Estimating HIV Incidence and Detecting Recent Infection among Pregnant Adolescent Girls and Young Women in Malawi - Working Together for an AIDS-free Future for Girls and Women

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

UNITY AND FREEDOM

UCSF
University of California San Francisco

Estimating HIV Incidence and Detecting Recent Infection among Pregnant Adolescent Girls and Young Women in Malawi - Working Together for an AIDS-free Future for Girls and Women

Malawi Ministry of Health, 2018

Implementing Partners:
University of California, San Francisco, Institute for Global Health Sciences; Global AIDS Interfaith Alliance

Supporting Partners:
Office of the Global AIDS Coordinator, United States Centers for Disease Control and Prevention, United States Agency for International Development

Funding source:
This project has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC) under the terms of the Cooperative Agreement Award #: U2GGH000977 "Technical Assistance Services to Countries Supported by the President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund to Fight HIV, AIDS, Tuberculosis and Malaria".

Suggested citation:
Ministry of Health, Malawi. Estimating HIV Incidence and Detecting Recent Infection among Pregnant Adolescent Girls and Young Women in Malawi, 2017-18: Final Report. Lilongwe, Ministry of Health. October 2019

Contact information:
Susie Welty
University of California, San Francisco
Susie.welty@ucsf.edu

Table of Contents

1.0 Acronyms

AGYW	Adolescent girls and young women
AIDS	Acquired immunodeficiency syndrome
ANC	Antenatal care
ART	Antiretroviral therapy
CDC	US Centers for Disease Control and Prevention
CHAM	Christian Health Association of Malawi
DBS	Dried blood spot
DREAMS	Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe
EDTA	Ethylene-diamine-tetra-acetic acid
EIA	Enzyme immunoassay
FRR	False recency rate
HIV	Human immunodeficiency virus
HTS	HIV testing services
LAg	Limiting antigen
MDRI	Mean duration of recent infection
MOH	Ministry of Health
MPHIA	Malawi Population-Based HIV Impact Assessment
ODn	Optical density, normalized
PMTCT	Prevention of mother-to-child transmission
POC	Point of care
RITA	Recent infection testing algorithm
RNA	Ribonucleic acid
RSE	Relative standard error
STI	Sexually transmitted infection
VL	Viral load
VLS	Viral load suppression

2.0 Executive Summary

The Malawi Ministry of Health (MOH) and its partners implemented a novel surveillance system to detect and characterize recent HIV infections and estimate HIV incidence among adolescent girls and young women (AGYW) receiving antenatal care (ANC) in four high-prevalence districts of Malawi. A description of the methods and key findings from the pilot implementation of this surveillance system – which was called the **Malawi Recency Study –** are described in this report.

The **Malawi Recency Study** was implemented in public health facilities in Lilongwe, Blantyre, Machinga and Zomba districts. During a six-month period in 2017 and 2018, AGYW aged 15-24 years who were pregnant and newly diagnosed with HIV at their first antenatal care (ANC) visit for their current pregnancy were invited to participate in the study. AGYW who agreed to participate completed behavioral interviews and provided blood samples. Blood samples were sent to the Kamuzu Central Hospital or Zomba Central Hospital laboratories, where they were tested for recent HIV infection using the recent infection testing algorithm (RITA), comprised of the Limiting Antigen (LAg) Avidity Enzyme Immunoassay (EIA) and viral load (VL) to confirm the LAg Avidity EIA result. A normalized optical density (ODn) value, which measures antibody avidity, and VL were used to classify infections as recent or long-term. A result designated as recent infection through the RITA was interpreted as an infection that had likely been acquired within the past 12 months. Recent infection test results were returned to ANC sites within about three to four weeks and given to participants with counselling at their next routine ANC or HIV treatment visit. The study measured the prevalence of recent infection among AGYW attending ANC, from which annualized rates of HIV incidence were estimated. The study also described demographic and behavioral characteristics of participants, examined correlates of recent infection, and monitored any adverse events that were reported.

An additional objective of the Malawi Recency Study was to evaluate the performance of the Asanté™ HIV-1 Rapid Recency™ assay, which is a rapid, "point-of-care" (POC) test for recent infection. Due to their relatively low cost and perceived ease of use, rapid tests for recent infection (RTRI) may be used to expand access to recency testing to settings where routine HIV testing services (HTS) are provided. Real-time data on recent infections can be used to strengthen partner notification and identify and intervene upon areas of high transmission in a timely manner. During the Malawi Recency Study, plasma samples were tested with the LAg-EIA based algorithm and then tested in parallel with the Asanté™ assay at the laboratories, using both an Asanté™ reader and visual interpretation. Participant blood samples were also tested with Asanté™ with visual interpretation performed by nurses at 14 selected ANC sites in Lilongwe district. The study compared the results of the Asanté™ (with and without VL) and LAg and LAg-based RITA, which is considered the current reference standard, to determine how well the Asanté™ could distinguish between recent and long-term infections.

The key findings of the Recency Study are as follows:

- A total of 54,643 pregnant AGYW aged 15-24 years attended a first ANC visit for their current pregnancy at 121 sites in Blantyre, Lilongwe, Machinga and Zomba during the six-month study period. After excluding 933 (1.7%) AGYW with unknown serostatus, HIV prevalence among AGYW attending first ANC visit was 4.3% overall. A total of 1,159 (2.2%) AGYW were newly diagnosed with HIV and, therefore, eligible to participate in the study. Among AGYW who were eligible, 589 (50.8%) were enrolled in the study and completed the LAg-based RITA.

- Among the 589 newly diagnosed AGYW who were enrolled and completed the RITA, 68 (11.7%) were identified to have likely acquired HIV within the last 12 months. Conversely, 88.3% of participating AGYW had infections that were likely acquired more than 12 months prior to their first ANC visit and not previously diagnosed. By district, the prevalence of recent HIV infection among newly diagnosed participants was higher in Blantyre (11.9%) and Lilongwe (13.7%) than in Machinga (10.7%) and Zomba (5.3%) districts. The difference in prevalence is not statistically significant (p=0.1).

- Annualized incidence of HIV infection was 0.59% [95% confidence interval (CI): 0.42-0.75] among pregnant AGYW attending ANC in the four districts. By district, incidence was significantly higher in Blantyre (1.11, CI: 0.74-1.48) than in Lilongwe (0.57, CI: 0.32-0.83, *P*=0.02), Machinga (0.32, CI: 0.00-0.60, *P* <0.001) and Zomba (0.23, CI: 0.01-0.40, *P* <0.001) districts. Incidence was also significantly higher among AGYW aged 20-24 years (0.71, CI: 0.51-0.92, *P*=0.04) relative to those aged 15-19 years (0.41, CI: 0.22-0.61).

- At the central laboratory, there was substantial agreement between the LAg-avidity EIA results and the Asanté™ reader results [91.2% agreement, kappa (k) =0.71] and visual results (92.5% agreement, k=0.75). Agreement increased slightly when a VL test was added to the Asanté™ reader (95.1%, k=0.79) and visual tests (95.4% agreement, k=0.82) and compared to the LAg-based RITA with VL. However, agreement between the Asanté™ performed by nurses at the ANC site and the laboratory-based LAg Avidity EIA was slight (69.2%, k=0.32), indicating need for better training and implementing site-level monitoring and continuous quality improvement strategies following the training. Agreement between the Asanté™ results at the ANC site with a laboratory-based VL test and the LAg-based RITA with VL was moderate (79.3%, k=0.45).

- Among the 589 participants, 463 (78.6%) received their results and post-test counseling at routine ANC or HIV treatment follow-up visits. Longer than expected turn-around time of test results from the laboratories was the primary contributing factor to non-return of results, especially when VL testing was needed to confirm recent infection; approximately 20% of results were not returned from the lab by the time AGYW participants returned to the clinic for their second routine ANC visit.

The Malawi Recency Study met each of its primary objectives: producing estimates of HIV incidence among AGYW in high-prevalence districts of Malawi, describing the prevalence and potential correlates of recent infection, and providing data about the feasibility of using POC tests for identifying recent infections. In doing so, the study demonstrated a replicable framework for surveillance of recent HIV infection that can be used in ANC and other facility-based settings where routine HTS is provided, in Malawi and elsewhere. Results from the Malawi Recency Study also provide important and timely information that can be used to improve and implement HIV testing, prevention and treatment interventions among AGYW and their male partners. The Malawi Recency Study's estimates of incidence can also be used as a baseline benchmark against which the Government of Malawi's current plans to reduce HIV incidence among AGYW by 70% by 2020 can be measured. Prevention and early diagnosis of HIV infection among AGYW and their partners are critical challenges that the government and its partners will need to address in its ongoing response to the epidemic.

In order to expand recency testing and surveillance to more clinical and community-based HTS settings, several technical and logistical challenges should be considered. The study's results demonstrated high correlation of

the Asanté™ rapid recency assay with the LAg avidity EIA (with and without VL) when performed by laboratory technicians in a laboratory setting. However, the results of the Asanté™ test when done by nurses in the ANC site had low agreement with the results of the same test performed by laboratory technicians, indicating potential user error and a need for additional trainings, guidance and oversight in order to ensure that rapid recency tests are performed, interpreted and reported accurately. Incorporation of standardized guidelines, training curricula and supervisory support structures for recency testing into those already in place for routine HTS could be an efficient and effective approach. Finally, a substantial proportion (26%) of the pregnant AGYW who were classified as newly diagnosed, enrolled in the study and initially classified as "probable recent infections" using the LAg-avidity EIA had suppressed VL and were subsequently reclassified as "long-term". It is likely that these AGYW misreported negative or unknown serostatus to ANC staff, when in fact they had been previously diagnosed and prescribed ART elsewhere. Future applications of recent infection surveillance should explore strategies to limit misclassification of serostatus among HTS clients. Therefore, systems for incorporation of laboratory-based VL tests to confirm probable rapid test for recent infection results will be an important, near-term component of expansion of this new and potentially impactful method for tracking and responding to emergent patterns in the HIV epidemic.

3.0 Background and Study Rationale

HIV continues to cause substantial disease burden in Malawi. Approximately 30,000 new HIV infections occur among adults aged 15-64 years annually, and one in ten Malawian adolescents and adults aged 15-64 years were living with HIV by the end of 2016 [1]. Although the epidemic is generalized and affects nearly all segments of the Malawian population, adolescent girls and young women (AGYW) aged 15-24 years bear a disproportionate burden of disease. Estimates suggest that almost 25% of all new infections that occur in Malawi each year are among AGYW [2].

AGYW also bear a disproportionate burden of disease worldwide. Globally, an estimated 380,000 AGYW become infected with HIV every year [3]. AGYW now comprise 15% of the total population of women living with HIV worldwide [3]. More than 80% of all AGYW living with HIV are in sub-Saharan Africa, where they may face significant challenges protecting themselves from HIV because of gender norms, gender-based violence and limited access to education and healthcare [4]. The global HIV/AIDS response is now focusing substantial resources on interventions aimed at reducing new infections among AGYW, which can contribute significantly to epidemic control.

DREAMS – which is an acronym that stands for "Determined, Resilient, Empowered, AIDS-free, Mentored and Safe" – is one of the largest programs designed specifically to control the HIV epidemic among AGYW. DREAMS is a program that seeks to reduce HIV infections among AGYW in 10 sub-Saharan African countries that account for almost 50% of the new infections in girls and women worldwide [4]. In Malawi, DREAMS interventions were launched in Machinga and Zomba districts in 2016 and in Blantyre and Lilongwe districts in early 2018. The DREAMS core package of evidence-informed interventions aims to strengthen families, reduce risk of AGYW sex partners, mobilize communities for change, and empower AGYW. Interventions include cash transfers, educational subsidies, HIV testing and counseling services, and social asset building and parenting/caregiving programs. Malawi's National HIV Prevention Revised Strategy (2018) has set the expectation that DREAMS and other prevention interventions will reduce new HIV infections among AGYW by 70% from 2015 to 2020 [2].

Routine estimates of HIV incidence – defined as the rate of new HIV infections in a population over time – are needed to demonstrate the impact of prevention interventions, including DREAMS. HIV incidence can be measured in cross-sectional sero-surveys using recent infection testing algorithms (RITA) that can distinguish recent from long-term HIV infections [5]. RITAs have been used for measuring population-level HIV incidence in national surveys, including Population-Based HIV Impact Assessments [6]. However, the inclusion of RITAs in routine HIV testing programs to estimate HIV incidence and characterize patterns of recent infection has been limited [7].

