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A Novel Point-Of-Care Cytokine Biomarker Lateral Flow Test for the Screening for Sexually Transmitted Infections and Bacterial Vaginosis: Study Protocol of a Multi-Centre Multi-Disciplinary Prospective Clinical Study to Evaluate the Performance and Feasibility of the Genital Inflammation Test (GIFT).

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ABSTRACT

Introduction

A prototype lateral flow device detecting cytokine biomarkers IL-1α and IL-1β has been developed as a Point-of-Care (POC) test – called the Genital InFlammation Test (GIFT) - for detecting genital inflammation associated with sexually transmitted infections (STIs) and/or bacterial vaginosis (BV) in women. In this paper, we describe the rationale and design for studies that will be conducted in South Africa, Zimbabwe, and Madagascar to evaluate the performance of GIFT and how it could be integrated into routine care.

Methods and analysis

We will conduct a prospective, multidisciplinary, multi-centre, and cross-sectional clinical study comprising two distinct components: a biomedical ("diagnostic study") and a qualitative, modelling, and economic ("an integration into care study") part. The diagnostic study aims to evaluate GIFT's performance in identifying asymptomatic women with discharge-causing STIs (*Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV) and *Mycoplasma genitalium* (MG)) and BV. Study participants will be recruited from women attending research sites and family planning services. Several vaginal swabs will be collected for evaluation of cytokine concentrations (enzyme-linked immunosorbent assay), STIs (nucleic acid amplification tests), BV (Nugent Score), and vaginal microbiome characteristics (16S rRNA gene sequencing). The first collected vaginal swab will be used for the GIFT assay which will be performed in parallel by a healthcare worker in the clinic near the participant, and by a technician in the laboratory. The integration into care study aims to explore how GIFT could be integrated into routine care. Four activities will be conducted: user experiences and/or perceptions of the GIFT device involving qualitative focus group discussions and in-depth interviews with key stakeholders; discrete choice experiments; development of a decision tree classification algorithm; and economic evaluation of defined management algorithms.

Ethics and dissemination

The study was approved by the ethical committees in Madagascar, South Africa and Zimbabwe.

Strenghts and limitations of this study

- A multicountry evaluation of a new low-cost rapid POC test detecting vaginal inflammation to be conducted in three sub-Saharan countries characterized by different STI/BV contexts.
- Multidisciplinary approach including social science, economic analysis and machine learning to inform how the GIFT device could be integrated into STI management algorithms for women.
- Sensitization on genital inflammation including STI/BV among young non-pregnant women.
- The budget does not permit STI diagnosis using POC NAAT testing in Madagascar, in contrast to South Africa and Zimbabwe, leading to presumptive treatment aligning with the usual routine care practices.
- The user evaluation of the GIFT device does not include the evaluation nor quality assessment of the specimen extraction method.

Introduction

Sexually transmitted infections (STIs) continue to pose a significant health challenge globally, with over one million new cases reported daily. In 2020, approximately 377 million new cases of the four most common STIs (*Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), syphilis, and *Trichomonas vaginalis* (TV)) were documented, with over 70% occurring in low- and middle-income countries (LMICs), particularly in Sub-Saharan Africa and Latin America [1].

STIs impact women more than men, as women endure long-lasting consequences. Additionally, women are more susceptible to STIs than men due to anatomical and immunological differences [2,3]. The composition of the vaginal microbiome also plays a crucial role in sexual and reproductive health. Optimal vaginal microbiota, predominantly composed of *Lactobacillus* species, maintains an acidic pH, sustaining the vaginal epithelial barrier's integrity and preventing pathogen colonisation and invasion. However, various genetic, behavioural, and environmental factors can modulate this microbial composition and lead to bacterial vaginosis (BV) characterised by a decrease in protective *Lactobacillus* species along with an increase in bacterial diversity and vaginal pH [4-9].

BV remains a persistent public health issue, with prevalences ranging from 20-30% globally, and higher rates observed in Black populations compared to Caucasian or Asian populations [4, 6, 10]. In Sub-Saharan African populations, prevalences are notably high (around 33%) [10].

Both STIs and BV induce elevated genital inflammation, marked by increased local production of pro-inflammatory cytokines and chemokines, irrespective of symptoms [7, 11, 12, 13-15]. Studies in Sub-Saharan African women cohorts have shown that common STIs such as CT, NG, and TV significantly and consistently increased the concentrations of key genital tract cytokine biomarkers, including interleukin (IL)-1α and β [14, 16, 17]. BV caused similar changes in these cytokine biomarkers, although was also shown to be associated with lower concentrations of the chemokine

interferon-γ-induced protein of 10kDa (IP-10) [12, 18, 19]. Genital inflammation caused by STIs and BV has in turn been associated with increased risk for the acquisition of other STIs, including HIV-1 [20-25]. This is particularly concerning in Sub-Saharan African countries, where a significant proportion of new HIV-1 infections occur. The HIV-1 prevalence among adults (15-49 years) was estimated at 17.8% and 11% in South Africa and Zimbabwe, respectively in 2022. Women are disproportionally affected by HIV than men, with young women (15-24 years) particularly vulnerable with prevalence rates estimated at 8.7% and 4.4% in South Africa and Zimbabwe, respectively [26].

Both STIs and BV are also associated with other serious sexual and reproductive health complications, such as pelvic inflammatory disease which can lead to infertility and ectopic pregnancy and adversely affect woman's reproductive plans. For prevalent bacterial STIs and BV, intrauterine infection and/or vertical transmission to the unborn child or during birth can result in adverse pregnancy and birth outcomes, including preterm delivery, maternal and neonatal sepsis, low birth weight, pneumonia, and conjunctivitis in the newborn [3, 27].

Despite the high prevalence of STIs and BV, and their frequently asymptomatic nature, syndromic approaches remain the mainstay of management in LMICs [28]. Immediate testing and results delivery are crucial to facilitate prompt diagnosis and accurate treatment, reducing both STI transmission and infection progression [29-31]. Consequently, the inclusion of POC tests has been highly recommended for the management of symptomatic STIs [28]. Recently, nucleic acid amplification-based near-patient STI diagnostics have become available [32, 33], but none of them fulfills the REASSURED (including user-friendly, rapid, robust, equipment-free, and deliverable to end-users) criteria set out by World Health Organization (WHO) for POC tests [34].

Genital inflammation has been associated with a significantly increased risk of HIV acquisition in women and the presence of STIs driving this inflammation has been well described among South African women [11, 16, 22, 25]. To address the need for POC tests to improve STI/BV

management for women in LMICs, Masson *et al.* have focused on developing a POC lateral flow test to detect a combination of inflammation biomarkers (IL-1α, IL-1β, and IP-10), measured in lateral vaginal wall swabs, to improve BV and STI case finding in women compared to clinical signs [20]. This Genital Inflammation Test (GIFT) screening tool aims to improve case detection to help identify women at increased risk of HIV infection and to enhance the sexual and reproductive health of women in countries where only syndromic management is available.

This study protocol outlines a study to be conducted in three countries (South Africa, Zimbabwe, and Madagascar) to evaluate the performance of the first-in-field GIFT prototype device and explore possible routes for integration into routine care.

Purpose and study objectives

This multidisciplinary, cross-sectional, prospective, and multi-centric study has two distinctive sub-studies, a diagnostic evaluation and an integration into care study. Each sub-study has several objectives (Table 1).

Table 1: GIFT study objectives

	Diagnostic study	Integration into care study
Primary objective	To assess the sensitivity and specificity of the GIFT device at the point-of-care in South Africa, Zimbabwe, and Madagascar.	To evaluate how the GIFT device could be integrated into routine care
Secondary objectives	• To assess the predictive values of the GIFT device at the point-of-care • To assess the performance of the GIFT device at the point-of-care • To assess the performance of the device versus syndromic management without any laboratory testing (standard of care in South Africa, Zimbabwe, and Madagascar) • To determine the robustness of the device by comparing results read by clinicians with those read by laboratory professionals, and with the results obtained using an automated reader • To evaluate the accuracy of the GIFT device by comparing the GIFT device results with ELISA results using previously validated concentration cut-offs as the gold standard, including validation of the GIFT cytokine concentration cut-offs for each cytokine biomarker.	• To qualitatively and quantitatively assess the user experience, usability, and acceptability of the GIFT device at the point of care; • To examine patient preferences for various STI management aspects (attributes) to inform the development of STI management algorithms that integrate the GIFT device; • To generate algorithms that integrate the GIFT device to optimise case finding and STIs/vaginal infection management in women, using the complete dataset from the study; • To determine the cost and budget impact of the identified screening or diagnostic algorithm with the GIFT device, and to model the cost-effectiveness of different strategies of integration of GIFT into care.
Exploratory objectives	• Determine if other determinants (such as intermediate microbiota (Nugent 4-6), age, parity, sexual activity) improve the prediction of STI/BV status in women • To use 16S rRNA gene sequencing and vaginal bacteria specific quantitative NAATs to evaluate the proportion of cases of genital inflammation explained by vaginal dysbiosis that was not diagnosed as an STI or BV by NAATs or Nugent scoring • To explore the performance of the device in the presence of vaginal *Candida spp* colonisation.	

The diagnostic study aims to evaluate the performance of GIFT in identifying women with inflammatory STIs (including CT, NG, TV and MG) and BV and who may, therefore, be at higher risk of STI and HIV infection and potential reproductive complications. Syndromic management in the absence of any diagnostic testing misses all asymptomatic reproductive tract infections by definition, and GIFT may detect the presence of asymptomatic subclinical inflammation. Syndromic management guidelines currently call for women with cervicovaginal discharge to be treated for all reproductive tract infections that might cause such discharge, but studies have shown that a substantial proportion of these women do not have any infection [29]. In this scenario, GIFT may prevent overtreatment of symptomatic women without infection.

The primary objective is to assess the sensitivity and specificity of the GIFT device at the point-of-care in non-pregnant sexually active women aged 18-35 years accessing family planning services in South Africa, Zimbabwe, and Madagascar against nucleic acid amplification tests (NAATs) for CT, NG, TV, and MG plus BV by Nugent scoring (Table 1). Secondary objectives include assessing the device's predictive values; assessing performance in each country separately; assessing performance against syndromic management; assessing test reproducibility by comparing clinician- and laboratory-read results (visual reading versus automated reader); and setting the device's concentration cut-offs against gold standard cytokine concentrations (determined by enzyme-linked immunosorbent assay (ELISA)).

The integrative part of the study aims to explore the feasibility, acceptability, and cost-effectiveness of integrating GIFT into routine care in LMICs. The specific objectives include: assessing the user-experience, usability, and acceptability of the GIFT device at the POC; examining patient preferences for various STI management aspects to develop STI management algorithms; generation of algorithms that integrate the GIFT device to optimise case finding and STI/vaginal infection management in women; and determine the cost-effectiveness and budget impact of the identified algorithms with the GIFT device.

Methods and design

Study design

The diagnostic study will target women attending family planning services in each of the three study sites in South Africa, Zimbabwe, and Madagascar. A series of vaginal swabs will be collected by a midwife or nurse (Figure 1).

The first swab will be used to test the performance of the GIFT device. For each woman, the GIFT will be performed twice and read four times: one GIFT will be performed at the clinical site near the participant and visually read by a midwife or study nurse and a second GIFT will be done and visually read by a technician in the laboratory. The GIFT assays carried out by the midwife/study nurse and the laboratory technician will secondly be read using an automated lateral flow reader (Axxin, Fairfield, Australia) (Figure 1). The GIFT device performance in detecting (1) inflammation will be determined against cytokine enzyme-linked immunosorbent assay (ELISA); and (2) STIs/BV will be determined against the NAAT reference tests for CT, NG, TV and MG and Nugent scoring for BV (Figure 1). All women will be offered treatment based on the on-site positive STI diagnostic results for CT, NG, and TV. Additionally, treatment for BV will be offered based on the Nugent score analysis and at the clinician's discretion.

The integration into care study consists of four activities to determine how GIFT can be integrated in a feasible, acceptable, and cost-effective way into routine care and national guidelines in South Africa, Zimbabwe and Madagascar (Figure 2): 1. User experiences and/or perceptions of the GIFT device involving qualitative focus group discussions (FGDs) and in-depth interviews (IDIs) with key stakeholders; 2. Discrete choice experiment (DCE); 3. Development of a decision tree classification algorithm; 4. Economic evaluation of defined management algorithms.

Study sites

The study will be performed in three countries: South Africa, Zimbabwe, and Madagascar. In contrast to South Africa and Zimbabwe which are countries bearing a high burden of STIs, including HIV, and BV [35-37], data on STI/BV prevalence are relatively scarce in Madagascar. BV, TV, and cervical infections (CT and NG) prevalences measured in a large population sample of symptomatic Malagasy women (N=1066) were high at 53%, 24%, and 17% respectively, suggesting a significant burden of STIs/BV in the island [38]. Finally, Madagascar is less affected by HIV with a prevalence <1% [39] compared to South Africa and Zimbabwe, which were found to have an HIV-1 prevalence among adults (15-49 years) in 2022 of 17.8% and 11%, respectively [26].

In South Africa, the study will be conducted at the Desmond Tutu Health Foundation (DTHF) Masiphumelele Youth Centre located in the South peninsula of Cape Town. The Masiphumelele Research Site has been recognised as a pioneer in HIV prevention and adolescent research. The STI and BV diagnostic testing of the South African participants will be performed in their on-site laboratory. In addition, vaginal swabs collected at all three study sites will be tested for selected cytokines including IL-1α, IL-1β, and IP-10 by ELISAs and for vaginal microbiome composition by 16S rRNA gene sequencing being conducted at the University of Cape Town (UCT), along with samples from the other two trial sites.

In Zimbabwe, the study will be conducted at the Spilhaus Family Planning Centre located in the capital Harare. The study site is run by the Zimbabwe National Family Planning Council (ZNFPC) in collaboration with the Zimbabwe Ministry of Health and Child Care. The study will be conducted by researchers from the Organization for Public Health Interventions and Development (OPHID), equipped with a skilled medical and technical team. The study site's laboratory will perform the STI and BV diagnostic testing for Zimbabwean study participants.

In Madagascar, the study will be conducted at the Centre Hospitalier Universitaire de Gynécologie et d'Obstétrique Gynéco-Obstétricaux de Befelatanana (CHU-GOB), a public university hospital that specialises in obstetrics located in the capital of Madagascar, Antananarivo. Outpatient services

include prenatal consultations, family planning, obstetrics, and gynaecology services. Due to the lack of recent data on STI/BV prevalences, a pre-study on CT, NG, TV and MG prevalences was conducted by the clinical and research team of IPM from March to December 2022 to confirm the feasibility of conducting the GIFT study in the CHU-GOB in Antananarivo. The pre-study confirmed the appropriateness of the clinical study site in terms of the study population's number and STI and BV prevalence rates (results will be presented elsewhere).

