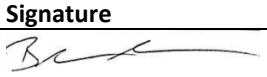
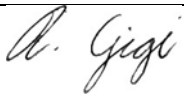
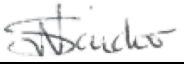
Clinical Performance Study Plan (CPSP)

	Name	Function	Date	Signature
Author	Birgitta Gleeson	Senior Scientist, FIND	08/08/2024	
Reviewer	Dr Ranjana Gigi	Medical oversight	28/10/2024	
Reviewer				
Approver	Mandisa Mdingi	Principal Investigator	28/10/2024	
Approver	Dr Sarita Naidoo	Sub-Investigator	15/04/2025	

Approval

Revision history

Version	Effective Date	Description of change	Author	Approver
1.0	22/10/2024			
1.1	15/04/2025	Any samples that have not been transferred to. the University of Pretoria will only be shipped to the FIND biobank in the United States upon receipt of approval from the Faculty of Health Sciences Research Ethics Committee and following the execution of a Material Transfer Agreement (MTA) between FPD and FIND, which will be submitted to the Faculty of Health Sciences Research Office. Change of study staff New Sub-Investigator: Dr Sarita Naidoo, FPD Previous Sub-Investigator: Dr Cecilia Ferreyra, FIND	Mandisa Mdingi, FPD	Birgitta Gleeson, FIND

Clinical Performance Study Plan (CPSP)

Evaluation of performance, acceptability and usability of a novel lateral flow assay for point-of-care detection of Neisseria gonorrhoeae infection among pregnant and symptomatic women in South Africa.

Type of performance study:	In vitro diagnostic (IVD) device clinical performance study
Study Design Type:	×Observational study X Cross-sectional design ☐ Longitudinal design ☐ Retrospective design ☐ Prospective design ☐ Prospective-retrospective design ☐ Interventional Study ☐ Using leftover/archived specimens ☐ Requiring surgically invasive sample-taking ☐ Involving additional invasive procedure
Registration:	clinicaltrials.gov
Reference number/Identifier:	AM007
Principal Investigator(s):	PI - Mandisa Mdingi Research Manager Foundation for Professional Development (FPD), Research Unit East London Office, 16 Surrey Road, Vincent, East London, South Africa mandisam@foundation.co.za Sub-I – Dr Sarita Naidoo Head of Department Foundation for Professional Development (FPD), Research Unit East London Office, 16 Surrey Road, Vincent, East London, South Africa saritan@foundation.co.za
Coordinating Principal Investigator	NA
Sponsor	**Foundation for Professional Development (FPD),** Knowledge Park, 173 Mary Road, The Willows, Pretoria FPD Research Unit East London Office, 16 Surrey Road, Vincent, East London, South Africa
IVD device:	NG LFA and NG LFA reader FIND developed prototype NG LFA and reader Reader Software version – FIND-Reader-beta-1.2.1
Manufacturer of the IVD device:	DCN Dx (No commercial manufacturer to date)

CPSP Version and Date:	Version 1.1 15/04/2025

CONFIDENTIALITY STATEMENT

The information contained in this document, especially unpublished data, is confidential and the property of FPD (e.g. the Sponsor). The information may not – in whole or in part – be transmitted, reproduced, published or disclosed to others than the applicable Competent Ethics Committee(s) and Competent Authority(ies) without prior written authorisation from FPD (e.g. the Sponsor) except to the extent necessary to obtain informed consent from those who will participate in the investigation.

SUMMARY OF THE REVISION HISTORY IN CASE OF AMENDMENTS

Version	Effective Date	Description of change	Reason for the change
1.0	22/10/2024		
1.1	15/04/2025	Any samples that have not been transferred to. the University of Pretoria will only be shipped to the FIND biobank in the United States upon receipt of approval from the Faculty of Health Sciences Research Ethics Committee and following the execution of a Material Transfer Agreement (MTA) between FPD and FIND, which will be submitted to the Faculty of Health Sciences Research Office. Change of study staff- New Sub-Investigator: Dr Sarita Naidoo, FPD Previous Sub-Investigator: Dr Cecilia Ferreyra	As per South African Good Clinical Practice, no samples may be shipped outside the country or shared with other researchers, if not approved by the Faculty of Health Science Research Ethics Committee and a signed MTA submitted to the Faculty of Health Science Research Ethics Committee. Both organisations, FIND and FPD agreed to have local Sub-Investigator

SIGNATURE PAGE(S)

ID number of the clinical performance study	AM007
Title	Evaluation of performance, acceptability and usability of a novel lateral flow assay for point-of-care detection of Neisseria gonorrhoeae infection among pregnant and symptomatic women in South Africa

Sponsor:

The Sponsor representatives and FIND have developed, reviewed, and approved the CPSP version 1.0 22/10/2024,and confirm hereby to oversee and coordinate the investigation according to the CPSP, the current version of the World Medical Association Declaration of Helsinki, the CIOMS International Guidelines for Health Research Involving Human Participants, ISO 20916 norm, ICH-GCP when applicable, and the applicable local regulatory requirements.

Primary contact person: Mandisa Mdingi

East London, 28 October 2024 — Signature

Place/Date

Medical Lead: Dr Ranjana Gigi

East London, 28 October 2024 — Signature

Place/Date

Sub investigator: Dr Sarita Naidoo

East London, 15 April 2025 — Signature

Place/Date

Principal Investigator: **Mandisa Mdingi**

I have read and understood this CPSP version 1.1 15/04/2025 and agree to conduct the investigation according to the CPSP, the current version of the World Medical Association Declaration of Helsinki, the CIOMS International Guidelines for Health Research Involving Human Participants, ISO 20916 norm, ICH-GCP when applicable, and the local legally applicable requirements.

East London, 15 April 2025

Place/Date

Signature

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SYNOPSIS

Title:	**Evaluation of performance, acceptability and usability of a novel Lateral Flow Assay (LFA) for point-of-care (POC) detection of *Neisseria gonorrhoeae* (NG) infection among pregnant and symptomatic women in South Africa.**
Short title / Study ID:	GO SA
CPSP, version and date:	Version 1.1 15/04/2025
Registration:	clinicaltrials.gov
Sponsor	FPD
Intervention:	Non-interventional
Name of the IVD device	FIND developed prototype NG LFA and NG LFA reader Reader Software version – FIND-Reader-beta-1.2.1
Stage of development:	Design frozen IVD
Background and rationale:	Gonorrhoea remains a significant public health challenge globally, particularly in South Africa, where it is considered endemic. Untreated gonorrhoea, which is now becoming more resistant to antibiotics, can lead to severe complications, including pelvic inflammatory disease, infertility, adverse pregnancy outcomes, and increased susceptibility to HIV. Pregnant women are especially vulnerable as untreated gonorrhoea can result in miscarriage, preterm birth, and neonatal infection. Early and accurate diagnosis is crucial for timely treatment and the prevention of these adverse outcomes. Nucleic acid amplification tests (NAATs) are considered the most reliable diagnostic tool for gonorrhoea. However, their high cost, long turnaround time and demanding infrastructure requirements often render them inaccessible in resource-constrained environments. The NG LFA, a rapid, point-of-care test developed by FIND, offers a potential solution for these settings. Previous NG LFA performance studies have shown promising results, with a sensitivity of 80% in asymptomatic women in East London[1]. However, a study in Zimbabwe found a lower sensitivity of 65% among pregnant women attending Antenatal Care (ANC). This discrepancy raises questions about the LFA's performance in this specific population and setting. The observed difference in sensitivity between asymptomatic women and pregnant women highlights the need to investigate the LFA's performance, specifically in the ANC population in East London. It has been hypothesised that pregnancy may induce physiological changes that affect the test's accuracy. Therefore, further investigation is needed to confirm this potential impact. If the NG LFA proves reliable and accurate in this setting, its widespread implementation in ANC clinics could significantly improve case detection and treatment rates, ultimately reducing the burden of gonorrhoea in the community.

	This study will also investigate the potential for self-collected samples. Self-collection offers several benefits, such as increased privacy and convenience, which could encourage more women to get tested. Additionally, it may reduce the workload for healthcare providers, especially in areas with limited resources. We will evaluate the feasibility and accuracy of self-collected samples compared to those collected by clinicians in the ANC population and symptomatic women who may prefer this method.
Objective(s):	Primary Objective 1. To determine the diagnostic accuracy of the NG LFA for the detection of NG in provider-collected vaginal samples from pregnant women attending ANC when compared to Xpert® CT/NG as the reference standard. Secondary Objectives 2. To determine the diagnostic accuracy of the NG LFA for the detection of NG in vaginal provider-collected samples among symptomatic non-pregnant women. 3. To compare the diagnostic accuracy of the NG LFA for the detection of NG in vaginal self-collected and provider-collected samples from pregnant women attending ANC. 4. To compare the diagnostic accuracy of the NG LFA for the detection of NG in vaginal self-collected and provider-collected samples from symptomatic non-pregnant women. 5. To compare the safety of self-collection of vaginal swabs vs provider collection among pregnant women attending ANC and symptomatic non-pregnant women. 6. To assess healthcare workers' acceptability, usability, and preferences regarding the NG LFA systems integration compared to the reference method. 7. To assess the acceptability and preference for self-collected vaginal samples compared to provider-collected samples among pregnant ANC attendees and symptomatic non-pregnant women and healthcare workers.
Outcome(s) / endpoint(s)	Primary endpoints – 1. Point estimates of sensitivity and specificity with 95% confidence intervals (Wilson's score method) among pregnant ANC attendees. Secondary endpoints - 2. Point estimates of sensitivity and specificity with 95% confidence intervals (Wilson's score method) among symptomatic non-pregnant women. 3. Point estimates of the percentage differences with 95% confidence intervals (Tango's score method) of the diagnostic performance metrics between self-collected and provider-collected vaginal samples for the detection of NG in pregnant ANC attendees. 4. Point estimates of the percentage differences with 95% confidence intervals (Tango's score method) of the diagnostic performance metrics between self-

	collected and provider-collected vaginal samples for the detection of NG in non-pregnant symptomatic women. 5. The number of adverse events reported following self-collection of vaginal swabs or collection by health service providers among pregnant women attending ANC and symptomatic non-pregnant women. 6. Mixed methods: Acceptability; distribution of acceptability questionnaire scores; usability; distribution of System Usability Scores; perceptions on acceptability, usability, and preferences of systems integration from qualitative semi-structured interviews and focus group discussions. 7. Mixed methods: Distribution of acceptability questionnaire score, perceptions on acceptability and preference from qualitative focus group discussions. The proportion of participants in each group who prefer self-collected samples over provider-collected samples.
Study Design Type and Rationale:	This is a cross-sectional, single time-point study with a paired design to determine the diagnostic accuracy of the NG LFA against the Xpert® CT/NG as a reference standard in vaginal samples collected from pregnant women attending ANC and to compare the accuracy and safety of self-collected and provider-collected samples in both non-pregnant symptomatic and pregnant ANC attendees. This design is appropriate as it allows for a direct comparison of the NG LFA's performance to the established gold standard in a real-world clinical setting. The inclusion of both sample types allows for an evaluation of the effect of the collection method on test performance, safety and acceptability.
Specimen type	High vaginal swabs (provider collected and self-collected)
Reference device	Xpert® CT/NG, which is the established gold standard for NG diagnosis on clinician-collected vaginal samples
Participants Inclusion/exclusion criteria:	**ANC population** **Inclusion -** • Women age ≥18 years. • Pregnant women. • Attending a study site for antenatal care. • Willingness to participate and signed informed consent form (ICF). **Exclusion -** • Self-reported use of antimicrobial therapy within 21 days preceding enrolment. • Use of vaginal douche or vaginal product in the previous 24 hours • Unable to provide specimens for testing. • A medical condition, serious illness, or other condition that could interfere with study procedures or jeopardise participant safety. **Symptomatic population** **Inclusion -** • Non pregnant women age ≥18 years.

<table>
<tr><td></td><td>

- Diagnosis of VDS. Diagnosis of VDS will be based on self-reported presence of symptoms consistent with VDS, such as increased or abnormal vaginal discharge.
- Willingness to participate and sign ICF.

Exclusion -

- Self-reported use of antimicrobial therapy within 21 days preceding enrolment.
- Use of vaginal douche or vaginal product in the previous 24 hours
- Unable to provide specimens for testing.
- A medical condition, serious illness, or other condition that could interfere with study procedures or jeopardise participant safety.

<u>Healthcare workers population</u>

Systems Usability Score Survey & TFA Acceptability Survey

- HCWs using the NG LFA during month 3 of the study.

Focus Group Discussions (FGD)

- HCWs actively involved in conducting the study

Focus Group Discussions (FGD) - Non-Study Participants

- HCWs working at the study site but NOT directly involved in the NG LFA study procedures.

</td></tr>
<tr><td>Measurements and procedures:</td><td>

Three provider-collected and one self-collected swab will be obtained from each study participant on day 1 for each of the following procedures -

Provider-collected:

1. NG LFA
2. Xpert® CT/NG
3. Discordance testing/storage

Self-collected:

1. NG LFA

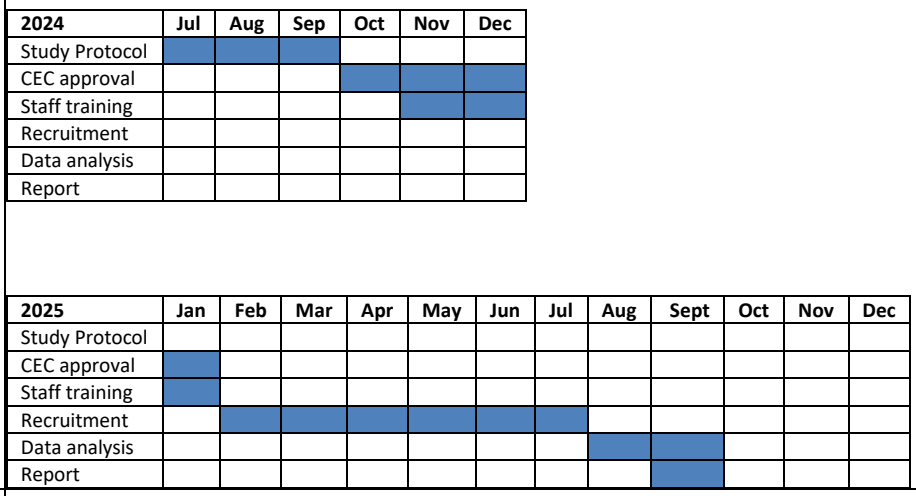
	GeneXpert	NG LFA	Storage/ Discordance
Provider-collected	**X**	**X**	**X**
Self-collected		**X**	

Operational research procedures:

The Theoretical Framework of Acceptability (TFA) acceptability survey relating to self-sampling among participants who are undergoing the sampling procedures will be administered after the sample collection procedures are completed.

