MeSH Consortium

Measurement & Surveillance of HIV Epidemics

Developing, testing & implementing innovative & efficient methods for routine HIV measurement & surveillance

The feasibility and utility of HIV recent infection testing in a range of routine service-provision contexts

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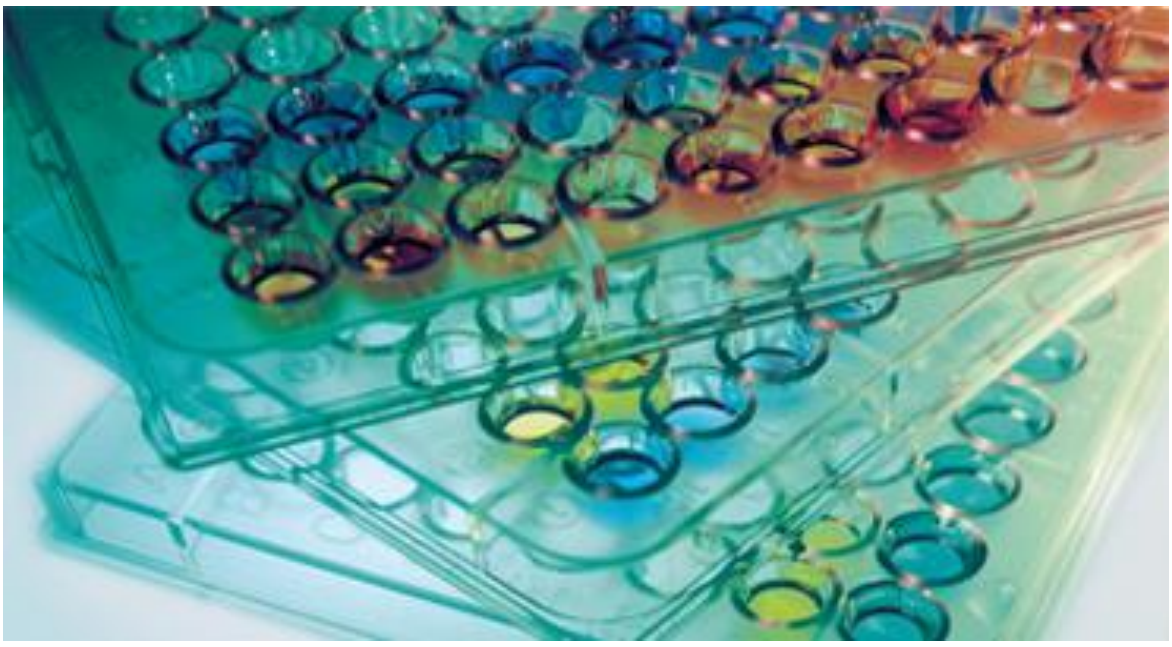

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Contents

Table of tables 5
Table of figures 6
Suggested citation 7
Acknowledgments 7
List of common abbreviations 7
Executive summary 8
Introduction 11
A changing landscape 11
Methods for HIV incidence estimation 11
Recent infection test & RITA 12
Use cases for RITAs 13
Incorporating a RITA as part of routine HIV service delivery 13
Objectives 15
Siaya County, Kenya: antenatal clinics providing PMTCT services 16
Nairobi, Kenya: routine HIV testing and counselling clinics 17
Zimbabwe: Sisters with a Voice programme 17
Zimbabwe: respondent driven sampling survey stored samples 18
Methods 19
Ethical approvals 19
Pilot design 19
Training, confidentiality, and consent to test 20
Return of test results and counselling 20
Qualitative research exploring the feasibility and utility of integrating RITA into routine settings 21
Quantitative research 25
Qualitative results 35
Background 36
Findings 38
Quantitative results 49
Siaya County, Kenya: antenatal clinics providing PMTCT services 50
Nairobi, Kenya: routine HIV testing and counselling clinics 59

Zimbabwe: Sisters with a Voice programme 66
Zimbabwe: RDS survey stored samples 72
Discussion & key findings 83
Feasibility of operationalising a RITA in routing setting 83
Acceptability and utility of using a RITA in routing setting 86
Yield and characteristics of people with recent HIV infection 88
HIV incidence estimates 91
Conclusions 92
References 94
Appendix 99
Appendix 1: Results from follow-up questionnaire after return of a positive recent infection test result 99
Appendix 2: Selection biases associated with the surveillance population 100
Appendix 3: Simulation modeling approach 102
Appendix 4: RITAs for the two excluded RDS surveys 105
Appendix 5: Sensitivity analysis RDS study 3 comparing tested samples to samples not tested for recent infection 107
Appendix 6: Laboratory testing of recent HIV infection 109

Table of tables

Table 1: Eight use cases for HIV RITAs (from FIND) 13
Table 2: Three pilots of HIV recent infection testing in routine service settings 19
Table 3: Details of the four RDS surveys 33
Table 4: Summary of qualitative research 35
Table 5: Summary results of the three pilots of HIV recent infection testing in routine service settings 49
Table 6: Characteristics of 2,365 women testing for HIV 51
Table 7: Estimate of false recent infection 52
Table 8: HIV and recent infection status by age and pregnancy status 52
Table 9: Characterisation of recent infections 53
Table 10: Comparison of women in the pilot to women in HDSS 54
Table 11: Incidence estimate by FRR, MDRI and ART status 57
Table 12: Characteristics of HTC participants testing for recent infection 60
Table 13: Return of recent infection test results (prior to ART metabolite testing) 61
Table 14: Participant characteristics by recent infection status 62
Table 15: Predictors of recent infection 63
Table 16: Predictors of recent infection, disaggregated by gender 64
Table 17: Index/partner testing 65
Table 18: Characteristics of FSWs tested for recent infection using plasma 67
Table 19: Characteristics of FSWs by HIV and recent infection status, and recency rates 68
Table 20: Return of recent infection test results 70
Table 21: Risk factors for testing positive for recent infection among FSWs (plasma; n=302) ... 71
Table 22: Summary results for RDS 1 and 3 74
Table 23: Characteristics of participants in RDS 1 and 3, by HIV and recent infection status 75
Table 24: Incidence calculations by RDS, MDRI and FRR 78
Table 25: Univariable and multivariable logistic regression analysis of risk factors associated with recent infection 79
Table 26: Clinical risk score for recent infection among HIV negative FSW 81
Table 27: Predicted risk of recent infection among HIV -ve clients based on their risk score 82
Table 28: Follow-up questionnaire to the return of a positive recent infection test result 99

Table 29: Comparison of characteristics of women who did not have enough plasma with those who did, among RDS study 3 samples that were eligible to be tested for recency (N=428) (RDS-II weighted) 107

Table of figures

Figure 1: Location of study sites for the three pilots 15
Figure 2: Recency testing algorithm (RITA) as applied in the three pilots 25
Figure 3: The practical process of Recency testing in Sisters with a Voice Clinics 38
Figure 4: Recruitment and testing flowchart (Maxim LAg plasma) 50
Figure 5: HIV prevalence in ANC attendees compared to HDSS in 2016 sero-survey 55
Figure 6: Simulation of HIV prevalence 55
Figure 7: HIV incidence estimates and rate ratios 56
Figure 8: Simulation of HIV incidence 58
Figure 9: Recruitment and testing flowchart (Maxim LAg DBS) 59
Figure 10: Recruitment and testing flowchart for the Maxim LAg plasma assay 66
Figure 11: Recruitment over time 69
Figure 12: Recruitment and testing flowchart for RDS study 1 73
Figure 13: Recruitment and testing flowchart for RDS study 3 73
Figure 14: Age distribution comparing ANC population to HDSS population 100
Figure 15: Parity comparison between ANC attendees and HDSS population 101
Figure 16: Simulation input 103
Figure 17: Recruitment and testing flowchart for RDS study 2 105
Figure 18: Recruitment and testing flowchart for RDS study 4 106

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List of common abbreviations

ANC: Antenatal clinic
CDC: Centres for Disease Control and Prevention
CeSHHAR: Centre for Sexual Health and HIV / AIDS Research - Zimbabwe
DBS: Dried Blood Spots
EDARP: Eastern Deanery AIDS Relief Program
FIND: The Foundation for Innovative New Diagnostics (FIND)
FSW: Female Sex Workers
HDSS: Health and Demographic Surveillance Site
HIV: Human Immunodeficiency Virus
HTC: HIV testing and counselling
KEMRI: Kenya Medical Research Institute
LAg Avidity EIA: Limiting Antigen Avidity Enzyme Immunoassay
LSHTM: London School of Hygiene and Tropical Medicine
PMTCT: Prevention of Mother To Child Transmission
RDS: Respondent Driven Sampling
RITA: Recent Infection Testing Algorithm

Executive summary

Distinguishing recently acquired infection from "long-standing" infection among persons newly diagnosed with HIV can help identify populations and geographic areas where current transmission is occurring; crucial information to inform programme planning. Given this, we set out to assess the feasibility and utility of various approaches to conducting HIV recent infection testing in a range of routine service-provision contexts; namely, in antenatal clinics providing PMTCT services (Siaya County, Kenya), routine HIV testing and counselling clinics (Nairobi, Kenya), and a programme for female sex workers (Zimbabwe).

To distinguish recently acquired infection from "long-standing" infection we used a Recent Infection Testing Algorithm that combined a Limiting Antigen Avidity Enzyme Immunoassay with clinical information (viral load; history of prior HIV diagnosis; exposure to ART). Maxim HIV-1 LAg-Avidity EIA dried blood spot and plasma kits were used for recency testing, with a rapid recency assay (Asante HIV-1 Rapid Recency Assay) also being applied in Siaya County. In Zimbabwe, we also ran the Maxim test kits on samples taken from female sex workers participating in a number of respondent driven sampling surveys.

For all three pilots we explored testing yields and described the characteristics of participants (and in Nairobi, their partners) testing recent infection positive. Additionally, we calculated HIV incidence rates (Siaya County and Zimbabwe), and assessed selection biases (Siaya County). In both Nairobi and Zimbabwe, recent infection test results were returned to clients, and interviews and questionnaires were conducted to explore the feasibility and acceptability of implementing a Recent Infection Testing Algorithm (RITA) from the perspective of health care providers and participants.

Having successfully developed protocols for incorporating recency testing, we experienced a number of significant barriers in their implementation. The main issue we experienced with implementation was poor adherence to 'Instructions for Use' in the laboratories. Our experiences highlight the importance of ensuring laboratories have dedicated equipment and trained staff for recency testing prior to scale-up. Other significant barriers we faced were the procurement of recency test-kits, complications in the delivery of test results back to participants (including delays in receiving test results back from the laboratory, and participants not returning for their results), and conducting antiretroviral metabolite testing (only successfully completed in Nairobi).

In Siaya County, among 424 pregnant women newly testing HIV positive, 10 (2.4%) were recent infection positive (one additional woman originally classified as recent was reclassified as "long-standing" due to evidence of having initiated ART). Women in their first trimester had a ten times increased odds of testing positive for recent infection compared to those testing in their second or third trimester. Incidence was estimated at 1.1 (95%CI: 0.29-1.9) per 100 person-years assuming a 0.003 false recency rate (FRR) and a mean duration of infection (MDRI) of 206 days. Longer MDRI and lower FRR led to higher incidence estimates (and vice versa). Among participants linked to a Health and Demographic Surveillance Site (HDSS) record at study enrolment, incidence between last HDSS negative HIV test and their ANC visit was 1.3 per 100 person-years (95% CI: 0.88-1.9).

In Nairobi, 40 (12.7%) women among 316 testing and 8 (3.7%) men among 216 testing were classified as positive for recent infection prior to ART metabolite testing. Two of these people (one woman and one man) were subsequently re-classified as having "long-standing" infection due to metabolite testing showing they had been in receipt of antiretroviral therapy. Therefore, 46 (8.6%) people (women: 39; 12.4% and men 7; 3.3%) were finally classified as positive for recent infection. Interestingly, higher yields were attained through partner testing. A significantly higher proportion of people aged under 25 years (compared to older groups), and women (compared to men), tested recent positive. Overall, three quarters of participants in Nairobi received their recency test result, although often following a delay arising from challenges in the laboratory.

In Zimbabwe, 33 (10.5%) FSW were recent infection positive among 314 tested. Overall, only one in ten FSW who tested for recent infection received their test result (the proportion was much lower among those classified as having "long-standing" infection compared to those with a recently acquired infection). Again, there were delays in getting the test results back to participants due to laboratory-based challenges.

The qualitative research conducted in Nairobi and Zimbabwe found responses to acceptability and utility of using RITA to be mixed. Acceptability was related to utility; if recency testing was assumed or understood to be useful, this encouraged acceptability. There was some confusion about whether recency test results could be used to inform treatment decisions. For some participants the logical individual outcome of recency testing was seen as to potentially identify the source of infection, and some saw this as useful. Others were not interested in identifying who infected them and found the possibility unhelpful, raising concerns about blame, risk of violence, and disruption to focusing on the future and treatment. There was some misunderstanding about how specifically a 'recent' infection related to a particular time period, or point in time. Utility is

likely to be lower for FSW than in the general population, due to a number of factors including sex worker mobility, and a reluctance to be contacted by clinic staff in general.

Despite challenges, in all settings we identified and characterised people classified as positive for recent HIV infection. In Nairobi and Zimbabwe we were also successful in returning test results back to some participants (although numbers were lower than anticipated, particularly in Zimbabwe), and in conducting qualitative research to explore understanding, acceptability, utility and risk and mitigation. In Siaya County and Zimbabwe, we were also successful in estimating HIV incidence (a process that would be made simpler through the availability of meaningful denominators, and consensus on MDRI and FRR), and in exploring potential biases in incidence estimates when using women attending ANC as the surveillance population.

Introduction

A changing landscape

In the era of test and treat (1, 2), increases in HIV prevalence will be a natural consequence of the success of treatment in extending life expectancy (3). This has led to a more complex relationship between HIV prevalence (the proportion of a population infected with HIV) and incidence (the occurrence of new HIV cases in a population over a specified period of time), making the need for identification of recently infected cases of HIV infection more important. Furthermore, the expanding armoury of interventions to prevent new infections and/or reduce the number of people living with undiagnosed HIV makes it increasingly difficult to interpret temporal and spatial trends in diagnoses. For example, an increase in diagnoses may be the result of a success in promoting HIV testing among those not previously tested, or of a failure in preventing new HIV infections.

Distinguishing recently acquired infection from "long-standing" (i.e. non-recent infection) infection among persons newly diagnosed with HIV can help identify populations and geographic areas where current transmission is occurring; crucial information to inform programme planning. As a disproportionate number of HIV transmissions originate from people who have early HIV infection (4), targeting effective prevention and behavioural interventions to populations where HIV infection is currently circulating could have a large impact on levels of transmission. Additionally, data on new infections may contribute to tracking the incidence of new infections over time, which can help assess whether control measures are working and understand better where prevention efforts should be targeted.

Methods for HIV incidence estimation

Various methods of estimating HIV incidence exist, all of which have potential biases. The most direct method is observation of a longitudinal cohort. This methodology involves recruitment and follow up of a well-defined cohort of persons who are HIV negative but who are at risk of acquiring infection over time. Several long-term cohort studies measuring incidence exist. The main limitations of such longitudinal studies are that they are time consuming, logistically challenging, the methods applied may influence outcome measurement, and expensive to implement (5, 6).

HIV incidence can also be measured indirectly, using mathematical models triangulating trends in HIV prevalence with data on births, deaths and other factors (7). Another modelling approach utilises CD4 cell counts at diagnosis to estimate HIV incidence through back calculation from time of diagnosis to probable time of infection (8-12). Outputs from such models require careful interpretation; this is particularly true when assessing temporal trends as the models are often reliant on data sources and assumptions that vary over time.

Recent infection test & RITA

A number of laboratory based assays have been developed that can identify recent HIV infections through the testing of blood specimens. An example, and one used in this study, is a Limiting Antigen Avidity Enzyme Immunoassay (LAg Avidity EIA). These assays look for specific antibody markers that evolve in the months following infection.

These assays are interpreted as part of a Recent Infection Testing Algorithm (RITA) that combines the test results with clinical information, such as viral load, history of prior HIV diagnosis, and exposure to anti-retroviral therapies, to distinguish recently acquired infection from "long-standing" infection among persons newly diagnosed with HIV (13). Linking clinical data to the results of the assays helps detect "long-standing" infections that have been misclassified as recent (7). Such laboratory based methods can be more efficient in time than the other methods described above as they generally do not require follow up of participants, and samples are collected as part of research or routine service delivery (14).

An independent evaluation of the five most commonly used tests for recent infection found that the Sedia LAg Avidity EIA approach was the most accurate assay across multiple subtypes for HIV infection, with an assay specificity of 98.5% (6). Two versions of the LAg assay exist: the Sedia assay produced by Sedia Biosciences Corporation (Portland, OR) and the Maxim assay produced by Maxim Biomedical (Bethesda, MD). Both companies produce kits for plasma specimens and dried blood spot eluates. A study comparing the performance of both plasma kits showed that the Maxim assay results in systematically lower ODn measurements and a slightly better reproducibility (15). For surveillance purposes, defined as the precision of incidence estimates, both tests were almost indistinguishable (15).

Use cases for RITAs

The Foundation for Innovative New Diagnostics (FIND) identified two families of use cases for RITAs: those where the ultimate aim is to estimate HIV incidence, and those where it is not. Table 1 presents five uses for RITAs where the aim is to estimate incidence, and three where it is not. In 2015, and in relation to HIV case surveillance, the WHO working group on HIV incidence assays concluded that currently available LAg assays met the minimum requirements for use cases 1, 2, 3 and 8 in certain populations where viral load information is incorporated, but did not meet the requirements for use cases 4 and 5, primarily due to the large sample sizes required (16).

Table 1: Eight use cases for HIV RITAs (from FIND)

		Use	Description of Use
Uses for RITAs related to estimating incidence	1	**National surveillance**	To provide national estimate of incidence; may be part of a broader demographic study
	2	**Program, prevention or trial planning**	To provide incidence estimate in sub-populations for planning, prioritizing, or other instances when an estimate of incidence is required. Often may be for only a city or region (Example: prioritize programs or investments, or identify sites for intervention trials)
	3	**Key or sentinel populations**	To provide incidence estimates in special sub-population using targeted sampling methods
	4	**Assessing the impact of population-level interventions**	To assess the impact of a population-level intervention (e.g., community-level intervention) by comparing incidence before and after the intervention
	5	**Case-based surveillance**	To provide national or regional incidence estimates via case-based reporting of newly identified HIV+ individuals
Uses for RITAs NOT related to estimating incidence	6	**Research purposes**	Identification of individuals with "recent" infections for multiple potential applications (e.g., recruitment of recently infected individuals into longitudinal cohort studies)
	7	**Individual patient management**	Identification of patients with recent infections to guide clinical management and/or public health programs (e.g., selecting therapy, and/or prioritizing contact tracing)
	8	**Targeted prevention planning**	To provide population-level data on recent infections to enable risk factors analysis or identify hot-spots to inform targeted prevention planning (no incidence estimate is obtained)

Source: amended from the Foundation for Innovative New Diagnostics (https://www.finddx.org/)

Incorporating a RITA as part of routine HIV service delivery

To date, RITAs have primarily been used in community-based HIV prevention trials, for example in Zimbabwe, Tanzania, South Africa and Thailand (17), and in national population-based HIV impact assessment (PHIA) surveys estimating national HIV incidence in twelve high-burden African countries (18). Additionally, the assay / algorithm has also been used as part of routine testing in the United States of America, the United Kingdom (where it forms part of the national HIV case surveillance system), and other high-income countries (19-21).

The inclusion of recency testing in routine HIV service delivery has several implications. First, programmes can anticipate additional costs resulting from test assays and logistics related to sample handling (22). For a client, an additional amount of blood may be required for laboratory-based testing (depending on whether a venous sample has already been taken for other purposes); additional time at the facility is also a consideration. Furthermore, unlike rapid HIV tests where clients receive their results at the same visit, currently available RITAs are partly laboratory-based and hence there is an expected delay between recency testing and return of results. Beyond returning of results timelines, programmes may need to make modifications to client flow in facilities, provide additional training to healthcare workers, and consider interventions resulting from recent infections such as contact tracing, community prevention initiatives, and psychological support systems.

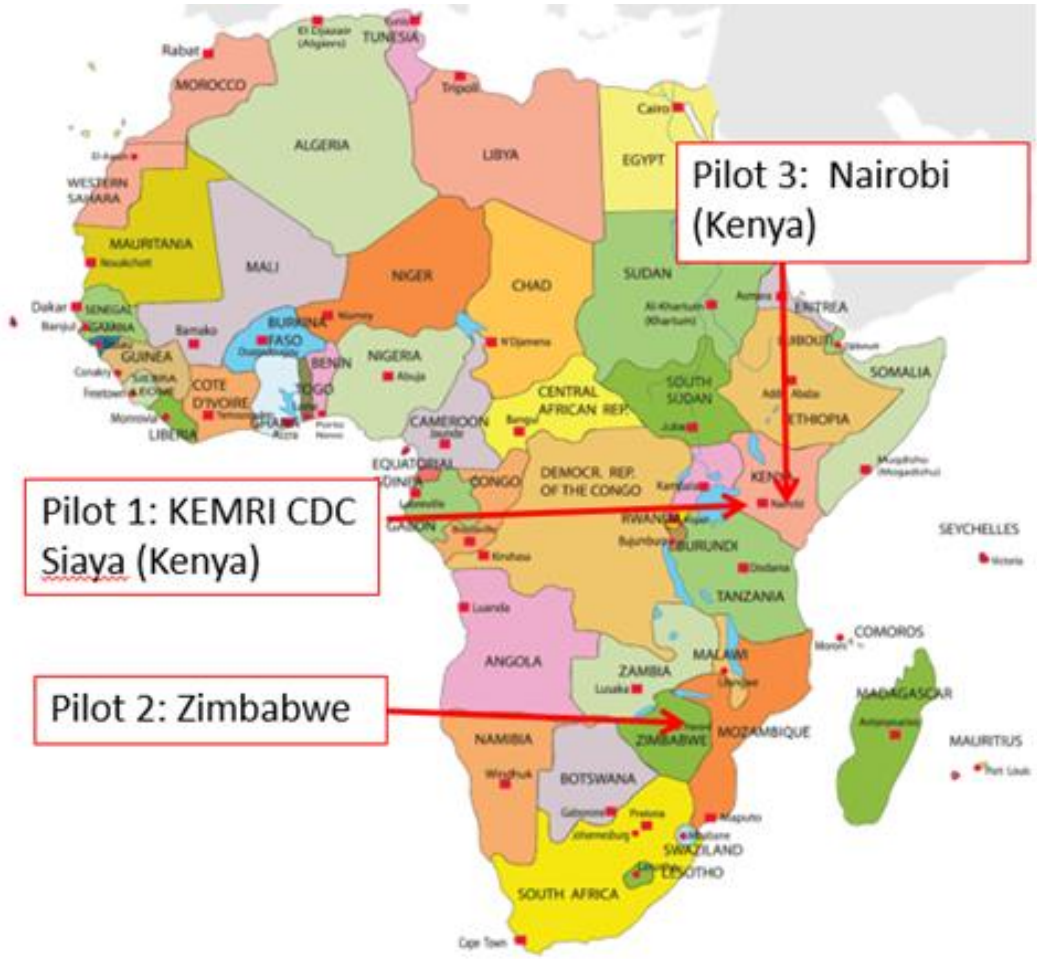
Psychosocial factors, such as discrimination and rejection, may arise from information suggesting when (within parameters), and potentially how, HIV may have been acquired. A study looking at psychosocial factors that influence uptake for HIV testing found fears of consequences, such as discrimination and rejection, hindered progress (23). The same study also found that despite these fears, testing is more likely to occur when an individual perceives more benefits from testing versus not testing (23). It is therefore important to make clear the benefits and potential adverse events attached to a recency test and to document factors that influence uptake. Programmes can also expect to experience challenges in running and interpreting the recency test itself, and in working their way along the RITA (for example, conducting and linking viral load and ART testing).

Exploring and better understanding these issues is currently of particular relevance. In 2019 the United States President's Emergency Plan for AIDS Relief called for recent infection surveillance, incorporating the return of the test result, to be implemented (using a point of care rapid recency test that is currently not validated for use in diagnostic procedures (24)) at scale across all sites, and among all persons newly diagnosed, in supported countries (25, 26). The Centers for Disease Control and Prevention in the USA is currently seeking pre-qualification approval from WHO to support the rollout of a rapid recency test. In this report, we present the results of three pilots where we integrated a RITA into routine programme activities in Kenya and Zimbabwe.

Objectives

To conduct three independent but linked prospective pilots in different routine service delivery settings in Kenya (two pilots - in antenatal clinics, and in an HIV testing and counselling (HTC) setting) and Zimbabwe (outreach testing services for female sex workers) to explore, principally, the feasibility and utility of the integration of RITA within routine programmatic activities (see Figure 1). In Zimbabwe, to also conduct recent infection testing on stored samples arising from four respondent-driven sampling surveys of female sex workers (FSW).

Figure 1: Location of study sites for the three pilots



Prevalence of HIV in Kenya and Zimbabwe is among the highest in the world. Prevalence in Kenya is estimated at 4.9% among persons aged 15-49 years based on estimates from 2017(27). Women had a higher prevalence (5.2%) than men (4.5%). In 2017, incidence among adults 15-49 years old was 0.19%(27). In Zimbabwe, HIV prevalence is estimated at 13.8% among women and

men aged 15 to 49 years. Prevalence is higher among women (16.7%) than men (10.5%)(28). In female sex workers aged 18 and above, HIV prevalence is estimated to be 57.8% (29).

Across the three pilots, our overarching objectives were:

1. Assess the feasibility and utility of integrating a RITA that includes a LAg avidity assay, viral load testing, and ART metabolite testing into routine service delivery;
2. Develop, adapt and pilot approaches to consent and results dissemination and counselling to clients;
3. Develop methods to use RITA test results to improve prevention and treatment programmes.

While the focus of these objectives was on feasibility and utility in the short term, each pilot was conducted with a longer-term aim in mind. The Kenyan antenatal clinic (ANC) settings pilot was orientated toward providing potentially useful information toward the wider adoption of RITA within ANC settings for surveillance purposes (use case 1 in table 1 above: national surveillance). Our pilot in Kenyan HTC settings was orientated toward providing useful information for the wider adoption of recency tests within such settings to inform patient care (use case 7: individual patient management). In Zimbabwe, our pilot aimed to inform the potential use of recency tests for both local programmatic decision making (use case 8: targeted prevention planning) and individual patient management (use case 7) among FSW (use case 3: key or sentinel populations).

To support the presentation of results, the pilot specific objectives below are presented in a different order / combination than in the protocols (which are available on request).

Siaya County, Kenya: antenatal clinics providing PMTCT services

Our pilot in Siaya County in western Kenya was conducted in collaboration with the Kenya Medical Research Institute (KEMRI) and the KEMRI/CDC Siaya Health and Demographic Surveillance Site (Siaya HDSS). The pilot aimed to implement HIV recent infection testing in antenatal clinics providing prevention of mother to child transmission (PMTCT) services in fourteen healthcare facilities. In the study area, HIV prevalence is high compared to the rest of the country. In 2017, HIV prevalence was estimated to be 21% (27), and HIV incidence 2% in some groups (30, 31). The fertility rate is >five children per woman, and almost all women (94%) access antenatal care at some point during pregnancy (32). PMTCT services are widespread in the study area, with 87% of women receiving HIV testing as a part of antenatal care (32).

ANC objectives:

1. Assess the feasibility of using RITA at ANC for HIV incidence surveillance, and identify the yield of women positive for recent infection;
2. Assess selection biases associated with the surveillance population (pregnant women using antenatal services);
3. Estimate the HIV incidence rate in this population, and compare with direct estimates from repeated tests for prevalent infection from the Siaya HDSS and ANC;
4. Compare two HIV recency assays: the Asanté™ HIV-1 Rapid Recency™ Assay and the HIV-1 LAg-Avidity EIA;
5. Validate ANC register ART status with a biomarker for ARV detection.