The unit of experimental bacteriology (UBEX) at the Institut Pasteur Madagascar (IPM) will be the study site's laboratory in Madagascar for diagnostic testing. UBEX is also designated as the reference laboratory in the GIFT project for BV and STI (CT, NG, TV and MG) comparator testing, as well as quantification of lactobacilli and BV-associated bacteria using quantitative PCR and molecular testing for the detection of *Candida* spp., and Y chromosome.

Study participants and recruitment

The diagnostic study will enroll its participants from sexually active women aged 18-35 years attending family planning services in each of the study's clinical sites, irrespective of symptoms. Prior to study initiation, advice will be sought from local Community Advisory Boards (CABs) or similar committees on the study material to be shared with potential study participants. The boards will be regularly consulted regarding language to use for behavioural and other sensitive questions included in the questionnaires. A CAB is currently not existing in Madagascar but will be installed as part of the study.

Due to the short recruitment period, sensitisation to the GIFT project will be extended in Antananarivo (Madagascar) to the family planning services of surrounding primary health care centres. In South Africa, the clinical study site offers family planning services to young women till the age of 25, and women outside of the study site will be recruited to enroll women between 25 and 35 years old.

Women interested in the diagnostic study will be invited to participate based on the inclusion and exclusion criteria detailed in Table 2. Each woman will only be enrolled once.

Table 2: In- and exclusion criteria for both diagnostic and integration into care studies

	Diagnostic study	Integration into care study
Inclusion criteria	• 18-35 years old • Willing and able to provide informed consent to participate in the study • Self-reported to be sexually active • Not pregnant (determined by pregnancy test) • Accessing family planning service	For all who are willing and able to provide informed consent to participate in the study. • User experiences/perceptions activity: - Local or regional policymakers, programmers, and other opinion leaders and decision-makers - Healthcare professionals at health facilities - Women who are eligible for the diagnostic study (including pregnant and menstruating women), but who are not part of the diagnostic study • Discrete choice experiments: Women who are eligible for the diagnostic study (including pregnant and menstruating women), who are either part of, or not part of, the diagnostic study • Decision tree classification algorithm: Data from diagnostic study participants • Economic evaluation: Healthcare professionals at health facilities involved in GIFT device implementation able to complete timesheets
Exclusion criteria	• <18 years or >35 years • Refusal by a participant to participate in the study • Treatment for any STI/BV in the past 30 days • Pregnancy • Enrolled in a study that does not allow co-enrolment in other studies	For all who are not willing or able to provide informed consent to participate in the study • User experiences/perceptions activity: - Non-relevant policymakers - Healthcare professionals at health facilities not included in diagnostic study - Women who are : ‣ part of the diagnostic study ‣ <18 years or >35 years ‣ Treated for any STI/BV in the past 30 days ‣ Pregnant ‣ Enrolled in a study that does not allow co-enrolment in other studies • Decision tree classification algorithm: Participants for whom there are no diagnostic study data • Economic evaluation: Healthcare professionals not at study sites, not involved in GIFT device implementation, and/or not able to complete timesheets

In addition to eligible women participating in the diagnostic study, participants for the integration into care study will include health care professionals (HCPs) including nurses, midwives, and matrons working at the clinical site where the diagnostic study is being conducted, women attending gynaecological consultations at the study clinic, local and regional policymakers, programmers, other opinion leaders, and decision-makers according to the inclusion and exclusion criteria (Table 2).

Sample size

A total number of 675 women (225 women per site) will be enrolled for the diagnostic evaluation of the prototype GIFT device. The sample size calculation is based on 7% precision, and an assumed sensitivity of 77% and a specificity of 71% of GIFT compared to NAATs for STIs and Nugent scoring for BV [40].

No sample size calculations were performed for the qualitative research. A purposive sampling strategy will be adopted to reflect maximum variation, as feasible, across the sample. It is anticipated that the proposed number of participants per activity (FGDs and IDIs), per country, will enable adequate data to be collected and saturation to be reached in the thematic analysis [41, 42]. Data collection may be finalised before the planned sample size is reached, if saturation is reached first.

For the DCE component of the study, using econometric criteria (including choice probability, confidence level, accuracy level, attrition rate, and number of choice tasks), subgroups of more than 30 individuals are required to conduct meaningful statistical analysis [43, 44]. Because the DCE study will undertake subgroup analysis on four to five groups (including respondent age group, socio-economic status, location of residence, experience with the GIFT device), up to 200 participants per site will be recruited for the DCE study, with a quarter of the participants recruited on the diagnostic study of the GIFT device, and reminder from women coming for gynecological consultations at the study clinic.

No sample size calculation is needed for the decision tree algorithm study, which is using data from the diagnostic study. Equally, there are no sample size requirements for the economic evaluation.

Study procedures and data collection

1) Diagnostic study

The diagnostic study participant and sample flow from screening to laboratory results is described in Figure 1 and detailed in Supplementary Figure 1 - Diagnostic study flow steps. After confirmation that participants were eligible for the study (Table 2) and written informed consent obtained, enrolled women will be interviewed using a structured questionnaire with questions including demographics, sexual behaviour, hygiene habits, current medical and reproductive history, medication, and symptoms. The data will be captured on paper or electronic case report forms (CRFs) (Figure 1). The clinician, midwife, or study nurse will perform a physical examination including a pelvic examination, and will collect finger prick blood for HIV testing and vaginal swabs using regular flocked swabs (Copan, Italy) (Figure 1 and Supplementary Figure 1 - Diagnostic study flow steps).

The first inserted swab will be eluted in buffer and the nozzle-sample (provided with the GIFT kit) will be applied to two prototype GIFT devices, one near the patient and the other in the laboratory (Figure 1). A second swab will serve for cytokine ELISA testing, and a third swab for pH testing using a pH indicator strip and to prepare a vaginal smear slide for BV (Figure 1). The next swabs will be collected and used for CT, NG and TV diagnosis in the laboratory, for reference STI testing including MG, quantitative NAAT of vaginal microbiome bacteria, the detection of *Candida sp* and Y chromosome for the presence of semen. The last two collected swabs will be used to characterise the vaginal microbiome by using 16S rRNA PCR and sequencing (Figure 1).

2) Integration into care study

The integration study is composed of four activities aiming to understand how GIFT can be integrated into STI control (Figure 2).

For the qualitative study assessing the user-experience, usability, and acceptability of the GIFT device at the point of care, data will be collected through IDIs and FGDs. Target groups will be: HCPs at the health facilities; local or regional policymakers, programmers, other opinion leaders, and decision-makers; and women who are eligible for the diagnostic study (Table 2), but who are not part of the diagnostic study. HCP participants will be identified through the diagnostic study sites and will be contacted directly to ask if they would be interested in participating. Policymakers recommended for inclusion through snowball sampling will be contacted and asked if they would be interested in participating. All women presenting at the family planning service facilities for inclusion in the diagnostic study will be informed about the integration study. For the DCE, the study team will seek to recruit women participating and not-participating in the GIFT diagnostic study to explore heterogeneity in patient preferences for various STI attributes. For all population groups, those who agree to participate will provide written informed consent, separate from the diagnostic study.

For the decision tree classification algorithm, data will be obtained from the diagnostic study participants, and no additional participants will be recruited. For the economic evaluation, healthcare professionals at health facilities involved in GIFT device implementation and able to complete timesheets will be recruited.

For the user experience activity (Figure 2, activity 1), up to 10-12 IDIs and/or 1-3 FGDs will be conducted with policy makers, up to 8-10 IDIs and/or 3 FDGs will be conducted with HCPs, and up to 12-15 IDIs and/or 3 FDGs will be conducted with women. FGDs will include participatory tools, such as card mapping. The FGDs with HCPs may additionally include role plays of how the providers would discuss the GIFT device with women, and how providers would discuss GIFT device results and subsequent care and management with women. IDIs will explore more detailed personal perceptions of the potential structures of integration.

User experience data (qualitative and FGDs) on the patient experience will help inform the development of attributes for the DCE (Figure 2, activity 2). The DCE aims to identify the relative

importance of factors influencing patient' preferences for the use of GIFT device. For the DCE study face-to-face interviews using a questionnaire will be conducted, with the questionnaire asking respondents to state preferences for hypothetical alternatives, each described by several attributes. A D-efficient design will be used to identify the choice tasks to present to respondents. Each choice task presents two STI screening test options that are defined using different levels of the attributes and then asks respondents to choose their preferred option, with an opt-out option available (to choose neither of the two options). The results of the DCE will inform the development of STI management algorithms that integrate the GIFT device, to increase the acceptability of the STI management programme.

A decision tree will be developed to classify symptomatic and asymptomatic women using the following attributes: socio-demographic information, signs and symptoms, medical history, sexual behaviour, the GIFT output, and vaginal pH. Different classifications will be tested by varying the combinations of the attributes and the number of levels of the tree. The algorithm will be developed using a training dataset (data from previous studies). After the development of the algorithm based on the training dataset, the algorithm will be tested on the data to be collected in the diagnostic study and refined if necessary. The algorithm will be designed to be as simple as possible (minimum number of nodes and minimum number of scenarios) and usable in routine practice. Finally, the management outcomes will be compared to those obtained with the WHO recommendations.

The economic evaluation (Figure 2, activity 4) will involve the collection of cost and service utilisation data from a provider's perspective at each of the clinical sites, using an ingredients-based approach. After obtaining approval from the facility and/or district, relevant facility management staff will be approached to share financial and other facility-level data. The cost and utilisation data will be collected from individual facility records and financial reports through cost data collection tools at each study site. The time that it takes to perform and analyse the GIFT assay will be obtained from clinical study staff. The time required for the delivery of routine family planning and

STI services will be assessed through timesheets completed by selected healthcare workers at the study sites. These data will be captured in the Provider Cost Data Collection Tool by data collectors. This activity will determine the cost and budget impact of the identified screening or diagnostic algorithm with the GIFT device and will model the cost-effectiveness of the different strategies of integration of GIFT into care.

Laboratory procedures

The BV diagnosis will be obtained from a vaginal smear carried out with the third swab collected during the gynecological examination (Figure 1). The slide will be transferred from the clinic to the respective on-site laboratory for Gram staining and Nugent scoring [40].

CT, NG, and TV diagnosis from collected vaginal swabs (Figure 1) will be performed using the diagnostic tests available in each of the three sites. In South Africa and Zimbabwe, GeneXpert tests (Cepheid, Sunnyvale, USA) will be used for CT and NG detection, and TV detection will be performed with the OSOM trichomonas rapid test or TV GeneXpert test, depending on the availability. In Madagascar, the vaginal swabs will be tested by UBEX (IPM) using validated qPCR protocols for CT, NG, and TV detection [45,46].

The swabs collected for STI reference testing in South Africa and Zimbabwe will be shipped to the IPM (Madagascar) for reference testing using validated qPCR [45-47], vaginal bacteria-specific quantitative qPCR testing, molecular detection of *Candida* spp. and presence of semen (Y chromosome testing).

Three swabs per participant will be sent to the University of Cape Town, for cytokine ELISA measurement and 16S rRNA gene PCR and sequencing. The results obtained with the prototype GIFT device will be compared to the cytokine concentrations resulting from the ELISA measurements. 16S rRNA gene sequencing and *Candida* spp. NAAT will be used to evaluate the proportion of cases of genital inflammation that could be explained by vaginal bacterial or fungal dysbiosis, respectively. The swabs will be stored in each study site at -80°C until shipment using dry ice to the respective laboratories as stipulated above.

Monitoring, quality assurance, and control

This study will be monitored in accordance with regulations applicable to diagnostic study, including International Committee on Harmonisation of Good Clinical Practice (ICH-GCP) and GCLP (Good Clinical Laboratory Practices) requirements, and sponsor-specific standard operating procedures (SOP). The diagnostic study will be monitored by the Epidemiology and Clinical Research Unit at the IPM. Routine monitoring will be conducted throughout the study and at the study closure. All laboratory activities including specimen transport, processing, testing, result reporting, and storage will be conducted in accordance with clinical trial quality requirements. The GCLP guidelines will be followed and the designated laboratories will perform testing according to the SOPs which will be documented in the analytical plan.

A batch of known negative and positive specimens for CT/NG, TV, pregnancy test and slides for Nugent scoring will be provided by the UBEX laboratory at IPM for external quality control. The external quality control will consist of three panels to be tested at the start, middle, and end of the study or every three months. Prior to the use of the GIFT devices, a lot validation will be performed using the quality control panel provided by the manufacturer according to the SOP.

Data management

The REDCap platform will be used as clinical data management system (CDMS) for the clinical study. Depending on the organisation in each study site data will be collected partially or fully using paper CRF (pCRF) before being entered into REDCap on a daily basis. In addition, data may be collected and entered in real-time into REDCap using tablets.

The GIFT database will be managed by the Epidemiology and Clinical Research Unit at the IPM. The quality and accuracy of CRF data transcription into the database will be verified weekly. Missing or overdue forms will be identified and tracked using the REDCap field comment log. A control script developed by the central data manager will check the double data entry, and any transcription differences found will be noted. Status reporting including the list of inconsistencies will be done weekly by the central data management team. The weekly reports will also resume the

progress of data collection and entry in each study site. All reports will be sent to the operational team for verification and correction of the data, if required.

External data sources not described on the CRF like cytokine ELISA data, the STI reference testing data, and 16S rRNA sequencing data will be reconciled against the CDMS at the end of the study.

For the user experience study, FGD and IDI data will be audio-recorded and electronically transcribed into the national and/or local language of the study sites. The DCE questionnaire data will be captured electronically using REDcap and securely stored on a password-protected computer. Data for the economic evaluation will be collected in a Provider Cost: Data Collection Tool, and timesheets will be completed in Excel by HCPs.

Data analysis

Diagnostic study

1) Primary analysis: Sensitivity and specificity estimated of the GIFT device with 95% confidence intervals using any positive etiological NAATs for STIs (CT, NG, TV and MG) or Nugent score result for BV as reference standards will be calculated. Visual readings of the GIFT device performed by the clinicians and technicians will be included separately in this analysis.

2) Secondary analysis: Positive and negative predictive values with 95% confidence intervals of the GIFT device will be compared with the likelihood ratios using NAAT and Nugent scoring as reference standards overall and in each country (which may have different STI prevalence that will impact these predictive values).

The performance of the GIFT device will be compared to the performance of syndromic management for detecting STIs. Both methods (GIFT and syndromic management) will be compared to any positive results of NAAT (for CT, NG, TV and MG) and/or BV detected by Nugent scoring.