The purpose of the **Malawi Recency Study** was to pilot a novel surveillance system to detect and characterize recent HIV infection among newly diagnosed AGYW seeking antenatal care (ANC) at public facilities in four high-prevalence districts of Malawi – Blantyre, Lilongwe, Zomba, and Machinga – where DREAMS or "DREAMS-like" interventions were planned or had been rolled-out. A RITA that included the Limiting Antigen Enzyme Immunoassay (LAg-EIA) and viral load (VL) testing was performed on plasma or dried blood specimens of participating AGYW, and results were used to calculate cross-sectional estimates of HIV incidence in this population. The estimates of incidence from the Malawi Recency Study are intended to serve as the foundation for establishing routine HIV incidence surveillance to monitor trends and measure impact of prevention programs among AGYW.

In addition, the Malawi Recency Study aimed to evaluate the performance of a rapid, point-of-care (POC) test for recent infection called the Asanté™ HIV-1 Rapid Recency Assay (Sedia Biosciences Corp., Portland, Oregon, USA). Due to their relatively low cost and perceived ease of use rapid tests for recent infection (RTRI) may be used to expand access to recency testing to settings where routine HTS is provided [8]. Although no clinical recommendations for use of RTRI currently exist, it is possible that immediately available results could enhance existing partner notification services. The Asanté™ HIV-1 Rapid Recency Assay has been validated in a controlled laboratory setting using a panel of well characterized specimens [9]; however, data on the performance of Asanté™ and other POC recency assays in field laboratory or clinical settings are limited and not yet published. This component of the Malawi Recency Study was intended to provide data to inform the broader application of rapid recency testing for surveillance in programmatic settings in Malawi and elsewhere.

4.0 Objectives

The primary objectives of the Malawi Recency Study were to:

- Generate cross-sectional estimates of HIV incidence among pregnant AGYW aged 15-24 years receiving ANC services in four high-prevalence districts of Malawi in which DREAMS or DREAMS-like interventions have been introduced or planned.
- Describe the demographic and behavioral characteristics that may be associated with a higher risk for recent HIV infection among newly diagnosed HIV-infected pregnant AGYW aged 15-24 years receiving ANC services.

The secondary objectives of the Malawi Recency Study were to:

- Evaluate the performance of the Asanté™ Rapid Recency Assay in a field laboratory and as a POC test in ANC clinical settings.
- Assess the feasibility of integrating recency testing and return of results into routine ANC service delivery for pregnant AGYW aged 15-24 years.

5.0 Methods

5.1 Study Design and Setting

A cross-sectional survey was integrated into routine ANC services in public health facilities in Blantyre, Lilongwe, Machinga and Zomba districts. All Ministry of Health (MOH) and Christian Health Association of Malawi (CHAM) health facilities offering ANC services in the four study districts were initially selected to be included as study sites. However, some facilities were eventually excluded if existing site staff were not available to work on the study. Of 155 public health facilities in the four districts, a total of 121 (78.1%) ANC sites were included in the study, including 29 of 41 in Blantyre, 44 of 55 in Lilongwe, 18 of 21 in Machinga, and 30 of 38 in Zomba. Study enrollment was conducted for six months in each of the selected sites, from November 2017 to May 2018 in Blantyre and Lilongwe and from February to July 2018 in Machinga and Zomba.

5.2 Eligibility Criteria

Persons who met the following criteria were eligible to participate in the Malawi Recency Study:

- Female, aged 15-24 years at the time of the first ANC visit for that pregnancy

- Attended first ANC visit at selected sites during the study period
- Confirmed to be pregnant
- Accepted routine antenatal HIV testing
- Received an HIV-positive result from routine antenatal HIV testing for the first time at that first ANC visit (AGYW who had received a diagnosis of HIV infection at any time prior to the first ANC visit were not eligible)
- Provided informed consent to participate in the study

5.3 Target sample size

The number of eligible AGYW expected to participate in the study in the four districts was based on routine ANC and prevention of mother-to-child transmission of HIV (PMTCT) program data collected from April to June 2016 (3 months) and adjusted to the six-month study period. We assumed a similar scale of HIV positivity among AGYW during implementation of the Malawi Recency Study. The projected sample size of approximately 650 newly diagnosed AGYW aged 15-24 years achieved over six months of data collection was powered to detect a change in HIV incidence of 25% across the four districts combined between the current study and a repeated study planned for implementation approximately one year later.

5.4 Study Procedures

5.4.1 Screening for eligibility and informed consent

During routine ANC first-visits, following completion of routine HTS, counsellors referred AGYW who met the eligibility criteria to trained ANC staff (either a nurse or a health diagnostic assistant) to undergo informed consent procedures for the study.

5.4.2 Risk factor interview

After providing informed consent, participating AGYW completed a brief risk factor interview with ANC staff. The risk factor interview included questions about each participant's demographic characteristics – age, education and employment – as well as questions about the participant's current pregnancy, reproductive health, sexual history, recent sexual behaviors, experiences with physical or emotional abuse and receipt of previous HIV testing and prevention interventions. A revised risk factor questionnaire administered to participants in Machinga and Zomba also included questions on access to and uptake of DREAMS interventions, including family planning.

5.4.3. Specimen collection

Participants provided a blood sample by venipuncture or finger-prick. The blood collection method was assigned to ANC sites based on their proximity to one of the four study laboratories and the expected number of samples to be collected at the site. In closer proximity, higher volume sites, participants provided approximately five mL of whole blood collected by venipuncture into ethylene-diamine-tetra-acetic acid (EDTA) vacutainers. In lower volume, distant sites, participants provided capillary blood by finger-prick, which was used to prepare three to five dried blood spots (DBS) of 70 µL each on Whatman™ 903 filter paper. In addition, if venipuncture was unsuccessful at sites performing venous blood collection, participants could provide a DBS sample. The LAg-Avidity EIA can be used in either plasma or DBS (as well as whole blood or serum), while the Asanté™ HIV-1 Rapid Recency™ can be used in plasma (as well as whole blood or serum) but not DBS. We allowed for two specimen types in order to maximize the number of sites that could contribute data to the primary outcome estimates of incidence and prevalence of recent infection while also ensuring sufficient plasma samples to

evaluate the performance of the Asanté™ against the LAg-avidity EIA at the laboratory. A unique participant identification number label was affixed to each specimen and a study laboratory requisition form at the time of specimen collection. The laboratory requisition form was used to collect basic clinical and demographic information for each participant, including age, gravidity, ANC client number and ART client number. Venous blood specimens were stored at the ANC site in a cooler box filled with single-use ice before transport to the laboratory, which occurred on the same day that the specimen was collected from the participant. DBS cards were left to dry completely for a minimum of four hours and stored in bags with desiccant and humidity indicator cards at ambient temperature for a maximum of six days before transport to the laboratory.

5.4.4 Specimen transportation and preparation at the satellite laboratory

Venous blood samples were transported from ANC sites to a satellite laboratory every day, while DBS specimens were transported from ANC sites to a satellite laboratory a minimum of once per week. Samples were transported with their corresponding laboratory requisition forms. All samples were transported by Riders for Health motorcycle couriers. Upon receipt at the satellite laboratory, venous blood specimens were centrifuged to separate plasma within 24 hours from the time of collection. Plasma was aliquoted and stored immediately at -20 degrees Celsius (C) until they were transported to the central laboratory for recency testing. DBS specimens were stored at 4 degrees C at the satellite laboratory until they were transported to the central laboratory.

5.4.5 Recent infection testing algorithm at the central laboratory

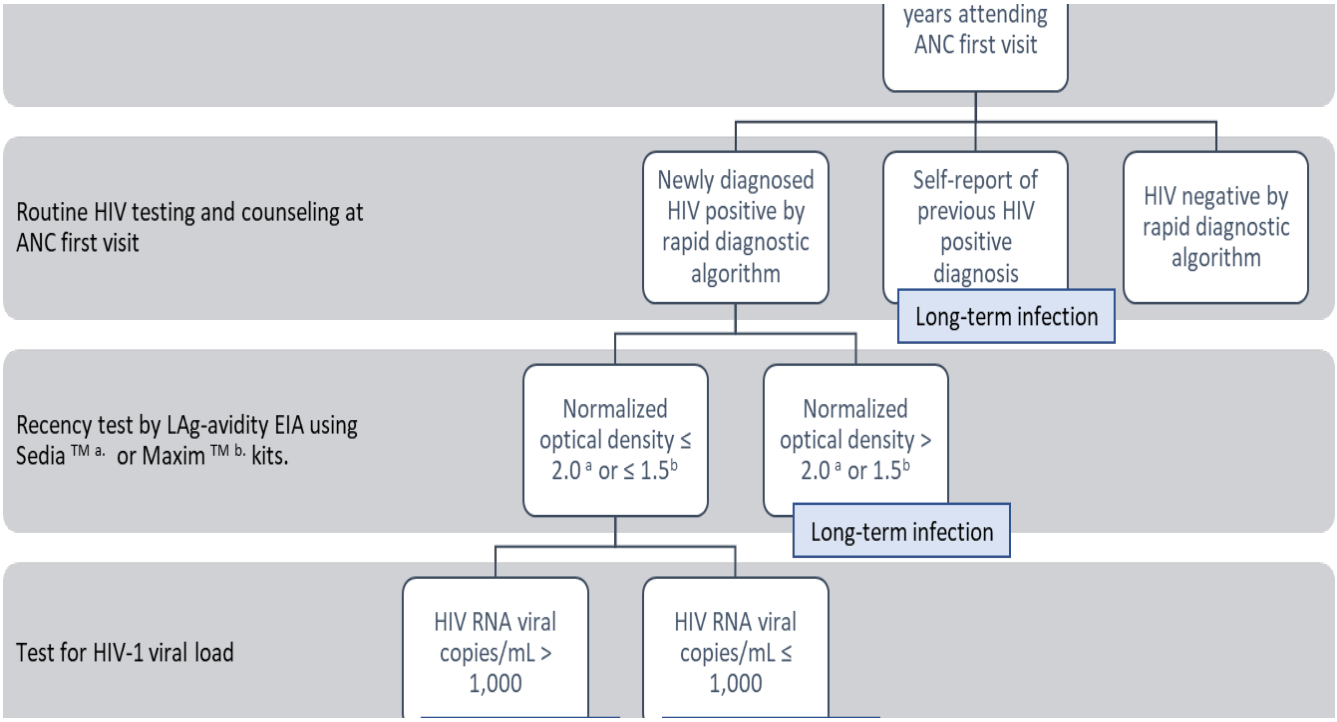
Samples were transported weekly from the satellite laboratories to the central laboratories. Testing for recent infection was performed at the Zomba Central Hospital or Kamuzu Central Hospital laboratories using a LAg-avidity (EIA)-based algorithm. Based on an independent evaluation of the five most commonly used tests for recent infection, the LAg Avidity EIA was the most accurate assay across multiple subtypes for HIV infection, with an assay specificity of approximately 98.5% [10]. The LAg-avidity EIA is based on the functional property, i.e. “avidity” (i.e., the binding strength) of antibodies. Antibody avidity increases with time since infection and is a robust parameter to distinguish recent from long-term infection. The LAg assay produces a normalized optical density (ODn) value as a measure of antibody avidity. Although a cutoff ODn of 1.5 with corresponding mean duration of recent infection (MDRI) of 130 days was used in the Malawi Population-Based HIV Impact Assessment (MPHIA), a cutoff of 2.0 was used in this study to match the corresponding transition from recent to long term-infection on the Asante assay [1]. Calibration of Sedia's LAg-avidity EIA (Sedia Biosciences, Corp., Portland, Oregon, USA) suggests that an overall MDRI of 161 days (95% CI 148-174) is appropriate at cutoff of 2.0 and should be used in an algorithm with VL (>1 000 copies/mL) to detect recent infections and calculate incidence [11].

Participant specimens underwent testing by the Sedia LAg-avidity EIA in singlet. Specimens with ODn values ≤ 2.0 during initial testing were confirmed by further testing of the samples in triplicate. The ODn was calculated by dividing the optical density (OD) for each specimen by the median OD of the calibrator. For specimens that had ODn values ≤2.0 and underwent triplicate testing for confirmation, the final ODn values for those specimen were the median values of the triplicate test results. Specimens that had final ODn values >2.0 when tested with Sedia LAg-avidity EIA were classified as “long-term” infections while specimens with ODn values ≤ 2.0 were classified as “probable recent” infections. Partway through implementation, the study began testing DBS specimens with Maxim HIV-1 LAg-Avidity EIA DBS Kits (Maxim Biomedical, Inc., Rockville, Maryland, USA), which has slightly different testing characteristics than the Sedia LAg-avidity kits [12]. Specimens that had final ODn values >1.5 when tested with the Maxim LAg-avidity kits were classified as “long-term” infections while

specimens with ODn values ≤ 1.5 were classified as "probable recent" infections. The different cutoffs for classification of recent infection for the two assays were utilized to enable the application of the same MDRI of 161 days to all specimens in the calculation of annualized incidence.