Nairobi, Kenya: routine HIV testing and counselling clinics

In Nairobi county, we collaborated with the Eastern Deanery AIDS Relief Programme (EDARP), to conduct HIV recent infection testing as part of routine HTC services at fourteen clinics. HTC has evolved over time to encompass stand-alone or facility based voluntary counselling and testing centres. EDARP has been providing community-based quality HIV and tuberculosis prevention, testing, treatment and care services in the eastern slums of Nairobi for the past 24 years (33). Nairobi county has an estimated prevalence of 6.1% and approximately 7,159 new infection in 2017 (27). The prevalence in the target population EDARP serves is estimated at 12% in certain urban settings (33).

HTC objectives:

1. Administer a follow-up questionnaire to study participants who test for recent HIV infection, and a survey to outreach workers, test counsellors and other healthcare providers;
2. Assess the feasibility and utility of integrating a RITA with return of recency results into routine HTC services at fourteen facilities in Nairobi, Kenya;
3. Identify and characterize where and among whom recent HIV infections are occurring;
4. Conduct index / partner testing.

Zimbabwe: Sisters with a Voice programme

The Sisters with a Voice programme for FSW operates in six static and thirty outreach sites across Zimbabwe, providing a range of services including testing, behavioural counselling, and referral to government ART services (34). The programme is run by the Centre for Sexual Health and HIV

AIDS Research in Zimbabwe (CeSHHAR - Zimbabwe) on behalf of the National AIDS Council and Ministry of Health and Child Care.

Aims of the programme include HIV prevention, diagnosis, provision of clinical services for STI and HIV, and referral to treatment. When a client tests HIV positive in one of the clinics, counselling is provided and the client is referred into the national ART system. Relevant confirmatory tests are conducted and treatment is started immediately. The population is at very high risk of HIV infection. Across sites, the prevalence of HIV is on average 58% (29, 35, 36). HIV incidence rates are poorly understood, but may be as high as 10% per year (37).

Sisters programme objectives:

1. Document experiences of women consenting and receiving results from a RITA, and explore the potential consequences of contact tracing and hotspot mapping;
2. Assess the feasibility and utility of integrating RITAs and return of recency results into routine programme service delivery for FSW, and identify the yield of women positive for recent infection;
3. Identify sociodemographic risk factors for recent HIV infection.

Zimbabwe: respondent driven sampling survey stored samples

The FSW programme undertook a number of respondent driven sampling (RDS) surveys in multiple locations across Zimbabwe between 2015 and 2017 (29, 36, 38-40). In 18 locations across the country, blood samples and comprehensive questionnaire data were available for at least 8,500 female sex workers, of whom over 3,500 were HIV positive. Three of the four surveys used dried blood spots and one survey used plasma samples.

RDS survey objectives:

1. Identify yield of clients testing positive for recent infection among RDS participants;
2. Estimate HIV incidence using RITA from surveys and compare with estimates of HIV incidence from repeat testers in the FSW programme;
3. Identify sociodemographic and behavioural risk factors of recent HIV infection;
4. Develop a risk-screening algorithm that could identify female sex workers at high risk of recent infection.

Methods

Ethical approvals

Local approval was provided by the ethical committee of Medical Research Council of Zimbabwe for the Zimbabwe pilot, by Kenyatta National Hospital-University of Nairobi Ethical Review Board for the pilot in Nairobi, and by KEMRI Scientific Ethics Review Unit (SERU application 3589) and London School of Hygiene & Tropical Medicine (LSHTM) (reference number 14458) for the pilot in Kisumu. Ethical approval was also obtained at the LSHTM for the Zimbabwe pilot (reference number 14542) and Nairobi pilot (reference number 14585).

Pilot design

Table 2 presents a summary of the three pilots of recency testing in routine service settings.

Table 2: Three pilots of HIV recent infection testing in routine service settings

Siaya County, Kenya	Nairobi, Kenya	Sisters with a Voice, Zimbabwe
Study population Pregnant women seeking antenatal care in selected medical facilities	**Study population** Clients attending any of the fourteen EDARP HTC facilities	**Study population** FSW attending any of the six static sites of the Sisters with a Voice Programme
Study period February – November 2018	**Study period** March – November 2018	**Study period** June – November 2018
Assays Maxim HIV-1 LAg-Avidity EIA venous blood & Asanté HIV-1 Rapid Recency Assay	**Assay** Maxim HIV-1 LAg-Avidity EIA DBS	**Assays** Maxim HIV-1 LAg-Avidity EIA venous blood & DBS
	Qualitative research Reasons for refusal; follow-up questionnaire; key informant interviews	**Qualitative research** Interviews with FSW; in-depth interviews with key informants; focus group discussions with sex workers
Inclusion criteria • Women aged 13 or older seeking antenatal care in one of the selected medical facilities in Siaya County • Provides informed consent • Received an HIV positive test result	**Inclusion criteria** • Aged 18 or older • Unknown HIV status prior to visit • Attending an EDARP HTC facility • Willing and able to provide informed consent • Received an HIV positive test result, or presumptive positive	**Inclusion criteria** • FSW aged 18 or older • Provides informed consent • Received an HIV positive test result
	Exclusion criteria • Indeterminate HIV result • Not willing to enrol on follow-up at facility • Taking pre-exposure prophylaxis	**Exclusion criteria** • Indeterminate HIV result • Prior history of testing HIV-positive (>1 year ago) • On ART

Training, confidentiality, and consent to test

All study staff underwent training prior to the commencement of the pilot. The training included good clinical practice, ethics training, and the handling of confidential information. Study staff were asked to sign a confidentiality agreement (some existing staff had previously signed such an agreement) to protect the identity of participants.

When clients were literate they were asked to read the consent/assent forms and got probed for their understanding. Study staff read consent/assent forms aloud to illiterate clients. These forms included information on study purpose, duration, potential harms and benefits, and procedures, including data collection, laboratory testing and, where relevant, return of test results. Consent forms were signed by eligible participants and stored for study records. Informed consent for illiterate participants was obtained in the presence of an independent witness. Each participant was provided a signed copy of the consent form, which included information about how to contact the study team in case they had any questions or concerns about the study.

In relation to qualitative research conducted in Zimbabwe and Nairobi, written informed consent was collected from all healthcare workers and eligible clients prior to data collection according to good clinical practice guidelines.

As per Kenyan research guidelines (41), for women aged 13 to 17 years who were pregnant, or were already parents in the Siaya county pilot, the need for parental consent was waived as they were considered emancipated and thus able to provide informed consent independently. Eligible women could consent only to participation in the study or record linkage between clinical and DSS information, or to both the record linkage and biomarker collection.

Informed consent had already been sought from RDS survey participants in Zimbabwe for incidence testing. Consent was provided for further testing on DBS samples for a period of up to five years from the time the RDS surveys were conducted. Further information on training, confidentiality and consent are available in the pilot specific protocols (available on request).

Return of test results and counselling

In Zimbabwe and Nairobi, recency test results were returned to participants who wanted them, irrespective of the recency test result. In these two setting, qualitative research was also conducted to understand the experiences of consenting to, and receiving results from, a RITA.

Participants choosing to receive their recency test result were asked to return to the testing facility after two weeks to collect their results. In Nairobi, the result was based on a LAg avidity assay for DBS and viral load. In Zimbabwe, it was based on a LAg avidity assay for plasma and viral load.

In both settings, health care workers returning recency test results to participants received training on counselling. The training also included guidance on returning recency results. Appropriate counselling on the recency result was provided to participants, opting to receive their results, at the follow-up appointment, or a subsequent visit if the return of the result was delayed. The counselling provided information on the meaning and implications of the result, including implications for partner notification, clearly stating the uncertainty in the test result, and highlighted the benefits of commencing treatment.

In Nairobi, participants who missed appointments or subsequent visits were contacted by a clinician through a phone call. Those not reached by a phone call, or those that continued to miss appointments after being reached by phone, were followed up by a community health worker through a home visit (as is routine within the programme). This is the standard operating procedure for patients who miss appointments at the facility. In Zimbabwe, participants received compensation to cover the cost of their time and transport costs for the follow up visit. Participants were required to provide locator information to ensure that clinic staff could contact them to come and collect their results. When participants chose to receive their result but did not show up at the next visit, phone calls were made by the nurses to follow-up with them but no home visits were conducted.

Qualitative research exploring the feasibility and utility of integrating RITA into routine settings

Our overall aim was to assess the feasibility and utility of integrating RITA into routine service delivery. The qualitative research component explored health care providers' and clients' experiences of the pilot processes in Kenya and Zimbabwe, including the delivery of recency test results and counselling. We collected data through in-depth interviews, focus group discussions and questionnaires.

Data Collection

Qualitative research was conducted separately for the two pilot sites.

Nairobi, Kenya: routine HIV testing and counselling clinics

In Nairobi, the following three approaches were applied to collect data about feasibility and utility:

1. Documenting reason for refusal to participate in the study among persons testing HIV positive during the study period (from July 2018 onward).
2. Administering a follow-up questionnaire to participants testing positive for recent infection at the next routine visit following the receipt of the RITA results. The questionnaire collected information on a person's experience of receiving a recency result, initiation on and adherence to ART, actions arising from the test result (for example, partner notification), and any reports of intimate partner violence after disclosure of a recency result. The questionnaire was administered on a tablet using Open Data Kit (ODK) platform.
3. Conducting key informant interviews with health professionals at EDARP facilities to assess their perspectives on feasibility and acceptability of including RITA in routine HTC procedures. Interviewees were purposively sampled from among HIV counsellors, outreach workers and healthcare providers at EDARP facilities. Interviews were facilitated by two members of the study team: one interviewer and one note-taker. Participants were required to sign the appropriate consent form. Discussion topics included:
    a. The acceptability and feasibility of including RITA in routine HTC services at EDARP facilities;
    b. Anecdotal information on adverse events that have resulted from recency testing and/or test results;
    c. The amount of additional time needed to be spent with each HTC client to perform RITA.

Interviews were digitally audio recorded and notes were taken during the interview. Recordings were fully or partially transcribed, and personal identifying information removed.

Zimbabwe: Sisters with a Voice programme

Three approaches to collect data about feasibility and utility were also applied in Zimbabwe:

1. In-depth, semi-structured interviews were conducted with FSWs who had received an HIV positive test result. The majority were conducted after recency testing before receipt of results. Participants were recruited by clinic staff and the researcher.

2. In-depth, semi-structured interviews were conducted with clinic nurses involved in piloting RITA at the static sites in Harare, Bulawayo, Gweru, Karoi, Mutare and Masvingo.
3. Focus group discussions (FGDs) were held with sex workers at four static sites. Convenience sampling was used by clinic staff to recruit eight to twelve participants for each FGD. The majority were female. In two sites (Harare and Bulawayo)) the clinic program had recently expanded to include male and transgender sex workers, and eight participants from these groups were included in two FGDs.

All data was collected by one researcher (see Research Context below). Interviews and focus group discussions were digitally audio recorded and transcribed, and personal identifying information removed.

Debriefing discussions

Debriefing discussions were held with study coordinators, laboratory managers, study implementers and other researches involved in this project across the three sites and the secretariat. All discussions were led by the same person (Mariken de Wit), focussing on experiences, challenges and solutions, and recommendations regarding different aspects of the project. Discussions were recorded digitally and by hand, and summary reports were prepared.

Data analysis

Qualitative data from Nairobi and Zimbabwe were first analysed separately, summarising responses to interview questions. Data relating to acceptability, laboratory work and delivery of recency testing were extracted from debriefing discussion notes. A second layer of thematic content analysis working across all data from both sites was then used to identify and categorise recurrent themes (42), and comparisons were drawn within and between accounts and sites.

Limitations

There were limitations to qualitative data collection which should be taken into account when interpreting results:

1. The qualitative data were collected by the same research team who had set up the study and had trained staff about conducting and promoting recency testing. In the Nairobi study, at the beginning of interviews health care workers were asked '*do you see the value of returning HIV recency results to clients for managing their care*?'. This question implied that there is a value, and that the interviewer would like to know whether the interviewee recognises that value. This set the tone of the interviews in a specific direction implying utility of recency testing.

2. The pilots were conducted in clinics which did not have stable long term funding. Staff may therefore have been keen to encourage and support externally funded programmes which could potentially financially support the clinics, and their salaried positions, in future. This could have made them reluctant to give negative feedback about recency testing.

3. All interviews with health care staff and sex workers were conducted on site in clinics, which may have influenced participants' responses. These limitations are related to power imbalances between the researcher and local participants, which can make it difficult for participants to give an independent evaluation of their experiences, and particularly to report negative views.

4. Logistical limitations influenced selection and availability of participants for interviews with FSWs in the Zimbabwe pilot. These were all conducted by one researcher travelling around the country by public transport, with limitations to how quickly she could travel between sites, and to what extent she could plan to interview specific participants at different points. An ideal scenario would have been to purposively sample participant's pre and post testing, following up some of the same participants, and interviewing similar proportions with positive and negative recency results. In practice it was difficult to arrange to interview participants who had received their recency test result as there were fewer participants than expected, and each stage of the process was uncertain (when results would be available, whether participants could be contacted, whether and when they would attend the clinic to receive their result).

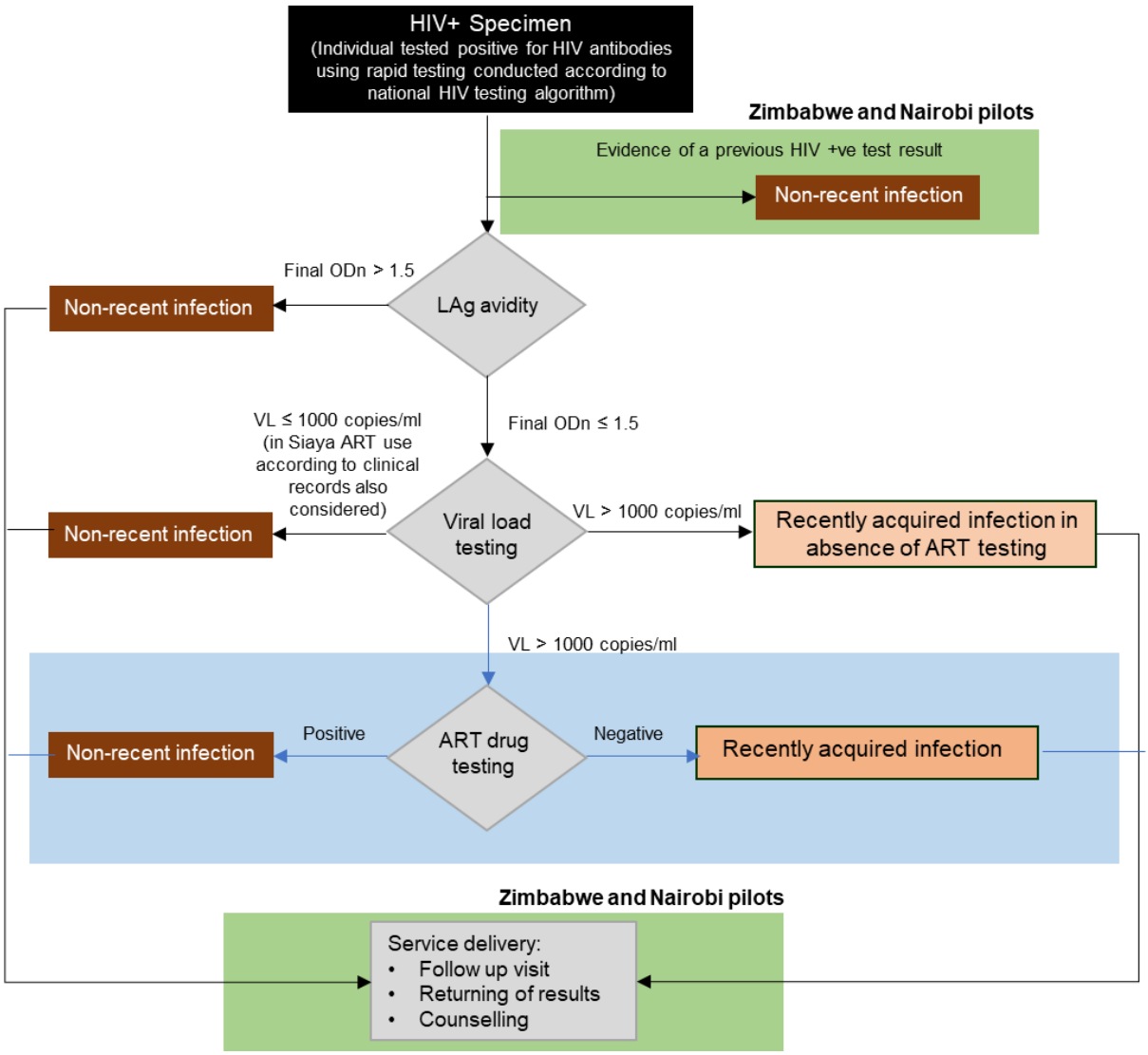
Quantitative research

The three pilot studies had certain aspects of their methods in common, but also had important differences. Common features are first presented first, followed by pilot specific information.

Recent Infection Testing Algorithm

Figure **2** presents the RITA applied in all three pilots. Further details on these three pilots, and the testing of Zimbabwe RDS stored samples, are provided below.

Figure 2: Recency testing algorithm (RITA) as applied in the three pilots



The RITA we integrated into routine service provision can be summarised as follows:

- Samples of participants who have tested HIV positive before, or who are on ART classified, classified as non-recent;

- LAg Avidity testing conducted on remaining participants;
- Samples with an ODn >1.5 classified as non-recent;
- If ODn ≤1.5, viral load testing conducted;
- Samples with a viral load ≤ 1000 copies/mL classified as non-recent;
- Samples with a viral load > 1000 copies/mL classified as potentially testing positive for recent infection;
- Samples classified as potentially testing positive for recent infection, tested for the presence of ART metabolites;
- Samples containing ART metabolites classified as non-recent;
- Samples free of ART metabolites classified as testing positive for recent infection.

LAg Avidity Enzyme Immunoassay (EIA)

The LAg Avidity EIA is based on the functional avidity or binding strength of antibodies. Antibody avidity increases with time since infection and is a robust parameter to distinguish recent from "long-standing" infection. The assay incorporates a recombinant protein (rIDR-M) containing the major variants of gp41 immunodominant regions among the HIV-1 group M viruses in order to minimize subtype bias. LAg Avidity EIA is a single-well avidity assay and provides a measure of antibody avidity as normalized optical density (ODn). The assay is optimized by coating plates with limiting amounts of rIDR-M which facilitates binding of only high avidity antibodies. Due to low density of antigen on coated wells, weak antibodies dissociate easily because of monovalent binding of antibodies. The single-well LAg Avidity EIA is different from the two-well avidity assay that is commonly used to calculate an avidity index. However, the end results are very similar and comparable. LAg Avidity EIA allows testing of more specimens per plate and is easier to perform.

The LAg testing protocol requires specimens with an ODn value ≤2.0 during initial testing to be retested in triplicate from a fresh dilution of the specimen to improve accuracy of the result. The ODn is calculated by dividing the OD for each specimen by the median OD of a calibrator specimen included with the assay. For specimens that have an ODn value ≤2.0 and undergo triplicate testing for confirmation, the final ODn value for that specimen is the median value of the triplicate test results. Anti-HIV serology was performed on specimens showing ODn value <0.4 to confirm HIV antibody positivity. The majority of laboratory results were reviewed remotely by Dr Gary Murphy (via the sharing of Excel template batch results) to check for issues and/or inconsistencies.

The Maxim HIV-1 LAg-Avidity EIA DBS Kit was used in Nairobi and Zimbabwe on DBS samples (http://www.maximbio.com/viewitem.php?itemID=92003&categoryID=11), with the Maxim HIV-1 LAg-Avidity EIA being used in Siaya county and Zimbabwe on venous blood (aka plasma (http://www.maximbio.com/viewitem.php?itemID=92001&categoryID=11). As the Maxim kits do not come with DBS filter paper cards, they were procured locally.

MDRI and FRR

The two key parameters of LAg testing are the mean duration of recent infection (MDRI), and the false recency rate (FRR). The MDRI varies by HIV subtype and viral load cut-off used. It is estimated to be between 130 to 206 days in this study, depending on the study location. Estimating the FRR is less reliable than estimating the MDRI, as it is highly dependent on the population surveyed. However, in our RITA we included viral load and, therefore, can be fairly confident that the FRR will be low in subtypes A, B, and C (D is currently more problematic), because the major source of false recent results in most situations is people on ART (or elite controllers), who have low viral load. To minimize false positive recent infections further, we planned to conduct laboratory tests for the presence of ART on samples with a positive RITA test result.

We did not seek to provide further information on MDRI or FRR for the assays we applied. However, in the Siaya county pilot and the Zimbabwe RDS survey study we used our results to make an initial estimate of the incidence rate, and to do this we required an MDRI and FRR. Reflecting that these figures remain under debate, we present incidence figures using a combination of plausible FRRs (0% to 1%) and plausible MDRIs (130 to 206 days).

Viral load testing

Viral load was measured using the Abbott m2000, Roche Cobas Ampliprep/Cobas Taqman, or similar automated platform according to the manufacturers' instructions. Internal quality control checks were run according to manufacturer's instructions.

ART testing

Up until 2015, WHO recommended to start ART when a person's CD4 cell count was below a certain threshold (43). This recommendation ensured only those with "long-standing" infection would be in receipt of treatment. In 2015, WHO recommended ART be initiated in everyone living with HIV regardless of CD4 cell count (2); the 'test and treat' strategy. In practice, some countries, among certain populations, had implemented a test and treat approach prior to 2015.

As the inclusion of information on exposure to ART could improve RITA performance through lowering false-recent misclassification (44), it was planned for samples assessed as recent positive in the three pilots to be sent to the Pharmacology Laboratory at the University of Cape Town to test for the presence of antiretroviral metabolites in the blood. Antiretroviral metabolites, including Lopinavir, Ritonavir, Nevirapine, Efavirenz, Indinavir, Saquinavir, Zidovudine, Lamivudine and Stavudine, were quantified by a robust simultaneous liquid chromatography/tandem mass spectrometry method. Laboratory quantifications were used to triangulate the results of the RITA. Additionally, in Siaya County, recency test results were linked to a women's clinical record to explore prior ART use. Should a sample be found positive for any of these drugs, it would be classified as a "long-standing" infection / false-recent.

Siaya County, Kenya: antenatal clinics providing PMTCT services

Sampling of participants

All pregnant women seeking antenatal care in the selected medical facilities between February 2018 and November 2018 were approached to assess eligibility.

Inclusion criteria

- Women aged 13 or older seeking antenatal care in one of the selected medical facilities in Siaya County;
- Provided informed consent;
- HIV positive.

Rapid recency (Asante)

In May, 2017 discussions commenced between the MeSH secretariat and the Centres for Disease Control and Prevention (CDC) to run samples collected for LAg avidity testing in Siaya County, Kenya using the Sedia Biosciences point of care Asanté™ HIV-1 rapid recency™ assay (http://www.sediabio.com/products/asante-rapid-hiv-1-recency-assay), to facilitate a comparison of results. The Asanté is a rapid recency test that PEPFAR intend to rollout for the surveillance of recent HIV infections (26).

Although designed to be a point-of-care test, for the purposes of our study the Asanté™ HIV-1 Rapid Recency® Assay was run in the laboratory on all samples after the Maxim HIV-1 LAg-Avidity EIA had been completed. Results were available in twenty minutes and were read visually. In three lines it shows whether the test is valid and properly performed, whether the specimen shows anti-HIV reactivity and whether it is a recent or "long-standing" infection (24). Results from

the Maxim LAg assay were compared to results from the Asante test with and without using viral load test results.

Specimen collection and testing

The testing algorithm for prevalent HIV infection used in all public ANC in Kenya is the third generation Alere DetermineTM HIV-1/2 (Alere Medical Co. Ltd, Chiba, Japan), followed by third generation First Response HIV 1-2.OTM (Premier Medical Corporation Ltd., Kachigam, India), with DNA polymerase chain reaction used to settle any discrepant results. When the patient was found to be HIV positive and consent had been given for biomarker collection, the nurse or laboratory phlebotomist drew a maximum of 10ml of venous blood that was used for all biomarkers. Samples were packed and transferred to the KEMRI-Centre for Global Health Research HIV Research Laboratory in Kisumu on a daily basis where they were tested (or stored for testing) using the following assays: (i) Maxim *HIV*-1 LAg-Avidity EIA and *Asanté*™ *HIV*-1 *Rapid* Recency™ Assay; (ii) viral load on cases with ODn≤1.5 or recent on Asante, (iii) ARV metabolites on those deemed "recent" on Maxim (University of Cape Town laboratory), and (iv) HIV subtype testing on those deemed "recent" on Maxim. The KEMRI Laboratory had conducted HIV recency testing in the past, but not with Maxim kits.

As part of routine practice, viral load test results were shared with the participant and the report kept in her treatment file to assist in clinical management. The rest of the tests were considered experimental and were not returned to the patient. The results of the recency tests were linked to the women's clinical record to assess prior ART use.

HIV subtype testing

Any sample classified as recent in the RITA were stored to undergo HIV subtype testing, to account for differences in MDRI of different subtypes. Samples were to be (at time of writing (July, 2019) testing was imminent) tested at the KEMRI/CDC Kisumu laboratory and classified into subtype. HIV-1 subtyping is based on amplification of a 1,084 base-pair region of the HIV-1 *pol* gene using RT-PCR. Consensus sequences will be analysed using the REGA v3.0 tool (45) to determine subtype.

Statistical analyses

First we described the flow of participants from initial clinic attendance to the final classification into three categories: recent infection, "long-standing" infection, HIV negative. Then we described characteristics of women by HIV (recency) status. Using multivariate models comparing HIV negative individuals with recently infected individuals, we determined factors predictive of recent

infection. Incidence was calculated for different MDRI and FRR values using the R package "inctools", an extension of Kasanjee *et a*l method for estimating incidence using cross-sectional assays (46). MDRI was calculated using the population HIV-1 subtype distribution (47) to weight subtype-specific MDRI estimates using a previously used approach (48).

Selection bias

Characteristics of women in the study were compared to women in the HDSS. Age distribution, parity (see Appendix 2) and HIV prevalence (ratios) by age were compared. A simulation study was undertaken to understand the anticipated relationships between HIV incidence and prevalence measured among currently pregnant women compared to the HIV incidence and prevalence in the underlying female population. A population of women aged 10 to 50 years was simulated. A second modelling approach was used to assess potential biases of using RITA in estimating ANC incidence using four individual-based simulation models representing different HIV epidemics. RITA based incidence estimates among ANC attendees were compared to incidence among 15 to 49 year old women and pregnant women. HIV incidence rate ratios were calculated by year. See Appendix 3 for more details about the modelling method.

Nairobi, Kenya: routine HIV testing and counselling clinics

Sampling of participants

All clients attending one of the fourteen EDARP HTC facilities in Nairobi, Kenya between March 2018 and November 2018 were approached to assess eligibility.

Inclusion criteria

- Aged 15 or older (protocol modified for 18 and older after start);
- Unknown HIV status prior to visit (i.e. never tested positive for HIV);
- Attending an EDARP HTC facility;
- Willing and able to provide informed consent;
- Received an HIV positive test result, or presumptive positive.

Exclusion criteria

- Subject with indeterminate HIV result;
- Not willing to enrol on follow-up at EDARP facility;
- Taking pre-exposure prophylaxis for HIV.

Specimen collection and testing

When a client tested HIV positive and consented, a blood sample was drawn and a follow-up visit scheduled. These samples were used to perform viral load testing at EDARP. DBS cards were prepared from the venous blood at the National HIV Reference Laboratory. Recency testing was conducted on DBS samples. A separate DBS card for samples testing positive for recent infection on RITA was stored to be shipped to the University of Cape Town for ARV metabolite testing at the end of the study. DBS cards were stored dry with desiccant at -20C or -80C.