Agreement between the GIFT device results read visually by both the clinician and the laboratory technician and on an automated reader will be determined.

The concordance of band intensity results from the GIFT device and cytokine ELISA measurements will be analysed. This analysis will be used to optimize the cytokine concentration cut-offs for interpretation of the GIFT device performance, in terms of sensitivity, specificity, and predictive values.

The impact of additional characteristics and/or determinants (such as intermediate microbiota, Candida, age, parity, and sexual activity) on the prediction of STI/BV status in women and the impact on the accuracy of the device will be determined.

Integration into care study

For the user experience study, iterative thematic analysis will be applied to the IDI and FGD data, conducted by the qualitative research team. Sekhon's framework of acceptability of healthcare interventions will be used [48].

For the DCE, data will be analysed in STATA using the utility function modelled from two alternatives: a systematic (explainable) component and a random (unexplainable) component. To estimate the trade-offs that respondents are willing to make between attributes, willingness to pay (WTP) for marginal improvements in attributes will be estimated for all attributes. WTP estimates will be calculated as the ratio of the coefficient of interest to the negative of the coefficient on the attribute with continuous variables. Subgroup analyses will be undertaken to investigate how preferences are influenced by demographic characteristics, residential area, socio-economic status, and experience of point-of-care testing.

The assessment of the decision tree classification algorithm will be based on the accuracy of the classification compared to the results of the gold standard methods (NAATs and Nugent score). Following this classification, different scenarios of management will be tested: immediate treatment, referral, re-evaluation, and no treatment. These scenarios will be optimised in order to avoid undertreatment and overtreatment by also considering immediate specific rapid diagnosis tests (if available) or risk scores for immediate presumptive treatment.

For the economic evaluation, models will be developed in Microsoft Excel. For the cost analysis, an ingredient-based model will be constructed, and for the budget impact analysis, an expenditure-based model will be applied. A decision-analysis model to estimate the cost and health outcomes associated with different GIFT device implementation strategies will be developed. The main outcome will measure the effectiveness of each approach in correctly diagnosing an STI and/or BV in women, proxied by the sensitivity measure of the diagnostic test or approach, in comparison to the gold standard NAATs and Nugent scoring. Secondary outcomes such as the performance of the device versus syndromic management and the variation in device results when determined by a clinician, laboratory technician or an automated reader, will be assessed by means of scenario analyses. Univariate sensitivity analyses will be carried out to test the robustness of the findings.

Patient and public involvement

Patients or the public were not involved in the formulation of the research question, study design, recruitment of the study. Findings will be reported to participating families and collaborators at the end of the study.

Ethics and dissemination

The study will be carried out according to the principles stated in the Declaration of Helsinki (as amended in Seoul in 2008) and any further updates, all applicable national and international regulations, and according to the most recent applicable principles of the GCP-ICH E6. All the participants will be informed about the diagnostic results. The choice of treatment for STIs or BV will be based on the current national guidelines in the three study sites. The protocol and all study documents such as informed consent forms were reviewed and approved by the University of Cape Town Human Research Ethics Committee (HREC reference 366/2022), Medical Research Council of Zimbabwe (MRCZ/A/2966), Comité d'Ethique pour la Recherche

Biomédicale de Madagascar (N° 143 MNSAP/SG/AMM/CERBM) and the London School of Hygiene and Tropical Medicine ethics committee (LSHTM reference 28046).

Prior to the start, this study has been submitted to the Clinicaltrials.gov public registry (NCT05723484).

Discussion

Here, we have described the diagnostic and integration studies that will be used to validate the GIFT device as a proof-of-principle prototype for implementing a novel POC cytokine test to detect vaginal inflammation associated with STIs and/or BV among women in differing clinical settings.

The development of low-cost rapid POC tests has become a major focus in the management of STIs/BV to replace or improve syndromic management widely adopted in Africa. The availability of these tests would offer all sexually active women the opportunity to be screened and treated. This may have particular benefit to women at high risk of STIs, including young women, pregnant women, female sex workers, and those with HIV.

The results from the prospective diagnostic study will be closely combined with the results of the qualitative research, modeling, and economic evaluation. The feasibility and acceptability studies will inform how the GIFT device could be integrated into national guidelines. The GIFT feasibility study consists of a "user experience and GIFT perceptions" prototype involving a large spectrum of people selected from the general population (HCPs, sexually active women, policymakers) that will help extend STI/BV screening into family planning services in primary health care services at affordable costs for LIMCs, with the aim that women should know about their health without delaying their treatment. The qualitative part of the study will improve our understanding of the different key factors contributing to the successful implementation of a novel screening device for STIs and BV in LMICs.

Besides evaluating a POC test for screening of STIs and BV, this study will be among the first of its kind, focusing on STI/BV prevalence and risk factors including up to 675 women mainly from urban areas in three sub-Saharan countries in different STI/BV contexts. The GIFT study will also improve our knowledge in both biological and molecular characterisation of the three most common STIs (CT, NG, and TV) and MG in association with the different states of the vaginal microbiota and will provide new insights into the interplay between STI and BV.

Our study protocol has some limitations. First of all, the user evaluation of the GIFT device, evaluating the performance of the device when applied by different types of users, does not include the evaluation of the extraction step. The extraction step of the vaginal swab will be performed by the medical staff only and not in parallel with a duplicate vaginal swab by a laboratory professional. Secondly, due to budget constraints, the STI diagnostic testing in Madagascar will be conducted by batch testing in the laboratory and not by POC NAAT testing as will be done in South Africa and Zimbabwe. Consequently, Malagasy participants will receive their results and treatment, if needed, after a delay of up to 10 days. This delay in treatment may facilitate further STI transmission. However, the study clinician may decide on presumptive treatment in alignment with the routine care practice in place.

In conclusion, the multidisciplinary study will be instrumental in developing strategies to improve STI and BV management in LMICs.

Study status

The study is ongoing. Data collection and data analysis are ongoing.

DECLARATIONS

Consent for publication

Not applicable

Availability of data and materials

Not applicable. The study is ongoing. Data collection and data analysis are ongoing.

Competing interests

The last authors, Jo-Ann Passmore and Lindi Masson, declare sharing a patent for the biomarkers for GIFT: patent number PCT/IB 2014/065740, October 2014. All other authors declared no potential conflict of interest.

Author Statement

SR and TC wrote the first draft of the manuscript. LM designed Figure 1, EHE and SF designed Figure 2. All authors contributed to the writing, and reviewed and approved the manuscript. LM and JAP validated the final version. All authors contributed to the elaboration of the study protocol.

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References

1. Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, Chico RM, Smolak A, Newman L, Gottlieb S, Thwin SS, Broutet N, Taylor MM. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ. 2019 Aug 1;97(8):548-562P. doi: 10.2471/BLT.18.228486.

2. Tuddenham S, Ravel J, Marrazzo JM. Protection and Risk: Male and Female Genital Microbiota and Sexually Transmitted Infections. J Infect Dis. 2021;223 (12 Suppl 2):S222-S235. doi: 10.1093/infdis/jiaa762.

3. Van Gerwen OT, Muzny CA, Marrazzo JM. Sexually transmitted infections and female reproductive health. Nat Microbiol. 2022;7(8):1116-1126. doi: 10.1038/s41564-022-01177-x.

4. Ness RB, Hillier S, Richter HE, Soper DE, Stamm C, Bass DC, Sweet RL, Rice P. Can known risk factors explain racial differences in the occurrence of bacterial vaginosis? J Natl Med Assoc. 2003;95(3):201-12.

5. Yotebieng M, Turner AN, Hoke TH, Van Damme K, Rasolofomanana JR, Behets F. Effect of consistent condom use on 6-month prevalence of bacterial vaginosis varies by baseline BV status. Trop Med Int Health. 2009;14(4):480-6. doi: 10.1111/j.1365-3156.2009.02235.x.

6. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol. 2013;209:505–23.

7. Anahtar MN, Byrne EH, Doherty KE, Bowman BA, Yamamoto HS, Soumillon M, Padavattan N, Ismail N, Moodley A, Sabatini ME, Ghebremichael MS, Nusbaum C, Huttenhower C, Virgin HW, Ndung'u T, Dong KL, Walker BD, Fichorova RN, Kwon DS. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. Immunity. 2015;42(5):965-76. doi: 10.1016/j.immuni.2015.04.019.

8. Jespers V, van de Wijgert J, Cools P, Verhelst R, Verstraelen H, Delany-Moretlwe S, Mwaura M, Ndayisaba GF, Mandaliya K, Menten J, Hardy L, Crucitti T; Vaginal Biomarkers Study Group. The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of

recent sex: a cross-sectional descriptive study across groups of African women. BMC Infect Dis. 2015;15:115. doi: 10.1186/s12879-015-0825-z.

9. Hickey RJ, Zhou X, Pierson JD, Ravel J, Forney LJ. Understanding vaginal microbiome complexity from an ecological perspective. Transl Res. 2012;160(4):267-82. doi: 10.1016/j.trsl.2012.02.008.

10. Peebles K, Velloza J, Balkus JE, McClelland RS, Barnabas RV. High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sex Transm Dis. 2019;46(5):304-311. doi: 10.1097/OLQ.0000000000000972.

11. Adapen C, Réot L, Menu E. Role of the human vaginal microbiota in the regulation of inflammation and sexually transmitted infection acquisition: Contribution of the non-human primate model to a better understanding? Front Reprod Health. 2022;4:992176. doi: 10.3389/frph.2022.992176.

12. Kyongo JK, Crucitti T, Menten J, Hardy L, Cools P, Michiels J, Delany-Moretlwe S, Mwaura M, Ndayisaba G, Joseph S, Fichorova R, van de Wijgert J, Vanham G, Ariën KK, Jespers V. Cross-Sectional Analysis of Selected Genital Tract Immunological Markers and Molecular Vaginal Microbiota in Sub-Saharan African Women, with Relevance to HIV Risk and Prevention. Clin Vaccine Immunol. 2015;22(5):526-38. doi: 10.1128/CVI.00762-14.

13. Doerflinger SY, Throop AL, Herbst-Kralovetz MM. Bacteria in the Vaginal Microbiome Alter the Innate Immune Response and Barrier Properties of the Human Vaginal Epithelia in a Species-Specific Manner. J Infect Dis. 2014;209 (12):1989–99. doi: 10.1093/infdis/jiu004

14. Masson L, Mlisana K, Little F, Werner L, Mkhize NN, Ronacher K, Gamieldien H, Williamson C, Mckinnon LR, Walzl G, Karim QA. Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study. Sex Transm infect. 2014;90(8):580-7.

15. Mtshali A, Ngcapu S, Mindel A, Garrett N, Liebenberg L. HIV susceptibility in women: The roles of genital inflammation, sexually transmitted infections and the genital microbiome. J Reprod Immunol. 2021;145:103291. doi: 10.1016/j.jri.2021.103291.

16. Masson L, Arnold KB, Little F, Mlisana K, Lewis DA, Mkhize N, Gamieldien H, Ngcapu S, Johnson L, Lauffenburger DA, Abdool Karim Q, Abdool Karim SS, Passmore JA. Inflammatory cytokine biomarkers to identify women with asymptomatic sexually transmitted infections and bacterial vaginosis who are at high risk of HIV infection. Sex Transm Infect. 2016;92(3):186-93.

17. Filardo S, Di Pietro M, Tranquilli G, Latino MA, Recine N, Porpora MG, Sessa R. 2019. Selected immunological mediators and cervical microbial signatures in women with *Chlamydia trachomatis* infection. mSystems 4:e00094-19. https://doi.org/10.1128/ mSystems.00094-19.

18. Jespers V, Kyongo J, Joseph S, Hardy L, Cools P, Crucitti T, Mwaura M, Ndayisaba G, Delany-Moretlwe S, Buyze J, Vanham G. A longitudinal analysis of the vaginal microbiota and vaginal immune mediators in women from sub-Saharan Africa. Sci. Rep. 2017;7(1):1-3.

19. Masson L, Barnabas S, Deese J, Lennard K, Dabee S, Gamieldien H, Jaumdally SZ, Williamson AL, Little F, Van Damme L, Ahmed K. Inflammatory cytokine biomarkers of asymptomatic sexually transmitted infections and vaginal dysbiosis: a multicentre validation study. Sex Transm Infect. 2019;95(1):5-12.

20. Galvin SR and Cohen MS. The role of sexually transmitted diseases in HIV transmission. Nat Rev Microbiol. 2004;2(1):33-42. doi: 10.1038/nrmicro794.

21. Borgdorff H, Tsivtsivadze E, Verhelst R, Marzorati M, Jurriaans S, Ndayisaba GF, Schuren FH, van de Wijgert JH. Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. ISME J. 2014;8(9):1781-93. doi: 10.1038/ismej.2014.26.

22. Masson L, Passmore JA, Liebenberg LJ, Werner L, Baxter C, Arnold KB, Williamson C, Little F, Mansoor LE, Naranbhai V, Lauffenburger DA. Genital inflammation and the risk of HIV acquisition in women. Clin. Infect. Dis. 2015;61(2):260-9.

23. McClelland RS, Lingappa JR, Srinivasan S, Kinuthia J, John-Stewart GC, Jaoko W, et al. Evaluation of the association between the concentrations of key vaginal bacteria and the increased risk of HIV acquisition in african women from five cohorts: a nested case-control study. Lancet Infect Dis. 2018;18:554–64. doi: 10.1016/S1473-3099(18)30058-6

24. Jarrett OD, Srinivasan S, Richardson BA, Fiedler T, Wallis JM, Kinuthia J, et al. Specific vaginal bacteria are associated with an increased risk of trichomonas vaginalis acquisition in women. J Infect Dis. 2019;220:1503–10. doi: 10.1093/infdis/jiz354.

25. McKinnon LR, Achilles SL, Bradshaw CS, et al. The evolving facets of bacterial vaginosis: implications for HIV transmission. AIDS Res Hum Retroviruses **2019**; 35:219–28.

26. GBD 2015 HIV collaboarators, Lancet HIV 2016; 3: e361–87. http://dx.doi.org/10.1016/S2352-3018(16)30087-X.

27. Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, et al. The Vaginal Microbiome and Preterm Birth. Nat Med. 2019;25(6):1012–21. doi: 10.1038/s41591-019-0450-2.

28. Guidelines for the management of symptomatic sexually transmitted infections. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

29. Verwijs MC, Agaba SK, Sumanyi JC, Umulisa MM, Mwambarangwe L, Musengamana V, Uwineza M, Cuylaerts V, Crucitti T, Jespers V, Van De Wijgert JH. Targeted point-of-care testing compared with syndromic management of urogenital infections in women (WISH): a cross-sectional screening and diagnostic accuracy study. Lancet Infect Dis. 2019;19(6):658-69.