As part of the RITA, probable recent infection specimens underwent VL testing for final confirmation of recency status. HIV-1 RNA VL copies/mL were measured using the Abbott m2000, Roche Cobas Ampliprep/Cobas Taqman (Roche Diagnostics USA, Indianapolis, Indiana, USA). Specimens in which VL was ≤ 1000 copies/mL were classified as "long-term" infections. Specimens in which VL was >1000 copies/mL were classified as "recent" infections. The Malawi Recency Study testing algorithm is displayed in Figure 1**.**

Figure 1: Routine antenatal care HIV screening and recent infection testing algorithm used to identify recent infections among newly diagnosed HIV positive pregnant adlosecent girls and young women receving antenatal care in Blantyre, Lilongwe, Machinga and Zomba Disticts in Malawi, 2017-18. (a) Sedia LAg-avidity EIA kits were used to test 121/210 DBS specimens and all 379 plasma specimens. (b) Maxim LAg-avidity EIA kits were used to test 89/210 DBS specimens. We used different cutoffs for classification of recent infection for the two assays because, at these cutoffs, the same MDRI of 161 days can be applied uniformly to all specimens in the calculation of annualized incidence based on the correlation between the two assays at those thresholds.



5.4.6 Evaluation of Asanté™ rapid recency assay performance

In addition to the LAg-based RITA, testing for recent infection was performed using the Asanté™ HIV-1 Rapid Recency™ assay at the central laboratories and at 14 high-volume ANC sites in Lilongwe district. The Asanté™ rapid recency assay is formatted as a lateral flow device with three lines: a control line, a diagnostic line, and a third line to distinguish recent or long-term infection [8, 9]. A limiting amount of antigen applied to the third line forms the basis for separation of recent (low-avidity antibodies) from long-term (high-avidity antibodies) infection with results available in under 20 minutes. The test can be interpreted both qualitatively and quantitatively. In qualitative or visual interpretation, the tester assesses the presence or absence of three lines on the test strip. Presence of only the control line indicates the client is HIV-seronegative, while presence of a

control and diagnostic lines indicates a seropositive HIV test with recent infection (i.e., one that was acquired within the past 12 months, approximately). The presence of all three lines indicates a seropositive HIV test with long-term infection (> 12 months). Determination of HIV diagnosis and recency of infection by quantitative interpretation involves inserting the test strip into a reader. The Asanté™ reader provides a quantitative value for the line intensity as a measure of antibody avidity, which can be compared to the ODn values produced by the LAg-avidity EIA for correlation.

Plasma specimens were tested with Asanté™ and interpreted both qualitatively (i.e., visual interpretation) and quantitatively using the test strip reader at the central laboratories. Additional VL testing was done on samples that tested recent on the Asanté™ rapid recency assays conducted in the central laboratories. At 14 ANC facilities in Lilongwe, the Asanté™ rapid recency assay was used on approximately 5 µL of the venous blood collected from participants for LAg-based recency testing. Asanté™ tests at the ANC sites were performed after the participant encounter in a private setting and results were interpreted qualitatively. DBS specimens were not tested with Asanté™ because the assay has not been validated for use with DBS.

5.5 Return of RITA Results

The study aimed to return results from the LAg-based RITA to ANC sites within four weeks after the date of participant enrollment, which is when AGYW newly diagnosed with HIV infection and initiating ART at the ANC first visit are routinely scheduled to return to the clinic for ART adherence counselling, treatment monitoring and continuing ANC services. ANC staff returned results of the LAg-based RITA and provided post-test counselling to participating AGYW when they returned for their routine follow-up. Participating AGYW were encouraged to discuss their recency test results with their partners. However, no data on partner disclosure were collected as part of the study. In addition to returning recency results, routine ANC and ART services were provided to AGYW per current national guidelines irrespective of the LAg-based RITA result. Asanté™ test results performed at the central laboratories and selected ANC sites in Lilongwe were not returned to participants.

5.6 Long-term Storage of Specimens

If consent was granted by the participant, any leftover samples remaining after the completion of the LAg-based RITA and Asanté™ tests were stored at -80^{o}C in a specimen repository. Participating AGYW could “opt-out” of this part of the study and still participate in the recency testing and risk-factor interview. Stored specimens were not linked to personal identifiers and cannot be traced back to the individual that provided the specimens. These specimens will remain in the repository for up to five years, maintained by the MOH and supporting partners for future studies in Malawi.

5.7 Data Management and Analysis

Data from the risk factor questionnaires, laboratory requisition forms and HIV testing registers were entered into a survey database by study coordinators and laboratory data clerks. Data from the different sources were merged and cleaned prior to analysis. Participants without sufficient quantity of specimen to complete the entire recent infection testing algorithm were excluded from the final analysis. Study coordinators abstracted data on age and HIV serostatus (based on prior HIV diagnoses and results of routine antenatal HIV test results) of all women attending ANC at participating sites from standard ANC registers to calculate the total number of women who were eligible to participate in the study and to estimate the prevalence and incidence of HIV among all AGYW attending ANC.

The demographic and behavioral characteristics of study participants were described using data from the risk factor questionnaires and laboratory requisition forms. The prevalence of recent infection among newly diagnosed study participants was calculated overall, by district, and by demographic and behavioral variables. Tests for statistical significance were calculated in bivariable models using Chi-square tests. These analyses were conducted using Stata/Intercooled C 12.1 (Statcorp, College Station, Texas, USA), weighted to account for survey non-response by age group (15-19 years vs. 20-24 years) and district [See Appendix 1a], and adjusted for clustering by the ANC site.

HIV incidence estimation

Incidence calculations were performed in *R* software version 1.1.442 (2009-2018 RStudio, Inc, Vienna, Austria) using *inctools* [14], which is a package designed to estimate HIV incidence from biomarker data in cross-sectional surveys based on the work of Kasanjee et. al [5]. Use of the *inctools*-incprops function requires that survey data be reduced to an estimate of HIV prevalence, an estimate of the prevalence of recent infection amongst those who are HIV infected and a variance/covariance matrix for these two prevalence estimates. Two prevalence estimates were calculated by combining recency test results and demographic data from survey participants with demographic and serostatus data from all AGYW attending ANC at study sites during the study period. Data on AGYW not enrolled in the study were abstracted from ANC registers. The estimates were calculated as follows:

- **HIV prevalence** = *number of all HIV positive AGYW attending ANC / number of all AGYW attending ANC excluding those with unknown serostatus.*

Because we used data from a census of all AGYW attending ANC, HIV prevalence was calculated without weighting for non-response. However, terms to account for clustering by ANC site were included in order to calculate robust standard errors.

- **Prevalence of recent infection** = *number of AGYW study participants classified as "recent" by RITA / (number of AGYW study participants classified as "recent" by RITA + number of AGYW study participants classified as "long-term" by RITA + number of AGYW who were classified as "previously diagnosed" with HIV prior to attending their current ANC first visit).*

In the calculation of the prevalence of recent infection, the study assumed that all previously diagnosed AGYW were long-term infections, although they were not enrolled in the study and not subject to recent infection testing. The prevalence of recent infection was weighted to account for unequal response by age group (15-19 v. 20-24 years), district, and previous HIV diagnosis (newly vs. previously diagnosed) [See Appendix 1b]. This estimate also accounted for clustering by ANC site.

Using the above estimates, the study calculated annualized rates of HIV incidence assuming a MDRI of 161 days (95% confidence interval [CI]: 145-177 days). The false recency rate (FRR) used for this analysis was based on previously published estimates. Findings from independent validations of LAg-only recency testing suggest that the FRR is 1.5% (95% CI: 0.3-4.4) [10]. However, the FRR is anticipated to be 1% or lower when VL is included in the RITA [13]. Because our sample of newly diagnosed individuals were assumed to not be on treatment, we

applied a FRR of 0.00 in the analysis in this study.[1] A design effect of 1.0 was also applied to the analysis. HIV incidence estimates were disaggregated by age group and by district.

Evaluation of the Asanté™ HIV-1 Rapid Recency Assay

The study evaluated the Asanté™ rapid recency assay by comparing its quantitative and qualitative results at the laboratory and at the selected ANC sites in Lilongwe against those of the LAg-avidity assay, with and without VL results. Correlation coefficients (r) for quantitative values, two-by-two tables and kappa statistics[2] from percent concordance were calculated. ODn cutoffs of ≤ 2.0 for the Sedia LAg-avidity assay, ≤ 1.5 for the Maxim LAg-avidity assay and line intensity units < 3.0 for the Asanté™ assay were used in these calculations. In addition, we assessed the overall concordance of the final HIV diagnosis from the national algorithm with the HIV diagnostic line on the rapid recency assay. If the control line did not appear during visual interpretation of the Asanté™ test or the reader had line intensity units < 2.8, the test was considered invalid and the specimen was excluded from the two-by-two comparisons shown in Table 17. If the positive verification line did not appear during visual interpretation of the Asanté™ test, the specimen was not considered HIV-positive and therefore excluded from the two-by-two comparisons shown in Table 17.

6.0 Training, Supervision and Quality Control

Training, supervision and quality control measures were applied to ensure that the implementation of the Malawi Recency Study was done according to protocol with a high degree of quality, accuracy, completeness and representativeness. Selected ANC site staff – including nurses, midwives and health diagnostic assistants – were nominated to work on the study by in-charges at each facility and trained in general principles of ethical conduct of human subjects' research and study procedures. Laboratory staff were nominated to work on the study by laboratory managers, and received training in procedures for receiving and processing specimens, VL, LAg avidity EIA, and Asanté™ rapid recency assay testing. Laboratory staff were certified following successful completion of training, which included competency panels with reference LAg values/classification established by the U.S. Centers for Disease Control and Prevention (CDC) International Lab Branch.

The in-country implementing partners and investigators provided regular supervision to ANC sites and laboratories to verify that the study protocols were being correctly implemented. Each ANC site was visited at least once per month and laboratories were visited weekly. At the end of the survey, the entire laboratory and risk factor datasets were checked for consistency against the paper-based forms; any discrepancies identified were investigated and corrected as necessary.

[1] Supplementary estimates of HIV incidence, which apply a FRR of 0.015, are presented in appendix 2 of this report.
[2] Kappa result be interpreted as follows: values ≤ 0 as indicating no agreement and 0.01–-0.20 as none to slight, 0.21–-0.40 as fair, 0.41– -0.60 as moderate, 0.61–-0.80 as substantial, and 0.81–-1.00 as almost perfect agreement [23].

7.0 Ethical Considerations

Eligible AGYW provided informed consent prior to participation. Consent procedures were conducted in English or Chichewa according to the AGYW's preference.

The probability and magnitude of harm or discomfort to participants were expected to be no greater than those encountered during the receipt of routine ANC services. The principal risk to the participant was breach of confidentiality or disclosure of individual level information collected, including HIV status. However, the study implemented human, physical and electronic procedures and protections at every stage to ensure the confidentiality and security of personal information. Study staff were trained to provide referrals to social support services for women who reported or who were suspected to have been experiencing emotional or physical abuse.

The study protocols were reviewed and approved by the Malawi National Health Sciences Research Committee, the the CDC Institutional Review Board (#6994), and the Committee on Human Research at the University of California, San Francisco. All data collected through this protocol are the property of the Malawi MOH.