Index /partner testing

Participants in the study, confirmed to be HIV positive, were counselled on index testing and asked to bring their sexual partners to the clinic to be tested.

Statistical analyses

First we assigned the flow of participants from showing up at the clinic to final classification into three categories: recent infection; "long-standing" infection; HIV negative. We then analysed the study population broken down by several demographic and epidemiological characteristics and compared characteristics of women with recent infection to women with "long-standing" HIV infection. Using multivariate models comparing participants with "long-standing" infection with those recently infected, we determined factors predictive of recent infection. We described the number and characteristics of partners brought in for HIV testing.

Zimbabwe: Sisters with a Voice programme

Sampling of participants

All female sex workers attending any of the six static sites of the Sisters with a Voice Programme between June 2018 and November 2018 were approached to assess eligibility.

Inclusion criteria

- FSW aged 18 years or older;
- Provided informed consent;
- Received an HIV positive test result

Exclusion criteria

- Indeterminate HIV test result at current clinic visit;
- Prior history of testing HIV-positive;
- Reported being on ART.

Specimen collection and testing

When a FSW tested HIV positive and consented to this study, a plasma and DBS sample was drawn. When a women preferred not to give a plasma sample, only DBS was taken. For women consenting to recency testing, a follow-up visit was scheduled. Both the plasma and DBS samples were shipped to the laboratory in Harare within 36 hours and stored at -20C or below for testing using the LAg avidity plasma and DBS assays. Results of plasma tests were to be made available at Sisters clinics within two weeks. It was intended for samples testing HIV antibody and recent positive to be sent to the University of Cape Town to test for the presence of ART.

Statistical analyses

Analysis was performed on plasma results. First we described the flow of participants from showing up at the clinic to the final classification into three categories: recent infection; "long-standing" infection; HIV negative. Then we described characteristics of women by HIV (recency) status and visually assessed clustering of testing in time. Using multivariate models comparing HIV negative individuals with recently infected individuals, we determined factors predictive of recent infection. Where both were available, we planned to compare recency test results from plasma samples to those from DBS samples (to calculate a Kappa statistic after cross-tabulating results; to plot ODn values from both assays; to calculate a regression coefficient and R^2).

Zimbabwe: RDS survey stored samples

Sampling of participants

Respondent driven sampling surveys were conducted for a range of different studies prior to the commencement of this recency study, to obtain representative samples of female sex workers. Sex workers in Zimbabwe are well networked with each other, which is a criterion for using the RDS survey method. In each cluster surveyed, geographical and social mapping was conducted to select women to act as "seeds". These women represented a range of ages, types of sex work, and geographical locations. The women (seeds) were interviewed and each given two coupons to distribute to two peers in the following two weeks. Women who received a coupon could attend an interview, and on interview completion were given two coupons for them to refer two peers. This process was repeated in five to seven waves after the initial recruitment of the seeds. Consent for recency testing was included in the individual study protocols of the four surveys which recruited participants using RDS. Financial compensation was given for completing the questionnaire and in addition for recruiting new participants. Details of the four RDS surveys are presented in Table 3. Further information on the four RDS surveys is available on request.

Specimen storage and testing

Blood from participants in RDS surveys I, II and IV were collected using DBS, whereas survey III used plasma samples. Participants enrolled in RDS surveys I and III, who tested HIV positive had viral load testing undertaken as part of those study protocols. HIV positive women recruited as part of RDS surveys II and IV had viral load testing conducted on DBS as part of this study. All four studies collected self-reports of knowledge of HIV status and whether or not a participant was in receipt of ART. All samples collected were linked to the study questionnaire data by a study specific alphanumeric identifier.

Table 3: Details of the four RDS surveys

RDS survey I **April-May 2016** **N=2883**	**RDS survey II** **April-July 2017** **N=2431**	**RDS survey III** **March-May 2017** **N=2713**	**RDS survey IV** **Oct-Nov 2017** **N=495***
Inclusion criteria			
- Currently working as a sex worker - Living or working in study site for at least 6 months - Age 18 years or older	- Young women selling sex - 18- 24 years old	- Female sex workers - Aged 18 years or older	- Young women selling sex - 16-19 years old
Sample collection			
DBS samples were collected by finger prick, air dried onto filter paper and stored at room temperature until they were transported to the laboratory. Viral load testing was done on all samples testing HIV positive using NucliSENS EasyQ HIV-1 assay (BioMe´rieux, Inc., Madrid, Spain). DBS were stored in gas permeable zip lock bags containing silica gel sachet at room temperature from time of collection	DBS samples were collected by finger prick, air dried onto filter paper and stored at room temperature. DBS were stored in gas permeable zip lock bags containing silica gel sachet at room temperature from time of collection.	Finger prick blood samples were taken for rapid HIV testing. All HIV-positive women had an additional finger prick blood sample taken for viral load testing. Viral load testing was conducted within 48 hours of collection using Xpert® HIV-1 Viral Load performed on the GeneXpert® Instrument Systems. All LAg avidity tests were done on plasma. Samples were stored at -20C at the laboratory.	Finger prick blood samples were taken for rapid HIV testing. All participants were asked for an additional finger prick blood sample to be taken as a DBS. These were air dried onto filter paper. DBS were stored in gas permeable zip lock bags containing silica gel sachet at room temperature from time of collection.
Location			
14 sites	6 sites	3 sites	2 sites

**Survey IV originally included four sites and 615 women; we include here only the two sites where RDS was applied*

Statistical analyses

First we assigned the flow of participants from showing up at the clinic to the final classification into three categories: recent infection; "long-standing" infection; HIV negative. We then described characteristics of women by HIV (recency) status using RDS II weighting, with women weighted by the inverse of the reported size of FSW contacts within their social network.

Using RDS weighted multivariate analysis with fixed terms for site and age (*a priori* potential confounders), we determined factors independently predictive of recent infection using data from RDS survey I. Data were collected through questionnaires on socio-demographic information (age, marital status, education, etc.), economic characteristics, sexual behaviour (with paying and non-paying partners), alcohol use, psychological health, physical and mental health, past history of sexually transmitted infections, sexual and social networks, social capital, utilisation of services including HIV testing, ART, PMTCT and family planning, stigma and experience of violence.

Incidence was calculated for different MDRI and FRR values using the Assay-Based Incidence Estimation toolbox version 3 (46). This tool is available from http://www.incidence-estimation.org/page/spreadsheet-tools-for-biomarker-incidence-surveys. Incidence estimates from the RDS surveys were compared to incidence calculated from programme data. We replicated the repeat HIV testing and HIV seroconversion cohort analysis of programme data conducted by Hargreaves *et al* (37) to estimate incidence for the period 2016 to 2017 (the period the RDS surveys were conducted). Incidence was calculated by dividing the number of seroconversions in the relevant time period by the person years of follow-up; women who had at least two HIV tests, of which at least one showed a negative result, were included.

Risk score development

A score predicting among whom the risk of HIV infection is likely to be highest was developed using data from RDS 1 and 3. We applied a scoring method similar to that of Sullivan *et al* (49). The predictive accuracy of the risk score was assessed by: (1) the link test to assess if the model was correctly specified; (2) the area under the receiver operating curve (AUROC) to assess predictive ability of the score; (3) Hosmer-Lemeshow goodness of fit test to see if the model was well calibrated. We selected all meaningful clinical risk factors through logistic regression with backward elimination. Age was considered an *a priori* risk factor. Factors significant at $P<0.1$ in univariate logistic regression models were considered for backward elimination. After determining factors predictive of recent infection, a screening model with clinical factors was developed.

Qualitative results

Objective 1 Nairobi: Administer a follow-up questionnaire to study participants who test for recent HIV infection, and a survey to outreach workers, test counsellors and other healthcare providers

Objective 1 Zimbabwe: Document experiences of women consenting and receiving results from a RITA, and explore the potential consequences of contact tracing and hotspot mapping

The results presented in this section relate to qualitative research conducted for Objective 1 of both the Zimbabwe Sisters programme and Nairobi HTC pilots. As described in the Methods section above, research was conducted separately in the two pilot sites by different research teams exploring similar aspects of parallel processes. Table 4 summarises our qualitative data.

Table 4: Summary of qualitative research

Site	Nairobi, Kenya: routine HIV testing and counselling clinics	Zimbabwe: Sisters with a Voice programme
Qualitative Data (data record code)	• 17 interviews with healthcare providers across 14 sites (RECN01-17) • 13 post-test questionnaires administered to participants after receipt of positive recency test (13 responses from 29 eligible participants, 45% response rate) (PT1-13) • Reasons for refusal documented for 23 potential participants who refused recency testing, of 96 potential participants who refused recency testing (24%) • Notes from debrief discussion with 4 researchers*	• 19 in-depth interviews with female sex workers across 6 sites, including 3 conducted post recency testing (RECZ01-11) • 6 in-depth interviews with healthcare workers (RECZ16-22) • 4 FGDs with 8-12 sex workers from 4 sites, combined total 40 participants of whom 32 were female, 4 male, 4 transgender. (2 sites all female, 2 sites mixed) • Notes from debrief discussion with 4 researchers*
Topics addressed	Clinical and epidemiological utility Acceptability Delivery Laboratory	Acceptability Delivery Laboratory

**Additionally, debrief discussions were conducted with two researchers in Siaya county and two at LSHTM*

The background section below describes the specific local study context and characteristics of respondents for each of the two sites. For the Zimbabwe site, a description is also provided of where the recency testing process 'sat' in relation to everyday clinic activities. Analysis of qualitative data from both sites identified four main themes: (1) understanding, (2) acceptability, (3) utility and (4) 'looking backwards': blame, anger and risk mitigation, discussed in turn.

Background

Nairobi, Kenya: routine HIV testing and counselling clinics

Research Context

The main qualitative research component in Nairobi aimed to explore experiences and perspectives of health care providers involved in piloting recency testing through individual interviews. Interviews were conducted by two researchers in English during November 2018, after study enrolment had finished. In addition, eligible people who refused to participate in the recency study (n=96) were asked their reason for refusal by clinic staff, with reasons recorded for 23 people. Participants who received a positive recent infection test result (n=29) were invited to complete a post-test questionnaire. This was completed by 13 participants (49%).

Characteristics of respondents

Interviews were conducted with 17 health care providers who had been involved in the implementation of the pilot and who spoke English. These included clinical officers, nurse counsellors, HIV counsellors and outreach workers.

The characteristics of other respondents (potential participants asked for reason for refusal and those completing post-test questionnaires) were not available.

Zimbabwe: Sisters with a Voice programme

Research Context

The qualitative component aimed to explore experiences and perspectives of clinic users and health care providers involved in recency testing. Data were collected between July and November 2018 by one researcher who travelled between the six Zimbabwe pilot clinics in cities across the central band of the country. The researcher spoke both predominant local languages (Ndebele and Shona), as well as English, and had substantial previous experience conducting peer support training within Sisters with a Voice programme clinics and qualitative data collection for a range of research studies with female sex workers over the preceding five years.

Characteristics of respondents

Sex workers: In total, 59 sex workers were interviewed or participated in focus group discussions. The majority of participating sex workers were female (n=51; 86.4%), four (6.8%) were transgender and four (6.8%) were male. Female participants were reported to have limited levels of education (partial secondary education), while the smaller number of male and transgender sex

workers who participated in the focus group discussions were described as having higher education levels (some to university level).

Female sex workers were characterised as being a mobile population, and mobility was frequently raised during interviews in discussion of individual HIV testing histories, for example:

'I would go to South Africa, Masvingo [south-east Zimbabwe] and get tested there or here and so forth' (RECZ01_Site 1)

Participants explained that they or other sex workers moved around to find clients, such as gold panners working in specific locations. One sex worker also reported moving location to avoid a previous sexual partner who she was afraid might harm her:

'I was now afraid so I ran away and went to Inyathi [near Bulawayo]'

(RECZ02_Site 1)

Sex worker mobility was identified as a challenge for enrolling and tracking study participants, and is discussed further below.

***Health care workers*:** The six health care workers interviewed were all female nurses who had obtained a diploma in general nursing and were registered as general nurses with additional qualifications in research and rapid testing for HIV. They all had previous experience working with female sex workers at CeSHHAR or with other NGOs, and at the time of interview were involved in data collection for the recency pilot study in Zimbabwe.

Practical process of Recency testing

For clinic users, recency testing occurred immediately between two unusual, significant events – being diagnosed with HIV and initiating HIV treatment. This context is important to situate study participants' engagement with, and response to, recency testing. It is described in Figure 3.

Figure 3: The practical process of Recency testing in Sisters with a Voice Clinics

1. On arrival at the clinic, eligibility for access to free clinic services was established via informal questions to establish that the attendee was involved in transactional sex;
2. The attendee was enrolled, allocated two identifier codes, and asked to provide their home address and telephone number;
3. In the waiting area, group health promotion talks were conducted, condom use demonstrated and condoms distributed, and HIV testing services introduced;
4. Attendees were asked if they wanted to test for HIV, and those wanting to opted for self-testing or provider initiated HIV testing. In a private consultation room they were screened by clinicians for STIs, and their history taken including discussion of HIV status. Pre-test HIV counselling, HIV testing, and post-test counselling were conducted. For those testing positive for HIV, ART treatment was discussed in post-test counselling and referral forms for ART treatment completed. Attendees testing positive for HIV were then referred to the Recency study nurse;
5. In a separate room, the recency study was explained including mention of reimbursement, pre recency counselling conducted, and the study consent form completed. Contact details, including two contact telephone numbers, were recorded in recency study records and a short demographic questionnaire completed. Blood was then drawn, and the attendee was given their reimbursement for time taken to participate in study (US$5);
6. Facilitated referral (a health worker accompanying the patient) was then completed to initiate ART treatment, at either a co-located or separate public sector clinic.

When the recency test result was returned to the clinic, (after 4 weeks or longer), staff attempted to contact the patient to invite them to come to the clinic and receive their result. This was often difficult as patients had not always given accurate names or contact details, and some who were contacted opted not to return to the clinic.

Findings

Theme 1: Understanding

Participants understanding of what 'recency testing' encompassed, what a 'recent infection' meant, and how results of recency testing could be applied were key factors underlying discussion of acceptability and utility. Understanding varied in several ways. The **period specified as 'recent'** was consistently understood differently by health care workers in the two pilot sites. In Zimbabwe, a recent infection was described as a new infection within the previous four months, while in

Nairobi it was described as a new infection within the previous six months. This one-third difference in the time window within which participants may be identifying potential sources of infection has implications for consideration of utility, discussed in Theme 3 below.

As this was a new test the **concept of recency testing** was unfamiliar to patients. As a health care worker explained '*if you explain to them they recently got infected with HIV they do not understand because it's a new thing to them, not so many people are talking about it*' (RECZ19_Site 5) Health care staff explained the concept carefully when inviting patients to join the study, but the information was given in the middle of a clinic visit when a patient had just received a positive HIV diagnosis. Immediately post diagnosis patients were likely to have been anxious or distracted, with some participants interviewed in Zimbabwe describing having felt extremely worried about their future, or suicidal, immediately after HIV diagnosis. For example, a focus group participant explained:

'*When you are HIV positive it's difficult to adjust. I think the first 24 hours* [after testing] *are the crucial…[..]…The next morning I will get in denial, in depression…*' (RECZ13_Site 3)

People describe not hearing another thing the nurse says after receiving a positive HIV result [personal communication]. At the point when recency testing was explained, participants may therefore have had limited capacity to engage with this new, conceptually unfamiliar information, potentially limiting their understanding of it.

In interviews and focus group discussions when sex workers were asked about recency testing they frequently confused HIV testing and recency testing. For example, in a focus group discussion after thorough explanation and discussion of recency testing, a participant asked for their view replied *' I think it would be helpful if I get tested and know I will then reduce unprotected sex'* (RECZ12_Site 1). The discussion leader then corrected the participant, explaining this she was not asking about HIV testing to determine HIV status, but instead '*now we are talking about this new one which can tell you whether you got infected these past four months or were infected a long time ago'*. Similarly, in an interview a sex worker who was specifically asked about recency testing, with an explanation of recency testing given, stated *' I think the test is good because it informs one of his/her status. Personally I am happy about it'* (RECZ07_Site 4). Despite being given an explanation of recency testing, this participant was referring to testing for HIV status. For some participants therefore the recency test was not understood as a separate process to HIV testing, so statements about their opinion of recency testing should therefore be interpreted cautiously.

Understanding of whether and how the results of recency testing would be used by clinicians managing treatment also varied. This is discussed below in Theme 3: Utility.

Theme 2: Acceptability

Recency testing was not taken up by all those who were eligible. In the Zimbabwe pilot, 72% of those eligible (367 of 511) underwent recency testing and in the Nairobi study 84.7% (532 of 628 eligible) consented to recency testing. Practical barriers to acceptability and contextual factors which facilitated acceptability were identified.

Practical barriers to acceptability

In analysis of data from Zimbabwe several practical barriers to acceptability were identified by participants. These were linked to the process experienced by patients at the clinic (Figure 3). Firstly, enrolment in the recency study required additional **time** to be spent at the clinic during an already long visit, prior to referral for ART initiation at another clinic, which discouraged some patients from enrolling. A health care worker explained that sex workers have competing demands on their time: *'most turn it down because of the time factor they will be rushing to go elsewhere maybe to pick up the kids from school or maybe to go and meet a client they will turn down recency to do that'* (RECZ16_Site 3). The same respondent explained that if a woman has come to the clinic accompanied by a partner or friend, and doesn't want them to know that she has received a positive HIV test result '*they are afraid that the partner may end up knowing something is happening*' (RECZ16_Site 3) if the process takes a long time.

The need to take additional **blood**, and the volume of blood required, was also identified as a potential barrier to acceptability in both pilots. Women undertaking recency testing in the Zimbabwe study gave five spots of blood for one test, usually from one fingerpick, and 7ml of blood for the other (if they also consented to the plasma test). The need to have this larger sample with plasma taken intravenously was reported to make some women nervous. As well as reluctance to undergo the practical process of having blood taken, one female sex worker cautioned that *'some may not be understanding when you remain with their blood samples.'* explaining that women *'might question where their blood is going'* (RECZ01_Site 1), related to local beliefs about bodily fluids and risk of witchcraft [personal communication]. Additionally, similarly to the issue of time taken for recency testing, having an extra blood sample tested may make it more evident to an accompanying partner/friend that they have tested positive to HIV.

The issue of the volume of blood required was also raised by health care workers from the Nairobi pilot. For example one noted that it was difficult for them to explain to the client '*the samples you need to get, because it's not only one, we have the other samples, the client feels they are…it's so much'* (RECN12_Site 10*)* . She went on to explain that while this was a challenge it was not necessarily a

barrier: '*as you start doing the blood, the client says, hey, this is a lot of blood, but we still manage*'. Another health care worker explained that a patient declined to participate because they did not want to have more blood taken after other samples had already been taken during their clinic visit:

'*when he or she comes, we prick twice, we prick again and then when the client now has accepted we are supposed to also do taking blood. So the client was saying "no you are pricking me a lot, so for many prickings, no". '* (RECN01_Site 1)

Concern about the amount of blood taken was also raised in researcher debriefing discussions in the Kisumu pilot, Kenya (not otherwise discussed in qualitative analysis). Difficulties were reported in obtaining approval from the sub-county health management team, a local level board who review all research in the community, due to concern about the amount of blood needed to be taken from pregnant women for this study.

A third practical barrier to acceptability identified in the Zimbabwe pilot was the need for sex workers to **provide contact details** for follow-up with recency testing results. The sex worker population is mobile, as discussed above, and sex work is illegal in Zimbabwe. Sex workers may not routinely use their true name, and may not want to provide their name, address or telephone number. Some avoided doing so by giving details which health care workers later found were incorrect when they attempted to contact them.

Contextual facilitators of acceptability

In the Zimbabwe pilot, where 72% of the eligible population participated in recency testing, two specific **contextual factors** contributing to acceptability were identified

Trust of clinic setting and staff

Sisters with a Voice clinics intentionally provide an unusual social and physical space for sex workers. They operate specifically for this 'key population', and new patients are screened to admit only those engaged in transactional sex (Figure 3). Peer educators from within the sex worker community contribute to clinic health promotion and outreach work. Staff make substantial efforts to make sex workers feel comfortable and welcome, and to remind them that the clinic is a safe space for them. For example, in closing an interview with a female sex worker who had recounted her initially suicidal feelings at receiving a positive HIV test result, the researcher emphasised that the clinic was for her and that clinic staff were always ready to support her:

'this clinic is yours right, if you feel like talking or crying you come here and do that. So you can come here and get any help you want right… this is your place and will be open from Monday to Friday and the nurses will be here to help you' (RECZ11_Site 2)

During interviews, participants expressed their gratitude for how they were treated at Sisters with a Voice clinics, and the importance of counselling and treatment they received there from staff who were not judgemental about their work. For example, when asked if she had anything else to add to the interview one participant replied:

'*I'm just happy with the way you and this programme welcomed me. We are grateful to this programme for us women who are not married and sell sex; we are free to tell you what's going on with our bodies, if I have an STI I am free to tell you about it. I am happy because we are free to tell you everything that is happening. I'm free to even come and collect my medication without fear of who will see us.*' (RECZ06_Site 3)

Not all sex workers visiting the clinics will have felt this way, but there was a consistent message from interview and focus group participants that they trusted Sisters with Voice clinic staff, and were grateful to have services provided specifically for sex workers. This may have influenced their willingness to participate in recency testing, when at a time of vulnerability immediately after receiving a positive HIV diagnosis they trusted that something proposed by clinic staff would be in their interests. Their decision to accept recency testing may not then have been primarily related to the purpose of the test, but to the context in which it was proposed, and who was suggesting it to them. This has potential ethical implications for how independently decisions about testing are made, and implications for take-up in other settings.

Financial reimbursement

In line with the precedent of previous research studies conducted in Sisters with a Voice clinics, study participants were given US$5.00 in recompense for their time. This equated to approximately the amount sex workers charged for one 'short time sex'. This was given to consenting study participants after their blood sample was taken, and also to interview and focus group participants. Staff felt that this reimbursement made recency testing more acceptable, particularly for younger women who were generally less engaged with clinic services and harder to reach.

Staff suggested that the reimbursement may have contributed to some sex workers coming forward for HIV testing who had previously received a positive HIV test result but pretended they hadn't, because they wanted to participate in the study and receive the reimbursement.

Theme 3: Utility

The question of utility was complex. It encompassed whether recency testing was seen to be useful, directly and indirectly, by whom, and for whom. Discussion drew on both what had happened during the study period and what participants expected may happen in the future if recency testing

became routine practice. Utility of recency testing was discussed by participants at population level, clinic level and individual level, presented here in turn.

Population level utility

Health care workers identified the **potential utility** of recency testing at population level to facilitate the identification of transmission hotspots so that they could target resources appropriately. For example a health care worker in the Zimbabwe pilot explained that by using data from the recency programme ' *we can say…in a certain area there are many recent infections so we can send resources and conduct health education talks, and quickly send intervention talks in that area to assist people. It also helps to know which age group is being affected the most*' (RECZ19_Site 5). However, this didn't happen in the pilot as numbers of recently acquired infections were small, and a system was not yet set up to feedback population level results to clinics.

The same healthcare worker noted the **complication of mobility** in the sex worker population for the utility of recency testing. The location where someone had been infected may be distant from where they were tested:

'*even if we test them here with the intention of finding out where the most recent infections are coming from, but then that sex worker has been working in Gweru or Mutare by the time they come back we won't be able to tell where the most [majority of] recent infection is concentrated'* (RECZ19_Site 5).

Timescales were also important for the potential utility of recency results in identifying transmission sites. Population level results need to be fed back to clinics quickly for interventions to be implemented before the situation on the ground changes. Some respondents suggested that a rapid recency test would be helpful to enable programmes react quickly.

When the programme level significance of recency testing was explained to sex workers in interviews and focus group discussions some could see an indirect benefit for the wider population of them individually participating in recency testing, noting for example '*now we know that it can benefit others even those who are not yet infected'* (RECZ15_Site 6).

Clinic level utility

Health care workers identified **mixed indirect utility** of recency testing at the clinic level, with variation between their experiences with individual patients and different clinic contexts.

As explained above the recency testing process took additional time which was suggested to sometimes deter people from both engagement in immediate HIV services, and engagement with wider clinic services. Conversely, additional time could sometimes be useful, giving health care

workers longer with people to emphasise aspects of counselling, develop rapport, answer questions and encourage partner notification where appropriate.

In the Nairobi pilot, laboratory delays led to a situation where the study was halted for six weeks and staff did not have recency results available for people when they expected them. In order to be able to give patients some results they began returning baseline viral load results. Health care workers reported that this was helpful, as some people were interested in their viral load and keen to reduce it through adherence to treatment. It then became difficult to separate discussion of specific utility of the recency test, from utility of the recency pilot process which included viral load reporting. For example, a health care worker explained that she found recency results useful '*because especially on the side of the viral load, because they are tied with the viral load actually, it will help us show the client where their levels are…*' (RECN03_Site 3).

Reports of patients' **motivations to initiate and adhere to treatment** in response to reporting of recent/long term test results varied, with health care providers giving examples of how they used both to emphasise the need for treatment. For example for a long-standing infection they could explain that the virus has been in the body for a long time, hence the need to begin treatment:

'you explain to them that since they have had the virus for a long their immune system is being weakened in turn this will give them pressure to take ARVs' (RECZ18_Site 1)

Conversely a test result indicating recent infection could be used to encourage conversation about viral load, disease progression, and the importance of adherence to treatment.

Some health care workers were wary of potential negative impacts of returning recency test results for treatment initiation and adherence, for example '*you are a bit sensitive and careful giving them the result, because maybe that can contribute to them defaulting'* (RECN05_Site 5). They explained there was a risk that patients with recent infection may delay initiating care.

Some health care workers also reported that the recency testing process helped them to identify patients who posed as new diagnoses, but had previously been tested and initiated treatment, for example *'it has been assisting us to capture the clients who have been on care elsewhere and now they come, pose as new'* (RECN03_Site 3). This could help clinic staff to link patient's current and previous records,

Health care workers also gave examples of situations where recency testing could help to facilitate partner referral, for example people with long term infections passing on details of former partners to health care workers for follow-up.. A health care worker in Kenya explained that when clients receive a long term result from recency testing they '*start thinking backwards'*, to identify previous

partners, enabling health workers to '*reach out to networks and get so many other clients*' (RECN05_Site 5).

Individual level utility

In the Zimbabwe pilot there was confusion among sex workers who participated in the qualitative study about whether and how results of recency testing **influenced treatment**. Some participants thought that recency test results gave health care workers information which was useful in planning their individual treatment, helping them to receive 'better' treatment. As discussed above, understanding of recency testing was mixed, and participants trusted health care workers to provide appropriate care. While participants were not told that the test would inform their individual treatment, some assumed that clinic staff were suggesting it because it would help them. For example, a participant explained:

'*I think there is some good because if it's known how long you have had the virus it can help health care workers to see how they can assist you'* (RECZ02_Site 1)

Focus group participants in Harare were unusual among respondents in reflecting more critically than some others on recency testing and other HIV programmes, and asking direct questions about utility for patients and for researchers. In response to discussion of recency testing, a participant asked specifically whether it influenced treatment:

'*Does it have any impact on treatment methods like because one has known that they have been infected 4 months ago does it mean that it will speed up your viral load suppression or it's going to remain the same…[…]… If it's beneficial in any way for treatment let us know that if one knows that they have been infected in 4 months ago then it will help in this way.'* (RECZ13_Site 3)

Focus group participants in Mutare similarly questioned the individual utility of recency testing for sex workers, with some suggesting that the programme would benefit the organisation more than the participants, and that they need to '*know the concrete benefits of this test'*, and what risks are associated with it (RECZ15_Site 6).