30. Garrett N, Mtshali A, Osman F, Masson L, McKinnon LR, Singh R, Mitchev N, Ngobese H, Kharsany ABM, Abdool Karim S, Mlisana K, Passmore JA, Rompalo A, Mindel A, Liebenberg L. Impact of point-of-care testing and treatment of sexually transmitted infections and bacterial vaginosis on genital tract inflammatory cytokines in a cohort of young South African women. Sex Transm Infect. 2021;97(8):555-565. doi: 10.1136/sextrans-2020-054740.

31. Asare K, Andine T, Naicker N, Dorward J, Singh N, Spooner E, Andriesen J, Osman F, Ngcapu S, Vandormael A, Mindel A, Abdool Karim SS, Bekker LG, Gray G, Corey L, Tomita A, Garrett

N. Impact of Point-of-Care Testing on the Management of Sexually Transmitted Infections in South Africa: Evidence from the HVTN702 Human Immunodeficiency Virus Vaccine Trial. Clin Infect Dis. 2023;76(5):881-889. doi: 10.1093/cid/ciac824. Erratum in: Clin Infect Dis. 2023;76(1):182.

32. Adamson PC, Loeffelholz MJ, Klausner JD. Point-of-Care Testing for Sexually Transmitted Infections: A Review of Recent Developments. Arch Pathol Lab Med. 2020;144(11):1344-1351.

33. The diagnostics landscape for sexually transmitted infections. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

34. Land, K.J., Boeras, D.I., Chen, XS. *et al.* REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol* **4**, 46–54 (2019). https://doi.org/10.1038/s41564-018-0295-3

35. Torrone EA, Morrison CS, Chen P-L, Kwok C, Francis SC, Hayes RJ, et al. (2018) Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. PLoS Med. 2018;15(2): e1002511.

36. Wand H, Reddy T, Dassaye R, Moodley J, Naidoo S, Ramjee G. Estimating prevalence and incidence of sexually transmitted infections among South African women: Implications of combined impacts of risk factors. Int J STD AIDS. 2020;31(11):1093-1101. doi: 10.1177/0956462420915388.

37. Delany-Moretlwe S, Mgodi N, Bekker L, et al. High prevalence and incidence of gonorrhoea and chlamydia in young women eligible for HIV pre-exposure prophylaxis in South Africa and Zimbabwe: results from the HPTN 082 trial. Sex Transm Infect. 2023;99:433-439.

38. Behets F, Andriamiadana J, Rasamilalao D, Ratsimbazafy N, Randrianasolo D, Dallabetta G, Cohen M. Sexually transmitted infections and associated socio-demographic and behavioural factors in women seeking primary care suggest Madagascar's vulnerability to rapid HIV spread. Trop Med Int Health. 2001;6(3):202-11.

39. Country factsheets, UNAIDS 2023. https://www.unaids.org/en/regionscountries/countries/madagascar.

40. Nugent RP, Krohn MA, Hillier SL.Reliability of diagnosing bacterial vaginosisis improved by a standardized method of gram stain interpretation. J Clin Microbiol. 1991;29:297e301.

41. Dworkin, S.L. specimen Size Policy for Qualitative Studies Using In-Depth Interviews. Arch Sex Behav 2012; 41, 1319–1320.

42. Guest G, Namey E, McKenna K. How Many Focus Groups Are Enough? Building an Evidence Base for Nonprobability specimen Sizes. Field Methods. 2017;29(1):3-22.

43. Louviere J, Hensher D, Swait J: Stated Choice Methods: Analysis and Application, First edition edn. Cambridge, United Kingdom: Cambridge University Press; 2000.

44 Ryan M, Gerard K, Amaya-Amaya M (eds.): Using Discrete Choice Experiments to Value Health and Health Care. Dordrecht, the Netherlands: Springer; 2008.

45. PRESTO CT/NG combined qualitative real time CT/NG assay (Kit # GM CG 160500). Goffin Molecular Technologies, the Netherlands

46. Crucitti T, Van Dyck E, Tehe A, Abdellati S, Vuylsteke B, Buve A, Laga M. Comparison of culture and different PCR assays for detection of Trichomonas vaginalis in self collected vaginal swab specimens. Sex Transm Infect. 2003 Oct;79(5):393-8. doi: 10.1136/sti.79.5.393. PMID: 14573835; PMCID: PMC1744760.

47. Müller EE, Venter JM, Magooa MP, Morrison C, Lewis DA, Mavedzenge SN. Development of a rotor-gene real-time PCR assay for the detection and quantification of Mycoplasma genitalium. J Microbiol Methods. 2012 Feb;88(2):311-5. doi: 10.1016/j.mimet.2011.12.017. Epub 2011 Dec 29. PMID: 22230235.

48. Sekhon, M., Cartwright, M. & Francis, J.J. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. BMC Health Serv Res. 2017;17, 88. https://doi.org/10.1186/s12913-017-2031-8.

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We are extremely grateful to the members of the scientific advisory board for the precious scientific discussions and suggestions for improving our study.

Figure Legend

Figure 1: Schematic of the GIFT diagnostic study

The recruited women who meet the inclusion criteria and consent to participate in the GIFT study will be administered a questionnaire. Data will be recorded on case report forms (CRF) in a paper or electronic (tablet) format by the clinical nurse followed by a gynecological exam. Vaginal swabs are collected: the first collected swab will be used for two GIFT assays: the first GIFT will be performed at the bedside by the midwife/nurse and the second GIFT by the lab technician. Each of them will read visually their own performed GIFT (naked eye reading). The two respective GIFT assays will then be read using a lateral flow automated reader. The other collected swabs will be used for different laboratory assays for the evaluation of the GIFT performance. HIV testing on fingerpick blood will be also included at the end of the medical exam. The figure was created with BioRender.

Figure 2: Integration study activities

Description of the four activities that will be performed for the GIFT integration into care study for improving the STI/BV diagnosis and control in women: 1) user experiences and/or perceptions of the GIFT device involving qualitative focus group discussions and in-depth interviews with key

stakeholders, 2) discrete choice experiments, 3) development of a decision tree classification algorithm and 4) economic evaluation of defined management algorithms. In addition to the women already participating to the GIFT diagnostic study, health care professionals (HCP), policy makers, key opinion leaders and patients coming for consultation will be included in the study.

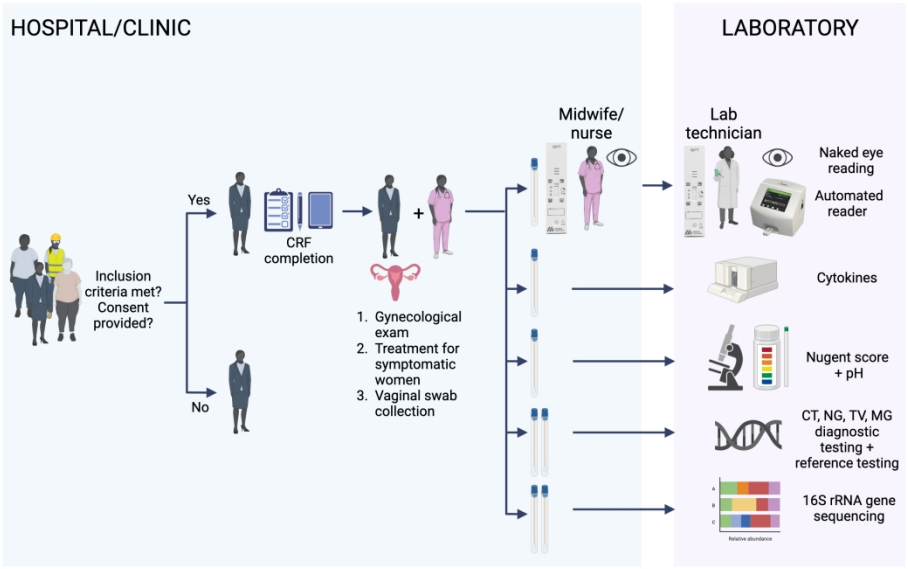
Additional files

Supplementary Figure 1: Diagnostic study flow steps

Description: Women attending family planning services will receive explanations on the GIFT study. After written consent and validation of the eligibility criteria and written informed consent, the enrolled woman will be subjected to a structured questionnaire. The data will be captured on paper or electronic case report form (CRF) by the clinical nurse. The CRF consists of 8 modules that will include all the information from sociodemographic information, behavioral risk assessment to medical information collected during the gynecological examination by the midwife or nurse.

Each CRF assigned to each participant will contain all the data collected through the diagnostic study including laboratory results that will be recorded in the REDCap GIFT database.

The vaginal swab sample collection will be done by the midwife/nurse after gynecological examination for : GIFT assays, pH measurement and microscopic analysis of a vaginal smear slide for BV diagnosis, and STI diagnosis. For this latter, a couple of vaginal swabs will be used for nucleic-acid amplification tests (NAAT) for CT, NG and TV to be performed locally on each study site. Based on the on-site positive diagnostic results for CT, NG, TV and BV a treatment will be offered by the clinic or hospital clinician to the participant. HIV will be tested on fingerprick blood.



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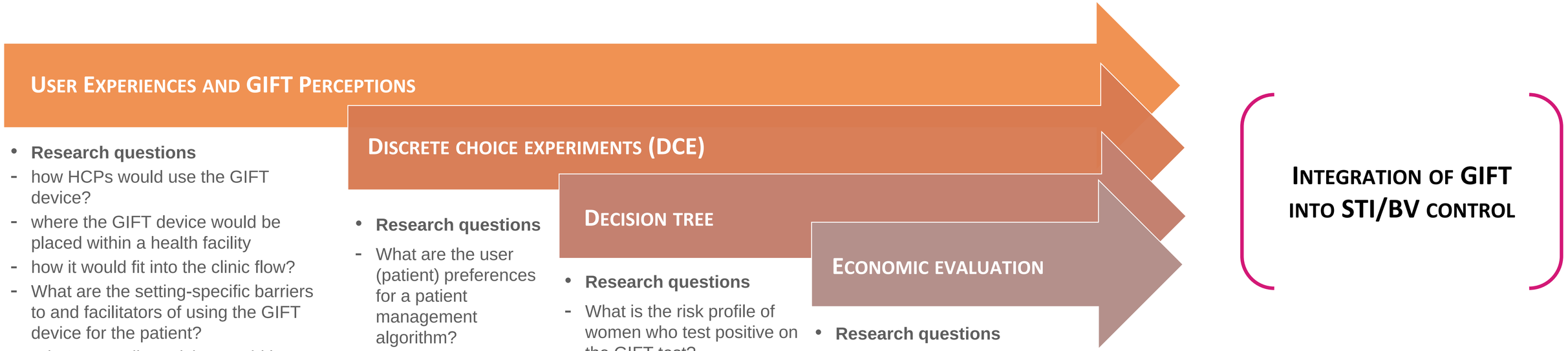
645x452mm (118 x 118 DPI)



- **Research questions**
- how HCPs would use the GIFT device?
- where the GIFT device would be placed within a health facility
- how it would fit into the clinic flow?
- What are the setting-specific barriers to and facilitators of using the GIFT device for the patient?
- What counseling advice would be needed when using the GIFT device?

- **Target groups**
- Health care professionals
- Policy makers
- Key opinion leaders
- Patients

- **Research questions**
- What are the user (patient) preferences for a patient management algorithm?
- What is the preference heterogeneity across users?
- Willingness to pay?
- **Target groups**
- Women participant to the diagnostic study
- Women non included in the diagnostic study

- **Research questions**
- What is the risk profile of women who test positive on the GIFT test?
- How do different testing strategies (immediate treatment after GIFT positive vs further re-evaluation) affect outcomes?
- If immediate treatment, which treatment would yield the best outcome?

- **Research questions**
- What are the costs and budget impact of the identified screening or diagnostic algorithm with the GIFT device?
- What are the costs of the different algorithms and strategies for GIFT device integration?

Explanation about the study to women attending family planning

- Verification of inclusion / exclusion criteria
- Consent signature
- Collection of urine sample for pregnancy test

Questionnaire : **Case Report Form (CRF)**

- Module 1 : Screening and Enrollment
- Module 2 : Sociodemographic Information
- Module 3 : Behavioral Risk Assessment
- Module 4 : General health and medical history including medication, smoke and alcohol
- Module 5 : Pregnancy and Contraception History
- Module 6 : HIV testing/status and treatment
- Module 7 : Gynaecological Physical Exam
- Module 8 : Sample collection and results

- Vaginal swabs sample collection
- pH and smear slide
- GIFT Test near the patient
- Finger prick blood sample collection for HIV test

- GIFT test in the laboratory -Test reading using a lateral flow test automated reader
- STI/BV tests : NAAT for CT/NG/TV
- Nugent scoring
- HIV test

- Treatment according syndromic approach OR appreciation of clinician
- Treatment of the participant and partners
- If positive HIV test : refer the participant to clinician

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any prespecified hypotheses	8-10
Methods			
Study design	4	Present key elements of study design early in the paper	11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	1-13
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	13-15
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	20-23
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	16-19
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	15
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA

		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	NA
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

A Novel Point-Of-Care Cytokine Biomarker Lateral Flow Test for the Screening for Sexually Transmitted Infections and Bacterial Vaginosis: Study Protocol of a Multi-Centre Multi-Disciplinary Prospective Observational Clinical Study to Evaluate the Performance and Feasibility of the Genital Inflammation Test (GIFT).

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Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Infectious diseases, HIV/AIDS
Keywords:	Sexually Transmitted Disease, BACTERIOLOGY, Public health < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES

A Novel Point-Of-Care Cytokine Biomarker Lateral Flow Test for the Screening for Sexually Transmitted Infections and Bacterial Vaginosis: Study Protocol of a Multi-Centre Multi-Disciplinary Prospective Observational Clinical Study to Evaluate the Performance and Feasibility of the Genital Inflammation Test (GIFT).

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Keywords: Sexually transmitted infections, bacterial vaginosis, lateral flow point of care test, cytokines, genital inflammation test.

ABSTRACT

Introduction

A prototype lateral flow device detecting cytokine biomarkers IL-1α and IL-1β has been developed as a Point-of-Care (POC) test – called the Genital InFlammation Test (GIFT) - for detecting genital inflammation associated with sexually transmitted infections (STIs) and/or bacterial vaginosis (BV) in women. In this paper, we describe the rationale and design for studies that will be conducted in South Africa, Zimbabwe, and Madagascar to evaluate the performance of GIFT and how it could be integrated into routine care.