Systems usability score survey and the TFA acceptability survey relating to the NG LFA will be administered to all healthcare workers who are manipulating the NG LFA at month 3 (projected mid-point of participant enrolment).

</td></tr>
</table>

	The TFA acceptability survey among healthcare workers regarding self-sampling will be administered to all healthcare workers conducting study-related testing procedures at month 3 (the projected mid-point of participant enrolment). At month 5, qualitative methods will be conducted among healthcare workers who are conducting the study and healthcare workers who work at the clinics where the study is taking place.
Number of participant participants/ specimens	**ANC – 778** **Symptomatic – 461** **Operational research:** All participants undergoing the testing procedure All healthcare workers who conduct the NG LFA testing 12 to 20 healthcare workers at the primary healthcare level who are not conducting the NG LFA testing
Overall study duration and duration for the individual participants:	Six months. The study is a cross-sectional study with a single time point.
Performance study schedule:	
Investigator(s)	Mandisa Mdingi – FPD Dr Sarita Naidoo – FPD
Study Site(s):	Multicentre performance study in five FPD satellite clinical sites in East London. **Clinal site locations in East London, South Africa -** 1. Nontyatyambo CHC, 15 Dunga Road, Nu 2, Mdantsane, 5219 2. Empilweni Gompo CHC, Dunoon Road, East London, 5209 3. Duncan Village Day Hospital, 24 Phoenix Street, Braelyn, East London, 5201 4. Grey Gateway Clinic, Lonsdale Road, King William's Town, 5601 5. Ndevana Clinic, Ndevana location, Zwelitsha, 5608

Statistical considerations, incl. rationale for sample size.	Diagnostic accuracy metrics will be estimated by comparing index test results against Xpert® CT/NG results from clinician-collected vaginal samples. Point estimates of sensitivity and specificity will be calculated as TP/(TP+FN) and TN/(TN+FP), respectively, with 95% CI using Wilson's Score. Tango's score method will be used to calculate 95% CIs while calculating paired differences. McNemar test will be used to compare sensitivities/specificities. In the ANC population, to estimate a sensitivity of 80% ±15% and a specificity of 98.1% ±5%, with alpha 5% and 80% power, 56 confirmed positives and 59 confirmed negatives are needed, respectively. To detect a difference <5% between the sensitivity/specificity of clinician-collected and self-collected samples with the assumption that the observed difference will be 1 % for both sensitivity/specificity, with alpha 5% and power 80%, 39 positive and 39 negative paired samples are needed. To obtain 56 confirmed positives in the population with a prevalence estimate of 8% and a power of 80%, 778 participants are needed to be screened. In the symptomatic population, to detect a difference <5%, with the assumption that the observed difference will be 1.5%, alpha = 5%, power 80%, 76 confirmed positive and negative pairs are needed. With a prevalence estimate of 18% and a power of 80%, 461 participants must be screened.
Compliance statement:	This performance study will be conducted in compliance with the CPSP, the current version of the Declaration of Helsinki, the CIOMS International Guidelines for Health Research Involving Human Participants, standard ISO 20916: 2019, ICH-GCP (as far as applicable), and all national regulatory requirements.

ABBREVIATIONS

AE	Adverse Event
ADE	Adverse Device Effect
AMR	Antimicrobial Resistance
ANC	Antenatal Care
ASADE	Anticipated Serious Adverse Device Effect
ASR	Annual Safety Report
CA	Competent Authority
CEC	Competent Ethics Committee
CPSP	Clinical performance study plan
CPSR	Clinical performance study report
CRF	Case Report Form (pCRF paper CRF; eCRF electronic CRF)
CT	*Chlamydia Trachomatis*
DD	Device Deficiency
DMC / DSMC	Data Monitoring Committee, Data Safety Monitoring Committee
FGD	Focus group discussion
FPD	Foundation for Professional Development
HCW	Healthcare worker
Ho, H1	Null hypothesis, Alternative hypothesis
ICF	Informed Consent Form
ICH-GCP	International Council for Harmonisation – guidelines of Good Clinical Practice
IEC	Independent Ethics Committees
IFU	Instructions For Use
IRB	Institutional Review Boards
ISF	Investigator Site File
ISO	International Organisation for Standardisation
ITT	Intention to treat
IVD	In Vitro Diagnostic
IVDR	In Vitro Diagnostic Device Regulation (EU) 2017/746 of 5 April 2017
MUS	Male Urethritis Syndrome
NAAT	Nucleic Acid Amplification Test
NG	*Neisseria gonorrhoeae*
NG LFA	*Neisseria gonorrhoeae* Lateral Flow Assay
PI	Principal Investigator

PMPF	Post-Market Performance Follow-up
POC	Point of Care
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SDV	Source Data Verification
SOP	Standard Operating Procedure
TFA	Theoretical Framework of Acceptability
STI	Sexually Transmitted Infection
USADE	Unanticipated Serious Adverse Device Effect
VDS	Vaginal Discharge Syndrome
WHO	World Health Organisation

INVESTIGATION SCHEDULE

Study Periods	Study visit	Notes
Visit	1	
Participant information	X	
Participant consent	X	
Demographics	X	
Medical history	X	
Routine genital examination	X	
Self-collected sample (1)	X	High vaginal swab
Provider-collected sample (3)	X	High vaginal swabs
NG LFA testing	X	
Xpert® CT/NG testing	X	
Storage of swab	X	
Treatment	X	
Adverse Events	X	
Routine STI counselling	X	
Partner notification leaflet	X	

1 INVESTIGATION ADMINISTRATIVE STRUCTURE

1.1 Sponsor

The Sponsor of this clinical performance study is:
Foundation for Professional Development (FPD)

1.2 Principal Investigator(s) and Study site(s)

Mandisa Mdingi
Research Manager, Foundation for Professional Development (FPD), East London Office, 16 Surrey Road, Vincent, East London, South Africa
mandisam@foundation.co.za

Clinical site locations in East London, South Africa -

1. Nontyatyambo CHC, 15 Dunga Road, Nu 2, Mdantsane, 5219
2. Empilweni Gompo CHC, Dunoon Road, East London, 5209
3. Duncan Village Day Hospital, 24 Phoenix Street, Braelyn, East London, 5201
4. Grey Gateway Clinic, Lonsdale Road, King William's Town, 5601
5. Ndevana Clinic, Ndevana location, Zwelitsha, 5608

1.3 Biostatistician

The following statistician/biostatistician will be involved in the clinical performance study:

Mikaela Watson, Associate, Biostatistician, FIND, Chem. du Pommier 40, 1218 Le Grand-Saconnex, Geneva, Switzerland
mikaela.watson@finddx.org

1.4 Medical oversight

Dr Ranjana Gigi, Physician, Foundation for Professional Development (FPD), East London Office, 16 Surrey Road, Vincent, East London, South Africa
ranjanag@foundation.co.za

1.5 Laboratory (for discordance testing)

The following laboratories will be involved in the clinical performance study:

Dr. Hyunsul Jung, Prinshof Campus, Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria.
wjdgustmf@gmail.com

1.6 Monitoring institution

Clinical Trials Unit, Clinical Affairs, FIND, Chem. du Pommier 40, 1218 Le Grand-Saconnex, Geneva, Switzerland

1.7 Data Safety Monitoring Committee and the Trial Steering Committee

This protocol, originally developed by FIND, outlines the procedures for evaluating the performance, acceptability and usability of a novel lateral flow assay for point-of-care detection of *Neisseria gonorrhoeae* infection among pregnant and

symptomatic non-pregnant women in South Africa. While FIND initiated the study design and protocol development, sponsorship has been transferred to FPD. This transition entails a complete handover of responsibilities, including ethical oversight, data management, and regulatory compliance. FIND will provide strategic oversight and guidance on trial-related issues by participating in the Trial Steering Committee (TSC) and Data and Safety Monitoring Board (DSMB).

The TSC will ensure the trial is conducted according to the protocol, contractual agreements, and applicable regulatory standards, including ICH-GCP and local regulations. The TSC will monitor trial progress, including recruitment, data collection, and analysis and will be responsible for reviewing and endorsing any proposed protocol amendments or changes in trial procedures. Additionally, the TSC will review results and provide guidance on the continuation, modification, and termination of the trial. It will also oversee the dissemination and publication of results. The TSC will comprise representatives from FIND, Sponsor, Investigators, statisticians, and independent experts. A charter outlining the TSC's roles, responsibilities, frequency of meetings, decision-making processes, and other key elements will be developed to ensure effective governance and oversight.

An independent DSMB will be established to protect the safety and well-being of study participants. The DSMB will operate independently of the Sponsor-Investigator and study teams, following a formal charter outlining its roles, responsibilities, and operating procedures. The DSMB will review accumulating study data for any safety concerns, monitor study conduct and progress, and ensure data quality and integrity. Based on its reviews, the DSMB may make recommendations to the TSC regarding the continuation, modification, or early termination of the trial, particularly regarding safety concerns or lack of efficacy (futility).

2 BACKGROUND AND RATIONALE

2.1 Background and rationale of the clinical performance study

The World Health Organization (WHO) estimates that over 80 million new gonococcal infections occur annually, with a disproportionate burden occurring in low- and middle-income countries[2]. Untreated gonorrhoea, which is now becoming more resistant to antibiotics, can lead to adverse maternal and neonatal outcomes[2]. Current diagnosis relies on NAATs like Xpert® CT/NG, which are sensitive and specific but expensive, require specialised equipment with a long turnaround time, and may not be readily available in resource-limited settings, particularly in primary healthcare facilities and ANC clinics. This lack of access has resulted in a reliance on syndromic management, leading to both over-treatment and undertreatment and consequently contributing to the spread of infection and the rise in AMR.

There is a pressing need for point-of-care (POC) tests for NG that are accurate, affordable, easy to use, and suitable for decentralised settings. POC tests would enable a shift from syndromic to etiological testing, allowing for correct diagnosis of NG and timely treatment initiation, potentially lowering transmission rates and improving patient outcomes. By providing rapid and accurate results at the point of care, these tests may enable prompt and appropriate treatment of infected women, reducing the duration of infection and curbing transmission rates[1].

Previous FIND-led NG LFA performance studies have shown promising results, with a sensitivity of 80% in asymptomatic women in East London (unpublished, Remco et al 2023). However, a study in Zimbabwe found a lower sensitivity of 65% among pregnant women attending ANC (unpublished, Martin et al 2023). This discrepancy raises questions about the LFA's performance in this specific population and setting. The observed difference in sensitivity between asymptomatic non-pregnant women and pregnant women highlights the need to investigate the LFA's performance, specifically in the

ANC population. It has been hypothesised that pregnancy may induce physiological changes affecting the test's accuracy. Therefore, further investigation is needed to confirm this potential impact.

This main research question is to determine if the NG LFA can accurately detect NG in vaginal samples collected from pregnant women attending ANC and non-pregnant symptomatic women and to determine if there is a difference in performance between self-collected and provider-collected samples. This study employs a cross-sectional design, comparing the NG LFA results to the Xpert® CT/NG reference standard in vaginal samples collected from women attending ANC and non-pregnant symptomatic women. This design is appropriate for assessing the diagnostic accuracy of the NG LFA in a real-world clinical setting. The inclusion of both self-collected and provider-collected samples allows for the evaluation of the impact of sample collection method on test performance and acceptability. This is important for understanding self-collection's feasibility, safety and potential benefits in expanding access to NG testing.

3 CLINICAL PERFORMANCE STUDY OBJECTIVES AND OUTCOMES (ENDPOINTS)

3.1 Study Objectives

This performance study aims to evaluate the diagnostic accuracy, acceptability, and user preference of the NG LFA for detecting NG in vaginal samples compared to the Xpert® CT/NG reference standard. The study will assess the performance of the NG LFA in both self-collected and provider-collected samples from pregnant women attending ANC and non-pregnant symptomatic women. By determining the accuracy and usability of the NG LFA, this study will inform decision-making regarding its potential adoption as a POC test for NG in resource-limited settings. Additionally, the study will assess the safety of self-collection of high-vaginal swabs for testing of NG. This will inform the future adoption of self-testing in routine practice.

Primary Objective

1. To determine the diagnostic accuracy of the NG LFA for the detection of NG in vaginal provider-collected samples from pregnant women attending ANC when compared to Xpert® CT/NG as the reference standard.

Secondary Objective(s)

2. To determine the diagnostic accuracy of the NG LFA for the detection of NG in vaginal provider-collected samples among non-pregnant symptomatic women.
3. To compare the diagnostic accuracy of the NG LFA for the detection of NG in vaginal self-collected and provider-collected samples from pregnant women attending ANC.
4. To compare the diagnostic accuracy of the NG LFA for the detection of NG in vaginal self-collected and provider-collected samples from non-pregnant symptomatic women.
5. To compare the safety of self-collection of vaginal swabs vs provider collection among pregnant women attending ANC and non-pregnant symptomatic women.
6. To assess healthcare workers' acceptability, usability, and preferences regarding the NG LFA systems integration compared to the reference method
7. To assess the acceptability and preference for self-collected vaginal samples compared to provider-collected samples among ANC attendees and symptomatic women and healthcare workers.

3.2 Study Outcomes (Endpoints)

Claims and Intended Performance of the NG LFA:
The NG LFA is a POC test designed to rapidly and accurately detect NG infection in male urine and female vaginal swabs. It aims to facilitate early diagnosis and treatment of NG infections, particularly in resource-limited settings. The device intends to be accurate, easy to use, and acceptable for self-collection and provider-collection samples.

Risks and Anticipated Adverse Device Effects:
As this study involves the use of a non-invasive diagnostic test, there are no specific safety objectives related to the use of NG LFA, which is a POC test. Treatment decisions will not be made according to the device under investigation and results will be collected for research purposes only. Collection of swab samples from study participants is minimally invasive and poses a low risk. Adverse effects associated with the collection of swabs could include discomfort or irritation during sample collection. Given the nature of the study, termination due to safety or other reasons is not anticipated.

Primary Outcome

1. Point estimates of sensitivity and specificity with 95% confidence intervals (Wilson's score method) among pregnant ANC attendees.

Secondary Outcome(s)

2. Point estimates of sensitivity and specificity with 95% confidence intervals (Wilson's score method) among symptomatic non-pregnant women.
3. Point estimates of the percentage differences with 95% confidence intervals (Tango's score method) of the diagnostic performance metrics between self-collected and provider-collected vaginal samples for the detection of NG in pregnant ANC attendees.
4. The number of adverse events reported following self-collection of vaginal swabs or collection by health service providers among pregnant women attending ANC and symptomatic non-pregnant women.
5. Point estimates of the percentage differences with 95% confidence intervals (Tango's score method) of the diagnostic performance metrics between self-collected and provider-collected vaginal samples for the detection of NG in symptomatic non-pregnant women.
6. Mixed methods: Acceptability; distribution of acceptability questionnaire scores. Usability; distribution of System Usability Scores. Perceptions on acceptability, usability, and preferences of systems integration from qualitative semi-structured interviews and focus group discussions.
7. Mixed methods: Distribution of acceptability questionnaire score, perceptions on acceptability and preference from qualitative focus group discussions. Proportion of participants in each group who prefer self-collected samples over provider-collected samples.