Recency test results did not influence treatment; everyone was initiated on the same ART regimen. It is important to be aware of the potential for patients to assume that recency testing is clinically beneficial, and and to ensure that patients do not consent to recency testing because they incorrectly believe that it will influence the treatment they receive. In the Nairobi pilot, a health care worker noted that learning that recency test results wouldn't influence their treatment was a reason given by eligible patients for declining to participate.

Recency testing prompted people to think about **who infected them** with HIV. This added an extra dimension to knowing their positive HIV status, in itself an emotionally complex situation to navigate. Participants' feelings about this were mixed across both pilot sites.

Some participants thought it was useful to potentially identify the source of their infection, in order to be able to 'feel closure' and move on. For some participants, learning that they had a longstanding infection showed that a new partner was not the source of their infection, and could enable them to take steps to protect their new partner from infection. Among the general population, identifying the potential source of infection was more likely than for sex workers. Among questionnaire respondents in Nairobi, 11 of 13 who were recently infected reported suspecting from whom they were infected (Appendix 1). For many sex workers the four month time period was too wide to identify a clear source of infection. As a female sex worker explained '*even if I think about it I won't be able to get the right answer because of the nature of the job I do'* (RECZ04_Site 1). Within the four month period, sex workers could have had 200-260 sexual partners [personal communication]. There was also some misunderstanding about how exact the 'recent' time period was, with additional explanation frequently required to clarify that it was within four months rather than exactly four months ago.

Some participants did not want to know whether their infection was recent or longstanding as it did not change their current situation, preferring to focus on beginning treatment and moving on.

For example, a female sex worker interviewed immediately after receiving her positive recency test result who was asked how useful the recency test was explained *'From my point of view I think it's just the same because I already have the virus…[..]…I am now just sticking to taking my pills and make sure I do not default and just try to prolong my life'* [RECZ04_Site 1]

Similarly , female focus group discussion participants suggested for a sex worker '*she doesn't have any reason for doing that test…..the question they are going to ask is that so if I know, then what?*' (RECZ15_Site 6). This resonates with the main reasons collected for refusal to consent to recency testing in Nairobi, (1) that the client did not want to know about recent infection (n=7), and (2) that they did not see the benefit of the study (n=5)[1].

[1] Reasons for refusal were collected from July 2018 for a small proportion of people testing HIV positive who did not consent to recency testing (n=23, of a total of 96 people refusing from March to October (24%); sub-total for July-October not available).

Theme 4: 'Looking backwards': blame, anger and risk mitigation

Two inter-related negative aspects of recency testing were identified by participants across the Nairobi and Zimbabwe sites. These were (i) the prompt to 'look backwards', reversing HIV treatment and counselling programme emphasis on acceptance, treatment adherence and moving on with life, and (ii) potential for blame, associated with risk of anger and violence. These are discussed together with strategies proposed by participants to mitigate them.

Looking backwards

Participating in recency testing encouraged individuals to think back to situations and sexual partners in their past in relation to their HIV diagnosis. As described above, receiving a positive HIV test result could be an unpleasant, frightening experience. HIV counselling services worked hard to help people to accept their HIV status, combat negative feelings, initiate treatment, and focus on the future. '*Looking backwards'* was identified as a negative aspect of recency testing by both sex workers and health care providers. A health care worker described reporting back recency results as '*reactivating a wound that healed*' (RECN11_Site 10), sometime after the patient had recovered from the initial shock of their HIV diagnosis. Creating a negative focus in the past by looking back to identify a possible source of HIV infection could disrupt the positive messages of HIV counselling. This made it more difficult to focus on acceptance, and moving forward with life.

Blame and anger

Thinking about who could have infected them could be upsetting for an individual, and could lead to blame and anger. Respondents discussed feelings of unhappiness, 'bitterness', 'stress' and 'un-forgiveness'. A study participant in Kenya who had received a positive recent infection test result explained in the follow-up questionnaire: '*I felt bad because I know who infected me and probably he did it knowingly….not helpful at all, not a good experience'* (PT2).

Within relationships, learning who has been infected for longer and potentially infected their partner could be a source of disruption and conflict. A different study participant in Kenya explained: '*knowing that I was recently infected gave me an idea of who infected me …I got so bitter and did not want to see him anymore. I cut my links with him and do not want to see him ever again*' (PT1).

Participants also associated blame and anger with risk of violence. For example a sex worker explained that someone may become angry, thinking '*this person did it to me'* and then want to do something bad to that person, *'they might stab that person with a knife'* (RECZ03_Site 1). Health care workers in both settings emphasised that knowledge of a recency result which enabled people to

identify who could have infected them could lead to violence. A health care provider in Kenya described a specific case of a man who was diagnosed with HIV and enrolled in the study. When he participated in recency testing and learned he had been infected within the last six months, he was able to identify who infected him. When he left the clinic "*the way he was expressing himself it's like he went to confront the lady'* RECN07_Site 7

Sex workers explained that as well as themselves being angry, they could be at risk of violence from angry clients if recency testing became available to clients. For example, if a man regularly had unprotected sex with one sex worker, did not have another partner and found that he had been recently infected, he would know that she was the source of his infection. This could lead to anger and violence: '*the danger would be that this man will come back and stab us in our homes'* (RECZ14_Site 4).

Risk mitigation

In order to mitigate potential risks associated with patient's emotional responses to thinking about who may have infected them with HIV, sex workers and health care providers from both pilot sites emphasised the importance of counselling services in supporting patients and preventing conflict. Some health care providers reported highlighting that the recency test does not tell patients specifically who infected them, for example: '*It is good to accept the result, and not go about trying to find who infected you so that they don't have those issues with having those fights or conflicts at home'* (RECN10_Site 9). Another health care provider explained that she advised patients '*sex is something you consented to, so no need to confront another person and then you'll end up in court or jail*' (RECN11_Site 10). The main message of counselling was to initiate and adhere to treatment '*moving on with life, not really focusing on past'* (RECN02_Site 2). Counselling could help people to move away from 'looking backwards', attributing blame and feeling angry, to looking forwards and moving on with their lives.

Quantitative results

In Table 5, we summarise the testing figures of the three pilots and the stored RDS analysis. To aid comparison, we present the results of the three pilots of recent HIV infection testing in routine settings before presenting the results arising from testing of the stored RDS samples. We align the results with the pilot specific objectives presented above.

Table 5: Summary results of the three pilots of HIV recent infection testing in routine service settings

	Siaya County, Kenya	Nairobi, Kenya	Sisters with a Voice, Zimbabwe	RDS surveys I & III, Zimbabwe
Inclusion criteria	• 13 years or older seeking ANC • HIV positive	• 18 years or older • Never tested positive for HIV • HIV positive • Not on PrEP	• FSW 18 years or older • HIV positive • Not on ART • Not having tested HIV positive	• FSW 18 years or older • RDS specific criteria presented in table 3
HIV positive	445	628	510	3198
LAg and viral load testing	426	532	314	623
Long standing infections	416	484	280	2828
Lag ≤1.5 and VL <1000	95	12	16	81
Lag >1.5	320	470	264	428
Self-reported evidence of long-standing infection / ART use	-	-	-	2319
Evidence of ART use	1*	2**	-	-
Recent infections	10	46	33	103
Recency rate (recent/HIV positive successfully tested for recency)	10 / 426 = 2.3%	46 / 530 = 8.7%	33 / 313 = 10.5%	103 / 2931 = 3.5%
Incidence (95%CI)	1.1 (0.3-1.9) ***	-	-	RDS 1 – 22.8 (16.0-29.6) RDS 3 – 1.7 (0.3-3.2)****

** Linking to the clinical record*
*** ART metabolite testing at UCT*
****Assuming FRR=0.003 & MDRI=206 days*
*****Assuming FRR=0.0 & MDRI=130 days*
In Siaya County, the denominator for the recency rate contains people having tested HIV positive before and people on ART. In Nairobi and in the Sisters with a Voice clinics in Zimbabwe people who have tested HIV positive before are excluded from the study so are also not counted in the denominator.

Siaya County, Kenya: antenatal clinics providing PMTCT services

Objective 1: Assess the feasibility of using RITA at ANC for HIV incidence surveillance, and identify the yield of women positive for recent infection

Over the study period 7th February 2018 to 30th November 2018, 2,410 eligible women presented at the fourteen participating antenatal clinics. Figure 4 presents the recruitment and testing figures among these women. In summary, of the eligible women, 2,365 (98.1%) consented to participate in the study. Of the 2,365 women, 1,807 (76%) were under 30 years of age, 1,213 (51%) sought ANC care at one of three largest sites (Akala, Aluor, and Wagai), 1,792 (76%) were married, and 1,158 (50%) had experienced three or more pregnancies (see Table 6). In total, 445 (18.9%) women tested positive for HIV (note: for six women the HIV status remained unknown). Of these 445 women, 426 (95.7%) had a LAg and viral load test, among whom eleven (2.6%) were classified as positive for recent infection (acquired in the past 206 days).

Figure 4: Recruitment and testing flowchart (Maxim LAg plasma)

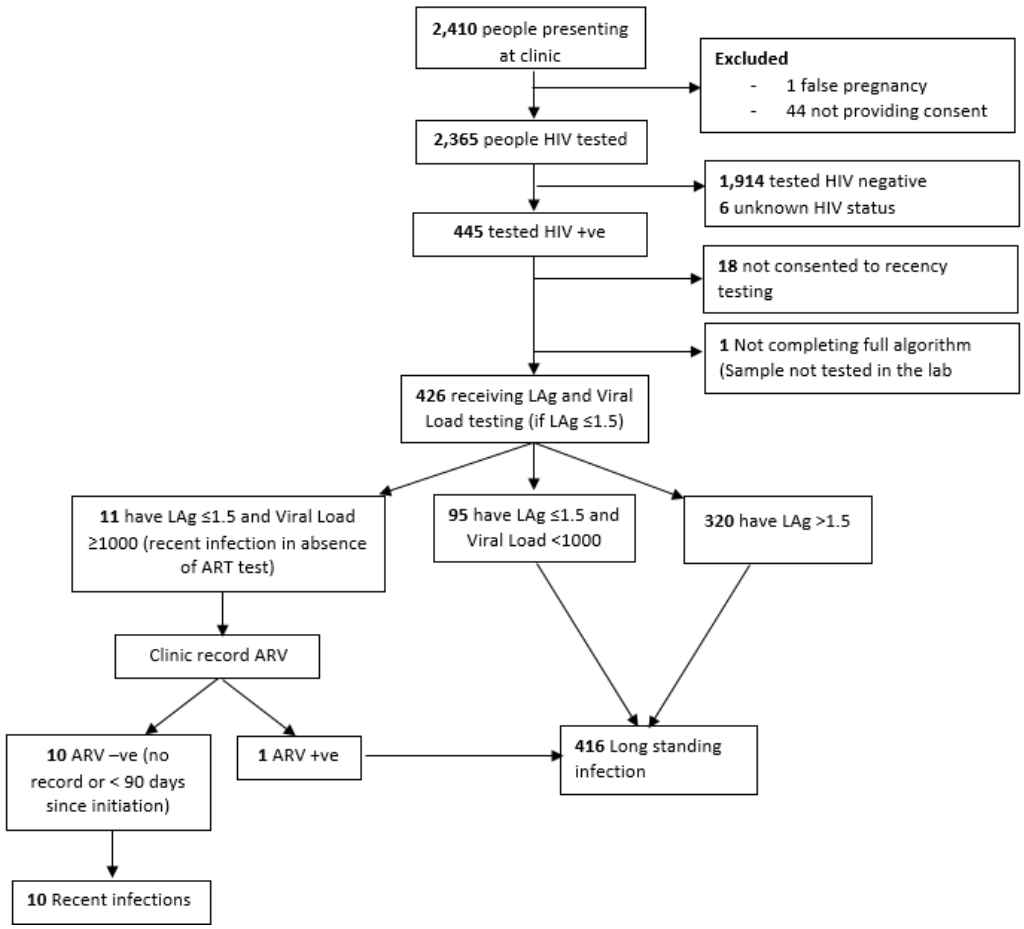


Table 6: Characteristics of 2,365 women testing for HIV

		N	%*
Age (in years)	<15	23	1
	15-19	481	20.4
	20-24	796	33.7
	25-29	507	21.5
	30-34	345	14.6
	35-39	176	7.4
	40+	35	1.5
	Missing	2	-
Site	Akala	526	22.1
	Aluor	374	16
	Asayi	86	3.7
	Bar-Sauri	3	0.1
	Dienya	153	6.6
	Gongo	85	3.5
	Masogo	195	8.3
	Nyawara	159	6.8
	Ogero	57	2.4
	Rera	153	6.4
	Siala	46	2
	Sirembe	149	6.3
	Uriri	66	2.7
	Wagai	313	13
Marital status	Married	1,792	75.8
	Single	501	21.3
	Separated / divorced	36	1.5
	Widowed	33	1.4
	Missing	3	-
Pregnancies (n ever)	1	663	28.3
	2	518	22.2
	3-4	754	32.2
	5-6	329	14.1
	7+	75	3.2
	Missing	26	-

** as among those for whom information was available*

Among the eleven women classified as recent, one had documentation (via linkage to the clinical record) of having initiated treatment almost four years prior to their sample collection date, and was therefore reclassified as not recent. Another woman had initiated treatment 78 days prior to her sample draw date. As her first known positive date was on the same date as ART initiation; she was classified as a recent infection. An additional woman had initiated ART three days prior to her sample draw; again she was classified as recent. Of the remaining women classified as recent, four initiated treatment on the same day as their sample draw, three initiated within sixteen days of their sample draw, and one did not have documented ART initiation.

To further assess whether any of the remaining ten women classified as recent were potentially false recent infections, we explored their testing history for prevalent infection from the HDSS and ANC record. Of these ten women, seven were linked to the HDSS and four had a prior HIV test date in the HDSS, all of which previously tested HIV negative (see Table 7). As such, there was no evidence of the ten women being misclassified as recent based on the HDSS. The 0% misclassification found in our small numbers in this study provides a lower bound for the proportion falsely recent using this RITA. Having accounted for all potential misclassification, ten (2.3%) women were classified as positive for recent infection.

Table 7: Estimate of false recent infection

Number of recent infection based on RITA	Recent infection with known prior positive date in HDSS	Proportion of RITA recent infections misclassified*
10	0 (4 linked w/prior neg HIV status)	0%

**represents the lowest bound of the false recency rate*

Table 8 shows nine of the ten women identified as positive for recent infection were under 30 years of age, and half (5/9; 55.6%) were in their first trimester of pregnancy. The ten recent positives were identified at seven different clinics and had sample collection dates ranging from 2 March 2018 to 12 November 2018, with dates fairly evenly distributed across the study period.

Table 8: HIV and recent infection status by age and pregnancy status

		HIV negative		HIV positive			
				non-recent		recent	
		N	%*	N	%*	N	%*
Age (in years)	<20	473	24.7	25	6.1	2	20.0
	20-24	694	36.3	94	22.8	4	40.0
	25-29	385	20.1	113	27.4	3	30.0
	30+	361	18.9	181	43.8	1	10.0
	Missing	1	-	1	-	0	-
Trimester	1st	215	11.5	69	17.6	5	55.6
	2nd	895	47.8	191	48.7	2	22.2
	3rd	763	40.7	132	33.7	2	22.2
	Missing	41	-	22	-	1	-

** as among those for whom information was available*

Table 9 shows women in their first trimester to have nearly a ten times increased odds of testing positive for recent infection compared to those in their second or third trimester. There were no notable differences in age between HIV-negative women and women testing positive for recent infection.

Table 9: Characterisation of recent infections

		N negative	N recent	Bivariable model	
				OR (95%CI)	p-value
Age (in years)	<20	473	2	0.74 (0.10-3.8)	0.72
	20-24	694	4	REF	-
	25+	746	4	0.93 (0.22-3.9)	0.92
	Missing	1	0	-	
Trimester	1st	215	5	9.6 (2.5-39.2)	<0.001
	2nd & 3rd	1658	4	REF	-
	Missing	41	1	-	-

The genotyping kits to conduct HIV sub-typing were received in Kisumu early May, 2019 (having been ordered late 2018). However, at time of writing (July, 2019) the extraction agents were being held in Germany awaiting inspection. As such, the testing of HIV subtype is pending.

Objective 2: Assess selection biases associated with the surveillance population

Women in the study (women in ANC) were compared to women in the KEMRI/CDC Siaya HDSS to assess possible selection biases. In table 10 the age, marital status and education distributions are shown for the women attending ANC and women in the HDSS at large. Median age of women of reproductive age in the HDSS was 27 years [IQR; 20-36 years], while for ANC participants it was 24 years [IQR; 20-29 years]. A lower proportion of the youngest (15-19 years) and oldest (>35 years) age groups were represented in the ANC relative to the HDSS, while a higher proportion of those attending ANC were 20-29 years of age than in the general HDSS. About two-thirds (65.3%) of women in the HDSS were married compared to three quarters (75.9%) of ANC attendees. Potential selection biases are further explored in Appendix 2.

Table 10: Comparison of women in the pilot to women in HDSS

		Women in ANC		Women in HDSS of reproductive age		Difference in %
		N	%	N	%	
Age (in years)*	15-19	481	20.6	5,673	27.2	-6.6
	20-24	796	34	3,431	16.4	17.6
	25-29	507	21.7	2,797	13.4	8.3
	30-34	345	14.7	2,912	13.9	0.8
	35-39	176	7.5	2,305	11	-3.5
	40-49	35	1.5	3,773	18.1	-16.6
	Missing	2	-	0	-	-
	Total±	2342	-	20891	-	-
Marital status§	Married	1,792	75.9	4,867	65.3	10.6
	Separated / divorced	36	1.5	75	1	0.5
	Single	501	21.2	1,956	26.2	-5
	Widowed	33	1.4	560	7.5	-6.1
	Missing	3	-	13,433	-	-
	Total	2365	-	20,891	-	-
Educational level attained§^	None	7	2.3	131	2.2	0.1
	Primary	253	81.4	4,476	74.9	6.5
	High School	48	15.4	1,258	21.1	-5.7
	Tertiary	3	1	110	1.8	-0.8
	Missing	966	-	13,433	-	-
	Total	1277		20,891		

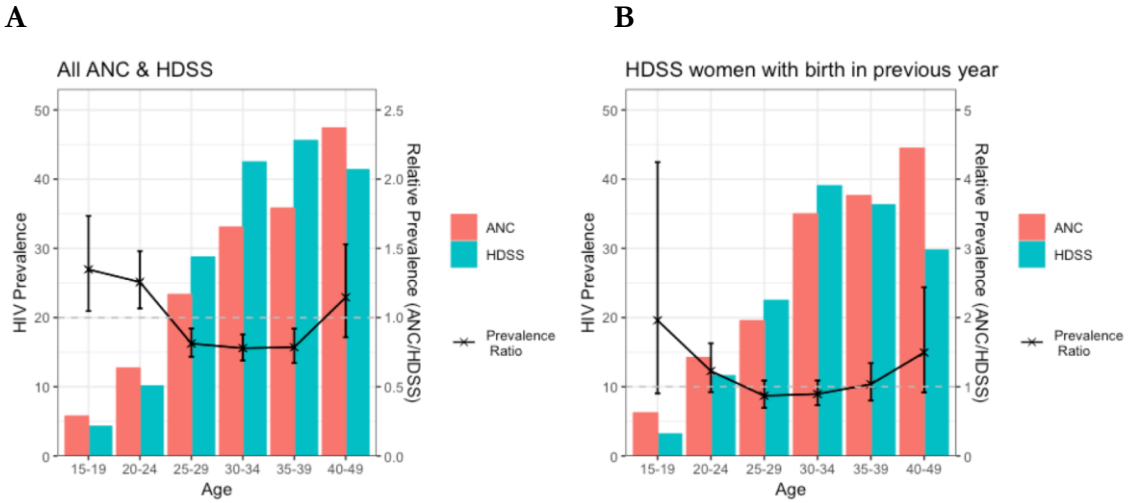
**Age distribution for HDSS for 1st July 2017 (the most recent stable estimate of the HDSS population).*
±Total for ANC age distribution excludes 23 participants <15 years of age.
§Marital status and education are only ascertained in the Longitudinal Bio-Behavioural Survey a nested cohort of approximately one-quarter of household within the HDSS in Gem, following ~15,000 individuals. Education is only ascertained at a single timepoint for individuals over 24 years of age.
^Education is not ascertained directly among ANC attendees, so is only available among ANC attendees linked to the HDSS.

Figure 5 compares HIV prevalence estimates among ANC attendees and women in the HDSS. Prevalence was higher among ANC attendees in the youngest age group than among the comparative group in the HDSS (prevalence ratio: 1.35 among 15 to 19 year olds; 1.26 among 20 to 24 year olds), and lower amongst those aged 25 to 39 years (prevalence ratio: 0.81 among 25 to 29 year olds; 0.78 among 30 to 34 year olds; 0.79 among 35 to 39 year olds) (Figure 5A). No differences were observed by age (all prevalence ratio 95% confidence intervals crossed 1) when comparing women attending ANC to women from the HDSS who had a birth in the year prior to their HIV test in the HDSS sero-survey (Figure 5B).

Figure 5: HIV prevalence in ANC attendees compared to HDSS in 2016 sero-survey

A) all ANC attendees compared to all 15 to 49 year old HDSS female participants

B) all ANC attendees compared to 15 to 49 year old HDSS female participants who reported a birth in the year prior to the sero-survey

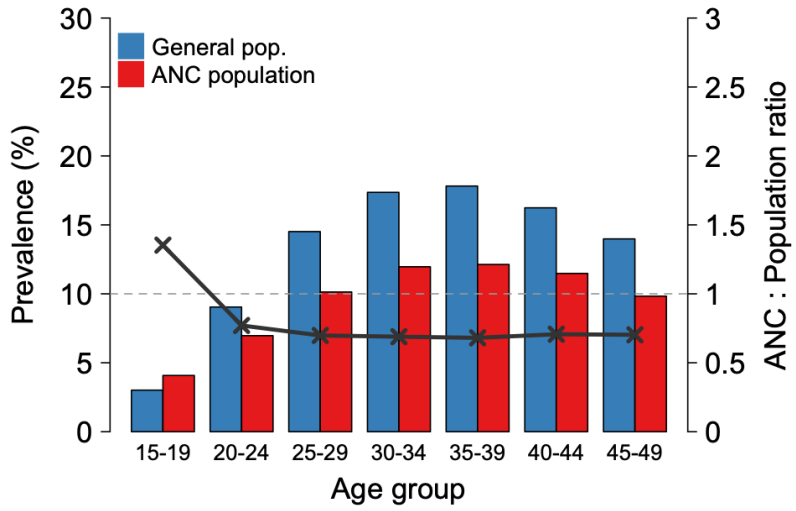


Data were collected at two different time points - ANC data were collected in 2018 and the HDSS data were collected in 2016

Due to non-repeat testing of HIV-positive participants in the HDSS sero-survey, previous HIV-positive status is carried forward in the HDSS prevalence estimates.

A simulation study, comparing ANC-based HIV prevalence with HIV prevalence in the general population (see Appendix 3 for more detailed methods), showed HIV prevalence in the ANC population to be higher than that in the general population in the youngest age group (15 to 19 years) (Figure 6). Among all other age groups, HIV prevalence was lower among ANC attendees than in the general population.

Figure 6: Simulation of HIV prevalence

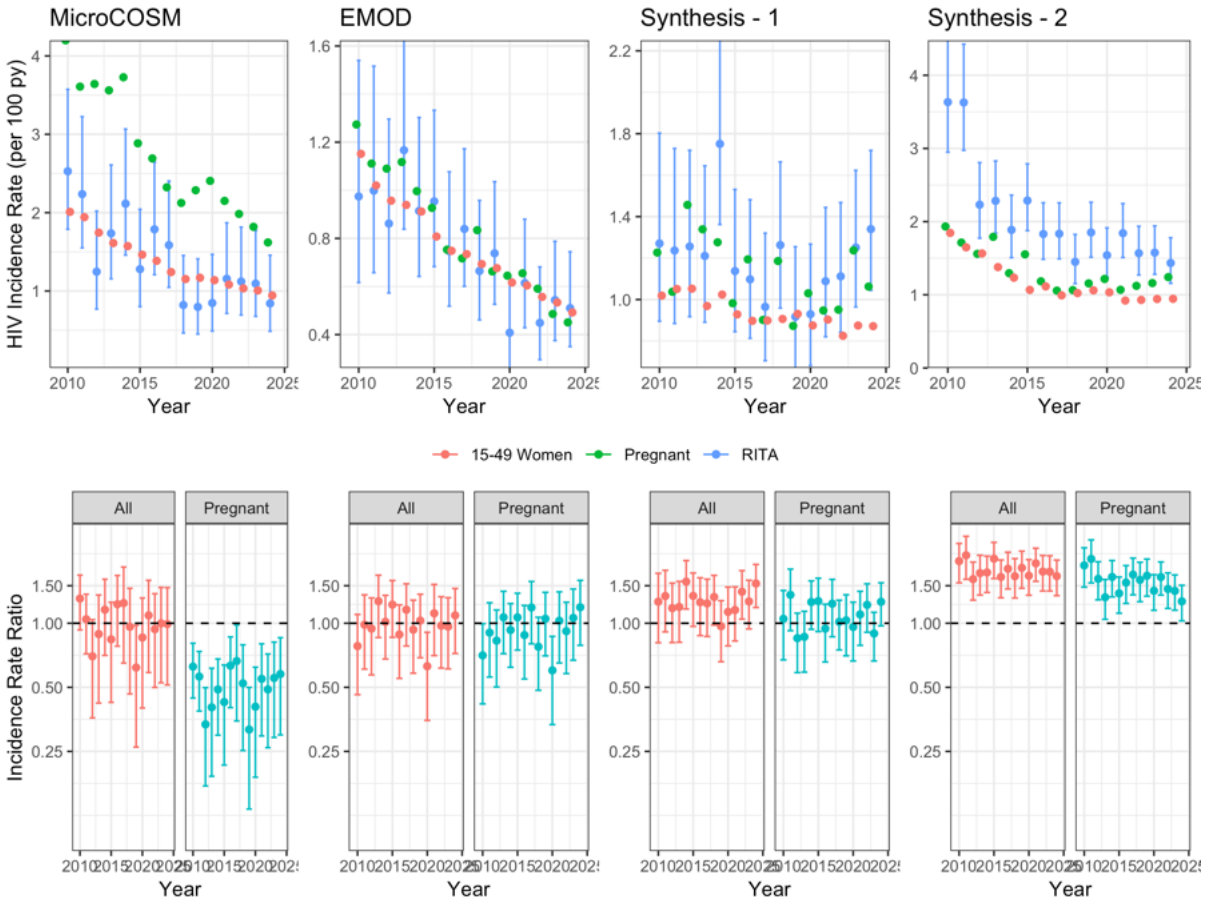


Using individual-based simulation models (see Appendix 3 for more details) we found bias in ANC RITA testing was variable across simulation models (Figure 7), and mainly driven by differences in ANC testing patterns across simulations. The ANC-based RITA underestimated incidence in pregnant women in MicroCOSM and overestimated incidence in Synthesis 2. ANC testing patterns varied between the settings with MicroCOSM having a higher proportion of pregnant HIV-negative women testing than HIV-positive women, with HIV-positive women testing in other venues and therefore becoming known positives and not contributing to ANC tests. Synthesis 2, in contrast, had a higher proportion of tests among acutely infected HIV-positive women, and a lower HIV-negative testing rate, thus biasing the RITA incidence estimate down.

Figure 7: HIV incidence estimates and rate ratios

Upper panel: RITA incidence estimated among ANC attendees in four individual-based simulation models, and incidence among 15 to 49 year old women and pregnant women

Lower panel: HIV incidence rate ratio comparing RITA incidence estimated among ANC new diagnoses to incidence in the 15 to 49 year old general population and pregnant women



Objective 3: Estimate HIV incidence

HIV incidence estimates using the RITA ranged from 0.62 to 1.1 per 100 person-years, depending on MDRI, FRR and classification of recent ART initiators (see Table 11; scenarios presented are described in the table footnotes). Longer MDRI, lower FRR, and allowing those with recent ART initiation dates (<90 days) to be classified recent, led to higher incidence estimates, while shorter MDRI, higher FRR, and allowing none on ART to be classified recent, led to lower incidence estimates.