Methods and analysis

We will conduct a prospective, multidisciplinary, multi-centre, cross-sectional, and observational clinical study comprising two distinct components: a biomedical ("diagnostic study") and a qualitative, modelling, and economic ("an integration into care study") part. The diagnostic study aims to evaluate GIFT's performance in identifying asymptomatic women with discharge-causing STIs (*Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV) and *Mycoplasma genitalium* (MG)) and BV. Study participants will be recruited from women attending research sites and family planning services. Several vaginal swabs will be collected for the evaluation of cytokine concentrations (enzyme-linked immunosorbent assay), STIs (nucleic acid amplification tests), BV (Nugent Score), and vaginal microbiome characteristics (16S rRNA gene sequencing). The first collected vaginal swab will be used for the GIFT assay which will be performed in parallel by a healthcare worker in the clinic near the participant, and by a technician in the laboratory. The integration into care study aims to explore how GIFT could be integrated into routine care. Four activities will be conducted: user experiences and/or perceptions of the GIFT device involving qualitative focus group discussions and in-depth interviews with key stakeholders; discrete choice experiments; development of a decision tree classification algorithm; and economic evaluation of defined management algorithms.

Ethics and dissemination

Findings will be reported to participants, collaborators and local government for the three sites, presented at national and international conferences, and disseminated in peer-reviewed publications. The protocol and all study documents such as informed consent forms were reviewed and approved by the University of Cape Town Human Research Ethics Committee (HREC reference 366/2022), Medical Research Council of Zimbabwe (MRCZ/A/2966), Comité d'Ethique pour la Recherche Biomédicale de Madagascar (N° 143 MNSAP/SG/AMM/CERBM) and the London School of Hygiene and Tropical Medicine ethics committee (LSHTM reference 28046).

Before the start, this study was submitted to the Clinicaltrials.gov public registry (NCT05723484).

Strengths and limitations of this study

Strengths

- A multicountry evaluation of a new low-cost, rapid POC test detecting vaginal inflammation to be conducted in three sub-Saharan countries characterised by different STI/BV contexts.
- Multidisciplinary approach, including social science, economic analysis, and machine learning to inform how the GIFT device could be integrated into STI management algorithms for women.

Limitations

- The budget does not permit STI diagnosis using POC NAAT testing in Madagascar, in contrast to South Africa and Zimbabwe, leading to delays in delivering the diagnostic results and providing treatment to the Malagasy study participants.
- The user evaluation of the GIFT device does not include the evaluation nor quality assessment of the specimen extraction method.

Introduction

Sexually transmitted infections (STIs) continue to pose a significant health challenge globally, with over one million new cases reported daily. In 2020, approximately 377 million new cases of the four most common STIs (*Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), syphilis, and *Trichomonas vaginalis* (TV)) were documented, with over 70% occurring in low- and middle-income countries (LMICs), particularly in Sub-Saharan Africa and Latin America [1].

STIs impact women more than men, as women endure long-lasting consequences. Additionally, women are more susceptible to STIs than men due to anatomical and immunological differences [2,3]. The composition of the vaginal microbiome also plays a crucial role in sexual and reproductive health. Optimal vaginal microbiota, predominantly composed of *Lactobacillus* species, maintains an acidic pH, sustaining the vaginal epithelial barrier's integrity and preventing pathogen colonisation and invasion. However, various genetic, behavioural, and environmental factors can modulate this microbial composition and lead to bacterial vaginosis (BV) characterised by a decrease in protective *Lactobacillus* species along with an increase in bacterial diversity and vaginal pH [4-9].

BV remains a persistent public health issue, with prevalences ranging from 20-30% globally, and higher rates observed in Black populations compared to Caucasian or Asian populations [4, 6, 10]. In Sub-Saharan African populations, prevalences are notably high (around 33%) [10].

Both STIs and BV induce elevated genital inflammation, marked by increased local production of pro-inflammatory cytokines and chemokines, irrespective of symptoms [7, 11, 12, 13-15]. Studies in Sub-Saharan African women cohorts have shown that common STIs such as CT, NG, and TV significantly and consistently increased the concentrations of key genital tract cytokine biomarkers, including interleukin (IL)-1α and β [14, 16, 17]. BV caused similar changes in these cytokine biomarkers, although was also shown to be associated with lower concentrations of the chemokine interferon-γ-induced protein of 10kDa (IP-10) [12, 18, 19]. Genital inflammation caused by STIs and BV has in turn been associated with increased risk for the acquisition of other STIs, including HIV-

1 [20-25]. This is particularly concerning in Sub-Saharan African countries, where a significant proportion of new HIV-1 infections occur. The HIV-1 prevalence among adults (15-49 years) was estimated at 17.8% and 11% in South Africa and Zimbabwe, respectively in 2022. Women are disproportionally affected by HIV than men, with young women (15-24 years) particularly vulnerable with prevalence rates estimated at 8.7% and 4.4% in South Africa and Zimbabwe, respectively [26].

Both STIs and BV are also associated with other serious sexual and reproductive health complications, such as pelvic inflammatory disease which can lead to infertility and ectopic pregnancy and adversely affect a woman's reproductive plans. For prevalent bacterial STIs and BV, intrauterine infection and/or vertical transmission to the unborn child or during birth can result in adverse pregnancy and birth outcomes, including preterm delivery, maternal and neonatal sepsis, low birth weight, pneumonia, and conjunctivitis in the newborn [3, 27].

Despite the high prevalence of STIs and BV and their frequently asymptomatic nature, syndromic approaches remain the mainstay of management in LMICs [28]. Immediate testing and results delivery are crucial to facilitate prompt diagnosis and accurate treatment, reducing both STI transmission and infection progression [29-31]. Consequently, the inclusion of POC tests has been highly recommended for the management of symptomatic STIs [28]. Recently, nucleic acid amplification-based near-patient STI diagnostics have become available [32, 33], but none of them fulfils the REASSURED (including user-friendly, rapid, robust, equipment-free, and deliverable to end-users) criteria set out by World Health Organization (WHO) for POC tests [34].

Genital inflammation has been associated with a significantly increased risk of HIV acquisition in women and the presence of STIs driving this inflammation has been welldescribed among South African women [11, 16, 22, 25]. To address the need for POC tests to improve STI/BV management for women in LMICs, Masson *et al.* have focused on developing a POC lateral flow test to detect a combination of inflammation biomarkers (IL-1α, IL-1β, and IP-10), measured in lateral vaginal wall swabs, to improve BV and STI case finding in women compared to clinical signs [20]. This Genital

Inflammation Test (GIFT) screening tool aims to improve case detection to help identify women at increased risk of HIV infection and to enhance the sexual and reproductive health of women in countries where only syndromic management is available.

This study protocol outlines a study to be conducted in three countries (South Africa, Zimbabwe, and Madagascar) to evaluate the performance of the first-in-field GIFT prototype device and explore possible routes for integration into routine care.

Purpose and study objectives

This multidisciplinary, cross-sectional, prospective, and multi-centric study has two distinctive sub-studies, a diagnostic evaluation and an integration into care study. Each sub-study has several objectives (Table 1).

Table 1: GIFT study objectives

	Diagnostic study	Integration into care study
Primary objective	To assess the sensitivity and specificity of the GIFT device at the point-of-care in South Africa, Zimbabwe, and Madagascar.	To evaluate how the GIFT device could be integrated into routine care

	Diagnostic study	Integration into care study
Secondary objectives	• To assess the predictive values of the GIFT device at the point-of-care • To assess the performance of the GIFT device at the point-of-care • To assess the performance of the device versus syndromic management without any laboratory testing (standard of care in South Africa, Zimbabwe, and Madagascar) • To determine the robustness of the device by comparing results read by clinicians with those read by laboratory professionals, and with the results obtained using an automated reader • To evaluate the accuracy of the GIFT device by comparing the GIFT device results with ELISA results using previously validated concentration cut-offs as the gold standard, including validation of the GIFT cytokine concentration cut-offs for each cytokine biomarker.	• To qualitatively and quantitatively assess the user experience, usability, and acceptability of the GIFT device at the point of care; • To examine patient preferences for various STI management aspects (attributes) to inform the development of STI management algorithms that integrate the GIFT device; • To generate algorithms that integrate the GIFT device to optimise case finding and STIs/vaginal infection management in women, using the complete dataset from the study; • To determine the cost and budget impact of the identified screening or diagnostic algorithm with the GIFT device, and to model the cost-effectiveness of different strategies of integration of GIFT into care.
Exploratory objectives	• Determine if other determinants (such as intermediate microbiota (Nugent 4-6), age, parity, sexual activity) improve the prediction of STI/BV status in women • To use 16S rRNA gene sequencing and vaginal bacteria specific quantitative NAATs to evaluate the proportion of cases of genital inflammation explained by vaginal dysbiosis that was not diagnosed as an STI or BV by NAATs or Nugent scoring • To explore the performance of the device in the presence of vaginal *Candida* spp colonisation.	

The diagnostic study aims to evaluate the performance of GIFT in identifying women with inflammatory STIs (including CT, NG, TV, and MG) and BV and who may, therefore, be at higher risk of STI and HIV infection and potential reproductive complications. Syndromic management in the absence of any diagnostic testing misses all asymptomatic reproductive tract infections by

definition, and GIFT may detect the presence of asymptomatic subclinical inflammation. Syndromic management guidelines currently call for women with cervicovaginal discharge to be treated for all reproductive tract infections that might cause such discharge, but studies have shown that a substantial proportion of these women do not have any infection [29]. In this scenario, GIFT may prevent overtreatment of symptomatic women without infection.

The primary objective is to assess the sensitivity and specificity of the GIFT device at the point-of-care in non-pregnant sexually active women aged 18-35 years accessing family planning services in South Africa, Zimbabwe, and Madagascar against nucleic acid amplification tests (NAATs) for CT, NG, TV, and MG plus BV by Nugent scoring (Table 1). Secondary objectives include assessing the device's predictive values; assessing performance in each country separately; assessing performance against syndromic management; assessing test reproducibility by comparing clinician- and laboratory-read results (visual reading versus automated reader); and setting the device's concentration cut-offs against gold standard cytokine concentrations (determined by enzyme-linked immunosorbent assay (ELISA)).

The integrative part of the study aims to explore the feasibility, acceptability, and cost-effectiveness of integrating GIFT into routine care in LMICs. The specific objectives include: assessing the user-experience, usability, and acceptability of the GIFT device at the POC; examining patient preferences for various STI management aspects to develop STI management algorithms; generation of algorithms that integrate the GIFT device to optimise case finding and STI/vaginal infection management in women; and determine the cost-effectiveness and budget impact of the identified algorithms with the GIFT device.

Methods and design

Study design

The diagnostic study will target women attending family planning services in each of the three study sites in South Africa, Zimbabwe, and Madagascar. A series of vaginal swabs will be collected by a midwife or nurse (Figure 1).

The first swab will be used to test the performance of the GIFT device. For each woman, the GIFT will be performed twice and read four times: one GIFT will be performed at the clinical site near the participant and visually read by a midwife or study nurse and a second GIFT will be done and visually read by a technician in the laboratory. The GIFT assays carried out by the midwife/study nurse and the laboratory technician will secondly be read using an automated lateral flow reader (Axxin, Fairfield, Australia) (Figure 1). The GIFT device performance in detecting (1) inflammation will be determined against cytokine enzyme-linked immunosorbent assay (ELISA); and (2) STIs/BV will be determined against the NAAT reference tests for CT, NG, TV, and MG and Nugent scoring for BV (Figure 1). All women will be offered treatment based on the on-site positive STI diagnostic results for CT, NG, and TV. Additionally, treatment for BV will be offered based on the Nugent score analysis and at the clinician's discretion.

The integration into care study consists of four activities to determine how GIFT can be integrated in a feasible, acceptable, and cost-effective way into routine care and national guidelines in South Africa, Zimbabwe, and Madagascar (Figure 2): 1. User experiences and/or perceptions of the GIFT device involving qualitative focus group discussions (FGDs) and in-depth interviews (IDIs) with key stakeholders; 2. Discrete choice experiment (DCE); 3. Development of a decision tree classification algorithm; 4. Economic evaluation of defined management algorithms.

Study sites

The study will be performed in three countries: South Africa, Zimbabwe, and Madagascar. In contrast to South Africa and Zimbabwe which are countries bearing a high burden of STIs, including HIV, and BV [35-37], data on STI/BV prevalence are relatively scarce in Madagascar. BV, TV, and cervical infections (CT and NG) prevalences measured in a large population sample of symptomatic

Malagasy women (N=1066) were high at 53%, 24%, and 17% respectively, suggesting a significant burden of STIs/BV in the island [38]. Finally, Madagascar is less affected by HIV with a prevalence <1% [39] compared to South Africa and Zimbabwe, which were found to have an HIV-1 prevalence among adults (15-49 years) in 2022 of 17.8% and 11%, respectively [26].

In South Africa, the study will be conducted at the Desmond Tutu Health Foundation (DTHF) Masiphumelele Youth Centre located in the South peninsula of Cape Town. The Masiphumelele Research Site has been recognised as a pioneer in HIV prevention and adolescent research. The STI and BV diagnostic testing of the South African participants will be performed in their on-site laboratory. In addition, vaginal swabs collected at all three study sites will be tested for selected cytokines including IL-1α, IL-1β, and IP-10 by ELISAs and vaginal microbiome composition by 16S rRNA gene sequencing being conducted at the University of Cape Town (UCT), along with samples from the other two trial sites.

In Zimbabwe, the study will be conducted at the Spilhaus Family Planning Centre located in the capital Harare. The study site is run by the Zimbabwe National Family Planning Council (ZNFPC) in collaboration with the Zimbabwe Ministry of Health and Child Care. The study will be conducted by researchers from the Organization for Public Health Interventions and Development (OPHID), equipped with a skilled medical and technical team. The study site's laboratory will perform the STI and BV diagnostic testing for Zimbabwean study participants.

In Madagascar, the study will be conducted at the Centre Hospitalier Universitaire de Gynécologie et d'Obstétrique Gynéco-Obstétricaux de Befelatanana (CHU-GOB), a public university hospital that specialises in obstetrics located in the capital of Madagascar, Antananarivo. Outpatient services include prenatal consultations, family planning, obstetrics, and gynaecology services. Due to the lack of recent data on STI/BV prevalences, a pre-study on CT, NG, TV, and MG prevalences was conducted by the clinical and research team of IPM from March to December 2022 to confirm the feasibility of conducting the GIFT study in the CHU-GOB in Antananarivo. The pre-study confirmed

the appropriateness of the clinical study site in terms of the study population's number and STI and BV prevalence rates (results will be presented elsewhere).

The unit of experimental bacteriology (UBEX) at the Institut Pasteur Madagascar (IPM) will be the study site's laboratory in Madagascar for diagnostic testing. UBEX is also designated as the reference laboratory in the GIFT project for BV and STI (CT, NG, TV, and MG) comparator testing, as well as quantification of lactobacilli and BV-associated bacteria using quantitative PCR and molecular testing for the detection of *Candida* spp., and Y chromosome.