Safety Outcome(s)

Any discomfort reported by participants during sample collection will be documented. Although the NG LFA and sample collection are considered of negligible risk, monitoring for any potential effects is essential to ensure participant safety. The safety of self-collection of high vaginal swab samples will be assessed as per secondary objective four above.

4 DESIGN OF THE CLINICAL PERFORMANCE STUDY

4.1 Study Design

This is a cross-sectional study with a paired design to determine the diagnostic accuracy of the NG LFA against the Xpert® CT/NG as a reference standard in vaginal samples collected in pregnant women attending ANC and to compare the accuracy of self-collected and provider-collected samples in both symptomatic non-pregnant women and pregnant ANC attendees. This design is appropriate as it allows for a direct comparison of the NG LFA's performance to the established gold standard in a real-world clinical setting. The inclusion of both sample types allows for an evaluation of the effect of the collection method on test performance and acceptability.

Justification for choice of the study population

This study will recruit pregnant women attending ANC and non-pregnant symptomatic women attending clinics for suspected STIs. These populations are at elevated risk for NG infection in South Africa and represent the target use case of the NG LFA in clinical practice. Approximately 57% of NG infections in women in South Africa are asymptomatic[3]. Without screening, these cases of NG infection can be left untreated. Pregnant women are especially vulnerable as untreated gonorrhoea can result in miscarriage, preterm birth, and neonatal infection. Early and accurate diagnosis is crucial for timely treatment and the prevention of these adverse outcomes. Efforts will be made to recruit a diverse sample of participants to ensure the study population is representative of the target population. Gender balance is not applicable as the study only focuses on female NG infection.

Inclusion and exclusion criteria for participants providing specimens.

ANC population

Inclusion -

- Women age ≥18 years.
- Pregnant women.
- Attending a study site for antenatal care.
- Willingness to participate and signed informed consent form (ICF).

Exclusion -

- Self-reported use of antimicrobial therapy within 21 days preceding enrolment.
- Use of vaginal douche or vaginal product in the previous 24 hours
- Unable to provide specimens for testing.
- A medical condition, serious illness, or other condition that could interfere with study procedures or jeopardise participant safety.

Symptomatic population

Inclusion -

- Non pregnant women age ≥18 years.
- Diagnosis of VDS. Diagnosis of VDS will be based on self-reported presence of symptoms consistent with VDS, such as increased or abnormal vaginal discharge.
- Willingness to participate and signed ICF.

Exclusion -

- Self-reported use of antimicrobial therapy within 21 days preceding enrolment.
- Use of vaginal douche or vaginal product in the previous 24 hours
- Unable to provide specimens for testing.
- A medical condition, serious illness, or other condition that could interfere with study procedures or jeopardise participant safety.

Healthcare workers population

Systems Usability Score Survey & TFA Acceptability Survey

- HCWs manipulating the NG LFA during month 3 of the study.

Focus Group Discussions (FGD)

- HCWs actively involved in conducting the study

Focus Group Discussions (FGD) - Non-Study Participants

- HCWs working at the study site but NOT directly involved in the NG LFA study procedures.

Justification for selection of study specimens

Vaginal swabs are the recommended sample type for NG testing in women and are easily obtainable through both self- and provider-collection methods. To date, the NG LFA has been validated for testing with female vaginal swabs and male urine only. Three provider-collected and one self-collected high vaginal swab will be collected from each participant. Study sites will record the required information for all collected swabs in a study sample log. The detailed specimen collection procedure for both self-collected and provider-collected samples is described in section 7.6.

Justification for the use of specimens collected specifically for the study purposes

The collection of new specimens is essential for this study due to several reasons:

1. Previous lab validation studies conducted by the developer, DCN, have indicated that using frozen samples for performance testing is unsuitable for the NG LFA. This practice has been shown to impact the sensitivity and specificity of the test negatively. The primary reason for this is believed to be the disruption of the vaginal matrix caused by the freezing process.
2. A key aspect of this study is evaluating the feasibility and accuracy of self-collected samples. New samples are required to compare self-collected and provider-collected specimens directly, ensuring a robust assessment of alternative collection methods.
3. The collection of new samples allows for establishing a biobank, which can be used for future research and development, such as developing a dual LFA for both *chlamydia trachomatis* and NG. This could have significant implications for improving the diagnosis and treatment of STIs globally.

4.2 Reference methods

Gold Standard(s) method(s)

The Xpert® CT/NG assay will be used as the gold standard reference method due to its high sensitivity and specificity for detecting NG in vaginal samples. The Xpert® CT/NG has been validated and widely used in clinical settings to diagnose NG infection. It is considered the best practice current standard of care for NG diagnosis due to its superior performance compared to other non-molecular based methods or syndromic management.

Routine Diagnostic method(s) (if applicable)

The study focuses on evaluating the NG LFA against the gold-standard Xpert® CT/NG rather than comparing it to existing routine diagnostic methods. In most low-resource settings, no diagnostics are available, and patients are treated according to a WHO-endorsed syndromic management approach.

4.3 Analytical Performance of NG LFA

The observed limit of detection with reference NG strains of the NG LFA was 5×10^3 CFU/ml. Inclusivity was demonstrated for 31 NG strains. Exclusivity testing showed no cross-reactivity with 28 non-*Neisseria* and nongonococcal *Neisseria* species; cross-reactivity was observed with *Neisseria meningitidis*, *Neisseria lactamica*, and *Neisseria polysaccharea*[4].

4.4 Clinical Performance

To date, three FIND-led clinical performance studies have been conducted (1 published and 2 unpublished).

1. Symptomatic study, East london[1]

	Male Patients (n=200)	Female Patients (n=200)
NG prevalence	128 (64%)	36 (18%)
Sensitivity	96·1% (91·2– 98·3)	91·7% (78·2–97·1)
Specificity	97·2% (90·4–99·2)	96·3% (92·2–98·3)

2. Asymptomatic study, East London (unpublished, Peters et al., 2023)

	Males (n=500)	Females (n=400)
NG prevalence	6.2%	8.3%
Sensitivity	80.6% (63.7% - 90.8%)	81.8% (65.6% - 91.4%)
Specificity	94.2% (91.8% - 96.0%)	98.1% (96.1% - 99.1%)

3. ANC study, Zimbabwe (Unpublished, Martin et al., 2023)

Variable	Sensitivity % (n/N)	95% CI	Specificity % (n/N)	95% CI
Overall	65·8% (25/38)	48·6 - 80·4%	99·2% (867/874)	98·4 – 99·7%

Clinical claims and intended clinical IVD device performance to be evaluated

The clinical claims for the NG LFA are centred around its potential to provide rapid, accurate, and accessible POC diagnosis for NG infection in non-pregnant and pregnant women. It is intended to be used for the detection of NG infections in vaginal swab specimens collected from both symptomatic non-pregnant women and women attending ANC. The NG LFA aims to offer several advantages over traditional testing, including:

1. Rapid Results: The LFA is designed to provide results within 30 minutes, enabling faster diagnosis and treatment decisions compared to laboratory-based tests that may take hours or days.
2. Ease of Use: The LFA is intended to be user-friendly, requiring minimal training and resources. This makes it suitable for use in various settings, including primary healthcare facilities and ANC clinics.

3. Affordability: The LFA is expected to be more cost-effective than current molecular diagnostic methods, potentially expanding access to testing in resource-limited settings.

Parameters of clinical performance to be determined

The primary parameters of clinical performance to be determined in this study include:

- Sensitivity: The proportion of true NG-positive samples correctly identified by the NG LFA.
- Specificity: The proportion of true NG-negative samples correctly identified by the NG LFA.
- Positive Predictive Value (PPV): The probability that a positive NG LFA result truly indicates NG infection.
- Negative Predictive Value (NPV): The probability that a negative NG LFA result truly indicates the absence of NG infection.

Justification for chosen of clinical performance parameters

The chosen clinical performance parameters are essential for assessing the diagnostic accuracy of the NG LFA in the target population. Sensitivity and specificity are key test performance measures, indicating its ability to identify infected and uninfected individuals correctly. PPV and NPV provide valuable information about the likelihood of a positive or negative test result accurately reflecting the true infection status. Determining these clinical performance parameters will have significant implications for individual health and public health management decisions. A highly sensitive and specific POC test like the NG LFA could enable rapid diagnosis and treatment of NG infection, potentially reducing transmission rates, improving patient outcomes, and mitigating the development of AMR. Furthermore, accurately identifying NG-infected individuals can help guide appropriate treatment decisions and prevent unnecessary antibiotic use. By establishing the clinical performance of the NG LFA in both self-collected and provider-collected samples from non-pregnant symptomatic women and pregnant women attending ANC, this study will provide valuable evidence to inform decision-making regarding its potential implementation as a POC test for NG in resource-limited settings.

5 IDENTIFICATION OF THE IVD DEVICE UNDER INVESTIGATION

5.1 Summary description of the IVD device under investigation

Item	Description
Manufacturer/Developer	DCN (Designed frozen RUO IVD – currently undergoing transfer to a manufacturer)
Manufacturer SRN Number	NA. DCN are a contract research organisation, not a manufacturer
Device identification and labels	The NG LFA device will be labelled with a unique identifier, including a lot number expiration date and clear instructions on the packaging that the device is for research use only.
Device traceability	Each NG LFA device will be traceable through a unique lot number linked to manufacturing records.
List of technical and functional features of the device	The NG LFA is a rapid, qualitative immunoassay that detects NG-specific antigens in female vaginal swabs and male urine specimens. The test is based on the lateral flow principle, where the sample migrates along a nitrocellulose strip containing capture antibodies specific for NG antigens. A result is interpreted by the presence or absence of a fluorescently labelled test line detected by a fluorescent reader.
Device analyte	Neisseria gonorrhoeae (NG) specific antigens.
Type of specimen	Male urine or vaginal swab. Only vaginal swabs will be evaluated in the current study.
Specimen handling and storage condition	Vaginal swab specimens should be collected using NG LFA-specific swabs and should be tested immediately by being placed into the provided buffer solution.
Intended use	Detection of NG in male urine or female vaginal swabs.

Intended clinical benefit	Rapid identification of NG infection at the point of care, timely initiation of appropriate treatment, potential reduction in transmission rates, and improved patient outcomes.
Intended users	Healthcare providers (e.g., doctors, nurses, midwives, healthcare assistants) trained in the collection of vaginal swab specimens and the interpretation of LFA results.
Intended use environment	Point-of-care settings include primary healthcare facilities, sexual health clinics, and ANC clinics.
Target population	Non-pregnant symptomatic women presenting with symptoms of vaginal discharge syndrome. Pregnant women attending ANC.
Indications	Suspected NG infection based on clinical symptoms or risk factors. Routine screening for NG infection in pregnant women.
Contraindications	None known
IVD device accessories	Vaginal swab Buffer solution Positive and negative controls Sample tube and dropper top Medicine dropper NG LFA NG LFA Fluorescent reader Timer and power source (not included)
IVD device software (Reader)	FIND-Reader-beta-1.2.1
IVD device mode of use	The NG LFA is a single-use, disposable device.
IVD device calibration	No calibration is required. Positive and negative controls should be used daily before testing on study participants.
IVD device handling and storage condition	The NG LFA and buffer should be stored at room temperature in their sealed pouch until use. The reader should also be stored at room temperature. The device should not be used after the expiration date. Stability testing of the NG LFA demonstrated that the NG LFA test signal remained stable at 40°C/70% relative humidity for 575 days (19 months) of real-time stability and at 2-8°C and 55°C for 70 days for accelerated stability testing.
Training and experience requirements	Users should be healthcare providers trained in the collection of vaginal swab specimens and interpretation of LFA results.

6 ETHICAL ASPECTS AND RISK EVALUATION

6.1 Ethical aspects

Registration of the investigation

After receiving ethical approval, this performance study will be registered on clinicaltrials.gov.

Competent Ethics Committee (CEC)

The PI submits the CPSP, the ICF, participants' recruitment procedures and any other information provided to participants, the PI's Curriculum Vitae to the CEC, and obtains ethical committee approval before the start of the performance study. The PI ensures that a copy of the final CEC approval letter is received, shared with the Sponsor, and filed in the Investigator's file & Sponsor Trial Master File before the performance study starts.

Reporting duties to the Competent Ethics Committee (CEC)

No changes will be made to the CPSP without prior Sponsor and CEC approval, except where necessary to eliminate apparent immediate hazards to participants. Refer to Chapter 8 for safety reporting.
Progress reports will be submitted to the Ethics Committee at the end of each year from the commencement. The Annual progress report form is available from the ethics website.
All serious adverse events will be reported to the CEC within 72 hours of first knowledge of the event. All protocol violations, protocol deviations and minor GCP violations will be reported to the EC as soon as the PI becomes aware of the violation. The PI will notify the CEC if the study is prematurely suspended or terminated. A summary will be sent to the CEC outlining the reasons for the suspension or termination before the anticipated termination date. The suspension or premature termination of the performance study will be reported to the CEC within 24 hours if due to safety reasons. The reasons for the study suspension or premature termination must be given.
A final report is submitted to the CEC within one year after the regular end of the performance study and within three months after a premature termination of the performance study. Competent Authorities (CA) approval is required. The report will also be submitted to the SA regulatory authorities. The study design and procedures will be submitted parallel to the relevant ethics committees , ensuring the protection of participants' rights and safety.

Reporting duties to the Competent Authorities (CA)

The study will be registered with the South African Health Products Regulatory Authority (SAHPRA), and a six-monthly progress report will be submitted following approvals. All serious adverse events (fatal or life-threatening related and unexpected) will be reported to SAHPRA within seven days of the PI's first knowledge. Other serious adverse reactions (unexpected, not fatal or life-threatening) will be reported within 15 calendar days of the PI's first knowledge of the event.

Ethical conduct of the investigation

The performance study will be carried out according to the CPSP and with principles enunciated in the current version of the Declaration of Helsinki, the CIOMS International Guidelines for Health Research Involving Human Participants, standard ISO 20916, ISO 14971, ICH-GCP as far as applicable, and South Africa’s regulatory authority’s requirements.

Declaration of Interests

The Sponsor and all Investigators involved in this performance study declare that they have no financial, intellectual, or other personal interests that could unduly influence their professional judgment or compromise the integrity of this research.