Table 11: Incidence estimate by FRR, MDRI and ART status

	FRR = 0.003				FRR = 0.01			
	MDRI = 206.3*		MDRI = 201**		MDRI = 206.3*		MDRI = 201**	
	ART1^	ART2^	ART1^	ART2^	ART1^	ART2^	ART1^	ART2^
Incidence estimate	1.1	0.98	1.1	1	0.75	0.62	0.77	0.64
95% CI	0.29-1.9	0.21-1.8	0.30-2.0	0.22-1.8	0-1.6	0-1.4	0-1.6	0-1.4

Eight scenarios are presented with combinations of:: 1) false recency rate (FRR) values of 0.003 (used in an in-press manuscript from the CEPHIA collaboration to describe a Kenya-like scenario where all treated participants are correctly classified as long-term) and 0.01 (used in the recent DREAMS evaluation in Malawi); 2) mean duration of recent infection (MDRI) of 206.3 days (based on an average of subtype-specific CEPHIA MDRI estimates for Maxim<1.5 ODn and VL>1000 copies/ml, weighted to the most recent available subtype distribution for Kisumu) and 201 days (the average CEPHIA MDRI estimates for Maxim<1.5 ODn and VL>1000 copies/ml); 3) ART assumptions among those with a CCC record indicating that at sample collection they had been on ART for >90 days classified as non-recent or >7 days classified as non-recent.

** MDRI = 206.3, based on subtype distribution in Yang et al 2004, AIDS and Human Retroviruses (70.9% A, 18.5% D, 5.7% C)*

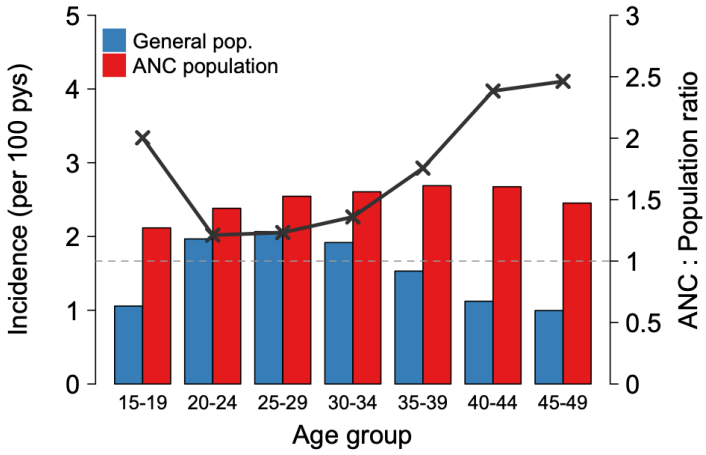
***MDRI = 201, overall MDRI estimated by CEPHIA for Maxim<1.5 & VL>1000*

^ ART1 = documented ART>90 days classified as "nonrecent"; ART2 = documented ART>7 days classified as "nonrecent"

Among the 1,970 women participating in the pilot who living in an HDSS area of residence, 1,277 (65%) were able to be linked to an HDSS record at study enrolment. Among these women, 532 (42%) had prior HIV sero-survey results, of whom 96 (18.0%) were HIV-positive on their most recent HIV test in the HDSS; the remaining 436 (82.0%) being HIV-negative. Among those whose last HIV test was HIV-negative, the HIV incidence between last HDSS HIV test and ANC visit was 1.3 per 100 person-years (95% CI=0.88-1.9) (based on 25 seroconversions among 434 women contributing 1,877 person years). This estimate is similar to the one obtained using RITA.

A simulation comparing ANC HIV incidence to HIV incidence in the general population, showed HIV incidence in the ANC population (measured using our recent infection test results) to be higher across all ages, though to a much lesser extent among women aged 20 to 34 years (see figure 8).

Figure 8: Simulation of HIV incidence



Objective 4: Evaluate the Asanté HIV-1 Rapid Recency Assay

Having run the Maxim LAg assay on the collected samples, the Asante HIV-1 Rapid Recency Assay point-of-care test was then subsequently run to assess HIV reactivity and recent infection for comparative purposes. Of the 445 women originally testing HIV positive, 373 (83.8%) also had the Asante assay run against their sample. The results arising from this comparison are currently awaiting CDC clearance.

Objective 5: Validate ANC register ART status with a biomarker for ARV detection

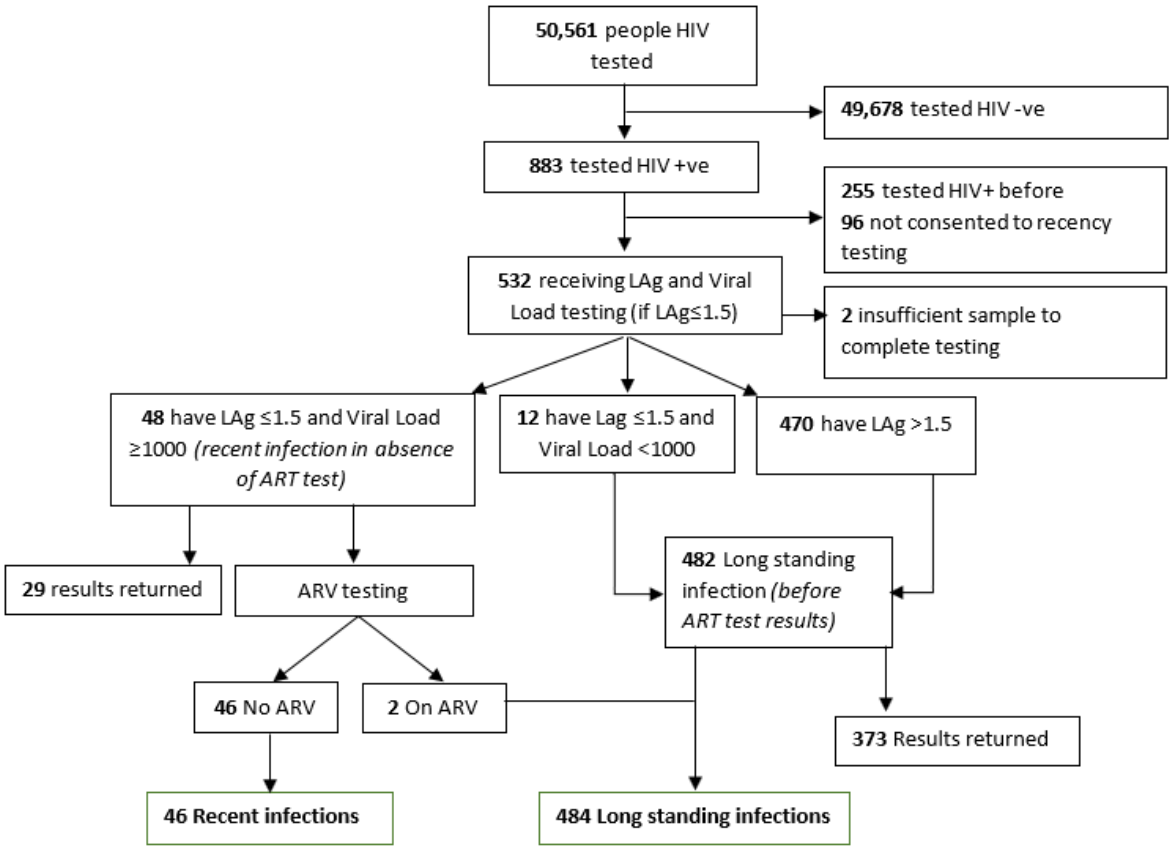
It was planned for samples assessed as recent positive in the three pilots to be sent to the University of Cape Town for testing the presence of antiretroviral treatment metabolites in the blood (the inclusion of information on exposure to ART improves RITA performance). Negotiations with regards this commenced in August, 2018. Between August, 2018 and May, 2019 there was regular communication between members of the MeSH working group and the Clinical Pharmacology Department at UCT. Despite these discussions, and despite the samples being ready to ship, as of writing this report (July, 2019) we await an import licence and contractual information from South Africa. As such, we were unable to address this objective within the timeframe of this work for this pilot.

Nairobi, Kenya: routine HIV testing and counselling clinics

Objective 2: Assess the feasibility of integrating RITAs and return of recency results into routine HTC services at fourteen facilities in Nairobi, Kenya

During the eight months implementation of the recency pilot from March 2018 to October 2018 50,561 men and women came to test at the fourteen EDARP facilities. Of these, 883 (1.75%) tested HIV positive. Of these 883, 255 were subsequently found to have tested HIV positive before, thereby, resulting in 628 being eligible for recency testing. Of these, 532 (84.7%) consented to participate in the recency study. Of these 532 men and women consenting, two had insufficient samples to complete the algorithm after the viral load failed (therefore they were not given a final classification). Figure 9 presents the recruitment and testing figures.

Figure 9: Recruitment and testing flowchart (Maxim LAg DBS)



Of those who enrolled and successfully tested for recency, 48 (9.1%) were classified as positive for recent infection prior to ART metabolite testing. Metabolite testing identified two (4.2%) (one woman and one man) of these 48 to have been wrongly classified as they were shown to have been in receipt of antiretroviral therapy. Therefore, in total, 46 (8.7%) people were classified as positive

for recent infection; of these, 39 (84.8%) were women. These 46 women and men are described under objective three below.

Of the 532 participants initially consenting to testing (includes two for whom there was insufficient sample to test), 316 (59.4%) were female, of whom 57 (18%) were pregnant. The majority of participants (341; 64.1%) had ever tested for HIV, with a third (179; 33.6%) having tested in the past twelve months. Table 12 presents additional participant characteristics.

Table 12: Characteristics of HTC participants testing for recent infection

		Tested for recent	
		N (532)	%
Sex	**Male**	216	40.6
	Female	316	59.4
Age (in years)	**15-19**	18	3.4
	20-24	96	18.0
	25-29	115	21.6
	30-34	108	20.3
	35-39	82	15.4
	40+	113	21.2
Residence in catchment	**Yes**	454	85.3
	No	78	14.7
Marital status	**Single**	114	21.4
	Married/co-habiting	293	55.1
	Separated	80	15.0
	Divorced	10	1.9
	Widowed	32	6.0
	Unknown	3	0.6
Highest level of education	**None**	9	1.7
	Primary	268	50.4
	Secondary	198	37.2
	Tertiary	55	10.3
	Unknown	2	0.4
Employment status	**Employed**	376	70.7
	Unemployed	154	28.9
	Unknown	2	0.4
Ever tested for HIV	**Yes**	341	64.1
	No	191	35.9
Tested for HIV in last 12 months	**Yes**	179	33.6
	No	351	66.0
	Unknown	2	0.4
Pregnancy status (n316)	**Pregnant**	57	18.0
	Not pregnant	259	82.0

Although the National HIV Reference Laboratory had performed LAg assay testing before, but not with DBS, the testing procedures proved more challenging than expected. Due to failing runs, recruitment was paused for a month. Clients were informed about the delay as it affected timelines for returning results. Several troubleshooting sessions were held and the laboratory was decontaminated and the service engineer contacted. Parallel runs were performed with the back-up laboratory at the Kenyatta National Hospital laboratory. This led to the conclusion that the washer and readers were not calibrated properly and did not function well. This problem was solved by borrowing the KNH equipment and having a dedicated washer for this study.

The majority, but not all, of test results arising from the taking of samples were received back at the testing site. The test results for 24 people were never received. As of all 532 participants tested for recent infection, results were received by the overwhelming majority (508; 95.5%). Table 13 provides a breakdown of the return of test results. Of the 48 clients originally classified as positive for recent infection, 29 (60.4%) received their results (one of these was subsequently reclassified as "long-standing" following ART metabolite testing); this percentage is lower than that among those originally classified as having "long-standing" infection (77.1%). Of the 19 participants who did not receive their recent infection positive test result, seven were lost to follow up, five travelled out of Nairobi and did not return, and seven said they did not want to receive results.

Table 13: Return of recent infection test results (prior to ART metabolite testing)

			N (532)	%
Recency test result returned to testing site		**Yes**	508	95.5
		No	24	4.5
Recency test result returned to participant		**Yes**	402	75.6
		No	130	24.4
Recency test result returned by recency status	**Long-term**	**Yes**	373	77.1
		No	111	22.9
	Recent	**Yes**	29	60.4
		No	19	39.6
Time to return result (n=402)		**Within two weeks (as in protocol)**	0	0.0
		>two weeks, but within one month	7	1.7
		Between 1 and 2 months	48	11.9
		Between 2 and 3 months	81	20.1
		More than 3 months	266	66.2

Due to challenges experienced in the laboratory, results were delayed. Two thirds of participants received their results more than three months after their initial test visit as a result of these delays. The objective was to return test results within two weeks of the initial test visit.

Objective 3: Identify and characterize where and among whom new HIV infections are occurring

Table 14 presents participant characteristics by their recent infection status, as well as recency rates. Among the 315 women tested for recent infection, 39 (12.4%) were classified as recent, whereas, among the 215 men this figure was 3.3%.

Table 14: Participant characteristics by recent infection status

N (530)*		Recent		Long-standing		Recency
		N (46)	%	N (484)	%	rate**
Sex	Male	7	15.2	208	43.0	3.3
	Female	39	84.8	276	57.0	12.4
Age (in years)	15-19	5	10.9	13	2.7	27.8
	20-24	16	34.8	79	16.3	16.8
	25-29	13	28.3	102	21.1	11.3
	30-34	7	15.2	100	20.7	6.5
	35-39	2	4.3	80	16.5	2.4
	40+	3	6.5	110	22.7	2.7
Residence in catchment	Yes	41	89.1	412	85.1	9.1
	No	5	10.9	72	14.9	6.5
Marital status	Single	15	32.6	97	20.0	13.4
	Married/co-habiting	24	52.2	269	55.6	8.2
	Separated	6	13.0	74	15.3	7.5
	Divorced	0	0.0	10	2.1	0.0
	Widowed	1	2.2	31	6.4	3.1
	Unknown	0	0.0	3	0.6	0.0
Highest level of education	None	2	4.3	7	1.4	22.2
	Primary	16	34.8	251	51.9	6.0
	Secondary	21	45.7	176	36.4	10.7
	Tertiary	7	15.2	48	9.9	12.7
	Unknown	0	0.0	2	0.4	0.0
Employment status	Employed	28	60.9	347	71.7	7.5
	Unemployed	18	39.1	135	27.9	11.8
	Unknown	0	0.0	2	0.4	0.0
Ever tested for HIV	Yes	35	76.1	305	63.0	10.3
	No	11	23.9	179	37.0	5.8
Tested for HIV in last 12 months	Yes	25	54.3	153	31.6	14.0
	No	21	45.7	329	68.0	6.0
	Unknown	0	0.0	2	0.4	0.0
Pregnancy status (n=316)	Pregnant	10	25.6	47	17.0	17.5
	Not pregnant	29	74.4	229	83.0	11.2

** Excludes two participants for whom there was insufficient sample to test*
** Calculated as recent / (recent & non-recent); the denominator only includes new diagnoses as repeat testers were excluded*

Around half (21; 45.7%) of recent infections were among participants aged under 25 years, participants who were married or co-habiting (24; 52.2%), and those who had tested HIV negative in the last twelve months (25; 54.3%). As expected, the percentage of testing in the past twelve months was higher in the recent positive group (25; 54.3%) as compared to those classified as having "long-standing" infection (153/484; 31.6%). The percentage of participants found to be recent positive varied across the fourteen health facilities. Five facilities had a percentage >10%, with the highest being 20%.

Predictors of recent HIV infection were first assessed using a logistic regression model. Individually, being a woman, being under 25 years old, having tested for HIV in the last 12 months, and presenting at the facility with the largest catchment area and the largest corresponding patient volume were shown to be individually predictive of recent infection. A generalized estimated equation (GEE) was developed to account for site clustering. Testing for interactions indicated an interaction between age at diagnosis and gender. Young women (15-29 years old) had 3.85 times the odds of recent infection than men in the same age group. As expected, clients who reported testing for HIV in the last 12 months had 1.72 times the odds of recent infection than those who reported last testing over 12 months ago. The output of the final GEE model is listed in Table 15. The output of the final GEE model, disaggregated by sex, is in Table 16.

Table 15: Predictors of recent infection

	N	OR (95%CI)	P-value
Male aged 15-29 years	58	1	-
Male aged 30+ years	158	0.98 [0.95, 1.01]	0.23
Female 15-29 years	171	3.85 [1.53, 9.70]	<0.01
Female 30+ years	145	1.15 [0.45, 2.92]	0.76
Tested over 12 months ago	351	1	-
Tested within last 12 months	179	1.72 [1.48, 2.01]	<0.01

Final GEE model includes age at diagnosis, gender, and HIV testing history (having tested for HIV in the last 12 months), and accounts for clustering at the site level

Table 16: Predictors of recent infection, disaggregated by gender

	Male			Female		
	N	OR (95%CI)	P-value	N	OR (95%CI)	P-value
Aged 15-29 years	58	1	-	171	1	-
Aged 30+ years	158	0.99 [0.94, 1.06]	0.87	145	0.23 [0.16, 0.32]	<0.01
Tested over 12 months ago	162	1	-	189	1	-
Tested within last 12 months	53	1.05 [1.01, 1.08]	0.01	126	2.12 [1.83, 2.45]	<0.01

Final GEE model, disaggregated by sex, includes age at diagnosis, gender, and HIV testing history (having tested for HIV in the last 12 months), and accounts for clustering at the site level

The average age at which women were first diagnosed with HIV infection was 14% lower than the age at which men in this study were ($p<0.01$). Female participants had 2.04 times the odds of testing within the last twelve months than did male participants and 3.12 times the odds of ever having tested ($p<0.01$). To confirm the study findings, HIV testing data should be analysed to determine whether men and women, particularly those under 30 years old, were tested in EDARP facilities at the same rate during the study period. Low rates of HIV testing among younger men could instead suggest that men face structural and cultural barriers to HIV testing. Further investigation may be needed to reassess their risk for infection.

Objective 4: Conduct index / partner testing

Just over a quarter (144; 27.1%) of participants, following an HIV positive test result and counselling, subsequently brought a sexual partner in to the clinic for HIV testing. Of these 144, two brought in two partners. Table 17 presents summary results relating to the 146 partners tested for HIV. Among the 146 sexual partners of index cases testing for HIV, 61 (41.8%) were positive. Among these 61 partners, 21 enrolled in the pilot, of whom five (23.8%) were classified as positive for recent infection.

Among all "non-partner" participants (i.e. less the 146 partners), 1.6% (822/50,417) were HIV positive, and 8.7% (46/530) were classified as positive for recent infection. The percentage of partners who were HIV positive and recent infection positive were significantly higher than among all "non-partner" participants ($p<0.001$ & $p=0.019$, respectively).

Table 17: Index/partner testing

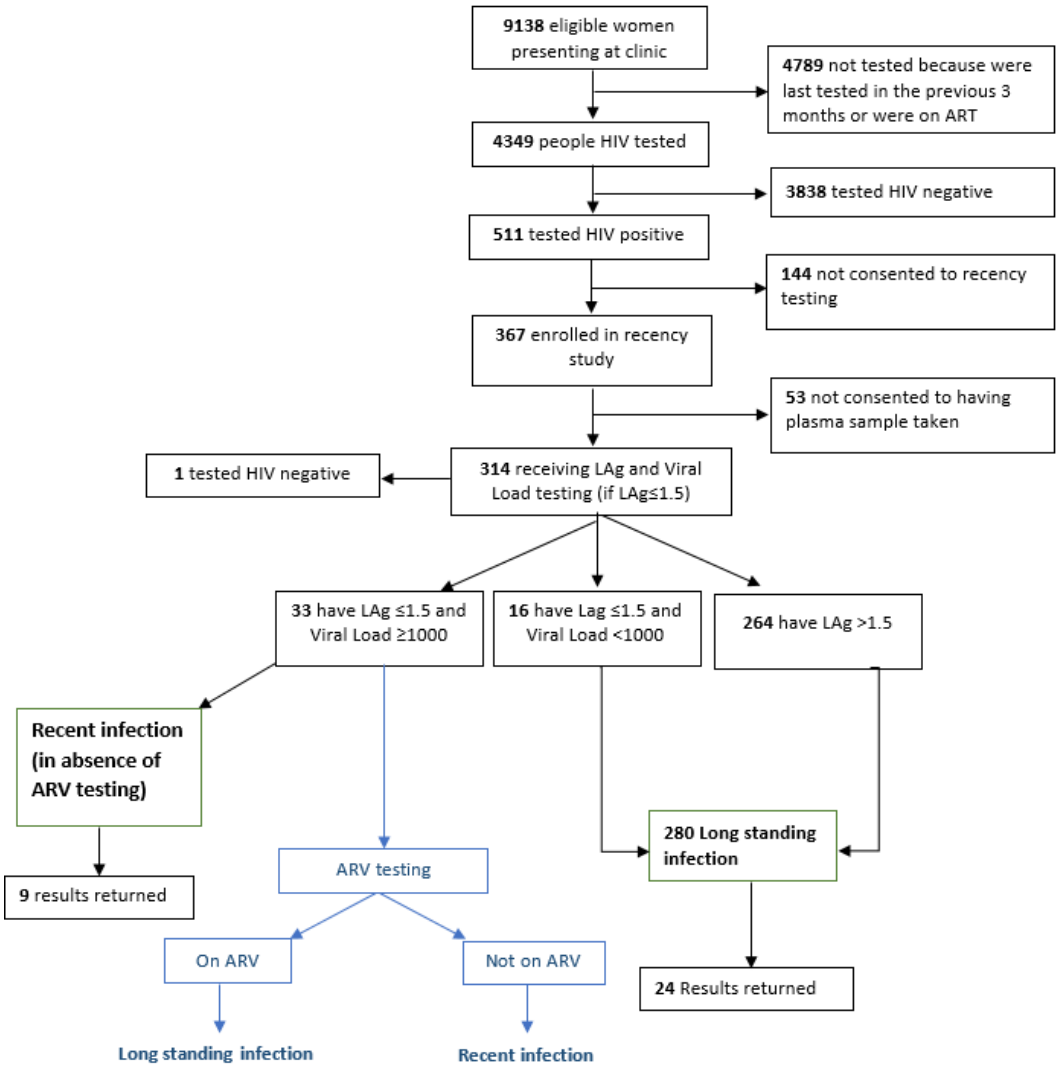
		N	%
Brought sexual partner for HIV testing (n=532)	**Yes**	144	27.1
	No	388	72.9
Number of sexual partners brought for testing (n=144)	**1**	142	98.6
	2	2	1.4
Sex of sexual partners (n=146)	**Male**	70	47.9
	Female	76	52.1
HIV status of sexual partners (n=146)	**Positive**	61	41.8
	Negative	85	58.2
Sex of partners among those HIV-infected (n=61)	**Male**	21	34.4
	Female	40	65.6
Enrolled into recency study (n=61)	**Yes**	21	34.4
	No	40	65.6
Recency status of sexual partners (n=21)	**Recent**	5	23.8
	Long-standing	16	76.2
Sex of partners by recency status (n=21)	**Male long-standing**	11	52.4
	Male recent	0	0.0
	Female long-standing	5	23.8
	Female recent	5	23.8

Zimbabwe: Sisters with a Voice programme

Objective 2: Assess the feasibility of integrating RITAs and return of recency results into routine programme service delivery for FSW, and identify the yield of women positive for recent infection

Between June 2018 and November 2018, 9,138 eligible women presented at the six participating static facilities. Figure 10 presents the recruitment and testing figures among these women using the plasma based Maxim assay. In summary, of the 4,349 (47.6%) women tested for HIV, 511 (11.7%) tested positive. Of these women, 314 (61.4%) agreed to have a plasma sample taken. Based on their LAg and viral load test, 33 (10.5%) women were classified as positive for recent infection.

Figure 10: Recruitment and testing flowchart for the Maxim LAg plasma assay



Of the 511 women testing HIV positive, 367 (71.8%) agreed to have a DBS sample taken and a viral load test (a slightly higher figure than for plasma). Among these women, 129 (35.1%) were classified as positive for recent infection based on their LAg and viral load test. This percentage greatly exceeds the figure based on plasma and from the other two pilots. Due to this, procedural investigations were conducted. These investigations revealed the DBS cards provided by the within country supplier had expired in 2014. This issue was not previously discovered due to the small size of the print presenting the expiry date. Due to this error it was not possible to compare the ODn values of the plasma and DBS samples. From hereon we present plasma results only.

Table 18 shows the characteristics of the 314 FSW providing a plasma sample. Of these women, 302 (96.2%) were successfully linked between the laboratory results and questionnaire data. Almost half (141; 46.7%) of these women were aged between 25 and 34 years. Four in ten of the women presented at one specific site (119; 39.4%). The majority of women (247; 81.8%) attained a secondary school education (highest form) and/or were separated or divorced (177; 58.6%).

Table 18: Characteristics of FSWs tested for recent infection using plasma

	N=302*	%
Age (years)		
18-19	24	7.9
20-24	93	30.8
25-34	141	46.7
35+	44	14.6
Study site		
1	55	18.2
2	28	9.3
3	119	39.4
4	34	11.3
5	46	15.2
6	20	6.6
Education		
None	3	1.0
Primary	51	16.9
Secondary	247	81.8
Tertiary	1	0.3
Marital status		
Single/ never married	87	28.8
Married/ living together as if married	24	8.0
Divorced/ separated	177	58.6
Widowed	14	4.6

**12 FSW not included in this analysis as recency test result was not linked to characteristics data*

Table 19 presents participant characteristics by HIV and recent infection status. It also presents recency rates as among those newly diagnosed with HIV. By age, women aged 18 or 19 years present the highest percentage of positivity for recent infection (21.7%). By education and marital status, elevated percentages of recent infections, as compared to the other groups, were respectively observed among those for whom secondary education was their highest attainment (12.6%), and those who were single or never married (19.5%). As compared to other study sites, the percentage of recent infections observed at study site 2 (3.6%) was low.