Study participants and recruitment

The diagnostic study will enroll its participants from sexually active women aged 18-35 years attending family planning services in each of the study's clinical sites, irrespective of symptoms. Before study initiation, advice will be sought from local Community Advisory Boards (CABs) or similar committees on the study material to be shared with potential study participants. The boards will be regularly consulted regarding language to use for behavioural and other sensitive questions included in the questionnaires. A CAB is currently not existing in Madagascar but will be installed as part of the study.

Due to the short recruitment period, sensitisation to the GIFT project will be extended in Antananarivo (Madagascar) to the family planning services of surrounding primary health care centres. In South Africa, the clinical study site offers family planning services to young women till the age of 25, and women outside of the study site will be recruited to enroll women between 25 and 35 years old.

Women interested in the diagnostic study will be invited to participate based on the inclusion and exclusion criteria detailed in Table 2. Each woman will only be enrolled once.

Table 2: In- and exclusion criteria for both diagnostic and integration into care studies

	Diagnostic study	Integration into care study
Inclusion criteria	• 18-35 years old • Willing and able to provide informed consent to participate in the study • Self-reported to be sexually active • Not pregnant (determined by pregnancy test) • Accessing family planning service	For all who are willing and able to provide informed consent to participate in the study. • User experiences/perceptions activity: - Local or regional policymakers, programmers, and other opinion leaders and decision-makers - Healthcare professionals at health facilities - Women who are eligible for the diagnostic study (including pregnant and menstruating women), but who are not part of the diagnostic study • Discrete choice experiments: Women who are eligible for the diagnostic study (including pregnant and menstruating women), who are either part of, or not part of, the diagnostic study • Decision tree classification algorithm: Data from diagnostic study participants • Economic evaluation: Healthcare professionals at health facilities involved in GIFT device implementation able to complete timesheets
Exclusion criteria	• <18 years or >35 years • Refusal by a participant to participate in the study • Treatment for any STI/BV in the past 30 days • Pregnancy • Enrolled in a study that does not allow co-enrolment in other studies	For all who are not willing or able to provide informed consent to participate in the study • User experiences/perceptions activity: - Non-relevant policymakers - Healthcare professionals at health facilities not included in diagnostic study - Women who are : ‣ part of the diagnostic study ‣ <18 years or >35 years ‣ Treated for any STI/BV in the past 30 days ‣ Pregnant ‣ Enrolled in a study that does not allow co-enrolment in other studies • Decision tree classification algorithm: Participants for whom there are no diagnostic study data • Economic evaluation: Healthcare professionals not at study sites, not involved in GIFT device implementation, and/or not able to complete timesheets

In addition to eligible women participating in the diagnostic study, participants for the integration into care study will include health care professionals (HCPs) including nurses, midwives, and matrons

working at the clinical site where the diagnostic study is being conducted, women attending gynaecological consultations at the study clinic, local and regional policymakers, programmers, other opinion leaders, and decision-makers according to the inclusion and exclusion criteria (Table 2).

Sample size

A total number of 675 women (225 women per site) will be enrolled for the diagnostic evaluation of the prototype GIFT device. The sample size calculation is based on 7% precision, and an assumed sensitivity of 77%, and a specificity of 71% of GIFT compared to NAATs for STIs and Nugent scoring for BV [40].

No sample size calculations were performed for the qualitative research. A purposive sampling strategy will be adopted to reflect maximum variation, as feasible, across the sample. It is anticipated that the proposed number of participants per activity (FGDs and IDIs), per country, will enable adequate data to be collected and saturation to be reached in the thematic analysis [41, 42]. Data collection may be finalised before the planned sample size is reached, if saturation is reached first.

For the DCE component of the study, using econometric criteria (including choice probability, confidence level, accuracy level, attrition rate, and number of choice tasks), subgroups of more than 30 individuals are required to conduct meaningful statistical analysis [43, 44]. Because the DCE study will undertake subgroup analysis on four to five groups (including respondent age group, socio-economic status, location of residence, and experience with the GIFT device), up to 200 participants per site will be recruited for the DCE study, with a quarter of the participants recruited on the diagnostic study of the GIFT device, and reminder from women coming for gynecological consultations at the study clinic.

No sample size calculation is needed for the decision tree algorithm study, which will use data from the diagnostic study. Equally, there are no sample size requirements for the economic evaluation.

Study procedures and data collection

1) Diagnostic study

The diagnostic study participant and sample flow from screening to laboratory results is described in Figure 1 and detailed in Supplementary Figure 1 - Diagnostic study flow steps. After confirmation that participants were eligible for the study (Table 2) and written informed consent obtained, enrolled women will be interviewed using a structured questionnaire with questions including demographics, sexual behaviour, hygiene habits, current medical and reproductive history, medication, and symptoms. The data will be captured on paper or electronic case report forms (CRFs) (Figure 1). The clinician, midwife, or study nurse will perform a physical examination including a pelvic examination, and will collect finger prick blood for HIV testing and vaginal swabs using regular flocked swabs (Copan, Italy) (Figure 1 and Supplementary Figure 1 - Diagnostic study flow steps).

The first inserted swab will be eluted in buffer and the nozzle-sample (provided with the GIFT kit) will be applied to two prototype GIFT devices, one near the patient and the other in the laboratory (Figure 1). A second swab will serve for cytokine ELISA testing, and a third swab for pH testing using a pH indicator strip and to prepare a vaginal smear slide for BV (Figure 1). The next swabs will be collected and used for CT, NG, and TV diagnosis in the laboratory, for reference STI testing including MG, quantitative NAAT of vaginal microbiome bacteria, the detection of *Candida* spp, and Y chromosome for the presence of semen. The last two collected swabs will be used to characterise the vaginal microbiome by using 16S rRNA PCR and sequencing (Figure 1).

2) Integration into care study

The integration study is composed of four activities aiming to understand how GIFT can be integrated into STI control (Figure 2).

For the qualitative study assessing the user-experience, usability, and acceptability of the GIFT device at the point of care, data will be collected through IDIs and FGDs. Target groups will be: HCPs at the health facilities; local or regional policymakers, programmers, other opinion leaders, and decision-makers; and women who are eligible for the diagnostic study (Table 2), but who are not part of the diagnostic study. HCP participants will be identified through the diagnostic study sites and will be contacted directly to ask if they would be interested in participating. Policymakers recommended for

inclusion through snowball sampling will be contacted and asked if they would be interested in participating. All women presenting at the family planning service facilities for inclusion in the diagnostic study will be informed about the integration study. For the DCE, the study team will seek to recruit women participating and not-participating in the GIFT diagnostic study to explore heterogeneity in patient preferences for various STI attributes. For all population groups, those who agree to participate will provide written informed consent, separate from the diagnostic study.

For the decision tree classification algorithm, data will be obtained from the diagnostic study participants, and no additional participants will be recruited. For the economic evaluation, healthcare professionals at health facilities involved in GIFT device implementation and able to complete timesheets will be recruited.

For the user experience activity (Figure 2, activity 1), up to 10-12 IDIs and/or 1-3 FGDs will be conducted with policy makers, up to 8-10 IDIs and/or 3 FDGs will be conducted with HCPs, and up to 12-15 IDIs and/or 3 FDGs will be conducted with women. FGDs will include participatory tools, such as card mapping. The FGDs with HCPs may additionally include role plays of how the providers would discuss the GIFT device with women, and how providers would discuss GIFT device results and subsequent care and management with women. IDIs will explore more detailed personal perceptions of the potential structures of integration.

User experience data (qualitative and FGDs) on the patient experience will help inform the development of attributes for the DCE (Figure 2, activity 2). The DCE aims to identify the relative importance of factors influencing patients' preferences for the use of GIFT devices. For the DCE study, face-to-face interviews using a questionnaire will be conducted, with the questionnaire asking respondents to state preferences for hypothetical alternatives, each described by several attributes. A D-efficient design will be used to identify the choice tasks to present to respondents. Each choice task presents two STI screening test options that are defined using different levels of the attributes and then ask respondents to choose their preferred option, with an opt-out option available (to choose neither of the two options). The results of the DCE will inform the development of STI management

algorithms that integrate the GIFT device, to increase the acceptability of the STI management programme.

A decision tree will be developed to classify symptomatic and asymptomatic women using the following attributes: socio-demographic information, signs and symptoms, medical history, sexual behaviour, the GIFT output, and vaginal pH. Different classifications will be tested by varying the combinations of the attributes and the number of levels of the tree. The algorithm will be developed using a training dataset (data from previous studies). After the development of the algorithm based on the training dataset, the algorithm will be tested on the data to be collected in the diagnostic study and refined if necessary. The algorithm will be designed to be as simple as possible (minimum number of nodes and minimum number of scenarios) and usable in routine practice. Finally, the management outcomes will be compared to those obtained with the WHO recommendations.

The economic evaluation (Figure 2, activity 4) will involve the collection of cost and service utilisation data from a provider's perspective at each of the clinical sites, using an ingredients-based approach. After obtaining approval from the facility and/or district, relevant facility management staff will be approached to share financial and other facility-level data. The cost and utilisation data will be collected from individual facility records and financial reports through cost data collection tools at each study site. The time that it takes to perform and analyse the GIFT assay will be obtained from clinical study staff. The time required for the delivery of routine family planning and STI services will be assessed through timesheets completed by selected healthcare workers at the study sites. These data will be captured in the Provider Cost Data Collection Tool by data collectors. This activity will determine the cost and budget impact of the identified screening or diagnostic algorithm with the GIFT device and will model the cost-effectiveness of the different strategies of integration of GIFT into care.

Laboratory procedures

The BV diagnosis will be obtained from a vaginal smear carried out with the third swab collected during the gynecological examination (Figure 1). The slide will be transferred from the clinic to the respective on-site laboratory for Gram staining and Nugent scoring [40].

CT, NG, and TV diagnosis from collected vaginal swabs (Figure 1) will be performed using the diagnostic tests available in each of the three sites. In South Africa and Zimbabwe, GeneXpert tests (Cepheid, Sunnyvale, USA) will be used for CT and NG detection, and TV detection will be performed with the OSOM trichomonas rapid test or TV GeneXpert test, depending on the availability. In Madagascar, the vaginal swabs will be tested by UBEX (IPM) using validated qPCR protocols for CT, NG, and TV detection [45,46].

The swabs collected for STI reference testing in South Africa and Zimbabwe will be shipped to the IPM (Madagascar) for reference testing using validated qPCR [45-47], vaginal bacteria-specific quantitative qPCR testing, molecular detection of *Candida* spp. and presence of semen (Y chromosome testing).

Three swabs per participant will be sent to the University of Cape Town, for cytokine ELISA measurement and 16S rRNA gene PCR and sequencing. The results obtained with the prototype GIFT device will be compared to the cytokine concentrations resulting from the ELISA measurements. 16S rRNA gene sequencing and *Candida* spp. NAAT will be used to evaluate the proportion of cases of genital inflammation that could be explained by vaginal bacterial or fungal dysbiosis, respectively.

All testing will be performed blinded to the clinical information and results obtained at each of the study sites, including the GIFT results.

The swabs will be stored in each study site at -80°C until shipment using dry ice to the respective laboratories as stipulated above.

Monitoring, quality assurance, and control

This study will be monitored per regulations applicable to diagnostic study, including International Committee on Harmonisation of Good Clinical Practice (ICH-GCP) and GCLP (Good Clinical

Laboratory Practices) requirements, and sponsor-specific standard operating procedures (SOP). The diagnostic study will be monitored by the Epidemiology and Clinical Research Unit at the IPM. Routine monitoring will be conducted throughout the study and at the study closure. All laboratory activities including specimen transport, processing, testing, result reporting, and storage will be conducted following clinical trial quality requirements. The GCLP guidelines will be followed and the designated laboratories will perform testing according to the SOPs which will be documented in the analytical plan.

A batch of known negative and positive specimens for CT/NG, TV, pregnancy test, and slides for Nugent scoring will be provided by the UBEX laboratory at IPM for external quality control. The external quality control will consist of three panels to be tested at the start, middle, and end of the study or every three months. Before the use of the GIFT devices, a lot validation will be performed using the quality control panel provided by the manufacturer according to the SOP.

Data management

The REDCap platform will be used as a clinical data management system (CDMS) for the clinical study. Depending on the organisation in each study site data will be collected partially or fully using paper CRF (pCRF) before being entered into REDCap daily. In addition, data may be collected and entered in real-time into REDCap using tablets.

The GIFT database will be managed by the Epidemiology and Clinical Research Unit at the IPM. The quality and accuracy of CRF data transcription into the database will be verified weekly. Missing or overdue forms will be identified and tracked using the REDCap field comment log. A control script developed by the central data manager will check the double data entry, and any transcription differences found will be noted. Status reporting including the list of inconsistencies will be done weekly by the central data management team. The weekly reports will also resume the progress of data collection and entry in each study site. All reports will be sent to the operational team for verification and correction of the data, if required.

External data sources not described on the CRF like cytokine ELISA data, the STI reference testing data, and 16S rRNA sequencing data will be reconciled against the CDMS at the end of the study.

For the user experience study, FGD and IDI data will be audio-recorded and electronically transcribed into the national and/or local language of the study sites. The DCE questionnaire data will be captured electronically using REDcap and securely stored on a password-protected computer. Data for the economic evaluation will be collected in a Provider Cost: Data Collection Tool, and timesheets will be completed in Excel by HCPs.

Data analysis

Diagnostic study

1) Primary analysis: Sensitivity and specificity estimated of the GIFT device with 95% confidence intervals using any positive etiological NAATs for STIs (CT, NG, TV and MG) or Nugent score result for BV as reference standards will be calculated. Visual readings of the GIFT device performed by the clinicians and technicians will be included separately in this analysis.

2) Secondary analysis: Positive and negative predictive values with 95% confidence intervals of the GIFT device will be compared with the likelihood ratios using NAAT and Nugent scoring as reference standards overall and in each country (which may have different STI prevalence that will impact these predictive values).

The performance of the GIFT device will be compared to the performance of syndromic management for detecting STIs. Both methods (GIFT and syndromic management) will be compared to any positive results of NAAT (for CT, NG, TV and MG) and/or BV detected by Nugent scoring. Agreement between the GIFT device results read visually by both the clinician and the laboratory technician and on an automated reader will be determined.

The concordance of band intensity results from the GIFT device and cytokine ELISA measurements will be analysed. This analysis will be used to optimize the cytokine concentration cut-offs for

interpretation of the GIFT device performance, in terms of sensitivity, specificity, and predictive values.