Patient Information and Informed Consent

1. The PI or its designee explains to each potential participant the nature of the performance study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any burden and discomfort it may entail in a language the participant best understands.
2. Each participant will be informed that participation in the performance study is voluntary, that she may withdraw from the performance study at any time, and that withdrawal of consent will not affect her subsequent medical assistance and treatment. The participants are also informed that authorised individuals other than their treating physician may examine their medical records.
3. When applicable, the participants will be informed that their personal physicians may be informed, with their approval, about their participation in the performance study.
4. All participants will be given a study information sheet and an ICF describing the performance study and providing sufficient information for the participants to make an informed decision about their participation in the performance study.

5. Participants will be informed that they can ask any question and consult with family members, friends, their treating physicians, or other experts before deciding to participate in the performance study. Enough time for reflection will be given to the participants.
6. A participant's formal consent is obtained before the participant undergoes any study procedure, using the ICF approved by the CEC.
7. The consent form will be signed and dated by the participant and the PI or her/his designee who obtained the informed consent.
8. The participant should read, understand, and voluntarily agree before signing and dating the ICF; the participant is given a copy of the signed document. The signed consent form is retained as part of the performance study documents.
9. Participants who cannot read or write will choose an impartial witness to attend the informed consent sessions and sign on behalf of the participant.
10. The participant's medical records are clearly marked to indicate that the participant is enrolled in the performance study.
11. The participant is provided with well-defined procedures for possible emergencies related to the performance study. The PI makes the necessary arrangements for emergency treatment.
12. Should individuals decide not to participate in the study, we will ensure they receive the standard treatment like anyone presenting with STI-associated symptoms.
13. Participants will be compensated with a voucher of R100 for the local grocery store (e.g. Shoprite, Spar, Checkers) for the time associated with their participation in the study.

Participant privacy and confidentiality

The Sponsor and the PI affirm and uphold the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. The anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual participant medical information obtained from this performance study is confidential, and disclosure to third parties is prohibited. Confidentiality will be guaranteed by assigning a unique participant identification number to each individual enrolled in the study. This unique identifier will replace any direct personal identifiers (such as names or addresses) in all study-related documentation and databases. The unique identification number will be generated using a secure, alphanumeric system that ensures no connection can be made to the participant's personal information. This system will be managed by authorised study personnel only, and access to the code linking identifiers to personal data will be strictly controlled. Study documentation is stored in a secure room in a lockable cabinet. Participant registers will be kept in a separate cabinet from any study data (e.g. laboratory results). All digital platforms are password protected; these passwords differ from those used to access the device (tablet, laptop). Research data can only be accessed by the appropriate research team members. No other individuals will be able or allowed to access data. For data verification purposes, authorised representatives of the Sponsor, the CA or a CEC may require direct access to parts of the medical records relevant to the performance study, including participants' medical history.

Dissemination of study findings and post-study access

The findings of this study will be shared through various channels to ensure they reach relevant audiences:

1. Scientific Publication: The results will be submitted for publication in peer-reviewed scientific journals to contribute to the broader knowledge base.
2. Conference Presentations: Findings may be presented at relevant scientific conferences to facilitate discussion and exchange of ideas among researchers.
3. Reports to Stakeholders: Study sponsors, funding agencies, and other relevant stakeholders will receive comprehensive reports detailing the research's outcomes and implications.

4. Public Dissemination: Depending on the nature of the findings, efforts may be made to share results with the public through press releases, media interviews, or accessible summaries.

All study data will be securely archived and de-identified to protect participant confidentiality. Anonymised data may be made available to other qualified researchers upon reasonable request and participants to appropriate ethical and legal considerations. Strict measures will be taken to ensure that no personally identifiable information is shared during the dissemination or data-sharing process. By disseminating findings and providing access to data, we aim to maximise the impact of this research and contribute to advancements in the relevant field.

Community engagement

FPD has a longstanding relationship with the South African Department of Health at the national, provincial, and district levels. Research projects are leveraged to conduct training and capacity-building activities supporting the district's service delivery and systems strengthening. FPD works closely with an extensive network of local stakeholders, non-governmental organisations, and community partners. Community advisory boards are in place to ensure that projects are designed and implemented in a locally relevant and culturally appropriate way. Quarterly reports are shared at the provincial and district levels. After the study, results will be shared with the stakeholders and local research organisations at the community level.

6.2 Risk evaluation

The proposed study evaluating the performance of the NG LFA for detecting NG infection among pregnant and symptomatic non-pregnant women presents a low overall risk profile. The procedures involved, primarily the collection of vaginal swabs, are routine in clinical practice and carry minimal physical discomfort. However, certain aspects warrant consideration.

1. The diagnosis of an STI, like gonorrhoea, may cause anxiety or stress. Measures will be in place to offer support and counselling to participants who require it. Routine counselling about STIs, including the occurrence and implications of asymptomatic infections, as well as condom use and safe sex, will be provided by the study team.
2. Considering confidentially concerns regarding STIs, strict data handling and anonymisation protocols will be followed to protect participant privacy.
3. Self-collected samples may introduce variability in specimen quality, potentially affecting test accuracy. Participants will receive clear instructions and training.
4. We understand that in some communities, STIs can be associated with social stigma. We are fully committed to ensuring that participants in this study do not experience any form of discrimination or judgement due to their involvement. To achieve this, we will implement several key measures:
    a. Participant confidentiality will be maintained at the highest level throughout the study. All data collected will be de-identified and handled in accordance with strict data protection regulations.
    b. Participants will be assigned unique identifiers, ensuring their anonymity in any data analysis or reporting.
    c. c. All interactions with participants will be respectful and nonjudgmental, emphasising the importance of their health and well-being.
    d. Participants will receive comprehensive information about the study, including its purpose, procedures, and potential risks and benefits. This will empower them to make informed decisions about their participation.

e. We will provide access to support and counselling services for participants who may experience any emotional distress concerns or relationship challenges, such as separation or partner violence, which may be related to their participation or STI diagnosis.

Anticipated clinical benefits

The NG LFA, if proven effective, has the potential to deliver substantial clinical benefits and positive diagnostic impact for patients, particularly in resource-constrained settings:

- The rapid, POC nature of the NG LFA enables prompt identification of NG infections. This facilitates early intervention and targeted treatment, reducing the risk of complications, onward transmission, and the development of AMR
- The NG LFA's ability to provide a definitive diagnosis of gonorrhoea allows for a shift away from syndromic management, which relies on symptom-based treatment. This targeted approach ensures that patients receive the most appropriate antibiotics, promoting better treatment outcomes and reducing the unnecessary use of broad-spectrum antibiotics, a key driver of AMR.
- The LFA's simplicity and portability make it suitable in various healthcare settings, including remote or underserved areas. This can significantly increase access to testing for vulnerable populations, such as pregnant women and those in rural communities.
- Early detection and appropriate treatment of gonorrhoea contribute to reducing the overall disease burden in the community and curbing the spread of AMR. This can prevent serious complications like pelvic inflammatory disease, infertility, and adverse pregnancy outcomes, while also safeguarding the effectiveness of existing antibiotics.
- The study's exploration of self-collected samples could further expand access to testing and empower individuals to take control of their sexual health, potentially leading to earlier detection and treatment.

Anticipated risks

Expected risks associated with IVD device use

Based on the nature of the NG LFA and the procedures involved, the anticipated adverse device effects are expected to be minimal**.**

It is important to note that the study team will closely monitor participants for any adverse events and provide appropriate care and support if needed. Discomfort or minor pain may occur during the vaginal swab collection process. Although the procedure is minimally invasive, some individuals may experience slight discomfort.

1. Anxiety or stress associated with undergoing testing for STI might be a potential psychological risk.
2. Potential for stigma or discrimination associated with STIs, although the study will take measures to protect participant confidentiality and provide support.

Expected risks associated with clinical performance study

Based on previous similar studies, the risks associated with this study are minimal [5] [1]. Risks associated with self-collection of high vaginal swabs in pregnant and non-pregnant women are minimal as self-collection is generally a low-risk procedure and is widely used in some countries like the UK. However, the following risks have been identified.

- Pain and discomfort
- Embarrassment associated with the collection of vaginal specimens
- Relationship issues may arise following a diagnosis of an STI

Steps to control or mitigate the risks

Training of research staff

All study research nurses and healthcare providers will receive comprehensive training on self-sampling procedures and how to use the NG LFA device and NG LFA reader. Adequate training ensures accurate sample collection, patient safety, and comfort. The duration of the training will typically last about half a day and will depend on the experience of the research nurses and healthcare providers.

Prior to any procedure-specific training, study protocol training will be provided by the FIND study team.
Both self-sampling and provider-sampling training will be conducted by experienced clinicians or nurses familiar with gynaecological and obstetrical procedures. The training will include in-depth theoretical instruction on sampling techniques and demonstrations using visual aids. Trainees will have the opportunity to practice guiding the procedure under supervision and receive feedback. All study research nurses and healthcare providers will be trained on the detection of potential side effects. They will be instructed to report those as per safety reporting requirements (refer to section 8 of the protocol).

Experienced FIND staff will provide Training on LFA testing to ensure research staff have the knowledge required to perform LFA testing accurately. Training will include the basic principles of the NG LFA and the NG LFA reader, identifying test components, and practical hands-on demonstrations and practice of the entire NG LFA testing process and controls. Troubleshooting and potential clinical scenarios or errors will also be addressed.

Retraining will be provided if we see errors in LFA testing, deviations from protocol or issues regarding self-sampling techniques. Indicators that could help identify issues include high invalid rates, inconsistent results (unexpectedly high or low positivity rates), participant or healthcare provider feedback and study monitoring. Indicators for issues with self-sampling techniques could be higher rates of side effects reported in those groups with self-collected samples. Self-sampling kits will contain both pictorial and written instructions to guide participants.
Results from the NG-LFA will not be used to make clinical management decisions.

Residual risks associated with clinical performance study

Some unforeseeable or unforeseen risks may remain even with all mitigation strategies in place.

Risk-to-Benefit rationale

Should the NG LFA show high performance, this test would provide a major milestone in addressing the global burden of NG infection and combatting the emergence of AMR. At the individual level, participants might benefit from pathogen-directed therapy, i.e. fewer antibiotics, based on the Xpert® CT/NG test result. Asymptomatic individuals benefit from receiving a free STI screen and subsequent treatment with health benefits, if positive, that they would normally not receive. Also, it provides an aetiological explanation of their symptoms, allowing for better counselling. Obtaining multiple vaginal swabs for STI testing has been shown acceptable and feasible in multiple studies across the world. Therefore, weighing the benefits and risks, we believe this study is ethically justifiable.

7 CLINICAL PERFORMANCE STUDY PROCEDURES

7.1 Recruitment of Principal Investigators and Study Sites

Principal Investigators recruitment procedure

The PI was selected based on previous experience conducting STI studies in East London, familiarity with NG LFA from previous work, and valuable understanding of the context in East London.

Study Site recruitment procedure

Potential sites were evaluated based on their capacity to enrol the target study population, availability of necessary resources and infrastructure, and previous experience with STI studies, including NG LFA studies. Sites located in East London were selected due to the high prevalence of NG and the substantial number of women attending ANC clinics. The selected sites will be responsible for implementing the study protocol, collecting data, and ensuring the safety and well-being of study participants.

7.2 Study participants: recruitment, screening, enrolment, withdrawal and discontinuation

Participant recruitment and screening procedure

The primary recruitment target population will be women attending ANC clinics and symptomatic non-pregnant women presenting at participating healthcare facilities. The study will aim to enrol 1,239 women. Recruitment will be conducted over six months. Potential participants will be screened based on their eligibility criteria.
If enrolment goals are not met within the specified timeframe, the following actions will be considered depending on funding available:

- Extending the recruitment period
- Expanding recruitment to additional healthcare facilities
- Implementing targeted recruitment strategies

Participant enrolment procedure

Clinic staff and the study team will identify women attending ANC or healthcare clinics who they think will meet the eligibility criteria. Research nurses or healthcare providers will approach eligible women, provide information about the study, and assess their willingness to participate. Women who express interest will be provided with a detailed explanation of the study, including potential risks and benefits. A brief questionnaire will be administered to confirm eligibility based on inclusion and exclusion criteria. Written informed consent will be obtained from those willing to participate. Enrolment will involve assigning a unique study identification number, collecting baseline demographic and clinical data, and providing instructions for sample collection. The nurse will perform a routine genital examination. Both self-collected and provider-collected vaginal samples will be obtained from each participant. If the participant is not willing to provide a self-collected sample, then provider-collected vaginal samples will be collected and used for performance testing and analysis only.

Identification of concomitant intervention procedure

If participants receive concomitant interventions that could potentially interfere with the study results (e.g. interfering substances associated with local practices), this information will be documented and considered during data analysis.

Informed Consent procedure

The patient will receive full oral and written information about the investigation using their preferred non-technical language (English or isiXhosa) and will be given enough time to decide whether to participate. The patient will then sign and date the written ICF before entering the investigation. The investigator will maintain the signed ICF in the clinical study file. A copy of the written information and the signed ICF will be given to each patient. An investigator or its designee will conduct the informed consent process. The person conducting the informed consent process will also sign and date the informed consent form. If important new information needs to be provided to the patient, the patient will be informed with a written document that the patient will date and sign to confirm he has understood the new information. The written information document must be approved by the Ethics Committees beforehand. Should individuals decide not to participate in the study, we will ensure they receive the standard treatment like anyone presenting with STI-associated symptoms. All participants who consented to the study and participate in the study will be provided with a voucher for R100 from the local grocery store (e.g. Shoprite, Spar, Checkers) for their participation. We prefer not to keep cash at the research facilities for security reasons.

Participant compliance with clinical investigation intervention

The research team will conduct NG LFA testing, not the participants. Therefore, monitoring participant compliance with the device's use is not applicable. As the study involves self-collected and provider-collected vaginal samples, compliance will be monitored through the return and documentation of sample collection kits and documentation of sample collection by the provider. Participants will be provided with clear instructions on how to collect vaginal samples correctly and will be encouraged to ask questions if needed. If the participant is not willing to provide a self-collected sample, then provider-collected vaginal samples will be collected and used for performance testing and analysis only.

Participant withdrawal or discontinuation procedure

Participants are free to withdraw from the study at any time for any reason without any consequences.

7.3 Accounting for Participants

As this is a single-visit study, there is no risk of loss to follow-up. Each participant's visit will be documented, and their participation status will be recorded as either:

- Completed: The participant completed all study procedures.
- Withdrew: The participant voluntarily withdrew from the study before completing all procedures. The reason for withdrawal will be documented.
- Discontinued: The study PI discontinued the participant's participation for a specific reason (e.g., a medical condition). The reason for discontinuation will be documented.

7.4 Methods for Minimising Bias

Study sites are selected from areas with a high prevalence of NG and a substantial number of women attending ANC clinics to ensure the study population is representative of the target population. Eligible participants will be enrolled consecutively to avoid cherry-picking (selection bias) and ensure a diverse range of participants.