Table 19: Characteristics of FSWs by HIV and recent infection status, and recency rates

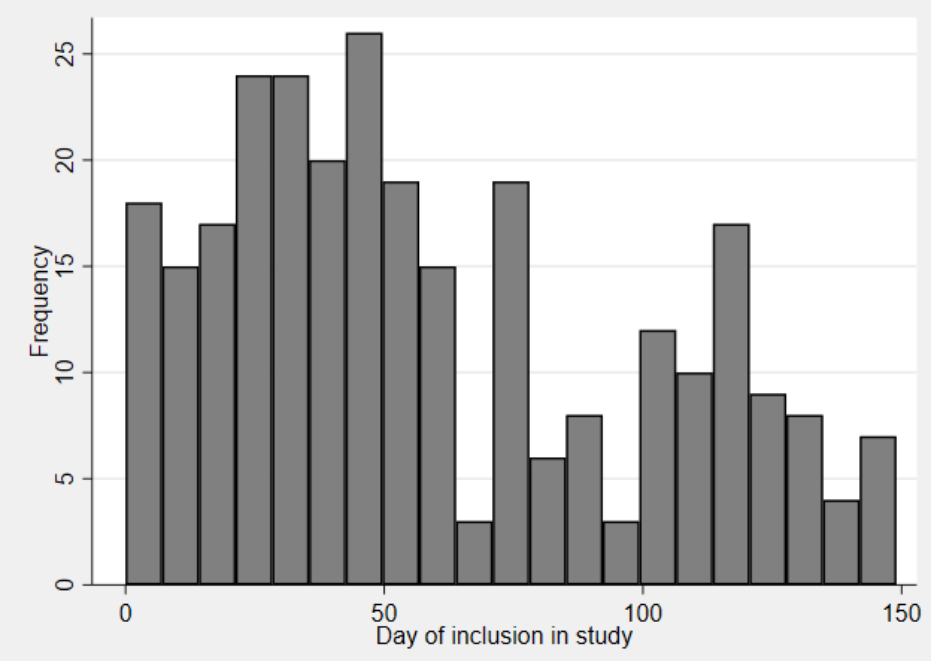
	HIV negative N=3839 n (%)	HIV positive (non-recent infection) N=268 n (%)	HIV positive (recent infection) N=33 n (%)	Recency rate* (recent infections / recent & non-recent infections) n/N = 33 / 301 = 11%
Age (years)				
18-19	367 (9.5)	18 (6.7)	5 (15.1)	21.7%
20-24	1167 (30.4)	80 (29.8)	13 (39.4)	14.0%
25-34	1447 (37.7)	128 (47.8)	13 (39.4)	9.2%
35+	598 (15.6)	42 (15.7)	2 (6.1)	4.5%
Missing	260 (6.8)	0 (0.0)	0 (0.0)	-
Study site				
1	655 (17.1)	49 (18.3)	6 (18.2)	10.9%
2	357 (9.3)	27 (10.1)	1 (3.0)	3.6%
3	1291 (33.6)	107 (39.9)	11 (33.3)	9.3%
4	320 (8.4)	29 (10.8)	5 (15.2)	14.7%
5	416 (10.8)	39 (14.6)	7 (21.2)	15.2%
6	800 (20.8)	17 (6.3)	3 (9.1)	15.0%
Education				
None	11 (0.3)	3 (1.1)	0 (0.0)	0.0%
Primary	539 (14.0)	49 (18.3)	2 (6.1)	3.9%
Secondary	2913 (75.9)	215 (80.2)	31 (93.9)	12.6%
Tertiary	61 (1.6)	1 (0.4)	0 (0.0)	0.0%
Missing	315 (8.2)	0 (0.0)	0 (0.0)	-
Marital status				
Single/ never married	1193 (31.1)	70 (26.1)	17 (51.5)	19.5%
Married/ living together as if married	119 (3.1)	22 (8.2)	2 (6.1)	8.3%
Divorced/ separated	2085 (54.3)	163 (60.8)	13 (39.4)	7.4%
Widowed	164 (4.3)	13 (4.9)	1 (3.0)	7.1%
Missing	278 (7.2)	0 (0.0)	0 (0.0)	-

***** *Women who have tested HIV positive before are excluded from the study so are also not counted in the denominator, the denominator therefore only includes new diagnoses*

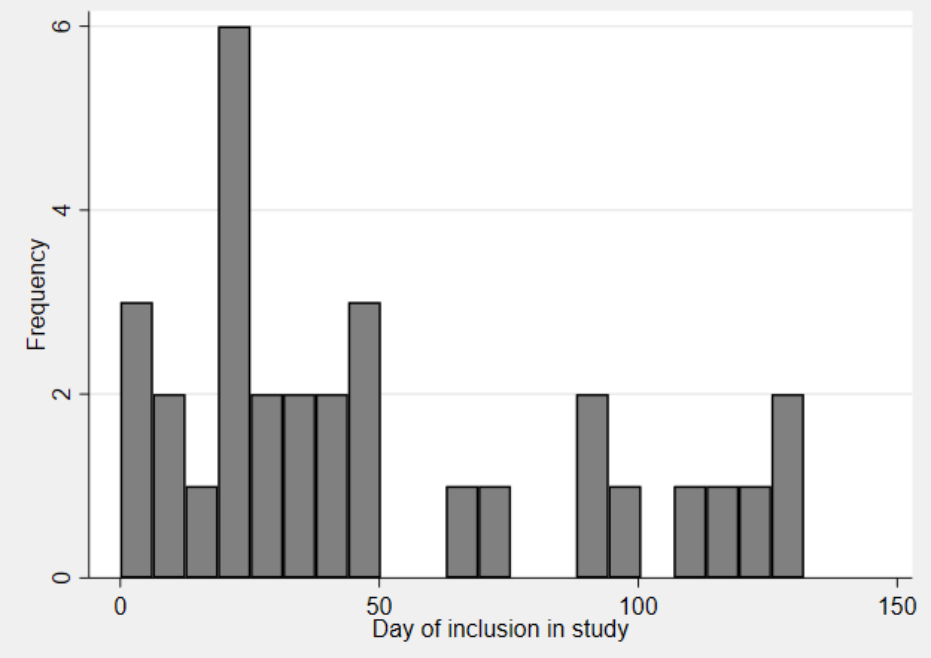
Figure 11 presents clustering in time according to (a) the number of women enrolled per week and (b) those testing positive for recent infection per week. Although Figure 11 a and b do not suggest the presence of clustering, Figure 11a does show a higher weekly number of women testing recent positive during the first fifty days of the pilot (68% of women were enrolled in the first half of the recruitment period), and Figure 11b shows that on one day six women tested positive for recent infection. Three of these women tested at the same site, and three were aged between 20 and 24.

Figure 11: Recruitment over time

a) Number of women enrolled by week



b) Number of women testing positive for recent infection by week



For the 314 women tested for recent infection, results were received back at the clinic for the majority (296; 94.3%). The test results for 18 women were not received. Table 20 provides a breakdown of the return of test results. Of the 33 women classified as positive for recent infection, 9 (27.3%) received their results. Among women classified as having "long-standing" infection this percentage was lower at 8.6% (24/280). No women stated they did not want to receive their results when they were enrolled into the study but in reality women frequently did not return to the clinic for results and when followed up had apparently given false contact information.

Due to challenges in the laboratory with the LAg Avidity assay, results were delayed. Most results were received back at the clinic following a period exceeding four weeks. The objective was to return test results within two weeks of the initial test visit. However, no women came to the clinic to collect results when the result was not available. The timeframe for returning results back to the women was variable (mainly due to variability in the time between visits to the clinic).

Table 20: Return of recent infection test results

			N=314	%
Recency test result returned to testing site		**Yes**	296	94.3
		No	18	5.7
Recency test result returned to client		**Yes**	33	11.1
		No	263	88.9
Recency test result returned by recency status	**Long-standing**	**Yes**	24	8.6
		No	256	91.4
	Recent	**Yes**	9	27.3
		No	24	72.7

Objective 3: Identify sociodemographic risk factors for recent HIV infection

Table 21 presents a risk factor analysis for testing positive for recent infection. It compares women testing positive for recent infection to HIV negative women. Results from the multivariable model were adjusted for age and study site. There was no strong evidence of an association between testing positive for recent infection and any of the variables.

Table 21: Risk factors for testing positive for recent infection among FSWs (plasma; n=302)

	n/N* (%)	Crude OR (95% CI)	P-value	Adjusted OR^ (95% CI)	P-value
Age (years)			0.37		0.30
18-19	5/372 (1.4)	1		1	
20-24	13/1180 (1.1)	0.82 (0.29-2.31)		0.79 (0.28-2.25)	
25-34	13/1460 (0.9)	0.66 (0.23-1.86)		0.60 (0.21-1.74)	
35+	2/600 (0.3)	0.24 (0.05-1.27)		0.22 (0.04-1.16)	
Study site			0.18		0.12
1	6/661 (0.9)	1		1	
2	1/358 (0.3)	0.31 (0.04-2.55)		0.33 (0.04-2.79)	
3	11/1302 (0.9)	0.93 (0.34-2.53)		1.04 (0.38-2.87)	
4	5/325 (1.5)	1.71 (0.52-5.63)		2.08 (0.62-6.94)	
5	7/423 (1.7)	1.84 (0.61-5.50)		2.08 (0.69-6.28)	
6	3/803 (0.4)	0.41 (0.10-1.64)		0.42 (0.10-1.69)	
Education			0.15		0.15
Primary or less	2/550 (0.4)	1		1	
Secondary or higher	31/3005 (1.0)	2.87 (0.68-12.01)		2.89 (0.68-12.24)	
Marital status			0.12		0.07
Single/ never married	17/1210 (1.4)	1		1	
Married/ living together as if married	2/121 (1.7)	1.18 (0.27-5.17)		1.82 (0.39-8.45)	
Divorced/ separated	13/2098 (0.6)	0.44 (0.21-0.90)		0.38 (0.17-0.84)	
Widowed	1/165 (0.6)	0.43 (0.06-3.24)		0.50 (0.06-4.18)	

**n=FSW testing positive for recent infection. N=FSW tested HIV negative and FSW testing positive for recent infection*

^Adjusted for age and study site

Zimbabwe: RDS survey stored samples

Data from four, previously conducted, RDS surveys were reviewed and analysed. Following extensive data cleaning, variable alignment, and analysis across the four surveys it was discovered two of the surveys had used expired DBS papers to conduct recent infection testing. It was necessary to exclude these two surveys (RDS 2 and 4 as presented in Table 3) from our analyses. For information only, the study flowcharts relating to these two surveys are presented in appendix 4.

Objective 1: Identify yield of clients testing positive for recent infection among RDS participants

Figure 12 and Figure 13 present the recruitment and testing figures among FSW participating in RDS surveys 1 (currently working as a sex worker; aged 18 years or over; recruitment April to May, 2016; n=2,883)and 3 (FSW; aged 18 years or over; recruitment March to May, 2017; n=2,713), respectively. In summary, in RDS1 1,686 (58.4%) of the women tested HIV positive, among whom 462 (27.4%) were tested for recent infection and viral load. Of these 462 women, 97 (21%) were classified as positive for recent infection. In RDS3, 1,512 (55.7%) of women were HIV positive, among whom 161 (10.6%) were tested for recent infection and viral load. Of these 161 women, 6 (3.7%) were classified as positive for recent infection.

Figure 12: Recruitment and testing flowchart for RDS study 1

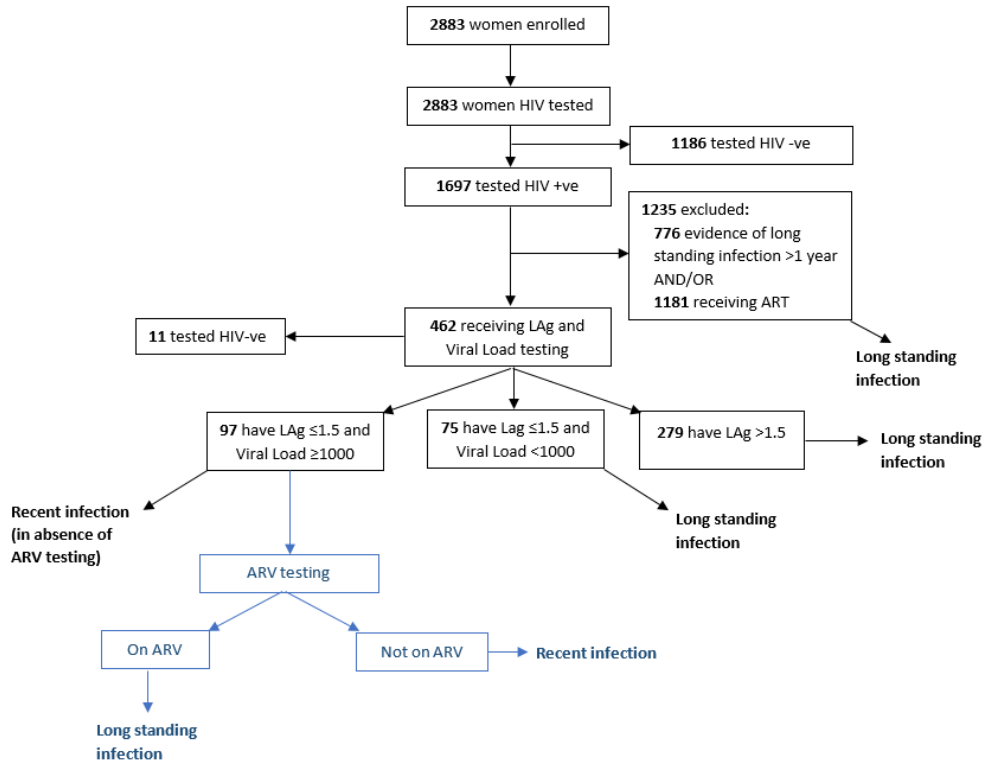


Figure 13: Recruitment and testing flowchart for RDS study 3

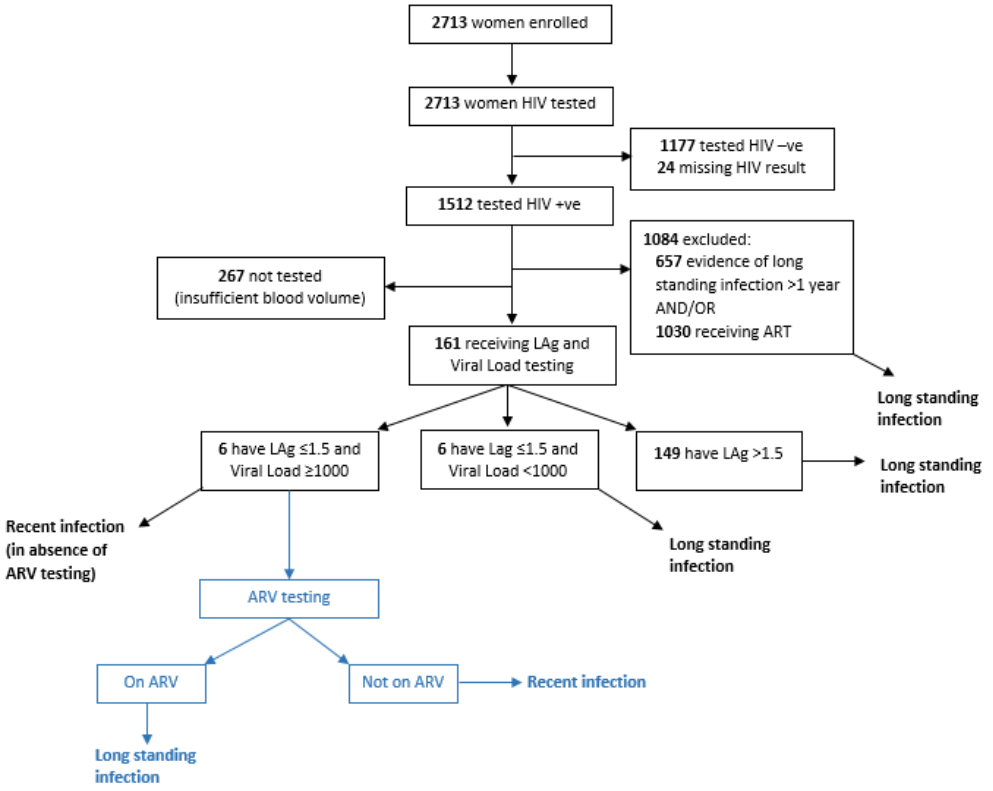


Table 22 presents the key results for RDS 1 and 3. In RDS 3, 267 women were not tested for recency. Sensitivity analysis found no important difference between these women and those tested (see Appendix 5). Women were of similar age, had similar testing history and similar HIV prevalence (32.7% among those not tested compared to 31.7% among those tested)

In RDS 1, the HIV recency rate (recently infected / HIV positive) was 5.8%. In RDS 3 the figure was much lower at 0.48% (denominator includes all people testing HIV positive). Based on an MDRI of 130 days and FRR of 0%, incidence was estimated to be 22.8 (95%CI 16.0-29.6) per 100 person-years in RDS 1. For RDS 3 incidence was estimated to be much lower at 1.7 (95%CI 0.3-3.2) per 100 person-years.

Table 22: Summary results for RDS 1 and 3

	RDS 1	RDS 3	Total
Study population			
Age in years	18+	18+	-
Study period	April-May 2016	March-May 2017	-
N	2883	2713	5596
HIV negative	1197 (41.5)	1177 (48.6)	2374
HIV positive			
Non-recent	1589 (55.1)	1239 (51.2)	2828
Recent	97 (3.4)	6 (0.2)	103
Not tested	0	267	267
Missing HIV status		24	24
Incidence per 100 person years (95%CI)*	22.8 (16.0-29.6)	1.7 (0.3-3.2)	

**Assuming MDRI=130 days and FRR=0%*

Table 23 presents the characteristics of FSW included in the two RDS surveys, and a breakdown of HIV status and recency test result. In both surveys, the majority of FSW were above 25 years (82.0% RDS 1, 71.1% RDS 3) and were divorced/separated (63.5% RDS 1, 69.9% RDS 3). Around half of the FSW had been in sex work for more than five years (56.1% RDS 1, 47.0% RDS 3). Whereas most women in RDS 1 had attended the Sisters with a Voice clinic in the past 12 months (79.2%), only 12.9% of women in RDS 3 had attended the clinic in that time period. The total number of women testing positive for recent infection was much higher in RDS 1 (n=97) than in RDS 3 (n=6).

Table 23: Characteristics of participants in RDS 1 and 3, by HIV and recent infection status

	RDS 1				RDS 3			
	Total	HIV negative	HIV positive: non-recent infection	HIV positive: recent infection	Total	HIV negative	HIV positive: non-recent infection	HIV positive: recent infection
	N=2883	N=1197	N=1589	N=97	N=2416^	N=1172	N=1238	N=6
	N (col%)	N (row%)	N (row%)	N (row%)	N (col%)	N (row%)	N (row%)	N (row%)
Age in years								
16-17	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
18-19	51 (2.1)	39 (84.6)	8 (11.2)	4 (4.2)	106 (4.9)	95 (89.3)	8 (9.9)	3 (0.8)
20-24	452 (16.0)	294 (64.3)	136 (30.8)	22 (4.8)	454 (21.0)	346 (76.6)	107 (23.3)	1 (0.07)
25-34	1312 (44.9)	569 (40.6)	693 (54.1)	50 (5.3)	1077 (44.7)	530 (50.8)	546 (49.2)	1 (0.03)
35+	1068 (37.1)	295 (30.2)	752 (68.1)	21 (1.7)	779 (29.4)	201 (21.1)	577 (78.9)	1 (0.05)
Site (differ by RDS survey)								
1	208 (7.1)	78 (34.0)	127 (62.6)	3 (3.4)	1332 (24.5)	660 (51.1)	669 (48.8)	3 (0.2)
2	208 (7.1)	70 (35.7)	123 (56.9)	15 (7.4)	719 (25.2)	355 (52.4)	361 (47.4)	3 (0.2)
3	206 (7.1)	86 (40.1)	111 (56.1)	9 (3.7)	179 (24.7)	89 (50.8)	90 (49.2)	0 (0.0)
4	210 (7.2)	93 (42.1)	111 (55.1)	6 (2.8)	186 (25.7)	68 (43.5)	118 (56.5)	0 (0.0)
5	202 (7.1)	83 (46.4)	111 (50.1)	8 (3.5)				
6	205 (7.1)	96 (48.5)	106 (50.2)	3 (1.3)				
7	209 (7.1)	129 (59.3)	73 (37.7)	7 (3.0)				
9	203 (7.1)	91 (44.6)	106 (52.4)	6 (2.9)				
10	205 (7.1)	66 (34.9)	133 (59.7)	6 (5.4)				
11	206 (7.1)	78 (38.0)	120 (56.9)	8 (5.1)				
12	205 (7.1)	109 (56.8)	94 (42.0)	2 (1.1)				
13	210 (7.1)	80 (33.1)	121 (63.5)	9 (3.4)				
14	202 (7.1)	88 (43.6)	108 (53.3)	6 (3.1)				
15	204 (7.1)	50 (22.9)	145 (69.1)	9 (8.0)				
Marital status								
Single/never married	427 (14.9)	239 (56.7)	175 (40.3)	13 (3.0)	492 (15.7)	319 (70.9)	170 (28.8)	3 (0.3)
Married/living together	42 (1.3)	20 (55.9)	22 (44.1)	0 (0.0)	29 (0.8)	15 (69.0)	14 (31.0)	0 (0.0)
Divorced/separated	1849 (63.5)	792 (42.2)	986 (53.0)	71 (4.8)	1540 (69.9)	772 (49.9)	766 (50.0)	2 (0.03)
Widowed	564 (20.3)	146 (27.1)	405 (71.0)	13 (1.9)	355 (13.7)	66 (21.0)	288 (78.9)	1 (0.1)
Missing	1 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Education								
None	106 (5.1)	40 (48.9)	63 (48.2)	3 (2.9)	706 (35.1)	260 (42.0)	444 (58.0)	2 (0.1)
Primary	789 (30.8)	278 (36.0)	485 (59.8)	26 (4.2)	115 (3.7)	46 (36.7)	68 (63.0)	1 (0.4)
Incomplete secondary	1009 (35.0)	400 (39.9)	576 (56.7)	33 (3.4)	1552 (59.8)	838 (54.6)	711 (45.3)	3 (0.06)
Secondary or higher	978 (29.1)	478 (47.5)	465 (48.2)	35 (4.2)	42 (1.3)	27 (45.9)	15 (54.1)	0 (0.0)
Missing	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)
Identify as sex worker								
No	226 (8.3)	103 (41.6)	117 (56.6)	6 (1.8)	*Variable not collected*			
Yes	2657 (91.7)	1094 (41.4)	1472 (54.5)	91 (4.0)				
Food insecurity*								
No	1686 (57.4)	730 (41.2)	887 (54.4)	69 (4.4)	1299 (53.2)	644 (49.6)	650 (50.0)	5 (0.4)
Yes	1197 (42.6)	467 (41.8)	702 (55.2)	28 (3.1)	1117 (46.8)	528 (47.3)	588 (52.6)	1 (0.1)
Age at first sex work in years								
<15	92 (3.6)	40 (51.3)	48 (45.9)	4 (2.8)	89 (3.4)	49 (69.6)	40 (30.4)	0 (0.0)
16-17	196 (7.2)	103 (63.0)	86 (36.1)	7 (0.9)	214 (9.7)	135 (66.1)	76 (33.4)	3 (0.5)
18-19	327 (10.7)	169 (53.3)	142 (42.3)	16 (4.4)	286 (12.3)	177 (70.0)	108 (29.9)	1 (0.05)
20-24	858 (28.8)	388 (42.4)	434 (50.4)	36 (7.2)	737 (31.0)	390 (50.8)	347 (49.2)	0 (0.0)
25+	1410 (49.7)	497 (34.5)	879 (63.2)	34 (2.3)	1090 (43.6)	421 (37.3)	667 (62.7)	2 (0.07)
Duration in sex work in years								
0-2	571 (21.6)	303 (51.1)	237 (42.9)	31 (6.1)	610 (28.7)	420 (68.8)	187 (30.7)	3 (0.5)
3-4	635 (22.3)	336 (50.5)	276 (45.7)	23 (3.8)	578 (24.3)	330 (57.1)	246 (42.6)	2 (0.3)
5+	1675 (56.1)	557 (34.1)	1075 (62.8)	43 (3.1)	1228 (47.0)	422 (34.4)	805 (65.6)	1 (0.08)
Missing	2 (0.0)	1 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number of clients in last week								
0-2	627 (24.0)	265 (44.5)	345 (52.6)	17 (2.9)	316 (19.0)	146 (46.2)	169 (53.7)	1 (0.03)
3-7	1345 (47.0)	573 (21.1)	724 (53.6)	48 (4.2)	921 (40.5)	429 (48.6)	488 (51.3)	4 (0.1)
8+	911 (28.9)	359 (37.8)	520 (58.2)	32 (4.0)	1179 (40.5)	597 (51.7)	581 (48.3)	1 (0.05)
Frequency of drinking 6+ drinks on one night								
Never	737 (45.5)	356 (47.5)	357 (49.0)	24 (3.5)	685 (37.1)	365 (51.8)	319 (48.2)	1 (0.05)
Once a month or less	206 (11.4)	70 (28.3)	129 (66.1)	7 (5.6)	233 (12.0)	109 (44.1)	123 (55.8)	1 (0.07)
2-4 times a month	245 (11.9)	79 (33.6)	155 (60.5)	11 (5.9)	200 (10.4)	102 (56.4)	98 (43.6)	0 (0.0)
2-3 times per week	270 (12.6)	106 (39.0)	152 (56.3)	12 (4.7)	323 (19.2)	164 (49.7)	158 (50.2)	1 (0.1)
4+ times a week	338 (18.6)	141 (42.0)	180 (51.6)	17 (6.4)	358 (21.3)	175 (56.1)	181 (43.7)	2 (0.2)
Missing	1087 (0.0)	445 (0.0)	616 (0.0)	26 (0.0)	617 (0.0)	257 (0.0)	359 (0.0)	1 (0.0)
Ever had sex without condom with steady partner in last month								
No	732 (44.0)	287 (40.0)	426 (57.5)	19 (2.6)	458 (36.8)	207 (43.6)	250 (56.4)	1 (0.03)
Yes	989 (56.0)	484 (51.0)	462 (44.0)	43 (5.0)	730 (63.2)	407 (53.3)	322 (46.7)	1 (0.04)
Missing	1162 (0.0)	426 (0.0)	701 (0.0)	35 (0.0)	1228 (0.0)	558 (0.0)	666 (0.0)	4 (0.0)

Ever had sex without condom with client in last month								
No	1645 (59.3)	691 (41.2)	905 (55.4)	49 (3.4)	2097 (88.6)	1020 (48.9)	1073 (51.1)	4 (0.06)
Yes	1029 (40.7)	444 (43.6)	543 (51.6)	42 (4.8)	319 (11.4)	152 (53.5)	165 (46.2)	2 (0.3)
Missing	209 (0.0)	62 (0.0)	141 (0.0)	6 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
STI symptoms in past year								
No	1832 (64.1)	862 (47.8)	906 (48.6)	64 (3.6)	1768 (76.0)	930 (52.2)	832 (47.7)	6 (0.1)
Yes	1048 (35.9)	333 (30.0)	682 (65.7)	33 (4.3)	646 (24.0)	240 (40.2)	406 (59.8)	0 (0.0)
Missing	3 (0.0)	2 (0.0)	1 (0.0)	0 (0.0)	2 (0.0)	2 (0.0)	0 (0.0)	0 (0.0)
Symptoms of mental health issues								
No	1657 (58.0)	708 (42.3)	883 (53.1)	66 (4.6)	1180 (55.6)	596 (49.4)	580 (50.5)	4 (0.1)
Yes	1217 (42.0)	485 (40.4)	701 (56.8)	31 (2.9)	1232 (44.4)	576 (49.6)	654 (50.3)	2 (0.05)
Missing	9 (0.0)	4 (0.0)	5 (0.0)	0 (0.0)	4 (0.0)	0 (0.0)	4 (0.0)	0 (0.0)
Attended the Sisters with a Voice clinic in past 12 months								
No	490 (20.8)	233 (48.8)	239 (46.6)	18 (4.6)	1853 (87.1)	918 (49.6)	930 (50.4)	5 (0.07)
Yes	2015 (79.2)	781 (38.4)	1173 (58.4)	61 (3.2)	560 (12.9)	253 (48.4)	306 (51.4)	1 (0.1)
Missing	378 (0.0)	183 (0.0)	177 (0.0)	18 (0.0)	3 (0.0)	1 (0.0)	2 (0.0)	0 (0.0)
Part of income from sex work								
Very little (>quarter)	220 (8.4)	82 (41.1)	133 (57.3)	5 (1.6)	335 (9.7)	139 (40.8)	196 (59.2)	0 (0.0)
Some (between ¼ and ½)	454 (15.1)	204 (44.1)	229 (50.3)	21 (5.6)	304 (10.5)	143 (46.6)	161 (53.4)	0 (0.0)
Most (more than half)	896 (30.4)	392 (43.4)	480 (53.4)	24 (3.2)	431 (16.4)	216 (45.7)	213 (54.1)	2 (0.2)
All	1313 (46.1)	519 (39.3)	747 (56.6)	47 (4.1)	1346 (63.4)	674 (52.1)	668 (47.8)	4 (0.08)
Times pregnant								
0	149 (5.8)	76 (53.4)	61 (36.7)	12 (9.9)	144 (5.5)	97 (73.3)	47 (26.7)	0 (0.0)
1-2	1457 (49.6)	685 (44.6)	718 (50.9)	54 (4.5)	1148 (48.3)	632 (55.5)	511 (44.4)	5 (0.1)
3-4	961 (32.6)	331 (36.2)	605 (61.6)	25 (2.3)	868 (34.0)	362 (44.5)	505 (55.4)	1 (0.04)
5+	316 (12.0)	105 (37.1)	205 (60.4)	6 (2.6)	256 (12.2)	81 (28.1)	175 (71.9)	0 (0.0)
Number of children								
0	246 (8.5)	119 (48.0)	111 (44.9)	16 (7.1)	244 (9.6)	139 (57.8)	105 (42.2)	0 (0.0)
1-2	1695 (58.8)	732 (41.4)	905 (54.2)	58 (4.4)	1380 (60.0)	710 (53.4)	665 (46.5)	5 (0.1)
3-4	792 (27.5)	288 (38.9)	483 (59.3)	21 (1.8)	689 (26.4)	285 (40.7)	403 (59.2)	1 (0.05)
5+	150 (5.2)	58 (43.1)	90 (54.1)	2 (2.8)	103 (4.0)	38 (26.4)	65 (73.6)	0 (0.0)

Percentages are RDS-II weighted

^ Of 2713, 6 had missing RDS recruiter information, 24 had missing HIV status, 267 HIV positive samples had insufficient volume for testing.