The impact of additional characteristics and/or determinants (such as intermediate microbiota, Candida, age, parity, and sexual activity) on the prediction of STI/BV status in women and the impact on the accuracy of the device will be determined.

Integration into care study

For the user experience study, iterative thematic analysis will be applied to the IDI and FGD data, conducted by the qualitative research team. Sekhon's framework of acceptability of healthcare interventions will be used [48].

For the DCE, data will be analysed in STATA using the utility function modelled from two alternatives: a systematic (explainable) component and a random (unexplainable) component. To estimate the trade-offs that respondents are willing to make between attributes, willingness to pay (WTP) for marginal improvements in attributes will be estimated for all attributes. WTP estimates will be calculated as the ratio of the coefficient of interest to the negative of the coefficient on the attribute with continuous variables. Subgroup analyses will be undertaken to investigate how preferences are influenced by demographic characteristics, residential area, socio-economic status, and experience of point-of-care testing.

The assessment of the decision tree classification algorithm will be based on the accuracy of the classification compared to the results of the gold standard methods (NAATs and Nugent score). Following this classification, different scenarios of management will be tested: immediate treatment, referral, re-evaluation, and no treatment. These scenarios will be optimised in order to avoid undertreatment and overtreatment by also considering immediate specific rapid diagnosis tests (if available) or risk scores for immediate presumptive treatment.

For the economic evaluation, models will be developed in Microsoft Excel. For the cost analysis, an ingredient-based model will be constructed, and for the budget impact analysis, an expenditure-based

model will be applied. A decision-analysis model to estimate the cost and health outcomes associated with different GIFT device implementation strategies will be developed. The main outcome will measure the effectiveness of each approach in correctly diagnosing an STI and/or BV in women, proxied by the sensitivity measure of the diagnostic test or approach, in comparison to the gold standard NAATs and Nugent scoring. Secondary outcomes such as the performance of the device versus syndromic management and the variation in device results when determined by a clinician, laboratory technician or an automated reader, will be assessed by means of scenario analyses. Univariate sensitivity analyses will be carried out to test the robustness of the findings.

Ethics and dissemination

The study will be carried out according to the principles stated in the Declaration of Helsinki (as amended in Seoul in 2008) and any further updates, all applicable national and international regulations, and according to the most recent applicable principles of the GCP-ICH E6. All the participants will be informed about the diagnostic results. The choice of treatment for STIs or BV will be based on the current national guidelines in the three study sites. The protocol and all study documents such as informed consent forms were reviewed and approved by the University of Cape Town Human Research Ethics Committee (HREC reference 366/2022), Medical Research Council of Zimbabwe (MRCZ/A/2966), Comité d'Ethique pour la Recherche Biomédicale de Madagascar (N° 143 MNSAP/SG/AMM/CERBM) and the London School of Hygiene and Tropical Medicine ethics committee (LSHTM reference 28046).

Findings will be reported to participants, collaborators and local government for the three sites, presented at national and international conferences and disseminated in peer-review publications.

Before the start, this study was submitted to the Clinicaltrials.gov public registry (NCT05723484).

Patient and public involvement

Before study initiation, we will engage with established local community advisory boards (CAB) or similar boards at each site. We will present the details of the study and ask for their input. According

to the specific procedures in place in each site, we may allow the CAB to comment on the written protocol, the flyers, and the posters. We will also ask the CAB for their feedback on the language used in the flyers, posters, and informed consent forms. During the study, the study investigators will regularly attend CAB meetings to update on the progress of the study. We will also ensure that the CAB and the community are the first to receive the results of the study.

Discussion

Here, we have described the diagnostic and integration studies that will be used to validate the GIFT device as a proof-of-principle prototype for implementing a novel POC cytokine test to detect vaginal inflammation associated with STIs and/or BV among women in differing clinical settings.

The development of low-cost rapid POC tests has become a major focus in the management of STIs/BV to replace or improve syndromic management widely adopted in Africa. The availability of these tests would offer all sexually active women the opportunity to be screened and treated. This may have particular benefit to women at high risk of STIs, including young women, pregnant women, female sex workers, and those with HIV.

The results from the prospective diagnostic study will be closely combined with the results of the qualitative research, modeling, and economic evaluation. The feasibility and acceptability studies will inform how the GIFT device could be integrated into national guidelines. The GIFT feasibility study consists of a “user experience and GIFT perceptions” prototype involving a large spectrum of people selected from the general population (HCPs, sexually active women, policymakers) that will help extend STI/BV screening into family planning services in primary health care services at affordable costs for LIMCs, with the aim that women should know about their health without delaying their treatment. The qualitative part of the study will improve our understanding of the different key factors contributing to the successful implementation of a novel screening device for STIs and BV in LMICs.

Besides evaluating a POC test for screening of STIs and BV, this study will be among the first of its kind, focusing on STI/BV prevalence and risk factors including up to 675 women mainly from urban areas in three sub-Saharan countries in different STI/BV contexts. The GIFT study will also improve our knowledge in both biological and molecular characterisation of the three most common STIs (CT, NG, and TV) and MG in association with the different states of the vaginal microbiota and will provide new insights into the interplay between STI and BV.

Our study protocol has some limitations. First of all, the user evaluation of the GIFT device, evaluating the performance of the device when applied by different types of users, does not include the evaluation of the extraction step. The extraction step of the vaginal swab will be performed by the medical staff only and not in parallel with a duplicate vaginal swab by a laboratory professional. Secondly, due to budget constraints, the STI diagnostic testing in Madagascar will be conducted by batch testing in the laboratory and not by POC NAAT testing as will be done in South Africa and Zimbabwe. Consequently, Malagasy participants will receive their results and treatment, if needed, after a delay of up to 10 days. This delay in treatment may facilitate further STI transmission. However, the study clinician may decide on presumptive treatment in alignment with the routine care practice in place.

In conclusion, the multidisciplinary study will be instrumental in developing strategies to improve STI and BV management in LMICs.

Study status

The study is ongoing. Data collection and data analysis are ongoing.

DECLARATIONS

Consent for publication

Not applicable

Availability of data and materials

Not applicable. The study is ongoing. Data collection and data analysis are ongoing.

Competing interests

The last authors, Jo-Ann Passmore and Lindi Masson, declare sharing a patent for the biomarkers for GIFT: patent number PCT/IB 2014/065740, October 2014. All other authors declared no potential conflict of interest.

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Author Contributions

Stéphanie Ramboarina and Tania Crucitti wrote the first draft of the manuscript. Lindi Masson designed Figure 1, Emma M Harding-Esch and Stéphanie Ramboarina designed Figure 2. All authors contributed to the writing, and reviewed and approved the manuscript. Stéphanie Ramboarina reformatted the manuscript. Lindi Masson and Jo-Ann Passmore validated the final version. All authors were involved in the conception of the study and contributed to the design of the study protocol. Tania Crucitti led the design of the diagnostic study with the participation of Katherine Gill Linda-Gail Bekker, Janneke H H M van de Wijgert, Aina Harimanana, Théodora Mayouya Gamana, Rindra Randremanana, Reziky Mangahasimbola, Chido Dziva Chikwar, Katarina Kranzer, Nicola Thomas, David Anderson, Fatime-Ramla Tanko, Monalisa Manhanzva, Micaela Lurie, Fezile Kumalo, Lindi Masson, Jo-Ann Passmore. Emma M Harding-Esch led the design of the integration into care study with the participation of Bich-Tram Huynh, Camille Fortas, Constance R S Mackworth-Young, Sarah Bernays, Edina Sinanovic, Ayako Honda, Suzanna Francis, Lindi Masson, Jo-Ann Passmore. The protocol development was facilitated by Tanya Pidwell; and validated by

Lindi Masson, Jo-Ann Passmore principal investigators of the study. All authors and all members of the study group contribute to the diagnostic and integration study.

References

1. Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, Chico RM, Smolak A, Newman L, Gottlieb S, Thwin SS, Broutet N, Taylor MM. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ. 2019 Aug 1;97(8):548-562P. doi: 10.2471/BLT.18.228486.

2. Tuddenham S, Ravel J, Marrazzo JM. Protection and Risk: Male and Female Genital Microbiota and Sexually Transmitted Infections. J Infect Dis. 2021;223 (12 Suppl 2):S222-S235. doi: 10.1093/infdis/jiaa762.

3. Van Gerwen OT, Muzny CA, Marrazzo JM. Sexually transmitted infections and female reproductive health. Nat Microbiol. 2022;7(8):1116-1126. doi: 10.1038/s41564-022-01177-x.

4. Ness RB, Hillier S, Richter HE, Soper DE, Stamm C, Bass DC, Sweet RL, Rice P. Can known risk factors explain racial differences in the occurrence of bacterial vaginosis? J Natl Med Assoc. 2003;95(3):201-12.

5. Yotebieng M, Turner AN, Hoke TH, Van Damme K, Rasolofomanana JR, Behets F. Effect of consistent condom use on 6-month prevalence of bacterial vaginosis varies by baseline BV status. Trop Med Int Health. 2009;14(4):480-6. doi: 10.1111/j.1365-3156.2009.02235.x.

6. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol. 2013;209:505–23.

7. Anahtar MN, Byrne EH, Doherty KE, Bowman BA, Yamamoto HS, Soumillon M, Padavattan N, Ismail N, Moodley A, Sabatini ME, Ghebremichael MS, Nusbaum C, Huttenhower C, Virgin HW, Ndung'u T, Dong KL, Walker BD, Fichorova RN, Kwon DS. Cervicovaginal bacteria are a major

modulator of host inflammatory responses in the female genital tract. Immunity. 2015;42(5):965-76. doi: 10.1016/j.immuni.2015.04.019.

8. Jespers V, van de Wijgert J, Cools P, Verhelst R, Verstraelen H, Delany-Moretlwe S, Mwaura M, Ndayisaba GF, Mandaliya K, Menten J, Hardy L, Crucitti T; Vaginal Biomarkers Study Group. The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of recent sex: a cross-sectional descriptive study across groups of African women. BMC Infect Dis. 2015;15:115. doi: 10.1186/s12879-015-0825-z.

9. Hickey RJ, Zhou X, Pierson JD, Ravel J, Forney LJ. Understanding vaginal microbiome complexity from an ecological perspective. Transl Res. 2012;160(4):267-82. doi: 10.1016/j.trsl.2012.02.008.

10. Peebles K, Velloza J, Balkus JE, McClelland RS, Barnabas RV. High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sex Transm Dis. 2019;46(5):304-311. doi: 10.1097/OLQ.0000000000000972.

11. Adapen C, Réot L, Menu E. Role of the human vaginal microbiota in the regulation of inflammation and sexually transmitted infection acquisition: Contribution of the non-human primate model to a better understanding? Front Reprod Health. 2022;4:992176. doi: 10.3389/frph.2022.992176.

12. Kyongo JK, Crucitti T, Menten J, Hardy L, Cools P, Michiels J, Delany-Moretlwe S, Mwaura M, Ndayisaba G, Joseph S, Fichorova R, van de Wijgert J, Vanham G, Ariën KK, Jespers V. Cross-Sectional Analysis of Selected Genital Tract Immunological Markers and Molecular Vaginal Microbiota in Sub-Saharan African Women, with Relevance to HIV Risk and Prevention. Clin Vaccine Immunol. 2015;22(5):526-38. doi: 10.1128/CVI.00762-14.

13. Doerflinger SY, Throop AL, Herbst-Kralovetz MM. Bacteria in the Vaginal Microbiome Alter the Innate Immune Response and Barrier Properties of the Human Vaginal Epithelia in a Species-Specific Manner. J Infect Dis. 2014;209 (12):1989–99. doi: 10.1093/infdis/jiu004

14. Masson L, Mlisana K, Little F, Werner L, Mkhize NN, Ronacher K, Gamieldien H, Williamson C, Mckinnon LR, Walzl G, Karim QA. Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study. Sex Transm infect. 2014;90(8):580-7.

15. Mtshali A, Ngcapu S, Mindel A, Garrett N, Liebenberg L. HIV susceptibility in women: The roles of genital inflammation, sexually transmitted infections and the genital microbiome. J Reprod Immunol. 2021;145:103291. doi: 10.1016/j.jri.2021.103291.

16. Masson L, Arnold KB, Little F, Mlisana K, Lewis DA, Mkhize N, Gamieldien H, Ngcapu S, Johnson L, Lauffenburger DA, Abdool Karim Q, Abdool Karim SS, Passmore JA. Inflammatory cytokine biomarkers to identify women with asymptomatic sexually transmitted infections and bacterial vaginosis who are at high risk of HIV infection. Sex Transm Infect. 2016;92(3):186-93.

17. Filardo S, Di Pietro M, Tranquilli G, Latino MA, Recine N, Porpora MG, Sessa R. 2019. Selected immunological mediators and cervical microbial signatures in women with *Chlamydia trachomatis* infection. mSystems 4:e00094-19. https://doi.org/10.1128/ mSystems.00094-19.

18. Jespers V, Kyongo J, Joseph S, Hardy L, Cools P, Crucitti T, Mwaura M, Ndayisaba G, Delany-Moretlwe S, Buyze J, Vanham G. A longitudinal analysis of the vaginal microbiota and vaginal immune mediators in women from sub-Saharan Africa. Sci. Rep. 2017;7(1):1-3.

19. Masson L, Barnabas S, Deese J, Lennard K, Dabee S, Gamieldien H, Jaumdally SZ, Williamson AL, Little F, Van Damme L, Ahmed K. Inflammatory cytokine biomarkers of asymptomatic sexually transmitted infections and vaginal dysbiosis: a multicentre validation study. Sex Transm Infect. 2019;95(1):5-12.

20. Galvin SR and Cohen MS. The role of sexually transmitted diseases in HIV transmission. Nat Rev Microbiol. 2004;2(1):33-42. doi: 10.1038/nrmicro794.

21. Borgdorff H, Tsivtsivadze E, Verhelst R, Marzorati M, Jurriaans S, Ndayisaba GF, Schuren FH, van de Wijgert JH. Lactobacillus-dominated cervicovaginal microbiota associated with reduced

HIV/STI prevalence and genital HIV viral load in African women. ISME J. 2014;8(9):1781-93. doi: 10.1038/ismej.2014.26.

22. Masson L, Passmore JA, Liebenberg LJ, Werner L, Baxter C, Arnold KB, Williamson C, Little F, Mansoor LE, Naranbhai V, Lauffenburger DA. Genital inflammation and the risk of HIV acquisition in women. Clin. Infect. Dis. 2015;61(2):260-9.