8.0 Results

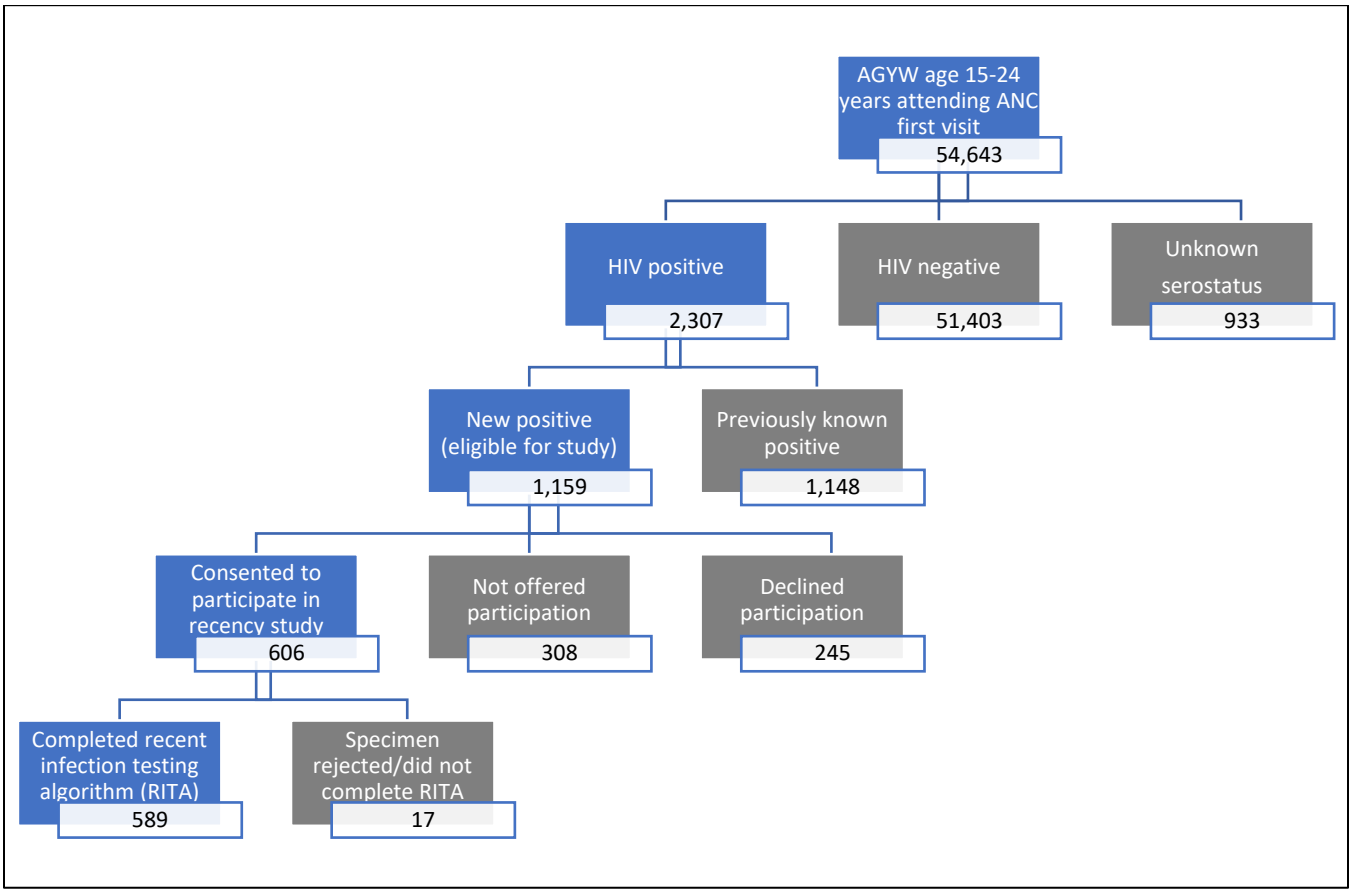
8.1 Summary of Eligibility and Enrollment

Summaries of study eligibility and enrollment are provided in Table 1 and Figure 2. There were a total of 54,643 pregnant AGYW aged 15-24 years who attended an ANC first visit at study sites in Blantyre, Lilongwe, Machinga and Zomba during the six-month study period. Among these, 933 (1.7%) had unknown HIV serostatus and were therefore excluded from the analysis. Among the remaining 53,710 AGYW, 51,403 (95.7%) were HIV uninfected and 2,307 (4.3%) were HIV infected. By district, HIV prevalence among pregnant AGYW attending an ANC first visit was 7.1% in Blantyre, 3.0% in Lilongwe, 4.0% in Machinga and 5.1% in Zomba. A total of 1,159 (2.2%) AGYW were newly diagnosed with HIV and therefore eligible to participate in the study. A larger percentage of AGYW were eligible in Blantyre (3.9%) relative to Lilongwe (1.8%), Machinga (1.3%) and Zomba (1.8%) districts. Among eligible AGYW, 589 (50.8%) were enrolled in the study and completed the LAg-based RITA. Levels of enrollment were higher in Machinga (69.7%) and Zomba (67.6%) than in Blantyre (42.8%) and Lilongwe (48.2%) districts. Enrollment was slightly higher among eligible AGYW aged 15-19 years (56.2%) than among those aged 20-24 years (49.0%).

Table 1: Summary of eligibility and enrollment in the Malawi Recency Study among adolescent girls and young women (AGYW) who were pregnant and attending antenatal care (ANC) firsts visit in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Overall		Blantyre		Lilongwe		Machinga		Zomba	
	#	%	#	%	#	%	#	%	#	%
AGYW (age 15-24 years) attending ANC first visit, *excluding those with unknown serostatus*	53,710		11,103		25,696		9,043		7,868	
AGYW HIV positive	2,307	4.3%	786	7.1%	760	3.0%	359	4.0%	402	5.1%
AGYW HIV positive who were newly diagnosed (eligible for study)	1,159	2.2%	430	3.9%	465	1.8%	119	1.3%	145	1.8%
AGYW who consented, provided a blood and completed recent infection testing algorithm (i.e. "enrolled" in recency study)	589	50.9%	184	42.8%	224	48.2%	83	69.7%	98	67.6%

Figure 2: Flow diagram of eligibility and enrollment in the Malawi Recency Study among newly diagnosed HIV positive adolescent girls and young women (AGYW) who were pregnant and attending antenatal care (ANC) for their first visit in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.



Reasons for non-enrollment among eligible AGYW are summarized in Table 2**.** Among the 570 eligible AGYW who were not enrolled in the study, 308 (54.0%) were not offered the opportunity to participate (i.e., "missed" by staff); 17 (3.0%) agreed to participate, completed the risk interview and provided a blood sample but were excluded because their sample was insufficient to complete the RITA; and 245 (43.0%) declined participation during the informed consent process. Among the 245 eligible AGYW who declined participation, 90 (36.7%) were not interested in the study, 39 (15.9%) had no time, 1 (0.4%) declined due to religious reasons, 88 (35.9%) needed to ask their partner's permission, 6 (2.4%) had a fear of needle/blood draw and 21 (8.6%) gave no reason for their refusal. The percentage of eligible AGYW that were missed by study staff indicates a need for frequent refresher trainings and more staff trained in recency procedures.

Table 2: Reasons for non-enrollment among eligible adolescent girls and young women (AGYW) who were not enrolled in the Malawi Recency Study in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Overall		Blantyre		Lilongwe		Machinga		Zomba	
	#	%	#	%	#	%	#	%	#	%
Reason for non-participation among eligible AGYW (n = 570)										
Eligible AGYW not offered opportunity to participate (i.e. "missed" by staff)	308	54.0%	99	40.2%	160	66.4%	28	77.8%	21	44.7%
Eligible AGYW declined participation	245	43.0%	139	56.5%	74	30.7%	6	16.7%	26	55.3%
Specimen not acceptable for recency testing	17	3.0%	8	3.3%	7	2.9%	2	5.6%	0	0.0%
Reasons provided by AGYW who declined participation (n = 245)										
Not interested	90	36.7%	47	33.8%	30	40.5%	3	50.0%	10	38.5%
No time	39	15.9%	19	13.7%	15	20.3%	1	16.7%	4	15.4%
Religious reasons	1	0.4%	0	0.0%	1	1.4%	0	0.0%	0	0.0%
Need partner permission	88	35.9%	60	43.2%	20	27.0%	2	33.3%	6	23.1%
Other	21	8.6%	12	8.6%	4	5.4%	0	0.0%	5	19.2%
Fear of needle/blood draw	6	2.4%	1	0.7%	4	5.4%	0	0.0%	1	3.8%

8.2 Demographic and Behavioral Characteristics of Participating AGYW

The demographic and behavioral characteristics of the 589 enrolled study participants who completed the RITA are described in Table 3**.** Nearly three quarters of participating AGYW were aged 20-24 years (72.3%). The median age of participants was 21 years. Younger women (aged 15-19 years) comprised a larger percentage of participants in Zomba (35.7%) than in the other three districts. Most participants were unemployed (67.1%). Unemployment was more common in Blantyre (77.2%) and Lilongwe (75.2%) than in Machinga (49.4%) and Zomba (44.9%), where the majority of employed participants reported that they worked as farmers. Only a small minority (7.5%) of participants had no formal education. However, the percentage of participants with no education was higher in Machinga district (27.7%) relative to the other three districts. The highest level of education completed by most participants was primary level (56.2%). The percentage of participants who completed secondary education was greater in Blantyre (43.7%) and Lilongwe (35.3%) than in Machinga (15.7%) and Zomba (25.8%). About three quarters of participants were currently married (75.7%), with the percentage of participants who were currently married ranging from 66.7% in Zomba to 79.5% in Lilongwe. Among those ever married, most were first married between the ages of 16-19 years (62.3%), and 10.5% were first married before the age of 16 years. Nearly one fifth of married participants had been married more than five years ago (18.9%).

Table 3: Demographic characteristics of newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in the Malawi Recency Study in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Blantyre		Lilongwe		Machinga		Zomba		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Age group (5 year interval)										
15-19 years	43	23.4	66	29.5	19	22.9	35	35.7	163	27.7
20-24 years	141	76.6	158	70.5	64	77.1	63	64.3	426	72.3
Age group (2 year interval)										
15-16 years	2	1.1	7	3.1	3	3.6	2	2.0	14	2.4
17-18 years	24	13.0	32	14.3	8	9.6	22	22.4	86	14.6
19-20 years	42	22.8	54	24.1	22	26.5	27	27.6	145	24.6
21-22 years	64	34.8	57	25.4	22	26.5	22	22.4	165	28.0
23-24 years	52	28.3	74	33.0	28	33.7	25	25.5	179	30.4
Currently employed										
No	142	77.2	167	75.2	41	49.4	44	44.9	394	67.1
Yes	42	22.8	55	24.8	42	50.6	54	55.1	193	32.9
Highest level of education completed										
No education	5	2.7	10	4.5	23	27.7	6	6.2	44	7.5
Primary/vocational	92	50.3	124	56.1	47	56.6	65	67.0	328	56.2
Secondary	80	43.7	78	35.3	13	15.7	25	25.8	196	33.6
University	6	3.3	9	4.1	0	0.0	1	1.0	16	2.7
Current marital status										
Never married	35	19.7	35	15.9	15	18.5	29	31.2	114	19.9
Currently married	135	75.8	175	79.5	61	75.3	62	66.7	433	75.7

Divorced, separated or widowed	8	4.5	10	4.5	5	6.2	2	2.2	25	4.4
Age when first married (if ever married)^										
12-15 years	11	9.2	13	8.2	7	13.2	11	15.7	42	10.5
16-19 years	75	63.0	88	55.3	38	71.7	49	70.0	250	62.3
20-24 years	33	27.7	58	36.5	8	15.1	10	14.3	109	27.2
Years since first married (if ever married)^										
0 to 1	34	31.2	63	42	11	21.2	22	31.4	130	34.1
2 to 5	52	47.7	64	42.7	30	57.7	33	47.1	179	47.0
6 or more	23	21.1	23	15.3	11	21.2	15	21.4	72	18.9
Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

The sum of the counts in strata of a given variable may be less than the total sample size because of missing responses. Unless indicated by ^, missing data for each variable is < 5%.

The characteristics of participants' current pregnancy at the time of enrollment are described in Table 4. Just under half (46.5%) of participants were pregnant for the first time – i.e., gravida one; 37.5% were pregnant for the second time, and 16.0% were pregnant for the third time or more. A greater proportion of AGYW in Machinga district had one or more previous pregnancies (38.8% gravida two and 18.8% gravida three or more). A large majority (71.3%) of participants came for their first ANC visit during the second trimester of pregnancy. About one in ten (9.3%) participants did not come to their ANC first visit until the third trimester, with the greatest percentage of third-trimester first-visits observed in Lilongwe district (12.9%). Most participants (72.8%) in Machinga and Zomba reported that their current pregnancy was planned. The question about planned pregnancy was not asked of participants in Blantyre and Lilongwe districts.

Table 4: Characteristics of current pregnancy among newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in the Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Blantyre		Lilongwe		Machinga		Zomba		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Gravidity										
1	77	44.3	101	47.4	34	42.5	47	52.2	259	46.5
2	66	37.9	84	39.4	31	38.8	28	31.1	209	37.5
3 or more	31	17.8	28	13.1	15	18.8	15	16.7	89	16.0
Trimester of pregnancy at ANC first-visit										
First trimester	36	19.6	40	17.9	20	24.1	18	18.4	114	19.4
Second trimester	135	73.4	155	69.2	57	68.7	73	74.5	420	71.3
Third trimester	13	7.1	29	12.9	6	7.2	7	7.1	55	9.3
Current pregnancy was planned^										
No	--	--	--	--	20	27	23	27.4	43	27.2
Yes	--	--	--	--	54	73	61	72.6	115	72.8

Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

The sum of the counts in strata of a given variable may be less than the total sample size because of missing responses. Unless indicated by ^, missing data for each variable is < 5%.