- Detailed, standardised protocols will be developed and followed for sample collection (self-collected and provider-collected) and testing (NG LFA and Xpert® CT/NG). Study personnel involved in sample collection and testing will undergo training to ensure procedure consistency and accuracy.
- Discordant results between the NG LFA and Xpert® CT/NG will be shipped to the University of Pretoria for further microbiological analysis
- Detailed data on potential confounding factors, such as age, gestational age, presence of symptoms, recent antibiotic use, and interfering substances, will be collected and analysed to assess their impact on the study results.
- Regular quality control checks will be conducted on the NG LFA and Xpert® CT/NG tests to ensure optimal performance. An independent monitor will monitor the study to ensure compliance with the protocol.
- Validated questionnaires will be used to assess the acceptability and usability of the NG LFA as well as the acceptability of self-sampling

7.5 Handling of the IVD device under investigation

IVD device labelling procedure

The NG LFA will be labelled with a study-specific identification number (e.g., batch/lot number), and the outer packaging will state that the IVD is “for research use only.”

IVD device supply procedure

FIND via DCN will directly ship the required NG LFA test kits to each participating study site. This will be done before the start of the study, ensuring that all sites have sufficient tests to begin enrolment and testing of participants. FIND will monitor the usage of NG LFA test kits at each site throughout the study. If a site runs low on kits, FIND will arrange for a re-supply to be shipped directly to the site. The re-supply quantity will be determined based on the site's enrolment rate and projected testing needs.

Xpert® CT/NG is a commercially available IVD. The study sites will be responsible for procuring their own test cartridges and supplies. They will use existing supply chains in South Africa and manage the re-supply of Xpert® CT/NG cartridges and supplies through their usual procurement channels. FIND will provide guidance and support as needed to ensure adequate availability of the comparator device throughout the study.

Each study site will maintain a detailed inventory log of the NG LFA and Xpert® CT/NG test kits, recording the receipt, usage, and remaining stock. This will facilitate re-supply requests and ensure adequate stock availability. All shipping and inventory records will be maintained in accordance with the study-specific documentation requirements.

IVD device storage procedure

The NG LFA and the Xpert® CT/NG will be stored in a dedicated, secure storage area with limited access within the study site.

Specific storage conditions for each device are as follows:

NG LFA and NG LFA reader should be stored in a cool, dry place away from direct sunlight. Extended stability testing has been performed with the NG LFA and buffer. Stability testing of the NG LFA demonstrated that

the NG LFA test signal remained stable at 40°C/70% relative humidity at 575 days (19 months) of real-time stability and at 2-8°C and 55°C at 70 days for accelerated stability testing. Stability testing of the reader has not been performed. DCN had advised to maintain the device between 15-40°C[4].

- Xpert® CT/NG: Refer to the manufacturer's instructions for specific storage requirements. Generally, Xpert® CT/NG cartridges should be stored in a cool, dry place away from direct sunlight. Sterility must be maintained during cartridge loading.

IVD device return or disposal procedure

NG LFA and NG LFA reader

- Upon study completion, all unused NG LFA test kits and readers will be returned to DCN via FIND.
- Used NG LFA test kits will be disposed of at the study site according to the site's standard operating procedures.
- In case of any NG LFA device deficiencies (malfunction, usability issues, labelling discrepancies), the affected device(s) will be returned to DCN via FIND for analysis. The study site will document the deficiency, including photographs if applicable, and follow FIND's instructions for device return and shipping.

Xpert® CT/NG

- As the Xpert® CT/NG is a commercially available IVD routinely used at the study site, unused cartridges or supplies will be managed according to the site's standard operating procedures.
- In the unlikely event of device deficiencies with the Xpert® CT/NG, the study site should follow the manufacturer's instructions for reporting and returning the affected device. FIND will be notified of any such occurrences.

NG LFA reader shipment

At the end of the study, any unused NG LFA test kits will be returned to FIND according to their instructions. Expired NG LFA test kits will be disposed of according to the study site's standard operating procedures. Any malfunctioning NG LFA test kits will be reported to FIND and returned according to their instructions.

To ensure the prototype fluorescent reader is shipped safely and arrives at its destination in good working condition, the following shipping instructions should be followed -

- Choose a box slightly larger than the reader, with enough space for ample cushioning.
- Use ample cushioning materials such as anti-static bubble wrap.
- Ensure the reader is surrounded and cannot move within the box.
- Clearly label the box with "Fragile" stickers on all sides, including arrows indicating the upright position.
- Close the box securely with packing tape, reinforcing all seams.
- If the box is not sturdy, consider placing it inside a slightly larger box with additional cushioning between the two boxes.
- Use a reputable carrier with experience in handling fragile items.
- Use a shipping service with tracking to monitor the package's progress.
- Clearly label the box with your and the recipient's contact information. Notify FIND when the shipment has been made so that the delivery time can be anticipated.

IVD device accountability procedure

To ensure the proper tracking and control of investigational devices throughout the study, the following procedures will be implemented:

All NG LFA test kits will be stored in a locked, limited-access area at the study site and will be accessible only to authorised study personnel. A detailed inventory log will be maintained at each site to record the receipt, usage, and disposal of NG LFA test kits. The NG LFA test kits are to be used exclusively for this clinical performance study and in accordance with the approved CPSP. Only trained and authorised study personnel will be allowed to handle and use the FPD will maintain a Device Accountability Log at their site to document the physical location of all NG LFA test kits from shipment to the study sites until their return or disposal.
PI or authorised designee at the study site will also maintain a Device Accountability Log. This log will document:

- Date of receipt of NG LFA test kits
- Unique identification of each test kit (e.g., batch/lot number, serial number)
- Expiry date of each test kit
- Physical storage location of the test kits
- Date(s) of use for each test kit
- Date of return or disposal of used test kits
- Date of return of unused, expired, or malfunctioning test kits
- Return/Disposal of Unused, Expired, or Malfunctioning Devices:

The accountability of the Xpert® CT/NG will be documented in the PI's Device Accountability Log. The documentation will include the date of use and the unique identification of each Xpert® CT/NG cartridge used in the study.

7.6 Specimen collection

Specimen collection procedure overview

This clinical performance study will involve the collection of fresh vaginal specimens specifically for the study purposes. Before sample collection, participants will be provided with clear instructions on how to provide a vaginal sample.

1. For self-collected samples, participants will be given a sterile NG LFA kit-specific swab and instructions on collecting the sample. Participants will be provided with a private space to collect their own vaginal sample using the provided swab and instructions. Always take the first sample as a self-collected sample, followed by provider collected.
2. A research nurse or research healthcare provider will collect three provider-collected high vaginal samples using separate sterile swabs- 1. one NG LFA kit specific swab, 2. one Xpert® CT/NG Vaginal/Endocervical Specimen Collection kit (Cepheid) and 3. one flocked swab. This will be done using standard clinical procedures to ensure consistency in the sample collection technique.
3. The order of collection of the first and second healthcare provider swabs will be randomly assigned utilising the randomisation functionality in RedCAP; the third swab is always used for storage/discordant analysis. Both self-collected and provider-collected swabs will be labelled immediately with a unique identifier to ensure traceability and avoid mix-ups.
4. The research team will test using the NG LFA and the Xpert® CT/NG reference standard at the bedside.

5. If there is no discordance testing required, one vaginal swab will be stored at -80°C for biobanking for future STI test development. Specimens with discordant results will be stored at -80°C before being shipped to the University of Pretoria for further microbiological analysis of discordant results and to test for other STI.

NG LFA kit-specific swab (self-collected)

Note: Always take the first sample as a self-collected sample, followed by provider collected.

a. Wash your hands before starting.
b. It is important to maintain a comfortable balance during the collection procedure.
c. Open the package containing the NG LFA-specific swab and remove the swab.
d. Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, request a new swab.
e. Carefully insert the swab into the vagina about 5 cm (two inches) inside the opening of the vagina and gently rotate the swab for 10 to 30 seconds. Ensure the swab touches the walls of the vagina so that the swab absorbs moisture.
f. Withdraw the swab without touching any surfaces.
g. Hand the swab to the healthcare provider for NG LFA testing.
h. Follow the Testing Procedure outlined below.

Xpert® CT/NG Vaginal Specimen Collection (provider collected)

a. Wash your hands before starting.
b. Open the outer peel pack (which contains the two-package kit) and identify the larger cleaning swab and discard it.
c. Open the package containing the pink-capped Xpert Swab Transport Reagent tube and individually wrapped collection swab. Set the tube aside before beginning to collect the sample.
d. Open the collection swab wrapper by peeling open the top of the wrapper.
e. Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, request a new collection kit.
f. Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft across the scoreline. Carefully insert the swab into the vagina about 5 cm (two inches) inside the opening of the vagina and gently rotate the swab for 10 to 30 seconds. Ensure the swab touches the walls of the vagina so that the swab absorbs moisture.
g. Carefully withdraw the swab and continue to hold it in your hand. While holding the swab in the same hand, unscrew the pink cap from the Xpert Swab Transport Reagent tube.
h. Do not spill the contents of the tube. If the contents of the tube are spilt, take a new collection kit.
i. Immediately place the specimen collection swab into the transport reagent tube.
j. Identify the scoreline on the collection swab shaft. Carefully break the swab shaft against the side of the tube at the scoreline and discard the top portion of the swab shaft. If needed, gently rotate the swab shaft to complete the breakage. Use care to avoid splashing contents on the skin. Wash with soap and water if exposed.
k. Re-cap the swab transport reagent tube and tighten the cap securely.
l. Label the transport tube with the sample identification information, including the collection date, as required.
m. Follow the Testing Procedure outlined below.

NG LFA kit-specific swab (provider collected)

a. Wash your hands before starting.
b. Open the package containing the NG LFA-specific swab.
c. Remove the swab, taking care not touching the tip or laying it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, take a new swab.
d. Carefully insert the swab into the vagina about 5 cm (two inches) inside the opening of the vagina and gently rotate the swab for 10 to 30 seconds. Ensure the swab touches the walls of the vagina so that the swab absorbs moisture.
e. Withdraw the swab without touching any surfaces.
f. Immediately insert the swab into the NG LFA buffer.
g. Follow the Testing Procedure outlined below.

Flocked swab (Storage/discordance) (provider collected)

1. Wash your hands before starting.
2. Open the package containing the NG LFA-specific swab and remove the swab.
3. Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, take a new swab.
4. Carefully insert the swab into the vagina about 5 cm (two inches) inside the opening of the vagina and gently rotate the swab for 10 to 30 seconds. Ensure the swab touches the walls of the vagina so that the swab absorbs moisture.
5. Withdraw the swab without touching any surfaces.
6. Place the swab back into the tube for storage at 4°C.

7.7 Specimen-ID assignment procedure

To ensure accurate identification and traceability of specimens throughout the study, a standardised allocated enrolment number will be used as the Specimen-ID.
Specimen storage procedure

Two provider-collected and one self-collected swab will be tested immediately at the site using the NG LFAs and Xpert® CT/NG. One provider-collected flocked swab will be stored in a labelled tube stored at 4°C at the clinical site. It will then be transported to a central facility for longer storage at -80°C.

1. If there are discrepancies between the NG LFA and Xpert® CT/NG results, this swab will be shipped to the Department of Medical Microbiology at the University of Pretoria for further analysis.

Specimen handling procedure

1. Swabs will be collected using aseptic techniques by qualified healthcare providers and instructed study participants.
2. A standardised specimen-ID assignment and labelling procedure will be implemented immediately after collection.
3. Swabs for immediate testing will be kept at room temperature - until the tests are performed.
4. The remaining flocked swab will be placed in a labelled tube containing and stored at 4°C in a designated refrigerator at the clinical site.

5. Before transport, specimens for biobanking and discordant samples will be carefully packaged to ensure they remain at the correct temperature and are protected from damage.

Specimen transport procedure

Samples will be transported to the central facility twice weekly under cold chain conditions. At the end of the study, samples for shipment to the University of Pretoria will be triple-packaged and shipped via a courier service under cold chain conditions. All samples that have not been transferred to the University of Pretoria will only be shipped to the FIND biobank in the United States upon receipt of approval from the Faculty of Health Sciences Research Ethics Committee, and following the execution of a Material Transfer Agreement (MTA) between FPD and FIND, which will be submitted to the Faculty of Health Sciences Research Office.

Specimen disposal procedure

All samples will be used for testing or stored for future research. As this study uses Xpert® CT/NG cartridges for reference standard testing, the disposal procedure for cartridges and used swab samples will adhere to the manufacturer's guidelines and local regulations for biohazardous waste.

Specimen accountability procedure

Each participant will be assigned a unique study ID. All collected specimens (vaginal swabs for both NG LFA and Xpert® CT/NG testing) will be labelled with this unique ID, ensuring anonymity and confidentiality.
A detailed sample log will be maintained, recording the following information for each specimen:

- Unique Study ID
- Date and time of collection
- Type of sample (self-collected or provider-collected)
- NG LFA identification number
- Test results (LFA from provider-collected samples and self-tested samples and Xpert® CT/NG from provider-collected samples)

All data collected, including specimen information and test results, will be entered into a secure electronic database with restricted access.

