection  - Drop-off | 3 (25.0%)  9 (75.0%) |
| Participant was trained in self-sampling at Enrolment | 12 (100%) |
| Study nurse thought participant seemed very comfortable with self-sampling at Enrolment | 12 (100%) |
| Participant reports collecting swabs on at least 12 time points over self-sampling period | 10 (83.3%) |
| Participant-reported reason of not handing in swabs at 12 time points\*\*  - “I simply forgot”  - “Someone close to me (husband) objected to me doing the self-sampling” | 1 (8.3%)  1 (8.3%) |
| Participant reported being comfortable with self-sampling after self-sampling period  - Very comfortable  - Somewhat comfortable | 11 (91.7%)  1 (8.3%) |
| Participant reports self-sampling becoming easier over time | 12 (100%) |
| Participant would do self-sampling again | 12 (100%) |
| What swab collection method would participant choose, after having completed self-sampling  - At home collection  - Drop-off | 6 (50.0%)  6 (50.0%) |
| Reasons for preferring home collection††  - More secure than drop-off centre  - Easier / less time-consuming | *N=6*  4 (66.7%)  3 (50.0%) |
| Reasons for preferring drop-off††  - Near home  - I might not be at home during home collection  - Easier (not otherwise specified) | *N=6*  2 (33.3%)  4 (66.7%)  1 (16.7%) |
| How often would participant wish to perform self-sampling††  - Every day  - Every other day  - Once per week  - Less than once per week | 3 (25.0%)  12 (100.0%)  11 (91.7%)  9 (75.0%) |

\*Corresponding to 131 out of 144 (91.0%) possible time points; this includes the woman who collected swabs at one time point only due to a social harm. Omitting this woman, collection occurred at 129 (97.8%) out of 132 (11 participants times 12 time points) possible time points as self-collection was skipped three times out of 132. A total of 258 swabs were handed in for the 131 time points at which collection occurred instead of the expected 262 (=131 times two swabs), as some women handed in one instead of two swabs at four different time points. One swab was chosen per time point (N=131) at random and underwent DNA extraction and sequencing, due to funding restrictions.

†N=46. A random subset of 46 out of 131 extracted samples were tested by Qubit directly after DNA extraction. Clinician-collected samples were not tested by Qubit directly after DNA extraction; hence, their concentration is not shown.

‡N=131. Results on Qubit that were returned as ‘too low’ (n=2) were set to zero.

§N=71. No results on Qubit were returned as ‘too low’. All participants had vaginal swabs taken by a clinician at the screening, enrolment, Day 7, Month 1, Month 2, and Month 6 visits. Only clinician-collected study visit swabs of the twelve participants involved in self-sampling were included. DNA concentration after the two PCR rounds did not differ significantly between self-sampled swabs and clinician-collected swabs (Kruskall Wallis p=0.484).

¶For the 20 occurrences in which clinician-sampled and self-sampled swabs were taken within one day of each other (sixteen times two paired swabs, and four times three paired swabs of which one clinician-sampled and two self-sampled; N=44). Self-collected and clinician-collected samples were never taken on the same day. See also supplementary figure 1 for a heatmap with more detailed information.

||N=24 self-sampled vaginal swabs and N=20 paired clinician-sampled swabs taken within one day of each other. Alpha diversity did not differ significantly between paired self-sampled and clinician-collected swabs (Kruskall Wallis p=0.795).

\*\*One of these women is the participant reporting the social harm. The other is a woman who reported having forgotten at least once; two other participants who did not hand in all 12x2 swabs as expected did not self-report this during the survey.

††Totals can add to more than 100% because participants were allowed to give multiple answers.

CI, confidence interval; IQR, inter-quartile range; PCR, polymerase chain reaction; rRNA, ribosomal RNA; VMB, vaginal microbiota.