Participants' sexual history and recent sexual behaviors are described in Table 5**.** The majority (46.9%) of participants reported first having sex between the ages of 15-17 years and being sexually active for more than four years (50.8%). One in ten (10.9%) participants became sexually active between the ages of 10-14 years. More participants first had sex between the ages of 10-14 years in Machinga (16.4%) and Zomba (17.6%) than in Blantyre (9.4%) and Lilongwe (7.1%). Most (80.8%) participants reported that their first sexual encounter was consensual (i.e., not forced). Participants reported a median of 2 (interquartile range: 1 to 3) lifetime sexual partners. Approximately one in four participants reported that they had only one partner in their lifetime (26.6%), and 10.2% reported that they have had four or more partners in their lifetime. Nearly all (83.2%) participants reported only one sexual partner during the past six months. One in ten participants had exchanged sex for money or gifts during the past 12 months (9.7%). Exchange of sex for money or gifts was most prevalent in Machinga, where 16.9% of participants reported that they had done so during the past 12 months. Over half (52.8%) of participants reported that they never used condoms during the past 12 months, with the remainder reporting that they had used condoms sometimes (43.5%) or always (3.7%). A large majority (86.3%) of participants reported that they had not used condoms since becoming pregnant.

Table 5: Sexual history and recent sexual behavior among newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in the Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Blantyre		**Lilongwe**		**Machinga**		**Zomba**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Age group at first sex^										
10-14 years	15	9.4	13	7.1	11	16.4	15	17.6	54	10.9
15-17 years	76	47.5	86	47.0	31	46.3	39	45.9	232	46.9
18-19 years	55	34.4	59	32.2	18	26.9	27	31.8	159	32.1
20-24 years	14	8.8	25	13.7	7	10.4	4	4.7	50	10.1
First sex was consensual										
No	33	18.8	48	22.6	10	12.7	17	17.9	108	19.2
Yes	143	81.2	164	77.4	69	87.3	78	82.1	454	80.8
Number of years sexually active^										
0 to 1	32	20.4	34	19.2	9	13.6	16	19.0	91	18.8
2 to 3	39	24.8	62	35.0	22	33.3	24	28.6	147	30.4
4 or more	86	54.8	81	45.8	35	53.0	44	52.4	246	50.8
Number of lifetime sex partners^										
1	47	29.2	55	28.6	13	19.1	21	23.1	136	26.6
2 to 3	99	61.5	113	58.9	49	72.1	63	69.2	324	63.3
4 or more	15	9.3	24	12.5	6	8.8	7	7.7	52	10.2
Number of sex partners in past 6 months^										

0	25	15.0	15	7.4	4	5.6	6	6.8	50	9.4
1	132	79.0	172	84.3	57	80.3	80	90.9	441	83.2
2 or more	10	6.0	17	8.3	10	14.1	2	2.3	39	7.4
Received money/gifts in exchanged for sex^										
Never	139	84.2	192	91.4	61	79.2	74	86.0	466	86.6
Within the past year	18	10.9	14	6.7	13	16.9	7	8.1	52	9.7
More than one year ago	8	4.8	4	1.9	3	3.9	5	5.8	20	3.7
Frequency of condom use in past 12 months										
Never	91	51.1	120	55.3	44	54.3	45	48.9	300	52.8
Sometimes	82	46.1	86	39.6	35	43.2	44	47.8	247	43.5
Always	5	2.8	11	5.1	2	2.5	3	3.3	21	3.7
Frequency of condom use since becoming pregnant										
Never	160	90.9	195	88.6	62	74.7	79	82.3	496	86.3
Sometimes	11	6.2	14	6.4	19	22.9	13	13.5	57	9.9
Always	5	2.8	11	5.0	2	2.4	4	4.2	22	3.8
Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

The sum of the counts in strata of a given variable may be less than the total sample size because of missing responses. Unless indicated by ^, missing data for each variable is < 5%.

The characteristics of participants' current main sexual partner are described in Table 6**.** A main partner refers to either a husband or steady boyfriend, or in the absence of either, the most recent male sexual partner. The relative age difference between participants and their partners varied. Overall, 29.7% had a partner that was younger, the same age or less than five years older, 26.0% had a partner that was five to 10 years older, 32.6% had a partner that was more than 10 years older and 11.7% didn't know their partner's age. Over half of participants' partners were circumcised (59.6%), with the majority of those circumcisions reported to be traditional (61.9%) as opposed to medical circumcisions (38.1%). Most (63.2%) participants did not know their partners' HIV status. About one in ten (11.0%) reported that their partner was HIV infected.

Table 6: Characteristics of current main sexual partner among newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in the Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Blantyre		Lilongwe		Machinga		Zomba		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Age of partner										
Younger, same age or < 5 years older	47	25.5	61	29.3	22	27.2	37	41.6	167	29.7
5-10 years older	58	31.5	54	26.0	11	13.6	23	25.8	146	26.0
>10 years older	65	35.3	56	26.9	39	48.1	23	25.8	183	32.6
Don't know	14	7.6	37	17.8	9	11.1	6	6.7	66	11.7
Partner is circumcised^										
No	61	37.2	124	61.1	4	5.4	23	27.4	212	40.4

Yes	103	62.8	79	38.9	70	94.6	61	72.6	313	59.6
Partner's circumcision type (if circumcised)^										
Traditional	50	61.0	27	42.2	50	76.9	39	68.4	166	61.9
Medical	32	39.0	37	57.8	15	23.1	18	31.6	102	38.1
Partners HIV status										
Negative	25	14.0	57	26.9	24	31.6	38	41.8	144	25.9
Positive	16	9.0	18	8.5	9	11.8	18	19.8	61	11.0
Unknown	137	77.0	137	64.6	43	56.6	35	38.5	352	63.2
Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

The sum of the counts in strata of a given variable may be less than the total sample size because of missing responses. Unless indicated by ^, missing data for each variable is < 5%.

Participants' use of alcohol and illicit drugs and experiences with physical or emotional abuse are described in Table 7**.** A minority (16.5%) of participants reported any alcohol use during the past six months or illicit drug use ever (3.0%). A quarter of participants (25.4%) had ever been physically or emotionally abused by a sexual partner, with the highest prevalence of abuse reported in Blantyre (30.2%). Fewer participants reported that they had been physically abused during the past 12 months (8.7%) or physically abused during their pregnancy (4.5%). About one in 20 (5.6%) participants reported that they had been forced to have sex against their will during the past 12 months. Nearly all (94.6%) participants reported that they were not currently afraid of being abused.

Table 7: Alcohol, drug use and history of physical or emotional abuse among newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Blantyre		Lilongwe		Machinga		Zomba		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Consumed any alcohol in past 6 months										
No	145	78.8	174	78.4	79	95.2	92	93.9	490	83.5
Yes	39	21.2	48	21.6	4	4.8	6	6.1	97	16.5
Ever used illicit drugs										
No	172	95.6	212	96.8	80	97.6	95	100	559	97
Yes	8	4.4	7	3.2	2	2.4	0	0	17	3.0
Ever abused by a sexual partner (physical or emotional)										
No	127	69.8	162	73.6	66	79.5	79	81.4	434	74.6
Yes	55	30.2	58	26.4	17	20.5	18	18.6	148	25.4
Abused during past 12 months										
No	165	89.7	198	90.4	79	95.2	91	92.9	533	91.3
Yes	19	10.3	21	9.6	4	4.8	7	7.1	51	8.7
Abused by person who got you pregnant										
No	173	94.0	209	95.9	80	96.4	95	96.9	557	95.5

Yes	11	6.0	9	4.1	3	3.6	3	3.1	26	4.5
Forced to have sex in past 12 months										
No	176	95.7	204	91.9	80	96.4	94	95.9	554	94.4
Yes	8	4.3	18	8.1	3	3.6	4	4.1	33	5.6
Currently afraid of being abused by someone										
No	156	96.3	194	95.6	0	0.0	1	33.3	351	94.6
Yes	6	3.7	9	4.4	3	100	2	66.7	20	5.4
Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

The sum of the counts in strata of a given variable may be less than the total sample size because of missing responses. Unless indicated by ^, missing data for each variable is < 5%.

Previous HIV testing, recent diagnosis and treatment of sexually transmitted infections (STI) and receipt of DREAMS interventions among participants are described in Table 8**.** Approximately one in three (30.6%) participants had never been tested for HIV prior to the ANC first visit when they enrolled in the study with the remainder having been tested within the past 12 months (33.2%) or more than 12 months ago (36.2%). Among participants in Machinga and Zomba who had ever been tested, the majority (85.7%) had been tested in a health facility as opposed to in a community-based setting. Reported symptoms or diagnosis of sexually transmitted infections (STIs) – including vaginal ulcers or discharge – in the past 12 months varied by district, ranging from 28.4% in Lilongwe to 43.2% in Zomba. Less than half of participants who reported symptoms or diagnosis of an STI in the past 12 months reported that they received treatment (41.7%). A previous diagnosis or treatment of syphilis infection was reported by 5.6% of participants. Few reported receiving a vaccine to prevent human papilloma virus and cervical cancer (1.9%). In Machinga and Zomba districts, a minority of participants had ever participated in a "Go Girls!" club (10.4%), another teen club (9.8%), or mother's club (14.5%). The questions about participation in clubs were not asked of participants in Blantyre and Lilongwe districts as these districts were not part of the DREAMS initiative the time of data collection.

Table 8: Previous HIV testing, sexually transmitted infections and receipt of DREAMS programs among newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in the Malawi Recency Study, Blantyre, Lilongwe, Machinga

	Blantyre		**Lilongwe**		**Machinga**		**Zomba**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Previously tested for HIV										
Never	49	26.8	75	33.8	26	31.3	29	29.9	179	30.6
< 12 months ago	67	36.6	74	33.3	27	32.5	26	26.8	194	33.2
≥ 12 months ago	67	36.6	73	32.9	30	36.1	42	43.3	212	36.2
Place of last HIV test (if ever tested)^										
Community-based	--	--	--	--	7	14.6	8	14.0	15	14.3
Facility-based	--	--	--	--	41	85.4	49	86.0	90	85.7
Symptoms of STI in past 12 months										
No	126	70.0	156	71.6	57	69.5	54	56.8	393	68.3

Yes	54	30.0	62	28.4	25	30.5	41	43.2	182	31.7
Received treatment for STI (if any symptoms)										
No	31	58.5	32	54.2	12	48.0	27	71.1	102	58.3
Yes	22	41.5	27	45.8	13	52.0	11	28.9	73	41.7
Ever diagnosed or treated for syphilis										
No	166	93.8	203	94.4	75	93.8	93	95.9	537	94.4
Yes	11	6.2	12	5.6	5	6.2	4	4.1	32	5.6
Ever received HPV vaccine										
No	177	100	214	98.6	77	93.9	91	96.8	559	98.1
Yes	0	0.0	3	1.4	5	6.1	3	3.2	11	1.9
Ever participated in teen club^										
No	--	--	--	--	63	78.8	67	80.7	130	79.8
Yes – Go Girls club	--	--	--	--	6	7.5	11	13.3	17	10.4
Yes – another club	--	--	--	--	11	13.8	5	6.0	16	9.8
Ever participated in mother's club^										
No	--	--	--	--	65	80.2	77	90.6	142	85.5
Yes	--	--	--	--	16	19.8	8	9.4	24	14.5
Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

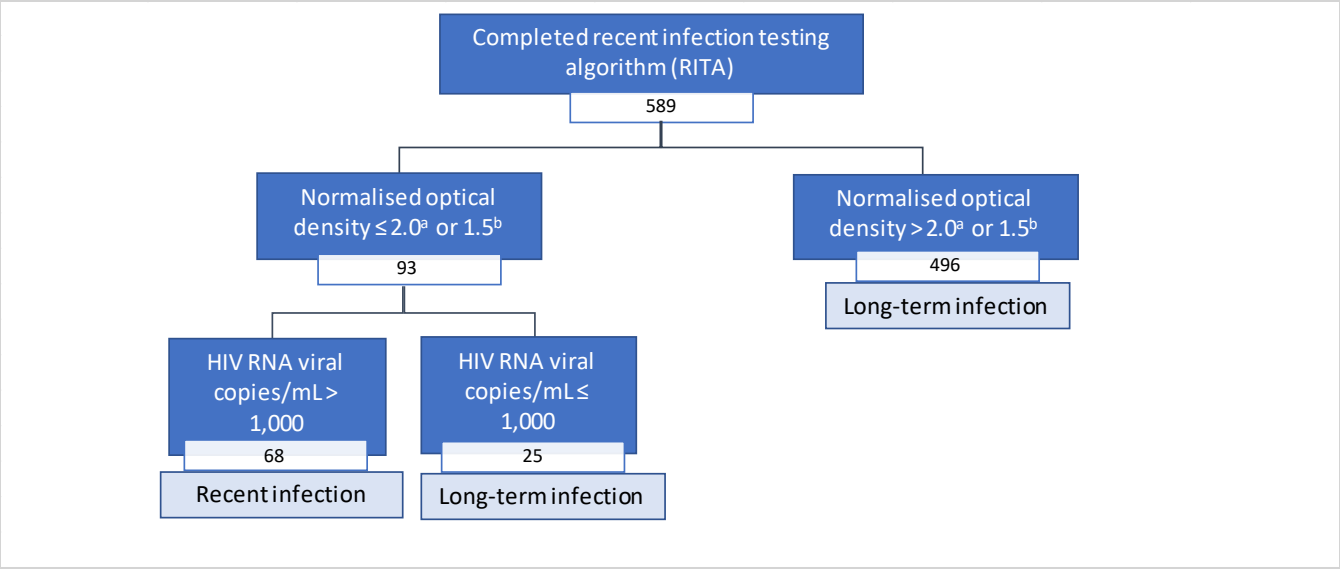
The sum of the counts in strata of a given variable may be less than the total sample size because of missing responses. Unless indicated by ^, missing data for each variable is < 5%.