7.8 Specimen assessment

Testing procedure

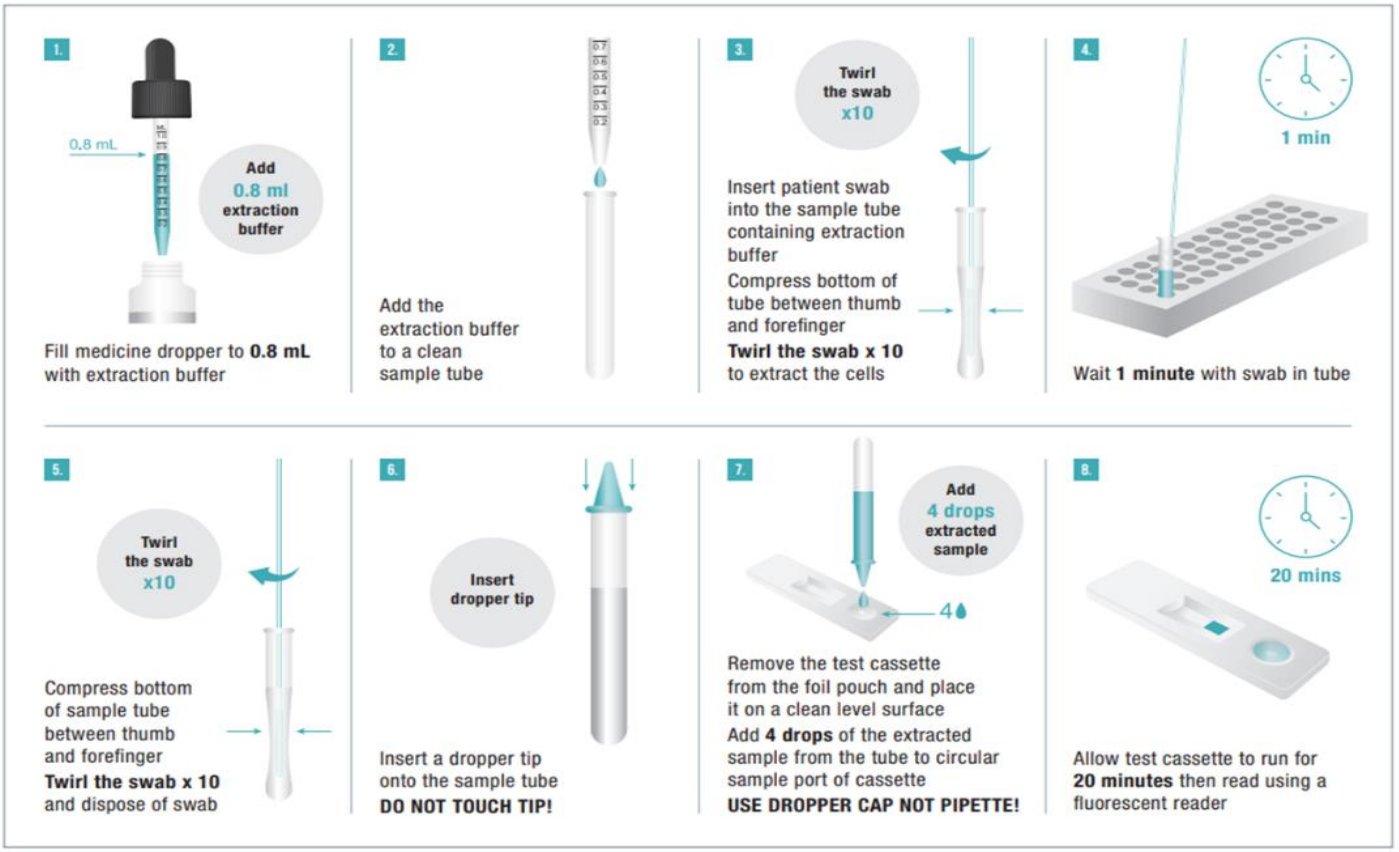
Testing procedure of the NG LFA for self-provider-collected samples (Figure 2)

1. Assemble all the required kit components, which include:
    a. Fluorescent NG LFA Reader
    b. Buffer with medicine dropper
    c. NG LFA
    d. Sample tube and dropper top
    e. Appropriately collected and labelled test sample
    f. Stand for the sample tube (not included in the kit)
    g. Timer (not included in the kit)

Note: Aseptic technique should be observed when all kits and materials are assembled.

Note: A reusable medicine dropper and a disposable transfer pipet may require separate training and practice for users with less experience transferring precise amounts of fluid.
Note: Care must be taken to ensure the sterility of the lysis buffer bottle, which is used for ~30 tests per bottle.

2. Using the medicine dropper, add 0.8ml of extraction buffer to a clean, unused sample tube.
3. Insert the provider-collected NG LFA kit-specific swabs into the sample tube containing the extraction buffer.
4. Gently compress the bottom of the sample tube between the thumb and forefinger and twirl the swab 10 times to extract the cells.
5. Wait 1 minute with the swab in the tube (use a timer).
6. Compress the bottom of the sample tube again between the thumb and forefinger and twirl the swab 10 times. Dispose of the swab according to local regulations for biohazardous waste.
7. Carefully attach the dropper tip to the sample tube, avoiding touching the tip.
8. Remove the test cassette from the foil pouch and place it on a clean, flat surface.
9. Using the dropper (not a pipette), four drops of the extracted sample from the sample tube are added to the circular sample port of the cassette.
10. Allow the cassette to run for 20 minutes (use a timer).
11. While waiting for the sample to run on the cassette. Ensure the reader is switched on and connected to a power source.
12. After 20 minutes, place the cassette into the cassette slot of the reader, facing upwards.
13. The reader will provide a result within 30 seconds. Record the result.
    a. NEGATIVE - a light will illuminate below the negative symbol
    b. POSITIVE - a light will illuminate below the positive symbol
    c. INVALID - a light will illuminate below the exclamation symbol
14. In the case of an invalid result, re-run the test by reinserting the cassette into the reader and recording the result.



Fluorescent microreader operation

TURN ON the battery pack (press the tiny button near the USB plug).
Ensure the battery is connected to the POC Reader with the USB cord.
TURN ON the FIND POC Reader by pressing the power button and holding for 2 seconds. The green light will turn ON.
TURN ON the battery pack (press the tiny button near the USB plug).
Ensure the battery is connected to the POC Reader with the USB cord.
TURN ON the FIND POC Reader by pressing the power button and holding for 2 seconds. The green light will turn ON.

INSERT CASSETTE into the POC Reader. All three lights will flash to show that a cassette has been detected. The scan should take < 5 seconds.

A single light will indicate the test result
NEGATIVE (-)
ERROR (!)
POSITIVE (+)

The testing procedure of the Xpert® CT/NG device for provider-collected samples

1. Obtain the following items:
   a. Xpert® CT/NG cartridge
   b. Transfer pipette (provided)
   c. Appropriately collected and labelled provider-collected test sample
2. Open the cartridge lid.
3. Gently invert the transport tube 3 to 4 times to ensure adequate sample and transport matrix mixing.
4. Unwrap the transfer pipette.
5. Open the transport tube lid, compress the bulb of the transfer pipette, insert the pipette into the transport tube, and release the bulb to fill the transfer pipette above the mark on the pipette shaft (Figure 1). Ensure the pipette is filled with no air bubbles present.
6. Empty the pipette's content into the sample chamber of the cartridge.
7. Close the cartridge lid.
8. See Xpert® CT/NG Package-Insert for details on how to run the test.

Procedure for assessment of the outcome

Procedure for assessment of outcomes related to IVD device performance

Two provider-collected swabs and one self-collected swab will be tested immediately at the bedside using the NG LFAs and Xpert® CT/NG test (see Testing procedure 7.10.1.1 and 7.10.1.2).
The results of the NG LFA will be recorded as positive, negative or invalid.

Results of the LFA will not be used for any treatment decision and will only be collected for research purposes.
This is a diagnostic accuracy study that does not use test results for clinical decision-making. All study personnel are made aware that the novel NG LFA under study is for research purposes only and cannot be used to determine whether to initiate treatment or for any other clinical management decisions.

Procedure for assessment of outcomes related to IVD device safety

The IVD under investigation will not be used for treatment decision making, and therefore, there are no anticipated adverse device effects. Any discomfort during sample collection will be documented and monitored.

7.8.1.1.1 Laboratory parameters procedure

In case the NG LFA and Xpert® CT/NG assay results are discordant, specimens will be shipped to the Department of Medical Microbiology at the University of Pretoria to identify potential cross-reactions with non-gonococcal *Neisseria* species. We will perform molecular detection of any *Neisseria* species, including *Neisseria gonorrhoeae*, *Neisseria meningitidis,* and specific other *Neisseria* species.

The following assays will be used for the discordant samples:
1) Neisseria genus-specific assay (melting curve analysis using hybridisation probes)
2) LightMix NG and CT kit
3) In-house N. meningitidis PCR assay (hydrolysis probe)
4) Species-specific PCR for N. lactamia and N.polysaccharea (this was done only for those that showed discordance between above 1 and 2)

7.8.1.1.2 Procedure for assessment of other outcomes

Outcome 5:

- Systems usability score survey will be administered to all healthcare workers who are manipulating the NG LFA during month 3. The survey (Annex 8) will be self-administered on paper forms which will then be entered into RedCap by the designated RA
- A TFA acceptability survey relating to the NG LFA will be administered to all healthcare workers who are manipulating the NG LFA during month 3. The survey (Annex 8 XX) will be self-administered on paper forms which will then be entered into RedCap by the designated RA
- Focus group discussions (FGD) among the healthcare workers who are conducting the study will be conducted during month 5 in groups of 4 to 8 HCWs. The FGD will be led by a qualitative researcher using the FGD discussion guide (Annex 8). The FGD will be recorded, transcribed, and coded using inductive and deductive coding, and the analysis will be thematic. The FGD will be conducted in English. Recordings will be deleted once the transcriptions are completed. The FGD will cover themes relating to Outcome 5 and Outcome 6
- Focus group discussions (FGD) among the healthcare workers who work at the study site but are not part of the study: the HCW will be consented and provided an education session by the study team regarding the NG LFA. The FGD will be conducted during month 5 of the study and will consist of at least 2 FGD of 4 to 8 HCWs for each FGD. The FGD will be led by a qualitative researcher using the FGD discussion guide (Annex 8). The FGD will be recorded, transcribed, and coded using inductive and deductive coding, and the analysis will be thematic. The FGD will be conducted in English. Recordings will be deleted once the transcriptions are completed

Outcome 6:

- TFA acceptability survey relating to self-sampling among participants undergoing testing procedures: Study staff will administer the TFA survey to each participant after the participant has undergone all sampling procedures (Annex 8). The survey administration should take between 10 and 15 minutes to complete. The paper versions of the CRF will be entered into Open Clinica by designated research staff.
- The TFA acceptability survey relating to perceptions on self-sampling by the HCW will be completed by all HCW conducting the study during month 3. The survey will be self-administered on paper forms and entered into RedCap by the designated research staff.

Mixed methods: Distribution of acceptability questionnaire score, perceptions on acceptability and preference from qualitative focus group discussions. The proportion of participants in each group who prefer self-collected samples over provider-collected samples.

Procedure for each visit

Visit: Enrolment, Sample Collection and Testing

1. **Participant Registration and Identification:**
    a. Obtain written informed consent from the participant after ensuring they have read and understood the study information sheet.
    b. Assign a unique participant identification number to maintain confidentiality throughout the study. This number will be used as an identifier for all study information, including questionnaires, interview guides, specimen labelling, and laboratory tests.
2. **Questionnaire Completion:**
    1. Assist the study participant in completing the short questionnaire.
    2. Ensure all socio-demographic, sexual, and clinical history questions are answered accurately
    3. Address any questions or concerns the participant may have regarding the questionnaire.
3. **Routine Genital Examination and Recording:**
    a. A qualified healthcare worker will conduct a routine genital examination, following standard clinical practices. Diagnosis of VDS will be based on self-reported presence of symptoms consistent with VDS or increased or abnormal vaginal discharge, sometimes with odour or irritation.
    b. Document the findings of the examination in the RedCAP platform, ensuring thoroughness and accuracy.
4. **STI Counselling and Safe Sex Education:**
    a. Provide comprehensive counselling to the participant STIs.
    b. Discuss the occurrence and implications of asymptomatic infections, emphasising the importance of regular testing.
    c. Educate the participant on the proper use of condoms and other safe sex practices to reduce the risk of STIs.
5. **Sample Collection:**

    Self-Collected Sample (x 1):

    a. Provide the participant with a kit-specific vaginal swab for NG LFA testing.
    b. Offer clear instructions on self-collecting the sample, ensuring they understand the procedure and feel comfortable performing it.
    c. If the participant is not willing to provide a self-collected sample, then provider-collected vaginal samples will be collected and used for performance testing and analysis only.
    d. Record any Adverse Events.

    Provider-Collected Samples (x 3):

    a. Explain the sample collection procedure to the participant, ensuring their comfort and understanding.
    b. Collect three high vaginal swabs using appropriate techniques:

- One kit-specific swab for NG LFA testing.
- One swab in Xpert® CT/NG Vaginal/Endocervical Specimen Collection kit (Cepheid).
- One flocked swab for further microbiological analysis if needed.

c. Record any Adverse Events.

6. **NG LFA and Xpert® CT/NG Testing:**

a. Perform the NG LFA test on the collected samples according to the protocol.
b. Conduct Xpert® CT/NG testing on the appropriate sample as per the assay protocol.
c. Record the test results accurately in source documents.

7. **Sample Storage:**

a. Store collected flocked swabs according to the study protocol and laboratory guidelines.
b. Ensure proper labelling and tracking of samples (flocked swabs) to maintain integrity and avoid confusion.

8. Treatment (if applicable):

All participants are provided with antimicrobial treatment at presentation if symptomatic. If the participant is willing to wait 90 minutes, results of the Xpert® CT/NG assay will be used for pathogen-directed therapy of *Neisseria gonorrhoeae* (500mg ceftriaxone intramuscular injection) and/or *Chlamydia trachomatis* (azithromycin 1g oral dose) in case of positive test result(s); these will be available within 90 minutes of sampling.

Symptomatic individuals with negative Xpert® CT/NG tests and those without a result available at the time of treatment will be provided with the standard syndromic management antibiotic regimen (azithromycin 1g start oral dose, ceftriaxone 250mg intramuscular injection, and metronidazole 2g stat dose) as per South African guidelines.

The contact details of asymptomatic individuals will be recorded, and if they are not willing to wait for the result, they will be contacted telephonically to discuss their Xpert® CT/NG result; individuals with a positive STI test will be provided with the same pathogen-directed treatment as symptomatic individuals.

Results of the LFA will not be used for any treatment decision and are only collected for research purposes. Only the results from Xpert® CT will be communicated to the individual.

9. **Partner Notification and Leaflet:**

a. Provide the participant with a partner notification leaflet explaining the importance of informing their sexual partners about the potential exposure to STIs.
b. Encourage participants to discuss testing and treatment options with their partners to prevent further transmission.

10. Study Visit Completion:

a. Provide a voucher of R100 for the local grocery store (e.g. Shoprite, Spar, Checkers) for their participation in the study.
b. Remind the participants of the study team's contact information in case they have any questions or concerns.

Once the participant receives Xpert® CT/NG result and treatment, all study-related procedures will be considered complete.

Study Periods	Consent (ICF)/Screening/Study visit	Notes
Visit	1	
Participant information	X	
Participant consent Form (ICF)	X	
Demographics	X	
Medical history	X	
Routine genital examination	X	
Self-collected sample (1)	X	High vaginal swab
Provider-collected sample (3)	X	High vaginal swabs
NG LFA testing	X	
Xpert® CT/NG testing	X	
Storage of swab	X	
Treatment	X	
Adverse Events	X	
Routine STI counselling	X	
Telephone number	X	
Partner notification leaflet	X	
Participant voucher	X	

Suspension or termination of the clinical performance study

Procedure for suspension or termination

The Sponsor, as per the recommendations of the TSC and DSMC, may terminate the performance study prematurely or at a particular study site or suspend the PI, according to the following circumstances

- ethical concerns
- insufficient participant recruitment.
- when the safety of the participants is doubtful or at risk.
- when monitoring identifies serious or repeated deviations on the part of a PI.

When suspension or premature termination occurs, the Sponsor justifies its decision in writing and promptly informs the other parties with whom it is in direct communication. When, for any reason, the Sponsor suspends or prematurely terminates the performance study at an individual study site, the Sponsor ensures that the CEC is notified. The PI and Sponsor keep each other informed of any communication from the CEC.