23. McClelland RS, Lingappa JR, Srinivasan S, Kinuthia J, John-Stewart GC, Jaoko W, et al. Evaluation of the association between the concentrations of key vaginal bacteria and the increased risk of HIV acquisition in african women from five cohorts: a nested case-control study. Lancet Infect Dis. 2018;18:554–64. doi: 10.1016/S1473-3099(18)30058-6

24. Jarrett OD, Srinivasan S, Richardson BA, Fiedler T, Wallis JM, Kinuthia J, et al. Specific vaginal bacteria are associated with an increased risk of trichomonas vaginalis acquisition in women. J Infect Dis. 2019;220:1503–10. doi: 10.1093/infdis/jiz354.

25. McKinnon LR, Achilles SL, Bradshaw CS, et al. The evolving facets of bacterial vaginosis: implications for HIV transmission. AIDS Res Hum Retroviruses **2019**; 35:219–28.

26. GBD 2015 HIV collaboarators, Lancet HIV 2016; 3: e361–87. http://dx.doi.org/10.1016/ S2352-3018(16)30087-X.

27. Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, et al. The Vaginal Microbiome and Preterm Birth. Nat Med. 2019;25(6):1012–21. doi: 10.1038/s41591-019-0450-2.

28. Guidelines for the management of symptomatic sexually transmitted infections. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

29. Verwijs MC, Agaba SK, Sumanyi JC, Umulisa MM, Mwambarangwe L, Musengamana V, Uwineza M, Cuylaerts V, Crucitti T, Jespers V, Van De Wijgert JH. Targeted point-of-care testing compared with syndromic management of urogenital infections in women (WISH): a cross-sectional screening and diagnostic accuracy study. Lancet Infect Dis. 2019;19(6):658-69.

30. Garrett N, Mtshali A, Osman F, Masson L, McKinnon LR, Singh R, Mitchev N, Ngobese H, Kharsany ABM, Abdool Karim S, Mlisana K, Passmore JA, Rompalo A, Mindel A, Liebenberg L.

Impact of point-of-care testing and treatment of sexually transmitted infections and bacterial vaginosis on genital tract inflammatory cytokines in a cohort of young South African women. Sex Transm Infect. 2021;97(8):555-565. doi: 10.1136/sextrans-2020-054740.

31. Asare K, Andine T, Naicker N, Dorward J, Singh N, Spooner E, Andriesen J, Osman F, Ngcapu S, Vandormael A, Mindel A, Abdool Karim SS, Bekker LG, Gray G, Corey L, Tomita A, Garrett N. Impact of Point-of-Care Testing on the Management of Sexually Transmitted Infections in South Africa: Evidence from the HVTN702 Human Immunodeficiency Virus Vaccine Trial. Clin Infect Dis. 2023;76(5):881-889. doi: 10.1093/cid/ciac824. Erratum in: Clin Infect Dis. 2023;76(1):182.

32. Adamson PC, Loeffelholz MJ, Klausner JD. Point-of-Care Testing for Sexually Transmitted Infections: A Review of Recent Developments. Arch Pathol Lab Med. 2020;144(11):1344-1351.

33. The diagnostics landscape for sexually transmitted infections. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

34. Land, K.J., Boeras, D.I., Chen, XS. *et al.* REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol* **4**, 46–54 (2019). https://doi.org/10.1038/s41564-018-0295-3

35. Torrone EA, Morrison CS, Chen P-L, Kwok C, Francis SC, Hayes RJ, et al. (2018) Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. PLoS Med. 2018;15(2): e1002511.

36. Wand H, Reddy T, Dassaye R, Moodley J, Naidoo S, Ramjee G. Estimating prevalence and incidence of sexually transmitted infections among South African women: Implications of combined impacts of risk factors. Int J STD AIDS. 2020;31(11):1093-1101. doi: 10.1177/0956462420915388.

37. Delany-Moretlwe S, Mgodi N, Bekker L, et al. High prevalence and incidence of gonorrhoea and chlamydia in young women eligible for HIV pre-exposure prophylaxis in South Africa and Zimbabwe: results from the HPTN 082 trial. Sex Transm Infect. 2023;99:433-439.

38. Behets F, Andriamiadana J, Rasamilalao D, Ratsimbazafy N, Randrianasolo D, Dallabetta G, Cohen M. Sexually transmitted infections and associated socio-demographic and behavioural factors in women seeking primary care suggest Madagascar's vulnerability to rapid HIV spread. Trop Med Int Health. 2001;6(3):202-11.

39. Country factsheets, UNAIDS 2023. https://www.unaids.org/en/regionscountries/countries/madagascar.

40. Nugent RP, Krohn MA, Hillier SL.Reliability of diagnosing bacterial vaginosisis improved by a standardized method of gram stain interpretation. J Clin Microbiol. 1991;29:297e301.

41. Dworkin, S.L. specimen Size Policy for Qualitative Studies Using In-Depth Interviews. Arch Sex Behav 2012; 41, 1319–1320.

42. Guest G, Namey E, McKenna K. How Many Focus Groups Are Enough? Building an Evidence Base for Nonprobability specimen Sizes. Field Methods. 2017;29(1):3-22.

43. Louviere J, Hensher D, Swait J: Stated Choice Methods: Analysis and Application, First edition edn. Cambridge, United Kingdom: Cambridge University Press; 2000.

44 Ryan M, Gerard K, Amaya-Amaya M (eds.): Using Discrete Choice Experiments to Value Health and Health Care. Dordrecht, the Netherlands: Springer; 2008.

45. PRESTO CT/NG combined qualitative real time CT/NG assay (Kit # GM CG 160500). Goffin Molecular Technologies, the Netherlands

46. Crucitti T, Van Dyck E, Tehe A, Abdellati S, Vuylsteke B, Buve A, Laga M. Comparison of culture and different PCR assays for detection of Trichomonas vaginalis in self collected vaginal swab specimens. Sex Transm Infect. 2003 Oct;79(5):393-8. doi: 10.1136/sti.79.5.393. PMID: 14573835; PMCID: PMC1744760.

47. Müller EE, Venter JM, Magooa MP, Morrison C, Lewis DA, Mavedzenge SN. Development of a rotor-gene real-time PCR assay for the detection and quantification of Mycoplasma genitalium. J Microbiol Methods. 2012 Feb;88(2):311-5. doi: 10.1016/j.mimet.2011.12.017. Epub 2011 Dec 29. PMID: 22230235.

48. Sekhon, M., Cartwright, M. & Francis, J.J. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. BMC Health Serv Res. 2017;17, 88. https://doi.org/10.1186/s12913-017-2031-8.

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Figure Legend

Figure 1: Schematic of the GIFT diagnostic study

The recruited women who meet the inclusion criteria and consent to participate in the GIFT study will be administered a questionnaire. Data will be recorded on case report forms (CRF) in a paper or electronic (tablet) format by the clinical nurse followed by a gynecological exam. Vaginal swabs are collected: the first collected swab will be used for two GIFT assays: the first GIFT will be performed at the bedside by the midwife/nurse and the second GIFT by the lab technician. Each of them will read visually their own performed GIFT (naked eye reading). The two respective GIFT assays will then be read using a lateral flow automated reader. The other collected swabs will be used for different laboratory assays for the evaluation of the GIFT performance. HIV testing on fingerpick blood will be also included at the end of the medical exam. The figure was created with BioRender.

Figure 2: Integration study activities

Description of the four activities that will be performed for the GIFT integration into care study for improving the STI/BV diagnosis and control in women: 1) user experiences and/or perceptions of the

GIFT device involving qualitative focus group discussions and in-depth interviews with key stakeholders, 2) discrete choice experiments, 3) development of a decision tree classification algorithm and 4) economic evaluation of defined management algorithms. In addition to the women already participating to the GIFT diagnostic study, health care professionals (HCP), policy makers, key opinion leaders and patients coming for consultation will be included in the study.

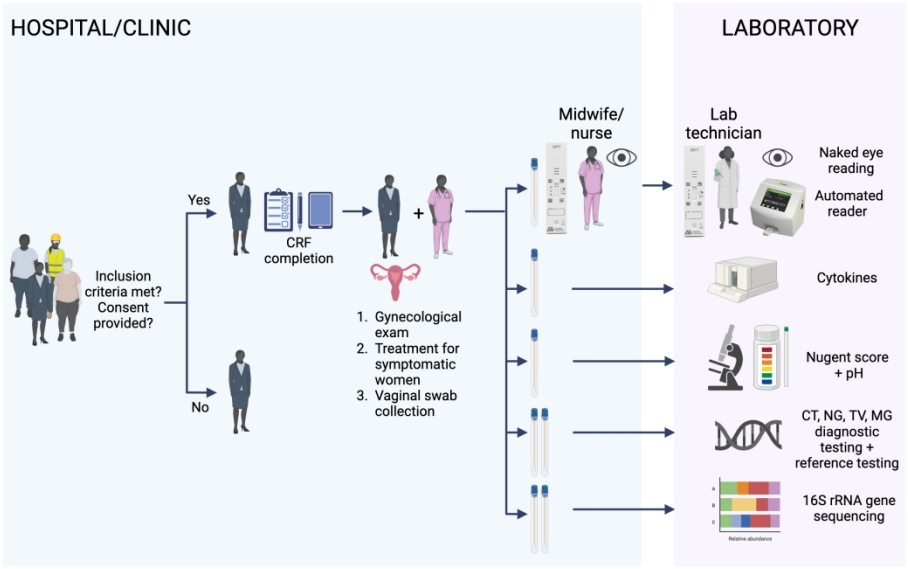
Additional files

Supplementary figure 1 : Diagnostic study flow steps

Description: Women attending family planning services will receive explanations on the GIFT study. After written consent and validation of the eligibility criteria and written informed consent, the enrolled woman will be subjected to a structured questionnaire. The data will be captured on paper or electronic case report form (CRF) by the clinical nurse. The CRF consists of 8 modules that will include all the information from sociodemographic information, behavioral risk assessment to medical information collected during the gynecological examination by the midwife or nurse.

Each CRF assigned to each participant will contain all the data collected through the diagnostic study including laboratory results that will be recorded in the REDCap GIFT database.

The vaginal swab sample collection will be done by the midwife/nurse after gynecological examination for : GIFT assays, pH measurement and microscopic analysis of a vaginal smear slide for BV diagnosis, and STI diagnosis. For this latter, a couple of vaginal swabs will be used for nucleic-acid amplification tests (NAAT) for CT, NG and TV to be performed locally on each study site. Based on the on-site positive diagnostic results for CT, NG, TV and BV a treatment will be offered by the clinic or hospital clinician to the participant. HIV will be tested on fingerprick blood.



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USER EXPERIENCES AND GIFT PERCEPTIONS
DISCRETE CHOICE EXPERIMENTS (DCE)
DECISION TREE
ECONOMIC EVALUATION
• Research questions
- how HCPs would use the GIFT device?
- where the GIFT device would be placed within a health facility
- how it would fit into the clinic flow?
- What are the setting-specific barriers to and facilitators of using the GIFT device for the patient?
- What counseling advice would be needed when using the GIFT device?
• Target groups
- Health care professionals
- Policy makers
- Key opinion leaders
- Patients
• Research questions
- What are the user (patient) preferences for a patient management algorithm?
- What is the preference heterogeneity across users?
- Willingness to pay?
• Target groups
- Women participant to the diagnostic study
- Women non included in the diagnostic study
• Research questions
- What is the risk profile of women who test positive on the GIFT test?
- How do different testing strategies (immediate treatment after GIFT positive vs further re-evaluation) affect outcomes?
- If immediate treatment, which treatment would yield the best outcome?
• Research questions
- What are the costs and budget impact of the identified screening or diagnostic algorithm with the GIFT device?
- What are the costs of the different algorithms and strategies for GIFT device integration?
INTEGRATION OF GIFT INTO STI/BV CONTROL

Explanation about the study to women attending family planning

- Verification of inclusion / exclusion criteria
- Consent signature
- Collection of urine sample for pregnancy test

Questionnaire : **Case Report Form (CRF)**

- Module 1 : Screening and Enrollment
- Module 2 : Sociodemographic Information
- Module 3 : Behavioral Risk Assessment
- Module 4 : General health and medical history including medication, smoke and alcohol
- Module 5 : Pregnancy and Contraception History
- Module 6 : HIV testing/status and treatment
- Module 7 : Gynaecological Physical Exam
- Module 8 : Sample collection and results

- Vaginal swabs sample collection
- pH and smear slide
- GIFT Test near the patient
- Finger prick blood sample collection for HIV test

- GIFT test in the laboratory -Test reading using a lateral flow test automated reader
- STI/BV tests : NAAT for CT/NG/TV
- Nugent scoring
- HIV test

- Treatment according syndromic approach OR appreciation of clinician
- Treatment of the participant and partners
- If positive HIV test : refer the participant to clinician

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1 Performance includes sensitivity, specificity, PPV, NPV, accuracy
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	4-5
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	6-8
	4	Study objectives and hypotheses	8-10; Table 1
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	10-13
Participants	**6**	Eligibility criteria	13-14; Table 2
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	12-14
	8	Where and when potentially eligible participants were identified (setting, location and dates)	12-14
	9	Whether participants formed a consecutive, random or convenience series	12-14, fig 1
Test methods	**10a**	Index test, in sufficient detail to allow replication	Not assigned as it is a study protocol, will be detailed in the paper reporting the results
	10b	Reference standard, in sufficient detail to allow replication	19, more details will be included in the paper reporting the results
	11	Rationale for choosing the reference standard (if alternatives exist)	20 - 21, 22
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	21, more details will be included in the paper reporting the results

	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	21, more details will be included in the paper reporting the results
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	19, reference performed later on and elsewhere than the study site
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	19
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	21-22
	15	How indeterminate index test or reference standard results were handled	Not assigned as it is a study protocol
	16	How missing data on the index test and reference standard were handled	Not assigned as it is a study protocol
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Not assigned as it is a study protocol
	18	Intended sample size and how it was determined	14-15
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Fig 1
	20	Baseline demographic and clinical characteristics of participants	Not assigned as it is a study protocol
	21a	Distribution of severity of disease in those with the target condition	Not assigned as it is a study protocol
	21b	Distribution of alternative diagnoses in those without the target condition	Not assigned as it is a study protocol
	22	Time interval and any clinical interventions between index test and reference standard	Not assigned as it is a study protocol
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Not assigned as it is a study protocol
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Not assigned as it is a study protocol
	25	Any adverse events from performing the index test or the reference standard	Not assigned as it is a study protocol
DISCUSSION			

	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	24
	27	Implications for practice, including the intended use and clinical role of the index test	23-24
OTHER INFORMATION			
	28	Registration number and name of registry	5, 23
	29	Where the full study protocol can be accessed	clinicaltrials.gov public registry (NCT05723484)
	30	Sources of funding and other support; role of funders	27

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.