8.3 Prevalence and correlates of recent HIV infection by the LAg-based RITA

A flow diagram of the results of the LAg-avidity EIA with and without inclusion of a VL to confirm "probable recent" results is shown in Figure 3. Among 589 enrolled participants who completed the LAg-based RITA, 93 had a "probable recent" result from the LAg-avidity EIA alone. However, 25 (26.9%) of these AGYW had a VL result <1,000 copies/uL and were ultimately classified as having "long-term" infections**.** After completion of VL testing, 11.7% (95% CI: 9.8-13.9) of HIV-infected participants had a confirmed recent HIV infection. Conversely, 88.3% of participants had a long-term infection (i.e., an infection that was probably acquired more than 12 months ago).

Figure 3: Results of recency testing using the LAg-avidity EIA, with and without inclusion of viral load to confirm probable recent infections, among newly diagnosed HIV positive pregnant adolescent girls and young women who participated in the Malawi Recency Study (2017-18) in Blantyre, Lilongwe, Machinga and Zomba Disticts. (a) Sedia LAg-avidity EIA kits were used to test 121/210 DBS specimens and all 379 plasma specimens using an ODn of less than 2.0 to classify recent infections. (b) Maxim LAg-avidity EIA kits were used to test 89/210 DBS specimens using an ODn of less than 1.5 to classify recent infections. We used different cutoffs for classification of recent infection for the two assays because, at these cutoffs, the same MDRI of 161 days can be

applied uniformly to all specimens in the calculation of annualized incidence based on the correlation between the two assays at those thresholds. Viral load was measured to confirm classification of recent infections.



Tables 9-14 show the prevalence of recent infection among survey participants tested with the LAg-based RITA (including VL) in the four districts, calculated by district and by the demographic and behavioral characteristics described in Section 8.1.[3] Prevalence estimates and confidence intervals reported in the text are weighted for non-response. The prevalence of recent infection by district was 11.9% (95% CI: 9.0-15.8) in Blantyre, 13.7% (95% CI: 10.9-17.1) in Lilongwe, 10.7% (95% CI: 5.2-20.9) in Machinga, and 5.3% (95% CI: 2.8-9.7) in Zomba. The prevalence of recent infection was 13.6% (95% CI: 9.0-20.1) among AGYW aged 15-19 years and 11.0% (95% CI: 8.9-13.6) among AGYW aged 20-24 years.

Table 9: Prevalence of recent infection among newly diagnosed HIV positive pregnant adolescent girls and young women , overall and by demographic and current pregnancy characteristics, Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Recent infection			P-val.
	#	%	95% CI	
District				
Blantyre (n=184)	22	12.0%	[9.0-15.8]	0.10
Lilongwe (n=224)	32	14.3%	[10.9-17.1]	
Machinga (n=83)	9	10.8%	[5.2-20.9]	

[3] RITA results are based on tests performed by Maxim or Sedia LAg kits. Maxim LAg-avidity EIA kits were used to test 89/210 DBS specimens. Sedia LAg-avidity EIA kits were used to test 121/210 DBS specimens and all 379 plasma specimens.

Zomba (n=98)	5	5.1%	[2.8-9.7]	
Age group (5 year interval)				
15-19 years (n=163)	22	13.5%	[9.0-20.1]	0.38
20-24 years (n=426)	46	10.8%	[8.9-13.6]	
Age group (2 year interval)				
15-16 years (n=14)	2	14.2	[3.4-43.7]	0.56
17-18 years (n=86)	13	15.2	[9.0-24.5]	
19-20 years (n=145)	17	11.5	[7.2-17.8]	
21-22 years (n=165)	21	13.1	[9.3-18.2]	
23-24 years (n=179)	15	8.9	[5.6-13.8]	
Currently employed				
No (n=394)	48	11.7	[9.3-14.7]	0.99
Yes (n=193)	20	11.8	[8.1-16.8]	
Highest level of education completed				
Primary or less (n=372)	38	10.3	[7.7-13.7]	0.22
Secondary or more (n=212)	30	14.1	[10.1-19.4]	
Current marital status				
Never married (n=114)	17	14.9	[9.4-22.8]	0.52
Currently married (n=433)	47	11.2	[8.8-14.2]	
Divorced, separated or widowed (n=25)	3	10.1	[3.7-24.7]	
Age when first married (if ever married)^				
12-15 years (n=42)	3	9.0	[3.0-23.7]	0.50
16-19 years (n=250)	23	9.2	[6.4-13.0]	
20-24 years (n=109)	14	13.2	[8.2-20.4]	
Years since first married (if ever married)^				
0-1 (n=130)	19	14.7	[9.7-21.7]	0.16
2-5 (n=179)	13	8.0	[4.9-12.8]	
6 or more (n=72)	6	9.0	[4.3-18.0]	
Total (n=589)	**68**	**11.7**	**[9.8-13.9]**	

Recent infection defined as having a LAg-avidity EIA result of normalized optical density < 1.5 using Maxim TM kits or < 2.0 using Sedia TM kits and an HIV-RNA viral load of >1,000 copies/mL. The sum of the counts in strata of a given variable may be less than the total number of recent infections because of missing responses. Unless indicated by ^, missing data for each variable is <5%. Counts are not weighted, while percentages and 95% CI are weighted for non-response.

Table 10: Prevalence of recent infection among newly diagnosed HIV positive pregnant adolescent girls and young women, overall and by characteristics of current pregnancy, Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Recent infection			P-val.
	#	%	95% CI	
Gravidity				

1 (n=259)	38	14.8	[11.1-19.6]	0.07
2 or more (n=298)	29	10.0	[7.8-12.9]	
Trimester of pregnancy at ANC first-visit				
First trimester (n=114)	16	15.6	[10.4-22.7]	0.02
Second trimester (n=420)	51	12.0	[9.7-14.8]	
Third trimester (n=55)	1	1.5	[0.2-10.1]	
Current pregnancy was planned^				
No (n=43)	3	6.9	[2.5-17.6]	0.72
Yes (n=115)	10	8.7	[4.3-17.0]	
Total (n=589)	**68**	**11.7**	**[9.8-13.9]**	

Recent infection defined as having a LAg-avidity EIA result of normalized optical density < 1.5 using MaximTM kits or < 2.0 using SediaTM kits and an HIV-RNA viral load of >1,000 copies/mL. The sum of the counts in strata of a given variable may be less than the total number of recent infections because of missing responses. Unless indicated by ^, missing data for each variable is <5%. Counts are not weighted, while percentages and 95% CI are weighted for non-response.

Table 11: Prevalence of recent infection among newly diagnosed HIV positive pregnant adolescent girls and young women, overall and by demographic and current pregnancy characteristics, Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Recent infection			P-val.
	#	%	95% CI	
Age group at first sex				
10-17 years (n=286)	30	10.5	[8.0-13.7]	0.10
18-24 years (n=209)	30	14.7	[11.1-19.3]	
First sex was consensual				
No (n=108)	9	9.1	[4.2-18.7]	0.41
Yes (n=454)	57	12.7	[10.5-15.3]	
Number of years sexually active^				
0-1 (n=91)	9	10.3	[4.4-22.2]	0.31
2-3 (n=147)	25	16.7	[11.0-24.4]	
4 or more (n=246)	25	10.6	[7.7-14.4]	
Number of lifetime sex partners^				
1 (n=136)	16	11.8	[7.5-17.9]	0.84
2-3 (n=324)	36	11.4	[8.8-14.6]	
4 or more (n=52)	7	13.7	[7.6-23.5]	
Number of sex partner in past 6 months^				
0 (n=50)	4	8.8	[3.8-19.2]	0.75
1 (n=441)	51	11.6	[9.3-14.5]	
2 or more (n=39)	5	13.7	[5.9-28.6]	
Received money/gifts in exchanged for sex^				

Never (n=466)	51	11.0	[8.8-13.7]	0.09
Within the past year (n=52)	10	20.3	[11.2-33.9]	
More than one year ago (n=20)	1	4.4	[0.6-26.4]	
Frequency of condom use in past 12 months				
Never (n=300)	31	10.5	[7.3-14.7]	0.62
Sometimes (n=247)	33	13.6	[9.5-19.2]	
Always (n=21)	2	11.2	[2.9-34.9]	
Frequency of condom use since becoming pregnant				
Never (n=496)	61	12.4	[10.2-15.0]	0.75
Sometimes (n=57)	5	8.8	[3.7-19.7]	
Always (n=22)	2	10.9	[2.8-34.0]	
Total (n=589)	**68**	**11.7**	**[9.8-13.9]**	

Recent infection defined as having a LAg-avidity EIA result of normalized optical density < 1.5 using MaximTM kits or < 2.0 using SediaTM kits and an HIV-RNA viral load of >1,000 copies/mL. The sum of the counts in strata of a given variable may be less than the total number of recent infections because of missing responses. Unless indicated by ^, missing data for each variable is < 5%. Counts are not weighted, while percentages and 95% CI are weighted for non-response.

Table 12: Prevalence of recent infection among newly diagnosed HIV positive pregnant adolescent girls and young women, overall and by characteristics of current partner, Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Recent infection			P-val.
	#	%	95% CI	
Age of partner				
Younger, same age or < 5 years older (n=167)	19	11.7	[7.9-17.2]	0.44
5-10 years older (n=146)	14	9.5	[5.2-16.8]	
>10 years older (n=183)	19	10.6	[6.8-16.2]	
Don't know (n=66)	12	17.5	[10.6-27.5]	
Partner is circumcised^				
No (n=212)	27	12.6	[9.1-17.3]	0.64
Yes (n=313)	35	11.3	[8.6-14.6]	
Partner's circumcision type (if circumcised)^				
Traditional (n=166)	14	8.5	[5.7-12.6]	0.69
Medical (n=102)	10	9.7	[5.9-15.4]	
Partner's HIV status				
Negative (n=144)	10	7.2	[4.0-12.7]	0.16
Positive (n=61)	8	14.6	[7.2-27.6]	
Unknown (n=352)	47	13.0	[10.7-15.7]	
Total (n=589)	**68**	**11.7**	**[9.8-13.9]**	

Recent infection defined as having a LAg-avidity EIA result of normalized optical density < 1.5 using MaximTM kits or < 2.0 using SediaTM kits and an HIV-RNA viral load of >1,000 copies/mL. The sum of the counts in strata of a given variable may be less than the total number of recent infections because of missing responses. Unless indicated by ^, missing data for each variable is <5%. Counts are not weighted, while percentages and 95% CI are weighted for non-response.