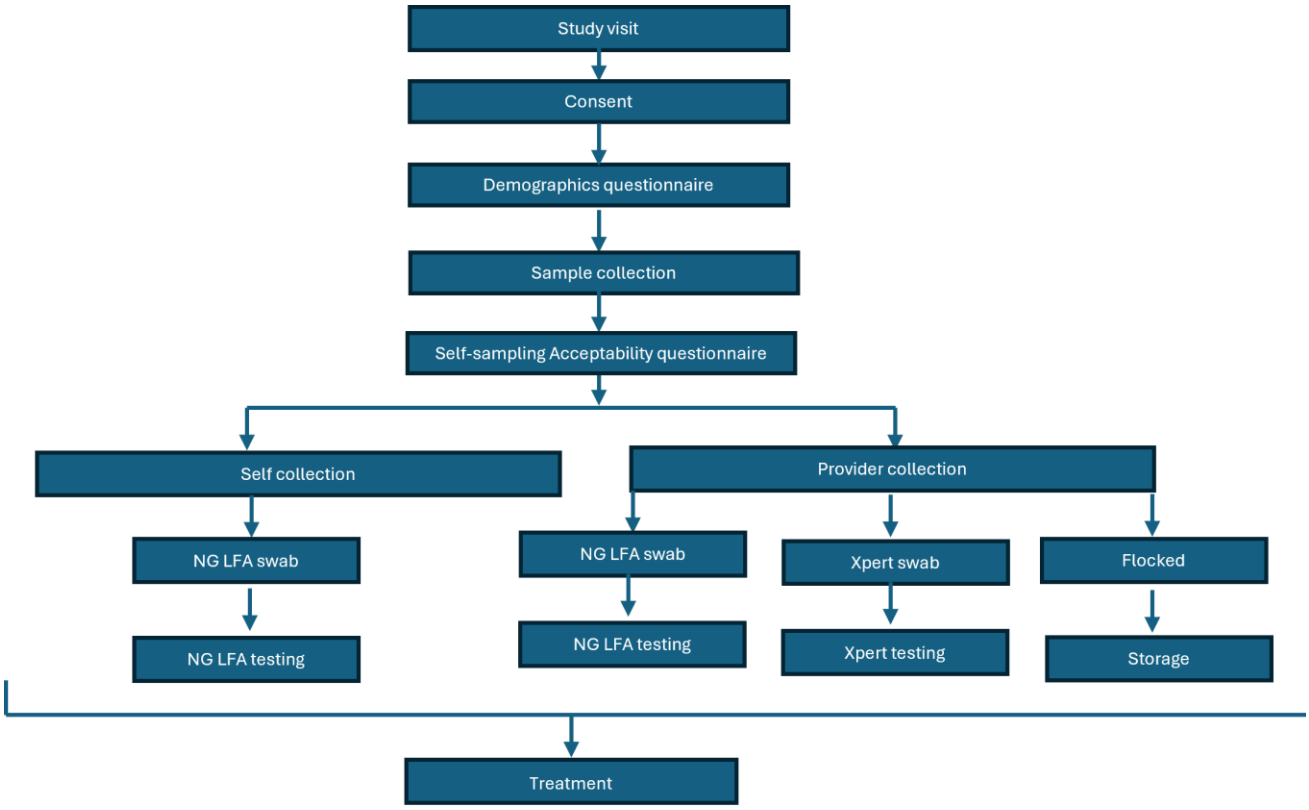
Procedure for resuming the clinical performance study after temporary suspension.

After analysing the reason(s) for the suspension and implementing the necessary corrective actions, the sponsor decides to lift the suspension. It informs the PI of the rationale and provides them with the relevant data supporting its decision. The Sponsor submits new safety information and any modified documents to the CEC. Upon receiving CEC approval, the performance study can resume.

Procedure for follow-up with participants after termination of the clinical performance study

NA.

7.9 Study duration and flowchart.



2024	Jul	Aug	Sep	Oct	Nov	Dec
Study Protocol						
CEC approval						
Staff training						
Recruitment						
Data analysis						
Report						

2025	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Study Protocol												
CEC approval												
Staff training												
Recruitment												
Data analysis												
Report												

8 SAFETY

The Principal Investigator and any qualified designees are responsible for the detection, medical management, documentation, recording and reporting of all events and the follow-up and reporting of all Adverse Events and Adverse Device Effects.

8.1 Terms and definitions

Adverse Event (AE) (Art. 2(60) IVDR)

Any untoward medical occurrence, unintended injury, or untoward clinical signs in study participants, users, or other persons in the context of a performance study, whether or not related to the device for the performance study.

Adverse Device Effect (ADE) (ISO 20916)

Adverse event related to the use of an IVD medical device under investigation.

Device Deficiency (DD) (Art. 2 Abs 62 MDR)

Inadequacy of a medical device is related to its inadequacy in the identity, quality, durability, reliability, safety, or performance for performance study, including malfunction, use errors, or inadequacy in information supplied by the manufacturer.

Device Deficiency with Serious Adverse Device Effect (SADE) potential (Art. 76 IVDR; ISO 20916)

Any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate.

Malfunction (ISO 20916)

Failure of an IVD medical device under investigation to perform in accordance with its intended use when used in accordance with the instructions for use or CPSP.

Serious Adverse Event (SAE) (Art. 2(61) IVDR)

Any adverse event that led to any of the following:

(a) a patient management decision resulting in death or an imminent life-threatening situation for the individual being tested or in the death of the individual's offspring,

(b) death,

(c) serious deterioration in the health of the individual being tested or the recipient of tested donations or materials, which resulted in any of the following:

(i) life-threatening illness or injury,

(ii) permanent impairment of a body structure or a body function,

(iii) hospitalisation or prolongation of patient hospitalisation,

(iv) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,

(v) chronic disease,

(d) foetal distress, foetal death or a congenital physical or mental impairment or birth defect.

Note: planned hospitalisation for a pre-existing condition or a procedure required by the CPSP without a serious deterioration of the participant's health status is not considered an SAE.

Serious Adverse Device Effect (SADE) (ISO 20916)

Adverse device effect (ADE) that has resulted in any of the consequences characteristic of a serious adverse event.

8.2 Expected Adverse Events and Mitigation Plans

Given that the NG LFA will not be used for treatment decisions and patients will be treated according to the standard of care in South Africa, the likelihood and severity of some adverse events may be reduced. Self-collection of high vaginal swabs is considered safe and accepted in pregnancy.

8.3 Solicited Adverse Events

After vaginal swab sample collection (self or physician-collected), participants will be asked if they have experienced any issues during and after the collection.

8.4 Categorization and Assessment of Adverse Events

All adverse events will be reported in a final clinical performance study report.

Casual relationships of SAE

A causal relationship towards the IVD medical device or the procedure of the investigation should be rated by the PI and the Sponsor as follows:

- **Not related**: The relationship to the device or procedures can be excluded.
- **Possible**: The relationship with using the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible.
- **Probable**: The relationship with using the investigational device seems relevant and the event cannot reasonably be explained by another cause.
- **Causal relationship**: The serious event is associated with the investigational device or with procedures beyond reasonable doubt.

Causality will be assessed using the WHO-UMC (WHO-Uppsala Medical Center) assessment system.

Adverse events categorization

The adverse events will be categorised by the PI and the Sponsor using the following algorithm:

- Does the AE meet the seriousness criteria?
  - No, it is not serious: AE
  - Yes, it is serious: SAE
- If AE, is it device related?
  - No, not-related: AE
  - Yes, related AEs: ADE
- If SAE, is it device related?
  - No, not-related: SAE
  - Yes, it is a possibility, probably, or causally related SAE: SADE
- If SADE, is it anticipated (within the expected type, severity and frequency of the complications)?
  - No, unanticipated SADE: USADE
  - Yes, anticipated SADE: ASADE

Adverse events severity grading

- Mild (Grade 1): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- Moderate (Grade 2): minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental (ADL).
- Severe or medically significant but not immediately life-threatening (Grade 3): hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care (ADL).
- Life-threatening consequences (Grade 4): urgent intervention indicated.
- Death (Grade 5)

8.5 Documentation and reporting

Device deficiencies (DD) and all **adverse events (AE),** including all **serious adverse events (SAE),** are collected, fully investigated and documented in the source document and appropriate CRF. Information about AEs will be collected during the study population period, i.e. from participant's informed consent until the last CPSP-specific procedure (at maximum 1 day 120 minutes).

Documentation and reporting responsibilities of the PI

- At the study visit, the participant will be directly asked about any experiences of discomfort, pain, bleeding, or other unusual symptoms following the vaginal swab procedure. Participants will be provided with contact information for the study team and encouraged to report any concerning symptoms
- The PI or designee records clinically relevant AEs together with an assessment as to whether the IVD device or sampling procedure was a cause of the event on a CRF.
- The documentation of AEs (including SAEs) by the PI includes information about the participant, diagnosis or symptoms, start and stop dates of event, event treatment, event resolution, the PI's assessment of seriousness, causal relationship to IVD device and/or study procedures, and expectedness.
- PI shall report to the Sponsor and TSC within 24 hours after becoming aware of the following events:
  - All SAEs and SADEs
  - Health hazards that require measures
  - All DDs that could have led to a SADE

SAEs and SADEs are reported to the Sponsor on an SAE report form (Annexes, chapter 17) within 24 hours of site awareness of the SAE/SADE.

Documentation and reporting responsibilities of Sponsor

- The Sponsor reviews the investigator's assessment of all AEs, determines and documents in writing their seriousness and relationship to the IVD medical device under investigation; in case of disagreement between the Sponsor and the PI, the Sponsor shall communicate both opinions to the Ethics Committee the TSC and CA if applicable.
- The Sponsor reviews all DD, determines and documents in writing whether they could have led to a SADE; in case of disagreement between the Sponsor and the Principal Investigator(s), the Sponsor shall communicate both opinions to the Ethics Committee, the TSC and CA if applicable.
- The Sponsor ensures that all SAEs and DDs that could have led to an SADE are promptly reported to the CEC and the TSC when required by the CEC.
- If applicable, the Sponsor will ensure to report to CA, without delay, all SAE that has a causal relationship with the device, the comparator or the study procedure or where such causal relationship is reasonably possible, as well as DD that could have led to a SADE if appropriate action had not been taken, intervention had not occurred, or

circumstances had been less fortunate. Furthermore, any new findings about those events will be reported to CA without delay. When necessary to ensure timely reporting, the Sponsor may submit an incomplete report followed up by a complete report.

- The Sponsor will, in the case of a multicentre clinical performance study, inform all PIs and TSC in writing of all SAE at all study sites that have been reported to the sponsor and ensure that they are reported to their CEC when required by national regulations or by the ethics committee, whichever is more stringent; this information shall be sent to all the PIs within a time frame established based on the perceived risk as defined in the risk analysis report.
- The Sponsor will, in the case of SADE and DD that could have led to SADE, determine whether the risk analysis needs to be updated and assess whether corrective or preventative action is required.
- The Sponsor will provide the CA, the CEC and TSC with the documentation at their request.

Medical management of SAEs and SADEs

The study team will ensure that study participants with SAEs receive standard medical care in the country. If treatment costs are extra, the study will cover those costs.

Time period to collecting SAEs information

For each participant, information on SAEs will be collected from the signing of the ICF until the end of the study. Investigators are not obligated to seek SAEs after the conclusion of the trial participation actively. However, if the investigator learns of any SAE, including death, at any time after a participant has been discharged from the trial, and he/she considers the event to be reasonably related to the IVD or trial participation, the investigator must promptly notify the Sponsor.

Follow up of SAEs and SADEs

All SAEs will be followed until resolution, stabilisation, the event is otherwise explained, or the participant is lost to follow-up. Prompt notification by the PI to TSC of an SAE/SADE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a Trial intervention under clinical investigation are met.
Where applicable, FPD has a legal responsibility to notify the local regulatory authority and other regulatory agencies about the safety of a trial intervention under clinical investigation.

Pregnancies: Pregnancy reporting is not applicable in this study.

Expedited and annual reporting to the Competent Ethics Committee and Competent Authority

The Sponsor reports the following events to the CEC and CA within 72 hours of awareness.

a) Any SAE that has a possible, probable or causal relationship with the investigational device, the comparator device or a test procedure;
b) Any DD that could have resulted in SAE under less propitious circumstances or if appropriate action or intervention had not taken place;
c) Any new findings relating to a reported incident.

In order to ensure the reporting is not delayed, an incomplete report may be provisionally provided.
If safety and health hazards require measures to be taken immediately during the conduct of the investigation, the Sponsor notifies the CEC and CA within 48 hours of these measures and the circumstances which made them necessary.

Periodic safety reporting

The sponsor submits an annual safety report (ASR) to the CEC and the CA. The ASR contains a list of all SADEs and DDs, a report on their degree of seriousness, causal relationship with the MD and procedure, and a report on participants' safety with information required by the CEC and CA.

8.6 Data management system

The study will use RedCap Version 14.7. This system is a password-protected online platform that ensures secure and efficient study data management. RedCap is hosted on a secure cloud service, with regular testing conducted before the study begins to verify its functionality and reliability. Data entry will be performed by trained project data entry clerks authorised by the Principal Investigator (PI)Data collection and entry.

Data will be recorded initially on pCRFs by the study team. These pCRFs will then be entered into RedCap by dedicated data entry clerks. To ensure accuracy and quality: Automated range checks within RedCap will help identify data entry errors by flagging values that fall outside of predefined ranges

Data review and database cleaning

Data will be cross-checked against source documents to confirm accuracy. Any discrepancies identified will be addressed and corrected as needed. Regular validation checks will ensure that RedCap adheres to required data processing and storage standards, thus maintaining system performance and data integrity.

Data verification

RedCap has advanced security features to ensure password protection, access control and audit trail: Each user will have individual accounts with unique passwords. Detailed records will be maintained, documenting user access, including dates and levels of privilege, to ensure secure data handling. An audit trail system will log all data entries, modifications, and queries, providing a comprehensive history of all data management activities.

Data analysing and archiving

Each enrolled participant will be assigned a unique study participant ID number to identify samples and information. The use of an anonymous participant ID will ensure the participant's confidentiality. To ensure that each clinical sample can be linked to clinical and laboratory results and tracked effectively from the collection site to the end-user, all lab request forms and sample collection will be labelled using the participant's ID. Post-analysis, data will be archived following the retention period specified in the data management plan. All records will be stored securely to guarantee their retrievability and protection against unauthorised access.