** Question asked differently across pilots: Study I-Food insecure if answer to following question is 'Yes' - In the past four weeks, was there ever no food to eat of any kind in your house because of lack of resources to get food?; Study III – Food insecure if answer to any of the following questions is 'Yes' - Sometimes we go to bed hungry / In the last week, have you had to go an entire day without eating because there was no food in your household?*

Objective 2: Estimate HIV incidence using RITA from surveys and compare with estimates of HIV incidence from repeat testers in the FSW programme

Table 24 presents incidence estimates for both surveys applying different MDRI and FRR values. The incidence estimates per 100 person years for RDS 1 are higher than for RDS 3. In both surveys, the youngest age group (18-19 years) presented the highest incidence for all MDRI and FRR combinations.

Analysing Sisters with Voice programme data for the period January 2016 to December 2017 (to match the time periods of the RDS surveys) we attained an estimated HIV seroconversion rate (incidence) of 6.4/100 person years (95% CI: 5.3 - 8.0). This estimate falls between our two RDS-based estimates.

Table 24: Incidence calculations by RDS, MDRI and FRR

		MDRI = 130 (11%)		MDRI = 200 (14%)	
		FRR= 0	FRR = 1 (20%)	FRR = 0	FRR = 1 (20%)
RDS 1	Overall	22.8 (16.0-29.6)	19.9 (13.2-26.7)	14.8 (9.7-19.9)	12.7 (7.8-17.5)
	Age in years				
	18-19	28.8 (0.0-59.1)	29.6 (0.0-61.4)	18.7 (0.0-38.7)	18.9 (0.0-39.3)
	20-24	21.0 (10.8-31.2)	20.7 (10.0-31.3)	13.7 (6.7-20.7)	13.2 (6.0-20.3)
	25-34	24.7 (15.8-33.6)	22.3 (13.2-31.3)	16.0 (9.6-22.4)	14.2 (7.9-20.4)
	35+	20.0 (10.2-29.8)	13.4 (3.4-23.4)	13.0 (6.2-19.8)	8.5 (2.0-15.1)
RDS 3	Overall	1.7 (0.3-3.2)	0 (0-0.2)	1.1 (0.2-2.1)	0 (0 -0.1)
	Age in years				
	18-19	16.9 (0.0-35.5)	17.3 (0-36.8)	11.0 (0.0-23.2)	11.0 (0-23.6)
	20-24	1.3 (0.0-3.8)	0 (0.0-2.6)	0.8 (0.0-2.5)	0 (0.0-1.6)
	25-34	0.7 (0.0-2.0)	0 (0.0-0.0)	0.4 (0.0-1.3)	0 (0.0-0.0)
	35+	1.6 (0.0-4.6)	0 (0.0-0.0)	1.0 (0.0-3.0)	0 (0.0-0.0)

Objective 3: Identify sociodemographic and behavioural risk factors of recent HIV infection

Table 25 presents analysis informing the development of a risk score to predict where risk of HIV infection will be highest. The table presents crude and adjusted odds ratios. Following adjustment for variables for which there was evidence of an association with testing positive for recent infection in univariate analysis, age at start of sex work ($p<0.001$), STI symptoms in past year ($p=0.04$) and number of children ($p=0.05$) remained associated in multivariable analysis.

Table 25: Univariable and multivariable logistic regression analysis of risk factors associated with recent infection

	HIV+ FSW recently infected with HIV					
	N=2477 N (%)	N=103 n (%)	Crude OR	P-value	Adjusted OR	P-value
Age in years				0.07		0.22
18-19	141 (4.1)	7 (4.7)	1		1	
20-24	665 (24.4)	23 (7.0)	1.51 (0.28-8.17)		0.47 (0.09-2.35)	
25-34	1153 (45.5)	51 (11.6)	2.64 (0.51-13.62)		1.08 (0.21-5.66)	
35+	518 (26.1)	22 (5.2)	1.10 (0.20-6.00)		0.77 (0.14-4.34)	
Marital status				0.10		
Single/never married	577 (19.7)	16 (5.0)	1			
Married/living together	35 (1.6)	0 (0.0)	0			
Divorced/separated	1639 (65.8)	73 (10.2)	2.16 (1.01-4.63)			
Widowed	226 (12.9)	14 (6.4)	1.31 (0.47-3.66)			
Education				0.75		
None	307 (5.8)	5 (5.5)	1			
Primary	351 (27.3)	27 (10.4)	2.00 (0.45-9.00)			
Incomplete secondary	1277 (33.6)	36 (7.9)	1.47 (0.33-6.48)			
Secondary or higher	540 (33.3)	35 (8.2)	1.53 (0.35-6.68)			
Food insecurity*				0.24		
No	1450 (57.9)	74 (9.7)	1			
Yes	1027 (42.1)	29 (6.9)	0.69 (0.37-1.28)			
Age at start of sex work (years)				<0.001		<0.001
<15	93 (4.3)	4 (5.3)	1		1	
16-17	248 (10.1)	10 (1.4)	0.26 (0.05-1.32)		0.21 (0.04-1.23)	
18-19	363 (13.6)	17 (7.7)	1.50 (0.33-6.80)		1.43 (0.41-4.94)	
20-24	817 (31.6)	36 (14.6)	3.08 (0.72-13.25)		2.70 (0.89-8.23)	
25+	956 (40.3)	36 (6.2)	1.18 (0.27-5.12)		1.10 (0.35-3.43)	
Duration in sex work (years)				0.51		
0-2	761 (27.3)	34 (10.6)	1			
3-4	691 (26.7)	25 (6.9)	0.63 (0.27-1.47)			
5+	1024 (46.0)	44 (8.2)	0.76 (0.40-1.44)			
Number of clients in last week				0.43		
0-2	429 (25.1)	18 (6.1)	1			
3-7	1055 (48.1)	52 (9.1)	1.54 (0.72-3.28)			
8+	993 (26.8)	33 (9.6)	1.64 (0.73-3.65)			
Frequency of drinking 6+ drinks on one night				0.28		
Never	746 (50.0)	25 (6.9)	1			
Once a month or less	187 (8.4)	8 (16.5)	2.67 (0.87-8.20)			
2-4 times a month	192 (10.2)	11 (15.1)	2.41 (0.88-6.62)			
2-3 times per week	284 (12.0)	13 (10.8)	1.64 (0.59-4.59)			
4+ times a week	331 (19.4)	19 (13.1)	2.04 (0.78-5.36)			

Condom-less sex with steady partner in the past month				0.32	
No	496 (36.7)	64 (6.1)	1		
Yes	955 (63.3)	44 (8.9)	1.48 (0.68-3.25)		
Condom-less sex with client in the past month				0.32	
No	1707 (54.1)	52 (7.5)	1		
Yes	702 (45.9)	45 (9.9)	1.35 (0.75-2.45)		
Failed to use a condom with client as a result of own drinking during the past 12 months				0.61	
No	1355 (81.9)	59 (10.5)	1		
Yes	392 (18.1)	17 (8.6)	0.81 (0.36-1.84)		
Failed to use a condom with client as a result of client's drinking during the past 12 months				0.57	
No	2241 (89.6)	94 (8.7)	1		
Yes	234 (10.4)	9 (6.8)	0.76 (0.30-1.94)		
STI symptoms in past year				0.03	0.04
No	1865 (72.9)	70 (7.0)	1	1	
Yes	608 (27.1)	33 (12.7)	1.95 (1.07-3.54)	1.86 (1.03-3.34)	
Symptoms of mental health issues				0.17	
No	1378 (60.0)	70 (9.9)	1		
Yes	1095 (40.0)	33 (6.6)	0.65 (0.36-1.19)		
Attended the Sisters with a Voice clinic in past 12 months				0.75	
No	1178 (25.3)	23 (8.5)	1		
Yes	1097 (74.7)	62 (7.7)	0.89 (0.43-1.82)		
Part of income from sex work				0.21	
Very little (> half)	227 (7.9)	5 (3.7)	1		
Some (¼ to ½)	369 (16.5)	21 (11.3)	3.33 (0.97-11.44)		
Most (> half)	636 (31.2)	26 (7.0)	1.96 (0.59-6.57)		
All	1245 (44.3)	51 (9.4)	2.72 (0.85-8.67)		
Times pregnant				0.14	
0	185 (8.1)	12 (15.7)	1		
1-2	1379 (53.8)	59 (9.2)	0.54 (0.23-1.29)		
3-4	720 (27.6)	26 (5.9)	0.34 (0.13-0.87)		
5+	193 (10.4)	6 (6.4)	0.37 (0.11-1.27)		
Number of children				0.05	0.05
0	274 (11.3)	16 (12.8)	1	1	
1-2	1509 (58.0)	63 (9.6)	0.73 (0.33-1.60)	0.55 (0.24-1.29)	
3-4	596 (24.8)	22 (4.5)	0.32 (0.13-0.79)	0.26 (0.10-0.70)	
5+	98 (5.9)	2 (6.1)	0.44 (0.09-2.17)	0.46 (0.08-2.61)	

** Question asked differently across pilots*

Study I – Food insecure if answer to following question is 'Yes' - In the past four weeks, was there ever no food to eat of any kind in your house because of lack of resources to get food?

Study III – Food insecure if answer to any of the following questions is 'Yes' - Sometimes we go to bed hungry / In the last week, have you had to go an entire day without eating because there was no food in your household?

Objective 4: Develop a risk screening algorithm that could identify female sex workers at high risk of recent infection

Our screening model, that included factors found to be associated with recent infection (age, age at start of sex work, STI symptoms and number of children), showed no evidence (p=0.72) the model was incorrectly specified and had a AUROC of 0.72, indicating good discrimination ability. However, the Hosmer-Lemeshow test indicated that model calibration was poor (p=0.02). The maximum score for each variable associated with recent infection was added up to form a clinical risk score (see Table 26). The total risk score ranges from 0 (lowest) to 11 (highest). The predicted probabilities of recent infection associated with each total score are presented in Table 27.

To illustrate the application of the risk score, consider a 25 year old woman who started selling sex at the age of 14, had STI symptoms in the past year, and has two children. The total risk score she gets is 2+3+1+2=8, which is associated with a 38% predicted probability of having been recently infected. However, working through such scenarios highlights that the clinical risk score is not sufficiently discriminating to be helpful. Further research is warranted.

Table 26: Clinical risk score for recent infection among HIV negative FSW

Characteristic		Score
Age in years	**18-19** **20-24** **25-34** **35+**	2 0 2 1
Age at start of sex work (years)	**<15** **16-17** **18-19** **20-24** **25+**	3 0 4 5 3
STI symptoms in past year	**No** **Yes**	0 1
Number of children	**0** **1-2** **3-4** **5+**	3 2 0 1
Maximum score		**11**

Table 27: Predicted risk of recent infection among HIV -ve clients based on their risk score

Total risk score	Predicted risk (%)
0	1.1
1	1.9
2	3.0
3	4.9
4	7.7
5	12.1
6	18.4
7	27.0
8	37.7
9	49.9
10	62.0
11	72.8

Discussion & key findings

We conducted three independent but linked prospective pilots to explore the feasibility, acceptability, and utility of integrating a RITA within routine programmatic activities (ANC and HTC in Kenya, and FSW outreach testing services in Zimbabwe), and to identify and characterize people with recently acquired HIV infection. We also conducted recent infection testing on stored samples arising from four RDS surveys of FSW in Zimbabwe.

Feasibility of operationalising a RITA in routing setting

In all three pilots, we assessed the feasibility of practically applying a RITA in routine service setting. For two of the pilots (Nairobi and Zimbabwe) the application included returning test results to participants.

Protocol development and implementation

We successfully developed and implemented protocols for incorporating RITAs into antenatal clinics providing PMTCT services (Siaya county, Kenya), routine HIV testing and counselling clinics (Nairobi, Kenya), and a programme for FSW (Zimbabwe).

During implementation, there were a number of deviations to the original protocols:

1. We originally planned to use the Sedia™ HIV-1 LAg-Avidity EIA for use with DBS specimens (50) in Nairobi and Zimbabwe, and the Sedia™ HIV-1 LAg-Avidity EIA (51) for use with plasma specimens in Siaya county and Zimbabwe (in Zimbabwe we planned to run both assays). Despite lengthy discussions with representatives of Sedia Biosciences Corporation, it was not possible to procure either assay. To avoid additional delay, we commenced procurement discussions with Maxim Biomedical. The Maxim HIV-1 LAg-Avidity EIA DBS Kit (52) was used in Nairobi and Zimbabwe on DBS samples, with the Maxim HIV-1 LAg-Avidity EIA (53) being used in Siaya county and Zimbabwe on venous blood (also referred to as plasma). A notable difference between the Sedia and Maxim kits was that with the Sedia kits included DBS filter paper cards. As the Maxim kits did not come with these papers, they were procured locally. This additional procurement process in Zimbabwe influenced, unexpectedly, our results (see below).

2. In Nairobi, we paused testing for a short period due to failed testing runs at the laboratory, the host partners requesting age of inclusion to be increased from 15 to 18 years, and to review the process for returning recency test results back to participants, which was exceeding the scheduled two week turn around (participants were informed about the pause and potential delays in returning test results).
3. It was only possible to consider information on ART metabolites in the blood to support final interpretation of test results for the Nairobi pilot (the test results being received May, 2019). Despite nine months of negotiations with the University of Cape Town, the timeframe provided insufficient to attain an import licence from the appropriate authorities in South Africa for the other two pilots. The time required to send batched samples to Cape Town, and receive results back at pilot facilities obviously greatly exceeds our original scheduled two week turn around for recency test result. To consider ART metabolites in the blood in the interpretation of a returned result it would be necessary to establish in-country laboratory facilities for such testing, which could prove to be expensive and impracticable.

Ethics approval and training

At the LSHTM, and within countries, ethics approval was sought and attained for implementing these protocols. To attain ethics approval we were required to address a number of queries raised by reviewing committees. These included:

- concerns / questions relating to the amount of blood required to draw;
- emancipated minors (Siaya county);
- whether it was ethical to return / or not return test results back to participants (all three pilots);
- whether sufficient counselling was in place (Nairobi and Zimbabwe).

All staff involved with the pilots received training in the handling of confidential information, and good clinical practice.

Laboratory testing for recent infection

Delays in procuring test kits reduced the period for laboratory preparation and training. Having received the test kits, the laboratory procedures were more challenging than expected and the complexity of obtaining high-quality outputs was underestimated. Transport also proved to be challenging in some cases, as it was necessary for whole blood samples to be centrifuged within six hours and stored in a freezer. In places where the clinic and laboratory were one or two hours

apart this required a good functioning transport system. None of the samples in our study were rejected because of low quality. Logistics were easier when working with DBS.

Appendix 6 presents a one page summary of how testing for recent HIV infection should be conducted in a laboratory. On commencement of testing, procedural errors were identified in all three pilots. These errors included:

- contamination and suboptimal room temperatures;
- poor performance of washers and readers (likely incorrect calibration, poor maintenance, or quality of product);
- elution issues;
- the use of out-of-date DBS filter paper cards (an issue we only identified by comparing DBS test results with those attained via plasma).

These errors could have been avoided by fully adhering to the company 'Instructions for Use' document. Unlike many standard Elisa's this assay has multiple pass/fail criteria with little scope for variance which means that any error in procedure may cause failure of the whole assay.

To address issues as they arose, and reduce the likelihood of further issues arising, we introduced on-site troubleshooting (members of the MeSH working group visiting the laboratories), bi-weekly conference calls, and the sharing via email of anonymised assay plate results. All members of the MeSH working group were invited to join the calls, which were organized by the MeSH secretariat and led by an agenda. The calls provided a platform to share learnings and to raise questions with our independent consultant (an expert in the development of, and application of, the laboratory testing of HIV recency assays). The sharing of initial test results identified a number of issues, in real-time, that could then be addressed in the laboratory. It is clear that all laboratories performing this assay should have bespoke training in the performance of the assay and partake in an external Quality Assurance scheme to provide confirmation of the laboratories ability to obtain accurate results.

Return of test results

Returning results to clients proved to be more challenging than expected, this was particularly true in Zimbabwe. In Zimbabwe, it was the view of the healthcare workers / interviewees that the women often did not see the value of the recency test result. A barrier to returning test results in Zimbabwe was women providing wrong contact details / not returning to the clinic. This may be

due to women not seeing the value in the test result, but may also result from women not wanting to be contacted by clinic staff generally, there being no reimbursement for returning, difficulties in travelling, and not wanting to 'look backwards' and disrupt acceptance of HIV status.

In both Nairobi and Zimbabwe the period for retrieving test results back from the laboratory often exceeded the planned two weeks (often due to the necessity to run batch testing). In Nairobi, this resulted in test results sometimes not being available on a person's next visit (in Zimbabwe, there were no such recorded instances). One person received a recency-positive test result that was subsequently reclassified as "long-standing" following ART metabolite testing. When returning of results to the client was delayed the results lost some of their value. An issue not reflected in our training or scripts was that if a test result was returned after, for example, three months, the period to which a positive recency test refers will have changed; i.e. at time of discussing the result it would not be possible for the participant to have acquired their infection in the past three months. Our proposed two-week turn-around provided to be unfeasible; on reflection an optimal turnaround period should have been chosen that facilitated batch testing and laboratory delays, maximised the meaning of the results, and worked within normal facility and patient timelines.

It is in relation, particularly, to the returning of recency test results that a number of groups have aired concern about the rollout of recency testing. These concerns include how the results are communicated to people, and the implications of such communication (for example, potential harm)(54). In terms of returning results that rely on laboratory-based testing, there is also a serious questions about the public health relevance of the test result as the window period for delivery widens (this issue would be negated by the availability and use of a rapid recency / POC assay).

Acceptability and utility of using a RITA in routing setting

The groups raising concern about whether and how recency test results are communicated have also aired concern that, based on current guidelines, there is no direct clinical benefit to the individual arising from a recency test result (54). Service users, health care providers and researchers participating in our pilot expressed similar concerns. In these programmes implementing test and treat, the added value of an individual patient or clinician knowing a recency test result was unclear.

In the Nairobi (HTC) and Zimbabwe (FSW programme) pilots we found that both acceptability and utility of using RITA were mixed. Acceptability was related to utility – if recency testing was assumed or understood to be useful, this encouraged acceptability.

Acceptability was influenced negatively by the additional time taken to enrol patients in recency testing and by the need to take additional blood. In the Zimbabwe pilot with FSWs women's trust in clinic staff, and reimbursement for time taken to participate in recency testing, were identified as important contextual factors facilitating acceptability.

Overall, utility was mixed. There was not a clear positive use of recency testing at individual level. There was confusion among recency test pilot participants (patients) about whether recency test results could be used to inform decisions about their treatment. Some patients in the Zimbabwe pilot assumed that test results would help clinicians to identify optimum treatment for them, and that therefore recency testing was of individual benefit to them, but this was not the case in either setting.

Participants associated recency testing with potentially identifying who had infected and some saw this as useful. Others were not interested in identifying who infected them and found the possibility unhelpful, raising concerns about blame, risk of violence, and disruption to focusing on the future and moving on from the traumatic experience of HIV diagnosis. Sex workers were less likely to be able to identify a potential source of infection as they would have had many partners during the 'recent' period.

For sex workers, qualitative findings from the pilot indicate that at the individual level acceptability of RITA was influenced by specific contextual factors (trust of clinic staff, reimbursement for participation), individual utility was limited or non-existent, and there was risk of disruption to 'moving on' from a HIV diagnosis, anger, and violence. The proportion of recency results returned to women in the Zimbabwe pilot was low (10.5%; n=33). This is likely to have been influenced by factors including sex worker mobility and reluctance to be contacted by clinic staff in general, but may indicate that there was little enthusiasm from this population to pursue the outcome of recency testing after they had received their reimbursement. For targeted prevention planning at the population level sex worker mobility needs to be considered as locations of infection and testing may differ. These findings suggest that if recency testing is rolled out to sex worker populations in other settings, contextual factors should be carefully considered and women may choose not to engage with receiving results.

Yield and characteristics of people with recent HIV infection

In all three pilots, and our Zimbabwe RDS stored sample study, we identified and characterised people classified as positive for recent HIV infection. In Nairobi, we also explored recent infection from the perspective of partner testing, and in Siaya county, as part of our characterisation, we assessed selection biases associated with pregnant women using antenatal services.

Testing and recent infection yield

Siaya county: of the 2,365 pregnant women tested for HIV, one in five (445) tested positive, among whom the overwhelming majority (426) tested for recent infection (LAg + VL). Among these women, eleven (2.6%) were classified as positive for recent infection. One woman was subsequently found, from the clinical record, to have been in receipt of ART (misclassification of 1/11 or 9.1%). Linking the records of the remaining ten women to the HDSS found no evidence of HIV status misclassification.

Nairobi: of the 50,561 women and men tested for HIV, 883 (1.75%) tested HIV positive, among whom 532 (60.2%) tested for recent infection. Two participants had insufficient sample to test. Among the remaining 530 women and men, 48 (9.1%) were originally classified as positive for recent infection prior to ART metabolite testing. Of these 48 participants, 29 (60.4%) received their test result. Of the 484 participants originally classified as having "long-standing" infection, this figure was 373 (77.1%). Following ART metabolite testing, two of the 48 were reclassified as "long-standing" due to evidence of having initiated treatment. One of these two had already received their recency-positive test result. Accounting for these two, 8.7% (46/530) of participants were finally classified as recent positive. Partner testing in Nairobi was shown to be effective with over a quarter of participants testing HIV positive subsequently bringing a sexual partner in for HIV testing. Among partners, four in ten were HIV positive (41.8%; 61/146), and among those participating in the study almost a quarter (23.8%; 5/21) were classified as recent positive; testing yields significantly higher than among "non-partner" participants.

Zimbabwe, sisters with a voice programme: of the 4,349 FSW tested for HIV, 510 (11.7%) tested positive, among whom 314 (61.4%) tested for recent infection. Among these women, 33 (10.5%) were classified as positive for recent infection. Of these 33 women, 9 (27.3%) received their test result. Among the 280 women with "long-standing" infection, the figure was low at 24 (8.6%).

When looking across all three prospective pilots, we saw that refusal for recency testing among HIV positive pregnant women attending ANC was lower than in the other two pilots. This may be due to recency testing being seen as just part of the routine antenatal service package. In all three prospective pilots, the observed percentage of people newly testing HIV positive (O) was similar to the expected (E) figure presented in the protocol and based on published and/or programme data (Siaya county: O 18.9%, E 18%; Nairobi: O 1.75% E 2.3%; Zimbabwe: E 10%, O 11.7%). In Siaya county fewer women overall tested for HIV than expected due to clinic caseloads being lower than initially reported. In Zimbabwe, funding for the Sisters programme transitioned between donors in 2018, which resulted in fewer nursing staff and stock outs of STI drugs. This reduced women's motivation to attend clinics, resulting in fewer clients than anticipated being eligible for HIV recency testing.

Zimbabwe, RDS: in RDS1, 58.5% 1,686/2,883) of women tested HIV positive, among whom 27.4% (462) were tested for recent infection, among whom 21% (97) were classified as positive for recent infection. In RDS3, the figures were 55.7% (1,512/2,713), 10.6% (161), and 3.7% (6), respectively.

How do we explain the difference in the percentage of women testing recent positive (21% v 3.7%)? The inclusion criteria for the two RDS studies only varied slightly in definition of a sex worker, and the variables duration in sex work, number of clients per week, and part of income from sex work, were similar. For RDS1, however, DBS samples were taken and stored in the laboratory at room temperature whereas, for RDS3, plasma samples were taken that were then stored at -20C in the laboratory. Subsequent to the commencement of our work, research was published suggesting DBS samples stored at room temperature had lower ODn values than those stored in the freezers, indicating decreased antibody avidity. The authors of the paper suggest that samples with low antibody may be more prone to misclassification that results in an overestimation of recent infection (55). The disparity in our results was surprising and may, in part, be due to such misclassification.

Characteristics (including ANC selection bias)

In all four branches of our research we characterised the people tested for recent HIV infection, and categorised as being recent. The key results from this work include the finding that women in Siaya county in their first trimester had ten times increased odds of testing positive for recent infection compared to those testing in their second or third trimester. While inference with such

small numbers is challenging, the higher than expected proportion of recent infections in their first trimester of pregnancy may be due to increased risk of HIV infection during unprotected sex leading to pregnancy, or may be due to lowered coital frequency among women later in pregnancy.

There are biases associated with ANC surveillance as all women are pregnant, and have recently been sexually active and do not use [comprehensively] contraceptives (56). Moreover, attendance is known to vary by factors associated with HIV (57), and HIV-infected women may be less likely to become pregnant (56-62).

As compared to women in the KEMRI/CDC Siaya HDSS, we found our participants were more likely to be aged between 20 and 29 years, and to be married. We also found HIV prevalence to be higher among ANC attendees in the youngest age groups (15 to 24 years) but lower amongst those aged 25 to 39 years. No such differences were observed when we compared our participants with women from the HDSS who had a birth in the year prior to their HIV test. Our simulation study only showed HIV prevalence in the ANC population to be higher in the youngest age group (15 to 19 years); in all other groups HIV prevalence was lower. Disparities between HIV prevalence estimates from the empirical data and simulation data may be partly attributable to the ANC and HDSS data representing two different time points (2018 and 2016, respectively, during which prevalence likely increased marginally), while the simulations are for the same time point.

Our individual-based simulation models suggested bias in ANC RITA testing was variable and mainly driven by differences in ANC testing patterns. When using RITAs in incidence surveillance for ANC populations, it is plausible that in settings where testing rates are uniformal across HIV status (such as EMOD or Synthesis 1) it is not necessary to account for HIV testing history, while in others where there is differential testing by status, it is of greater importance. Estimators incorporating HIV testing history are promising alternatives (63, 64) but rely strongly on self-reported testing history, which is likely subject to misreporting.

In Nairobi, a higher proportion of people aged under 25 years (compared to older groups), and women (compared to men), tested recent positive. The observed difference by sex is probably due to women presenting earlier in their course of infection than men. Women, as compared to men, were younger at age of HIV diagnosis, and were more likely to have ever tested and to have tested within the last twelve months. We were unable to investigate why women may presented earlier than men.

In Zimbabwe, we did not find strong evidence of any association with testing positive for recent infection in the Sister with a Voice programme. In our RDS study, age at start of sex work, STI symptoms in past year, and number of children were shown to be associated with a recent infection. These variables, along with age, were incorporated in to a screening model. Unfortunately, the outputs from this model, and an accompanying predicted probability of recent infection, discriminated insufficiently to be helpful.

HIV incidence estimates

In Siaya county we estimated HIV incidence using a RITA and from the HDSS data. In Zimbabwe we estimated incidence using a RITA and programme data.