Table 13: Prevalence of recent infection among newly diagnosed HIV positive pregnant adolescent girls and young women, overall and by alcohol, drug use and physical or emotional abuse, Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Recent infection			P-val.
	#	%	95% CI	
Consumed any alcohol in past 6 months				
No (n=490)	53	11.1	[8.8-14.0]	0.39
Yes (n=97)	15	14.6	[8.8-23.1]	
Abused alcohol in past 6 months				
No (n=530)	59	11.3	[9.3-13.8]	0.26
Yes (n=43)	8	17.4	[8.5-32.3]	
Ever used illicit drugs				
No (n=559)	63	11.6	[9.5-13.9]	0.67
Yes (n=17)	3	15.0	[4.4-40.6]	
Ever abused by a sexual partner				
No (n=434)	50	12.0	[9.6-14.8]	0.71
Yes (n=148)	17	10.9	[6.9-16.6]	
Abused during past 12 months				
No (n=533)	62	12.0	[9.9-14.5]	0.38
Yes (n=51)	5	8.1	[3.4-18.0]	
Abused by person who got you pregnant				
No (n=557)	66	12.1	[10.0-14.5]	0.12
Yes (n=26)	1	2.9	[0.4-19.2]	
Forced to have sex in past 12 months				
No (n=554)	62	11.4	[9.4-13.7]	0.32
Yes (n=33)	6	17.5	[7.5-35.7]	
Currently afraid of being abused by someone				
No (n=351)	45	12.5	[9.8-16.0]	0.27
Yes (n=20)	4	21.1	[8.7-43.0]	
Total (n=589)	**68**	**11.7**	**[9.8-13.9]**	

Recent infection defined as having a LAg-avidity EIA result of normalized optical density < 1.5 using MaximTM kits or < 2.0 using SediaTM kits and an HIV-RNA viral load of >1,000 copies/mL. The sum of the counts in strata of a given variable may be less than the total number of recent infections because of missing responses. Unless indicated by ^, missing data for each variable is <5%. Counts are not weighted, while percentages and 95% CI are weighted for non-response.

Table 14: Prevalence of recent infection among newly diagnosed HIV positive pregnant adolescent girls and young women, overall and by previous HIV testing, STI and receipt of DREAMS programs, Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Recent infection			P-val.
	#	%	95% CI	
Previously tested for HIV				
Never (n=179)	17	10.4	[6.8-15.7]	0.18
< 12 months ago (n=194)	29	14.8	[11.3-19.1]	
≥ 12 months ago (n=212)	21	9.7	[6.7-13.9]	
Place of last HIV test (if ever tested)^				
Community-based (n=15)	0	0.0	[0.0-21.8]	0.14
Facility-based (n=90)	12	13.1	[7.3-22.3]	
Symptoms of STI in past 12 months				
No (n=393)	45	11.6	[9.0-14.7]	0.81
Yes (n=182)	22	12.2	[8.5-17.2]	
Received treatment for STI (if any symptoms)				
No (n=102)	12	11.7	[6.7-19.8]	0.71
Yes (n=73)	10	14.0	[7.2-25.3]	
Ever diagnosed or treated for syphilis				
No (n=537)	63	12.0	[9.8-14.6]	0.67
Yes (n=32)	5	14.3	[6.6-28.1]	
Ever received HPV vaccine				
No (n=559)	67	12.1	[10.1-14.4]	0.19
Yes (n=11)	0	0.0	[0.0-28.5]	
Ever participated in teen club^				
No (n=130)	8	6.2	[3.2-11.7]	0.62
Yes – Go Girls club (n=17)	1	5.3	[0.7-31.2]	
Yes – another club (n=16)	2	11.9	[3.1-36.2]	
Ever participated in mother's club^				
No (n=142)	10	7.0	[3.9-12.3]	0.33
Yes (n=24)	3	12.5	[3.9-33.4]	
Total (n=589)	**68**	**11.7**	**[9.8-13.9]**	

Recent infection defined as having a LAg-avidity EIA result of normalized optical density < 1.5 using MaximTM kits or < 2.0 using SediaTM kits and an HIV-RNA viral load of >1,000 copies/mL. The sum of the counts in strata of a given variable may be less than the total number of recent infections because of missing responses. Unless indicated by ^, missing data for each variable is < 5%. Counts are not weighted, while percentages and 95% CI are weighted for non-response.

In bivariable models, the prevalence of recent infection was significantly higher among AGYW who were in their first trimester of pregnancy (15.6%, 95% CI: 10.4-22.7, *P* = 0.02) than in their second (12.0%, 95% CI: 9.7-14.8) or

third trimester (1.5%, 95% CI: 0.2-10.1) (Table 10). No other variables were significantly associated with recent HIV infection among pregnant, HIV-infected AGYW enrolled in the study.

8.4 HIV Prevalence and Annualized Estimates of HIV Incidence

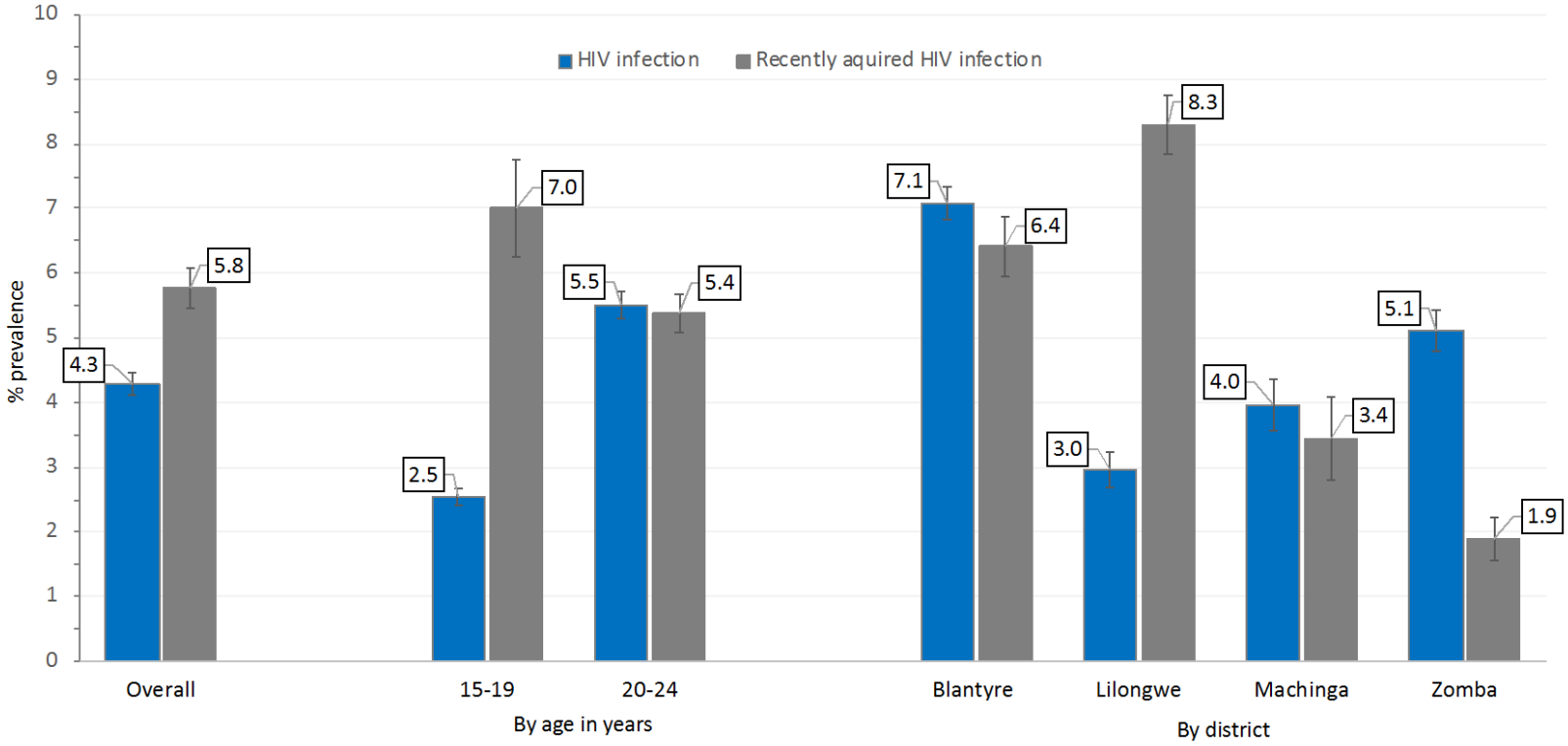
HIV prevalence was 4.3% overall, ranging from 3.0% in Lilongwe to 7.1% in Blantyre. Table 15 and Figures 4-5 show the annualized HIV incidence for pregnant AGYW attending ANC in the four districts, calculated using LAg RITA results among participants in combination with aggregate data of HIV status of all AGYW attending ANC. Among all AGYW attending ANC, the prevalence of recent infection was 5.8% (RSE: 10.5%) overall, 7.0% (RSE: 21.5%) among those aged 15-19 years and 5.4% (RSE: 11.2%) among those aged 20-24 years. By district, the prevalence of recent infection among all AGYW attending ANC was 6.4% (RSE: 14.5%) in Blantyre, 8.3% (RSE: 11.1%) in Lilongwe, 3.5% (RSE: 37.2%) in Machinga, and 1.9% (RSE: 35.3%) in Zomba.

Table 15: Estimates of HIV incidence among pregnant adolescent girls and young women in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Prevalence of HIV	Relative standard error (RSE)	Prevalence of recent infection	RSE	Annual-ized Incidence	95% CI low	95% CI high	*P*-val.
Overall	4.29	7.83	5.77	10.52	0.59	0.42	0.75	
By age								
15-24 years	2.54	10.20	7.00	21.51	0.41	0.22	0.61	Ref.
20-24 years	5.51	7.37	5.38	11.18	0.71	0.51	0.92	0.04
By district								
Blantyre	7.08	7.15	6.41	14.45	1.11	0.74	1.48	Ref.
Lilongwe	2.96	18.27	8.30	11.09	0.57	0.32	0.83	0.02
Machinga	3.97	20.27	3.45	37.20	0.32	0.00	0.60	<0.001
Zomba	5.11	12.42	1.90	35.32	0.23	0.01	0.40	<0.001

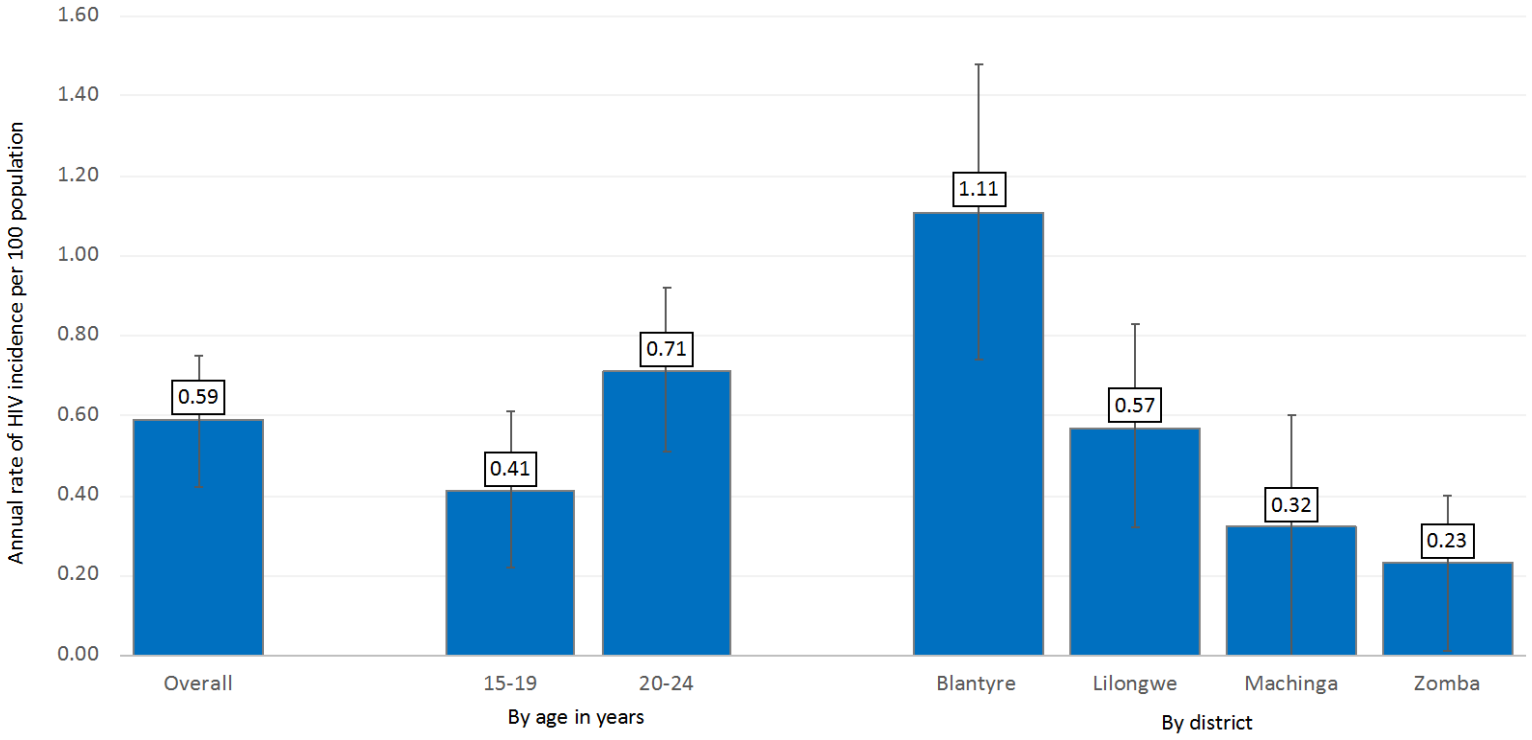
Incidence calculations were performed in R software version 1.1.442 (2009-2018 RStudio, Inc) using -inctools-, which is based on the published work by Kassanjee et al (2012). The following input parameters were used: LAg ODn cutoff < 1.5 or < 2.0 normalized optical density for specimens tested with Maxim and Sedia LAg kits, respectively; 2-year post-infection cutoff time; 161-day mean duration of recent infection; 0.00 false recency rate, and 1.06 design effect. HIV prevalence was calculated based on a census of data from all ANC attendees age 15-24 years at all sites during the study period and was therefore not weighted. Although they were not subject to recency testing as part of the study, all ANC attendees who were classified as previously diagnosed positive were assumed to be non-recent infections. Estimates are adjusted for non-response by age, district and HIV diagnosis (new or previously diagnosed) and clustering when estimating HIV incidence.