Electronic database validation
The validation procedures for RedCap will adhere to a strict data management protocol. Detailed documentation will define the data receipt, transfer, and processing criteria, ensuring that all data sent to the Sponsor is de-identified, with no personal identifiers included. Routine consistency checks will confirm that the system meets the established requirements and functions as expected. Measures will be implemented to ensure that all data entered into the system is traceable, complete, reliable, consistent, and logically structured. Reports generated by RedCap will undergo thorough

review to verify accuracy and alignment with the source data. Comprehensive audit trails will be maintained for all data entries and modifications, ensuring a permanent record of changes and preventing data deletion. The system will be secured through robust measures to prevent unauthorised access, safeguarding the confidentiality and integrity of the data. A current and up-to-date record of all personnel with access to the database, including their access levels, dates, and privileges, will be maintained. All finalised documents will be signed by the PI or an authorised designee, ensuring accountability and compliance with regulatory standards.

Data quality assurance

9 STATISTICAL METHODS

A dedicated statistical analysis plan (SAP) will be developed before the study's initiation. The document will describe in detail the procedures to evaluate each of the study outcomes. Therefore, this section only provides a high-level summary of the analysis strategy.

9.1 Determination of sample size

The sample size has been determined using a previously published formula [Equations 6.1 and 6.3] (ref). In the ANC population, to detect a sensitivity of 80% [65% - 95%] and a specificity of 98.1% [93.1% - 100%] with 80% power, 56 confirmed positives and 59 confirmed negatives are needed, respectively. To detect a difference <5% between the sensitivity/specificity of clinician-collected and self-collected samples in this population, with the assumption that the observed difference will be 1% for both sensitivity/specificity, with alpha 5% and power 80%, 39 positive and 39 negative paired samples are needed (ref). To obtain 56 confirmed positives with a prevalence estimate of 8% and a power of 80%, 778 participants needed to be screened.

In the symptomatic population, to detect a difference <5%, with the assumption that the observed difference will be 1.5% for both sensitivity/specificity, with alpha 5% and power 80%, 76 confirmed positive and 76 confirmed negative paired samples are needed (ref). To obtain 76 confirmed positives with a prevalence estimate of 18% and a power of 80%, 461 participants need to be screened.

Sample size for operational research aspects

- SUS and TFA among HCW conducting the NG LFA: given that there is a limited number of HCW who will be manipulating the NG LFA all HCW will be included in the survey. The analysis will be descriptive
- Qualitative aspects: the sample size will be defined based on the saturation point, meaning that all substantive themes have been identified and no new, substantiative themes emerge from additional interviews. It must be noted that due to the limited number of HCW who have experience with the NG LFA there is a risk that saturation may not be reached.

9.2 Statistical Analysis Plan

This section summarises the planned statistical analyses of the primary and secondary endpoints.

Populations for analyses

For analysis, the following populations are defined:

Populations for analyses

Population	Description
Enrolled/Intention-To-Test (ITT)	All participants who sign the Informed Consent Form (ICF)
Modified-Intention-To-Test (MITT)	All participants in ITT for whom at least one test result is available i.e., participants with incomplete results (e.g., reference test results is available, but index test not done)
Per Protocol Population (PP)	All participants in ITT for whom results for all tests are available (complied with the protocol)
Per Protocol Modified Population (PPM)	All participants in ITT for whom results for all tests on provider-collected vaginal samples are available

If the participant is not willing to provide a self-collected sample, then provider-collected vaginal samples will be collected and included in the analyses of Primary Objective 1 and Secondary Objective 1.

General Methodology

Table below summarises the usual methodology to report results of diagnostic tests when compared with a reference standard.

Definition of test results and classifications

	Reference standard classification			
Index Test Result		Positive	Negative	Total
	Predicted positive	a	b	(a + b)
	Predicted negative	c	d	(c + d)
	Total	(a + c)	(b + d)	(a + b + c + d)

a = True Positives	Sensitivity = a / (a + c)
b = False Positives	Specificity = d / (b + d)
c = False Negatives	Positive Predictive Value = a / (a + b)
d = True Negatives	Negative Predictive Value = d / (c + d)

Point estimates of sensitivity and specificity, with 95% confidence intervals based on Wilson's score method, will be calculated and reported in tables and graphically (forest plot).

Primary outcome analysis

Outcome	Analysis Population	Statistical Analysis Methods
Point estimates of sensitivity, specificity, positive and negative predictive value (PPV and NPV respectively) with 95% confidence intervals	Pregnant women attending ANCs in the per protocol modified population	As defined in the general methodology section

Secondary outcomes analyses

Outcome	Analysis Population	Statistical Analysis Methods
Point estimates of sensitivity, specificity, positive and negative predictive value (PPV and NPV respectively) with 95% confidence intervals	Symptomatic non-pregnant women in the per protocol modified population.	As defined in the general methodology section
Point estimates of the percentage difference in Sensitivity/Specificity between self-collected and provider-collected vaginal samples with 95% confidence intervals	Pregnant women attending ANCs in the per protocol population.	The percentage differences in diagnostic performance will be calculated as: $DIFFsens_{SELF-PROVIDER} = SENS_{SELF} – SENS_{PROVIDER}$ $DIFFspec_{SELF-PROVIDER} = SPEC_{SELF} – SPEC_{PROVIDER}$ 95% confidence intervals will be calculated based on Tango's score method. McNemar Test will be performed to assess the null hypothesis that the proportions of discordant pairs between the two index tests (self-collected vs provider-collected) are equal.
Point estimates of the percentage difference in Sensitivity/Specificity between self-collected and provider-collected vaginal samples with 95% confidence intervals	Symptomatic non-pregnant women in the per protocol population.	As outlined for secondary endpoint 2
Number of adverse events reported following self-collection of vaginal swabs or collection by health service providers among pregnant women attending ANC and symptomatic non-pregnant women.	All participants who provided at least one sample type.	Descriptive statistics represented by absolute numbers and proportions per population (ANC vs symptomatic). The statistical significance of the differences may be assessed using Chi^2 or Fisher's Exact Test.

Mixed methods: Distribution of acceptability questionnaire score and system usability score, thematic analysis of SSIs and FGDs.	SUS and TFA: all HCWs who are involved in preforming the NG LFA testing Qualitative: the above plus HCW working at the clinic the study is taking place in but not involved in the study	Descriptive statistics represented by absolute numbers, proportions, or means (where applicable). Thematic analysis to analyse free-response questions to identify key themes and messages.
Mixed methods: Distribution of acceptability questionnaire score, the proportion of participants in each population who prefer self-collected to provider-collected samples, thematic analysis of FGDs.	LFA survey: All participants who undergo the sampling procedure LFA survey HCW: HCW involved in the study Qualitative: the above + HCW working at the clinic the study is taking place in	Descriptive statistics represented by absolute numbers, proportions, or means (where applicable) within each population (ANC, symptomatic, and HCWs). Thematic analysis to analyse free-response questions to identify key themes and messages.

Subgroup analyses

Subgroups to be defined in the detailed SAP.

Descriptive Statistics

Descriptive statistics tables will be generated to summarise the characteristics of the participants in the ITT, MITT, PP and PPM populations. The number of participants included and excluded will be reported. Among the included participants, this information will be broken down by the previously defined subgroups.

Categorical variables will be presented in absolute numbers and percentages. Continuous variables will be summarised with means, standard deviations, and interquartile ranges.

Safety Analyses

See secondary endpoint 4.

Additional Analyses

Additional exploratory analyses will be described in the SAP.

Missing data

Missing data will not be imputed and therefore excluded from the analysis. This will be reported in the descriptive statistics tables.

Statistical software

The analysis will be performed using the R statistical language (version 3.4 or higher) and Microsoft Excel (version 16.16 or higher).

10 QUALITY ASSURANCE AND REGULATORY ASPECTS

10.1 Quality assurance and control

The PI ensures that experience and scientific or clinical knowledge are guaranteed and documented for all members of the study team at the study site in order to adequately conduct the performance study, including specific training as applicable to each role. All members of the study team receive specific training on the performance study, and such training is documented in the Study Site File.

Study documentation

The following documentation must be maintained throughout the study period.

Source data and source documents

The investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the site's study participants (source data). Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable by adding initials and a date, should not obscure the original entry, and should be explained if necessary.

Source documents provide evidence for the participants' existence and substantiate the integrity of the data collected. They are filed at the investigator's site. The investigator must maintain accurate documentation that supports the information entered in the CRF.

The definition of what constitutes source data can be found in the table below.

Participant data will include Demographics, clinical examination, and laboratory testing. Observations from the clinical examination will be recorded directly in the paper CRF.

Electronic CRF (direct data capture) will be used to ease data entry by non-clinical and non-laboratory staff whenever possible. If this is not feasible, a paper CRF will be used to mirror the design of the electronic CRF. The paper CRF will consist of some data that has been entered directly (e.g., source data) and some that has been transcribed from other sources, such as the lab report. Refer to the table below. Any source data directly entered into the paper CRF must be signed and dated by the person who generated the data.

Type of Data	***Original Place of Entry***
Study Details	*CRF*
Demographics	*CRF*
Clinical presentation	*CRF*
Physical examination	*CRF*
NG LFA and GX Testing	*CRF*
Partner notification leaflet	*CRF*
Qualitative questionnaire	*CRF*

The Investigator must permit study-related monitoring, audits, institutional review board/independent ethics committee (IRB/IEC) review, and regulatory agency inspections and provide direct access to participant medical records and source documents used for this study

Case Report Forms

CRFs will be developed to capture the data for each enrolled participant as required by the CPSP. The CRFs shall include information on each participant/specimen at commencement and during the clinical performance study, including use of the IVD device, adverse events, and any other relevant information.
Data is first captured on paper-CRF, then entered into RedCap electronic database by single data entry by authorised personnel who are listed on the study delegation log. No participant's personally identifiable information shall be included within the CRF.

Storage of biological material and related health data

Coded specimens and associated personal data will be stored in a FIND biobank only if participants have explicit consent for further use. This consent is obtained independently of the participant's decision to enrol in the study. Specimens will be transferred onwards to partner test development organisations, with whom FIND will partner for developing LFAs and molecular diagnostic tests for NG and CT. FIND will remain the ultimate custodian when samples are sent to third-party laboratories.

Specimens and personal data of participants who do not consent to further use will be stored at FPD's storage facility for 5 years after the study's completion. After this period, specimens will be destroyed, and data will be anonymised.

Archiving of essential clinical investigation documents

All performance study documents must be archived for a minimum of 10 years after regular or premature termination of the study.

10.2 Monitoring and monitoring plan

Study monitoring will be performed according to the Monitoring Plan.
The Sponsor will be allowed access to all source documents needed to verify the entries in the CRFs and other CPSP-related documents, provided that participant confidentiality is maintained in accordance with applicable laws.
The Sponsor will be responsible for inspecting the CRFs at regular intervals throughout the study and verifying the adherence to the CPSP and the completeness, consistency, and accuracy of the data being entered. The monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs/SADEs and the recording of the main efficacy, safety, and performance endpoints.
Once the study starts, an initiation visit will be performed at the study site. A routine visit will be performed every year or as required, and a final visit will be performed after the last patient has finished the study in the site. During each monitoring visit, risk-based monitoring of new data will be performed. The Sponsor will perform monitoring activities according to Sponsor's internal SOP on monitoring clinical performance studies.
Each visit will be documented in a monitoring report. The sponsor may decide to make additional monitoring visits based on the quality of the data.

10.3 Audits and inspections

The PI or institution supports the Sponsor in performing auditing activities and, when relevant, ethics committee reviews and RA inspections.
The PI or institution provides direct access to source data during and after the performance study for Sponsor audits, ethics committee reviews and RA inspections.

As required, the PI or institution obtain permission for direct access to source documents from the participant and/or hospital administration before starting the performance study.

10.4 Regulatory aspects

Clinical Performance Study Plan amendments

Substantial amendments are implemented only after approval by the TSC, CEC and the CA. The use of waivers from the CPSP is prohibited. Under emergency circumstances, deviations from the CPSP to protect the rights, safety and well-being of the participants may proceed without prior approval by the Sponsor, the TSC, the CEC and the CA, but a notification is required. Such deviations shall be documented and reported to the Sponsor, the CEC, and the CA within 2 days.
All non-substantial amendments are communicated to the TSC and CEC together with the Annual Safety Report (ASR). The ASR shall include any deviations from the CPSP that may have affected the rights, safety or well-being of the participant or the scientific integrity of the clinical performance study.

Deviation from the Clinical Performance Study Plan

The Investigator is not allowed to deviate from the CPSP. Any deviation from the CPSP must be documented, reported, and analysed according to the following procedures. The Investigator must promptly report any CPSP deviation to the Sponsor and the TSC within one week of its occurrence. The Sponsor, in collaboration with the TSC and the DSMB, if applicable, will analyse the impact of the deviation on the study participants, data integrity, and overall study results. For any CPSP deviation, the Investigator must implement appropriate corrective actions to mitigate any immediate risks to participants and prevent recurrence of the deviation. Corrective actions must be documented and reported to the Sponsor and TSC. For any CPSP deviation, the Investigator must implement appropriate corrective actions to mitigate any immediate risks to participants and prevent recurrence of the deviation. Corrective actions must be documented and reported to the Sponsor and TSC.

11 CONFIDENTIALITY AND DATA PROTECTION

The Sponsor and the PI affirm and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.
For data verification purposes (monitoring) the PI or institution provide direct access to source data during and after the performance study to the Sponsor's personnel, or to other personnel designated and authorised by the Sponsor.
The PI or institution provide direct access to source data during and after the performance study for Sponsor audits, CEC review and RA inspections.

12 PUBLICATION AND COMMUNICATION

The Sponsor retains ownership of the study results. However, the TSC will oversee the publication process to ensure adherence to the following principles –

Authorship eligibility should be based on ICMJE criteria (substantial contributions, drafting/revision, approval, accountability). FIND encourages in-country partners to take the first authorship position, where appropriate, and generally prefers to be listed as the last author for FIND-supported projects. Study data should be submitted for journal publication within 12 months of completion unless otherwise specified by the TSC. All manuscripts reporting study data

should be published in an open-access, peer-reviewed journal, preferably with a CC-BY license. The target journal should be agreed upon by all authors in consultation with the TSC, with a focus on reputable journals and avoidance of predatory publishing practices. Any observed gender effects, or lack thereof, will be published in the CPSR and subsequent publications. Publications should be accurate, complete, balanced, and transparent, adhering to Good Publication Practice guidelines and Relevant reporting standards (e.g., CONSORT, PRISMA, STARD, and SAGER guidelines) should be consulted and followed. Clinical trial registration numbers should be included when available.

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Study results will be communicated to study participants, healthcare providers, the public, and other relevant stakeholders, as guided by the TSC.

13 FUNDING AND SUPPORT

13.1 Funding

Studies are funded through by the Canadian Government through the Department Of Foreign Affairs, Trade And Development (DFATD).

14 INSURANCE

This performance study involves minimal risk of injury to study participants and therefore insurance has not been obtained. The approval of the CEC will be sought for this.

15 REFERENCES

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