Siaya county

Depending on MDRI, FRR and ART initiators, RITA-based incidence estimates in Siaya county ranged from 0.62 (95% CI: 0-1.4) to 1.1 (95% CI: 0.3-2.0) per 100 person-years. Longer MDRI, lower FRR, and allowing those with recent ART initiation dates (<90 days) to be classified recent, led to higher incidence estimates, while shorter MDRI, higher FRR, and allowing none on ART to be classified recent, led to lower incidence estimates. Among participants we linked to an HDSS record at study enrolment and whose last HIV test was negative, incidence between last HDSS HIV test and their ANC visit was 1.3 per 100 person-years (95% CI: 0.88-1.9). The HDSS point estimate is higher than those using RITA (although we should note the confidence intervals of the estimates are wide and overlap). The small difference between our point estimates may reflect lower coital frequency during late trimester pregnancies, or RITA-related estimation difficulties, such as changes in subtype-distribution leading to a shorter MDRI.

Our incidence simulation showed HIV incidence in the ANC population, as compared to that in the general population, to be higher across all ages, though to a much lesser extent among women aged 20 to 34 years. This may be due to differences in sexual activity across ages between pregnant women and general population women, with the youngest (aged 15 to 19 years) and older (aged 35 to 49 years) women in the general population being less likely to engage in sexual activity than the women attending ANC, and thus having a lower risk of HIV infection. In contrast, a higher proportion of the women at ages 20 to 34 years are likely to be sexually active regardless of whether pregnant, and thus at more similar risk of HIV infection.

Zimbabwe, RDS & programme data

As with the percentage of FSW classified as having recent infection, our incidence estimates per 100 person years varied greatly between the two RDS surveys, with the estimates for RDS1 (central estimate range: 12.7 to 22.8) greatly exceeding those for RDS3 (central estimate range: 0 to 1.7). It is likely that these differences are due mainly to potential misclassification in RDS1 arising from samples being stored at room temperature. For RDS3 (where samples were stored at -20C), incidence was much higher among the 18 to 19 year old group compared to older groups. Applying an FRR of one resulted in incidence estimates of zero for RDS3. These findings are both surprising and difficult to explain.

Falling between our two RDS estimates, is our estimate of 6.4/100 person years (95% CI: 5.3 - 8.0) from the programme data 2016 to 2017. A previous analysis (37) of the Sister with a Voice programme data for 2009 to 2014 presented an estimated HIV seroconversion rate of 9.8/100 person years (95% confidence interval: 7.1 to 15.9).

MDRI / FRR

We show, unsurprisingly, our incidence estimates to vary according to different MDRI and FRR combinations. To further explore FRR we had hoped to carry-out ART metabolite testing in all three pilots; this proved only to be feasible for the Nairobi pilot. In Nairobi, two (4.2%) of the original 48 people classified as recent infection positive were reclassified as "long-standing" following metabolite testing. Our exploration of clinical data in Siaya country found one (9.1%) of the eleven women classified, to that point, as recent infection positive to have been in receipt of ART and therefore be a false recent. Evidence of misclassification of HIV (65) or ART status (as highlighted in our pilot studies) emphasizes the need to make further enquiries, using clinical data and/or ART metabolite testing, to accurately assign the status of these two factors and correctly interpret recency test results.

Conclusions

We found that, despite a number of significant challenges, it was feasible to implement HIV recency testing in routine settings in Kenya and Zimbabwe. Analysing samples at required quality in the laboratory, as well as returning test results to participants, were our main challenging. There seems to be a larger benefit for the population than for the individual, and the potential for harm

should not be overlooked. In considering the roll-out of the surveillance of recent HIV infections, there are a number of challenges that need to be addressed, and the utility of the test in tracking the incidence of new infections over time should be assessed against alternative direct and indirect surveillance methods.

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Appendix

Appendix 1: Results from follow-up questionnaire after return of a positive recent infection test result

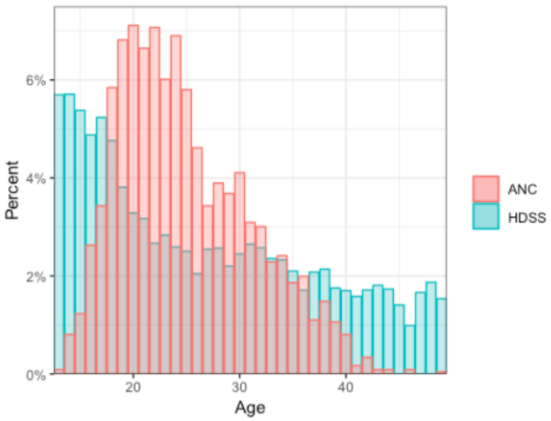
Table 28: Follow-up questionnaire to the return of a positive recent infection test result

		N (13)
Client suspects from whom he/she was infected	Yes	11
	No	2
Client discussed recency test result with partner suspected as source of infection	Yes	6
	No	6
	Refused to disclose	1
Client disclosed HIV status and/or recency test result with any sexual	Yes	7
	No	6
Sexual partners knew their HIV status	None of them did	3
	Some of them did	2
	All of them did	2
	No response	6
Client recommended partners get tested	Yes	10
	No	3
Number of partners clients recommended get tested	1	9
	2	1
	No response	3
Partner went for HIV testing	Yes	6
	No	7
Disclosure of HIV status or recency test result changed relationship	No change	5
	Strengthened relationship	1
	Had negative impact on relationship	7
Experienced violence or stigma following disclosure	Did not experience violence or stigma	9
	Was beaten	1
	Was shunned	0
	Other	3
Knowledge of recency status changed health-seeking behaviors	Yes, went to get treatment sooner	12
	No, no change in behavior	1
Recommends scale-up of recency testing in Kenya	Yes	12
	No	1

Appendix 2: Selection biases associated with the surveillance population

Figure 14 shows the age distribution of the ANC population and the HDSS population for comparison.

Figure 14: Age distribution comparing ANC population to HDSS population



HDSS shows age distribution among women resident in Gem in the HDSS in mid 2017 (1st July 2017).

In comparing HDSS Gem residents and ANC participants, we found that parity was similar in the two younger age-groups (aged 15 to 30 years) but lower in the ANC than the HDSS for older age groups (aged 30 to 49 years) (Figure 15A). This is potentially due to older women in the HDSS having largely already achieved desired family size, while older women in the ANC (who are actively pregnant) are seeking to have more children. While parity is directly ascertained by nurses providing care in the ANC, the HDSS does not directly ask women about their lifetime number of live births. The HDSS measure of parity is constructed from cumulating all individuals ever linked to a woman as the mother of a resident child/adult. Thus, the measure of parity presented here can be considered to be a lower bound for the HDSS women. As such, as a sensitivity to potential missed links for women who migrate into the HDSS without their children and therefore have lower parity using this measure than in truth, we additionally present parity for women who have been resident in the HDSS continuously since the age of 18 years, and thus would have had all children identified (though we may still miss infants who die before enumeration, if not reported). Since the HDSS started in 2001, we can only make this comparison up to women aged 35 years. This constraint decreases the HDSS sample nearly size six-fold (20,891 to 3,617). In this

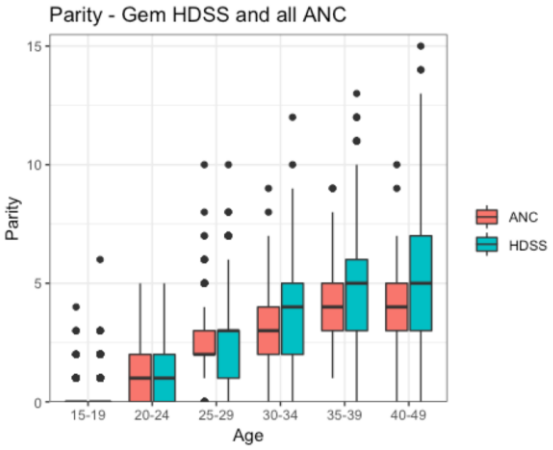
subset, ANC parity is slightly higher in the younger age groups (aged 15 to 29 years) than HDSS parity, but lower among those aged 30 to 34 years (Figure 15B).

Figure 15: Parity comparison between ANC attendees and HDSS population

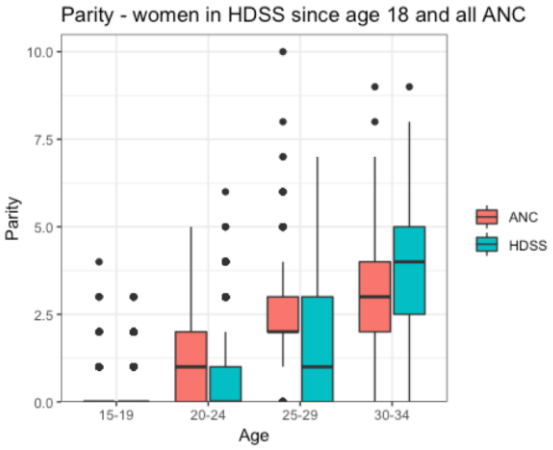
A) All Gem HDSS women

B) Only women who have lived in the HDSS since age 18 and currently resident in Gem.

A



B



Appendix 3: Simulation modeling approach

Simulation study

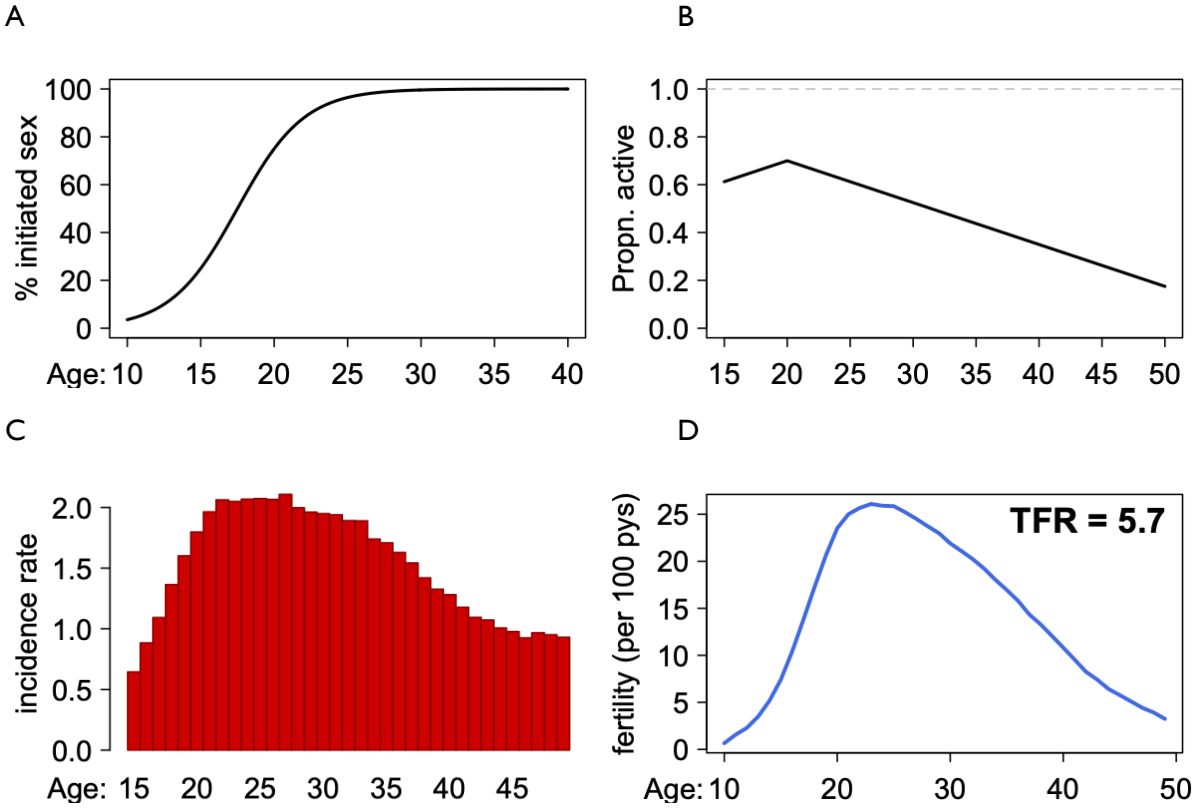
A population of women was simulated from age 10 to 50 years. Young women were simulated transitioning from not sexually active to sexually active with a median age of sexual debut in the simulated data of 17.5 years, following a logistic distribution (Figure 16a). Among women who were sexually experienced, about 60% of women were sexually active in unprotected acts at any time, with the proportion increased to age 20 years and decreased steadily thereafter, consistent with patterns of recent sexual activity reported in DHS interviews (Figure 16b). HIV negative women who were sexually active were exposed to a risk of HIV infection. The resulting pattern of HIV incidence increased sharply from age 15 to 23 from about 0.5 per 100 person years to 2.0 per 100 person years and decreased in older ages (Figure 16c). Women who were sexually active in each time step were also exposed to become pregnant. The simulated total fertility rate (TFR) was 5.7, with age-specific fertility rates varying by age with a peak between 20-25-year-olds (Figure 16d). The rate of becoming pregnant for HIV positive women depended on the stage of HIV infection based on estimates derived from estimates for fertility by duration of infection derived from general population HIV cohort studies(66). Simulations assumed that all pregnant women attended ANC. The time of first ANC visit was simulated from a truncated normal distribution with median first ANC visit at 4.5 months and 35% of women attending their first ANC during their first trimester. The model simulated LAg assay, which had a mean duration of recent infection (MDRI) of 130 days and false recency rate (FRR) of 1%.

Figure 16: Simulation input

A) Percent of simulated population who had initiated sex by age

B) Proportion of simulated population sexually active by age

C) Incidence rate in simulated population (per 100 person-years) by age; D) Fertility (per 100 person-years)



Individual level modelling

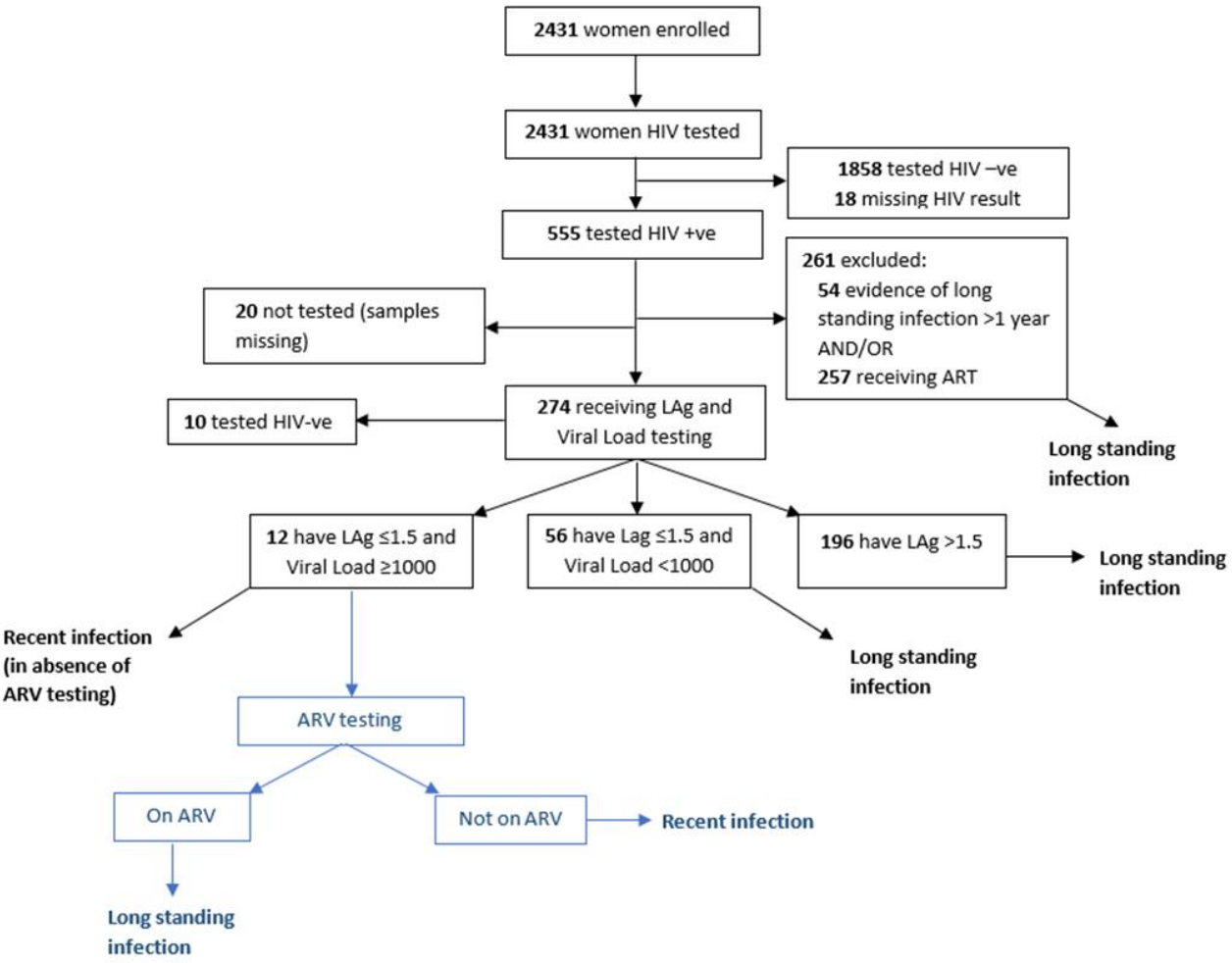
We assessed the potential bias of using a RITA in estimating ANC incidence applied to the results of four individual-based simulation models, roughly similar to the HIV epidemics in South Africa (MicroCOSM), Zambia (EMOD), Malawi (Synthesis 1) and Zimbabwe (Synthesis 2). We applied a RITA to ANC HIV tests among the four individual-based simulations, at first ANC visit. For the RITA, we use Kassanjee et al's method (67) to simulate a biomarker (such as limiting antigen avidity (LAg)) indicating recency that follows a sigmoidal curve over time following infection where the mean duration of recent infection (MDRI) is 186 days. An individual's biomarker value is randomly sampled from the sigmoidal curve based on their number of days post-infection at their test date. We assume that there are no false recent infections using this assay (false recency

ratio, FRR = 0). In a setting of 0 FRR, using Kassanjee et al 2012's (46) method, the incidence rate estimator simplifies to $\frac{R}{N*MDRI}$, where R represents the number classified as recent infections and N is the number who test negative. We limit to first HIV-positive test for repeat testers. We conduct 100 bootstrap resamples to account for stochastic variability in the draw of the sigmoidal biomarker based on days post-infection.

Appendix 4: RITAs for the two excluded RDS surveys

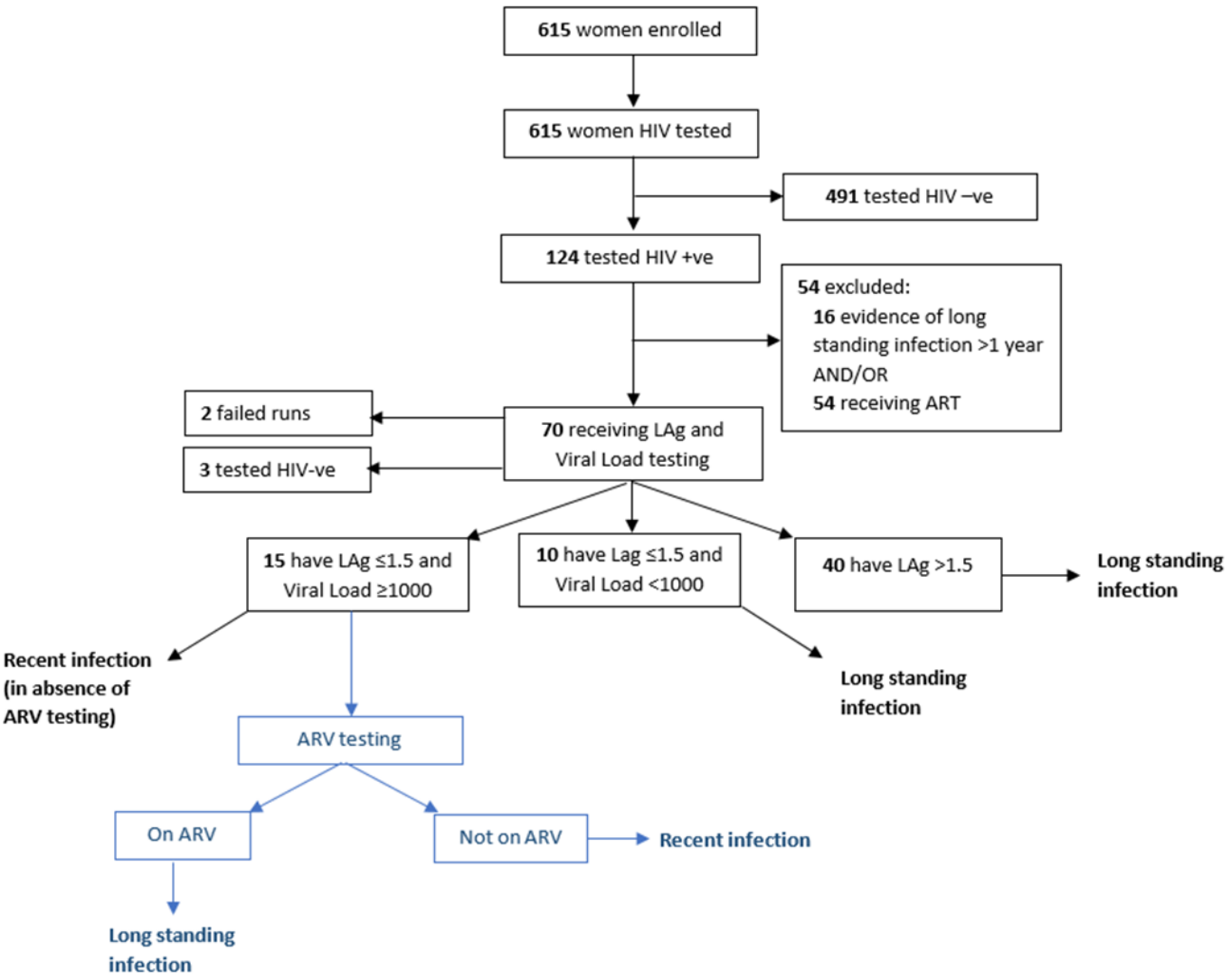
RDS study 2

Figure 17: Recruitment and testing flowchart for RDS study 2



RDS study 4

Figure 18: Recruitment and testing flowchart for RDS study 4



Appendix 5: Sensitivity analysis RDS study 3 comparing tested samples to samples not tested for recent infection

Table 29: Comparison of characteristics of women who did not have enough plasma with those who did, among RDS study 3 samples that were eligible to be tested for recency (N=428) (RDS-II weighted)

Characteristic	Plasma volume Insufficient (N=267) n (%)	Sufficient (N=161) n (%)	Comparison p-value^
Age at survey			0.53
18-19	10 (3.3)	5 (4.1)	
20-24	60 (30.4)	35 (23.4)	
25-34	136 (46.7)	75 (41.3)	
≥35	61 (19.6)	46 (31.2)	
Marital status			0.01
Single/ never married	56 (27.5)	29 (12.2)	
Married/ living together as if married	5 (0.9)	1 (0.1)	
Divorced/ separated	177 (56.4)	108 (79.6)	
Widowed	29 (15.2)	23 (8.1)	
Highest level of education			0.77
None	4 (3.1)	3 (1.9)	
Primary	67 (34.2)	48 (30.4)	
Secondary	195 (62.4)	110 (67.7)	
Tertiary	1 (0.3)	0 (0.0)	
Number of children			0.77
0	45 (19.9)	23 (14.9)	
1-2	133 (46.7)	88 (53.2)	
3-4	77 (31.9)	44 (29.8)	
≥5	12 (1.5)	6 (2.1)	
No food for one day in the past month			0.61
No	147 (60.5)	99 (65.2)	
Yes	120 (39.5)	62 (34.8)	
Age at start of sex work			0.16
≤15	12 (3.9)	1 (1.0)	
16-17	18 (8.7)	11 (7.9)	
18-19	31 (6.5)	24 (12.1)	
20-24	91 (37.4)	41 (21.1)	
≥25	115 (43.5)	84 (57.9)	
Duration in sex work (years)			0.28
0-2	79 (40.6)	42 (26.8)	
3-4	66 (16.7)	46 (38.6)	
≥5	122 (32.7)	73 (34.6)	
Number of clients in the last week			0.25
0-2	43 (15.8)	24 (27.1)	
3-7	98 (37.6)	70 (40.9)	
≥8	126 (46.7)	67 (32.0)	
Relationship with other female sex-workers in one's location			0.90
Good	178 (61.4)	96 (65.3)	
Neither good nor bad	71 (29.2)	50 (27.2)	

Bad/ no relationship	18 (9.4)	15 (7.5)	
No. of female sex-workers who are close friends			0.06
≤1	99 (39.2)	65 (54.3)	
2-3	107 (39.7)	70 (38.2)	
≥4	61 (21.1)	26 (7.5)	
Condom-less sex with steady partner in the past month			0.55
No	48 (33.2)	19 (26.4)	
Yes	110 (66.8)	59 (73.6)	
Condom-less sex with client in the past month			0.53
No	223 (87.9)	129 (85.0)	
Yes	44 (12.1)	32 (15.0)	
Frequency of drinking 6+ drinks on one night in the past 12 months			0.001
Never	76 (46.3)	45 (33.6)	
Once a month or less	20 (4.8)	22 (31.1)	
2-4 times per month	25 (9.7)	12 (11.9)	
2-3 times per week	43 (22.7)	16 (8.9)	
4 or more times per week	36 (16.5	24 (14.5)	
Symptoms of STIs in the past 12 months			0.48
No	172 (73.6)	114 (78.7)	
Yes	95 (26.4)	47 (21.3)	
Symptoms of common mental disorder			0.09
No	123 (53.5)	89 (68.6)	
Yes	143 (46.5)	72 (31.4)	
Last HIV test			0.37
Never	13 (4.1)	16 (9.5)	
≤6 months	146 (52.6)	76 (43.7)	
>6 months	108 (43.3)	69 (46.8)	
Results of most recent HIV test			0.94
Negative	181 (67.3)	108 (68.3)	
Positive	71 (32.7)	36 (31.7)	
Knowledge of HIV positive status*			0.83
No	196 (68.9)	125 (71.4)	
Yes	71 (31.1)	36 (28.6)	

*Proportion reported HIV positive among those who tested HIV positive during the survey

^Chi-squared

Appendix 6: Laboratory testing of recent HIV infection

1. It is important to provide thorough training before a study starts. Training should be centrally organised to ensure the content is similar for all sites;
2. Methods should be properly validated before any sample testing commences;
3. Maintenance records should be up-to-date and an overview of available equipment and reagents should be drawn up;
4. To ensure high quality outputs laboratories should be signed up to EQAPOL, a network of laboratories working on HIV recency testing providing external validation of test results by exchanging samples. Creating a network of laboratories contributes to better communication and collaboration;
5. To prevent laboratory issues from delaying the entire study it is useful to have a back-up laboratory than can perform the tests for a certain period to ensure continuity of care;
6. The limiting antigen assay is sensitive to procedural changes so it is critical to follow the procedure as described in the manufacturers project insert;
7. Assay performance should be evaluated prior to starting testing with patient samples;
8. To confirm performance as a minimum, the assay should be performed two or three times with control material from the kit and these runs must meet the pass criteria described in the product insert; the outputs from these runs should be shared with the wider research group for review to confirm the assay is working as expected;
9. If possible the laboratory should also run anonymous HIV positive samples alongside the control material to demonstrate reproducibility sample across runs (if the laboratory has previously been trained by CDC they will have be given a set of samples of known reactivates that they could run to confirm that the assay is working);
10. The laboratory should if possible include an Internal Quality Assurance Sample in each run (an HIV positive sample that can be used in every run to show that the assay is performing similarly for each run);
11. Once testing has begun the laboratory should look carefully at the values of the screening and confirmatory runs to check for concordance;
12. Throughout the period of operational testing there should be regular communication with the laboratories and testing results should be routinely reviewed as part of data monitoring.