Figure 4: HIV prevalence and percentage of HIV infections that were recently acquired among pregnant adolescent girls and young women in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.



HIV prevalence was calculated based on a census of data from all ANC attendees age 15-24 years at all sites during the study period and was therefore not weighted. Although they were not subject to recency testing as part of the study, all ANC attendees who were classified as previously diagnosed positive were assumed to be non-recent infections. "Recently acquired HIV infection" calculated among HIV infected AGYW attending ANC only (i.e., not all AGYW attending AGYW). Estimates are adjusted for non-response by age, district and HIV diagnosis (new or previously diagnosed) and clustering when estimating HIV incidence. Error bars represent upper and lower limit of survey adjusted standard error.

Figure 5: Estimates of HIV incidence among pregnant adolescent girls and young women in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.



Incidence calculations were performed in R software version 1.1.442 (2009-2018 RStudio, Inc) using -inctools-, which is based on the published work by Kassanjee et al (2012). The following input parameters were used: LAg ODn cutoff < 1.5 or < 2.0 normalized optical density for specimens tested with Maxim and Sedia LAg kits, respectively; 2-year post-infection cutoff time; 161-day mean duration of recent infection; 0.00 false recency rate, and 1.06 design effect. Error bars represent exact 95% confidence intervals around the point estimate.

Overall annualized incidence was 0.59% (95% CI: 0.42 – Overall annualized incidence was 0.59% (95% CI: 0.42-0.75) among pregnant AGYW in the four districts. By district, incidence was significantly higher in Blantyre (1.11, CI: 0.74 – -1.48) than in Lilongwe (0.57, CI: 0.32-0 .83, *P*=0.02), Machinga (0.32, CI: 0.00-0.60, *P*=0.02), and Zomba (0.23, CI: 0.01-0.40, *P* <0.001) districts. Incidence was also significantly higher among AGYW aged 20-24 years (0.71, CI: 0.51-0.92, *P*=0.04) relative to those aged 15-19 years (0.41, CI: 0.22-0.61). Supplementary estimates of HIV incidence, which apply a FRR of 0.015, are presented in Appendix 2.

8.5. Return of Recency Results

Table 16 shows summary statistics about return of recency test results from the laboratory to the ANC sites and participants. Of the 589 LAg-avidity-based RITA that were completed, 586 results were returned to the ANC sites. Among these 586 results, 425 (72.5%) were returned to the ANC site within 28 days from the date that the participant was enrolled in the study. The percentage of results that were returned within 28 days was lowest in Lilongwe (62.1%), relative to Blantyre (73.4%), Machinga (84.1%) and Zomba (85.4%) districts. The percentage of participants that returned to the ANC sites and received their recency results was 78.6% overall, 72.3% in Machinga, 74.1% in Lilongwe, 83.7% in Blantyre and 84.7% in Zomba. Delays in the return of recency results were primarily attributed to participants not returning for their scheduled follow-up appointments.

Table 16: Return of recency test result from testing laboratories to ANC sites and newly diagnosed HIV positive pregnant adolescent girls and young women who enrolled in the Malawi Recency Study, Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

	Blantyre		Lilongwe		Machinga		Zomba		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Recency results reported to be returned to ANC site										
No	0	0	0	0	1	1.2	2	2	3	0.5
Yes	184	100	224	100	82	98.8	96	98	586	99.5
Recency results returned to ANC site within 28 days										
No	49	26.6	85	37.9	13	15.9	14	14.6	161	27.5
Yes	135	73.4	139	62.1	69	84.1	82	85.4	425	72.5
Recency results returned to participant										
No	30	16.3	58	25.9	23	27.7	15	15.3	126	21.4
Yes	154	83.7	166	74.1	60	72.3	83	84.7	463	78.6
Total	**184**	**100**	**224**	**100**	**83**	**100**	**98**	**100**	**589**	**100**

8.6. Evaluation of Asanté™ HIV-1 Rapid Recency Assay™ Performance

Table 17 shows the agreement between the Asanté™ HIV-1 Rapid Recency Assay (by reader or visual interpretation at the central laboratory and by visual interpretation at the ANC site) and the LAg-avidity EIA, with and without the inclusion of VL testing to confirm recent infection. Results in each cell of the matrix are displayed as the number of specimens that completed both tests or algorithms, their classification (i.e., recent or long-term) by both tests, the overall percent agreement between the two tests or algorithms, and the *kappa (k)* statistic of agreement between the two tests or algorithms.

Table 17: Agreement of LAg avidity EIA and Asanté™ HIV-1 Rapid Recency™-based algorithms performed on specimens collected from newly diagnosed HIV positive pregnant adolescent girls and young women who were enrolled in the Malawi Recency Study in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18.

		LAg avidity EIA		LAg-avidity EIA with viral load		Asanté™ reader at laboratory		Asanté™ reader at laboratory with viral load		Asanté™ visual at laboratory		Asanté™ visual at laboratory with viral load	
		Long-term	*Recent*	*Long-term*	*Recent*	*Long-term*	*Recent*	*Long-term*	*Recent*	*Long-term*	*Recent*	*Long-term*	*Recent*
Asanté™ reader at laboratory	*Long-term*	286	16										
	Recent	17	54										
	n (agreement), kappa	373 (91.2%), k= 0.71											
Asanté™ reader at laboratory with viral load	*Long-term*			309	12								
	Recent			6	40								
	n (agreement), kappa			367 (95.1%), k= 0.79									
Asanté™ visual at laboratory	*Long-term*	291	16			301	7						
	Recent	12	56			2	64						
	n (agreement), kappa	375 (92.5%), k=0.75				374 (97.6%), k=0.92							
Asanté™ visual at laboratory with viral load	*Long-term*			312	12			322	2				
	Recent			5	41			1	44				
	n (agreement), kappa			370 (95.4%), k=0.82				369 (99.2%), k=0.96					

Asanté™ visual at ANC site *	*Long-term*	71	7			71	5			71	5		
	Recent	34	21			30	21			30	21		
	n (agreement), kappa	133 (69.2%), k=0.32				127 (72.4%), k=0.38				127 (72.4%), k=0.38			
Asanté™ visual at ANC site * with viral load at laboratory	*Long-term*			79	5			78	4			74	9
	Recent			20	17			21	16			21	16
	n (agreement), kappa			121 (79.3%), k=0.45				119 (79.0%), k=0.44				120 (75.0%), k=0.36	

*Result in each cell of this matrix are displayed as follows: number of specimens that completed testing by both recent infection testing algorithms (the overall percent agreement between the two tests or testing algorithms in their classification of an infection as either recent or long term), k = kappa statistic of agreement between the two algorithms. The total number of tests performed is not identical accross cells because some specimens could not undergo all tests due to insufficient sample. The cutoffs used to define recent infection were ≤ 2.0 normalized optical density (ODn) for the Sedia LAg EIA and < 3.0 line intensity units for the Asanté™ reader. VL cutoff for conifrmation of recent infection is >1,000 copies/mL for LAg and Asanté™-based algorithms. * The Asanté™ test was done at a subset of 14 ANC sites in Lilongwe. Asanté™ at the ANC site was performed on whole blood specimens, whereas all other tests were performed on plasma at the central laboratory. Specimens were HIV negative by the Asante test and excluded from comparisons if the Asanté™ positive verification line was not present by visual interpretation or had line intensiy units <2.8 by the reader.*

There were 379 plasma specimens that underwent an Asanté™ test at the two central laboratories. However, the quantity or quality of some specimens was not sufficient to complete all of the indicated tests. Consequently, the number of specimens that completed both tests is not uniform across all cells of the table. Additionally, 132 of 133 specimens initially tested with Asanté™ at the Kamuzu Central Hospital laboratory underwent a second Asanté™ test at the Zomba Central Hospital Laboratory due to quality concerns about the initial test. For these specimens, only data from the second tests are included in Table 17 and Figure 7**.** There were also four specimens that had positive verification line intensity units < 2.8 by the Asanté™ reader or a positive verification line that was absent by visual interpretation; these tests were considered invalid and therefore excluded from any 2x2 comparisons.

Among tests performed at the central laboratory, the Asanté™ reader results agreed substantially with the LAg-avidity EIA (91.2%, k=0.71). The Asanté™ reader with VL results also agreed substantially with the LAg-avidity EIA with VL (95.1%, k=0.79). Also at the central laboratory, the Asanté™ result by visual interpretation agreed substantially with the LAg-avidity EIA (92.5%, k=0.75). The Asanté™ visual interpretation with VL results had almost perfect agreement with the LAg with VL (95.4%, k= 0.82). The Asanté™ reader and Asanté™ visual results also had almost perfect agreement (97.6%, k=0.92).

A total of 133 blood specimens were tested with Asanté™ by nurses at 14 ANC sites in Lilongwe. Overall agreement between the ANC-based Asanté™ visual interpretation and the LAg-avidity EIA at the lab was slight (69.2%, k=0.32). Agreement between the LAg-RITA and the ANC-based Asanté™ became moderate when a VL test was added (79.3%, k=0.45). Agreement between the Asanté™ visual interpretation at the ANC site with the Asanté™ reader at the laboratory was slight (72.4%, k=0.38) as was the agreement with Asanté™ visual at laboratory (72.4%, k=0.38).

Figure 6 shows a scatterplot of the LAg-avidity quantitative test results (x-axis) against the Asanté™ reader quantitative test results (y-axis) for each specimen that completed both tests (n=373). The cutoff thresholds for recency classification (ODn ≤2.0 for LAg and line intensity units <3.0 for the Asanté™ reader) are displayed as black-dashed lines intersecting the two axes. Four specimens (all from the Zomba lab) in which the line intensity units were <2.8 by the Asanté™ reader (which indicates an invalid test) were excluded from the comparison. The Spearman's *r* coefficient from results shown in Figure 6 was 0.71, indicating a strong positive correlation between the two tests.[4] Figure 7 and Figure 8 display the subsets of data from tests performed at the Kamuzu Central Hospital (n=133) and Zomba Central Hospital laboratories (n=242), respectively. The plot in Figure 6 includes the initial test data from the Kamuzu Central Hospital laboratory of the 132 that were later retested at the Zomba Central Hospital laboratory due to quality issues. The correlation of LAg and Asanté™ results was less

[4] The r correlation coefficient measures the strength of a linear relationship between two variables, for example the AsanteTM reader's line intensity units and LAg-EIA ODn values. In general, the r coefficient can be interpreted as follows: 0 (no linear relationship); 0.1– -0.29 (very weak linear relationship); 0.30 – -0.49 (weak linear relationship); 0.50-0.69 (moderate linear relationship); 0.70-0.99 (strong linear relationship); 1 (perfect linear relationship.).

at Kamuzu Central Hospital (*r*=0.56) relative to the correlation between the two tests at Zomba Central Hospital (r=0.75).

Figure 6: Scatter plot of recency test results from the LAg-avidity EIA and Asante Rapid Recency Assay non-recent line reader, all plasma specimens (n = 373). The cutoff thresholds for recency classification (≤ 2.0 ODn for LAg and <3.0 line intensity units for the Asanté™ reader) are displayed as black lines intersecting the two axes. Blue circles represent specimens classified as "recent" by the LAg-based algorithm (LAg EIA ≤ 2.0 ODn + viral load > 1,000 copies/mL) and blue triangles represent specimens classified as "non-recent" (LAg EIA > 2.0 ODn + viral load ≤1,000/mL copies). The red triangles that appear in the bottom left quadrant of the plot were classified as "potential recent" by LAg, but were reclassified as non-recent by the VL result. Four specimens in which the line intensity units were < 2.8 by the Asante reader (which indicates an HIV negative test) were excluded from the comparison.

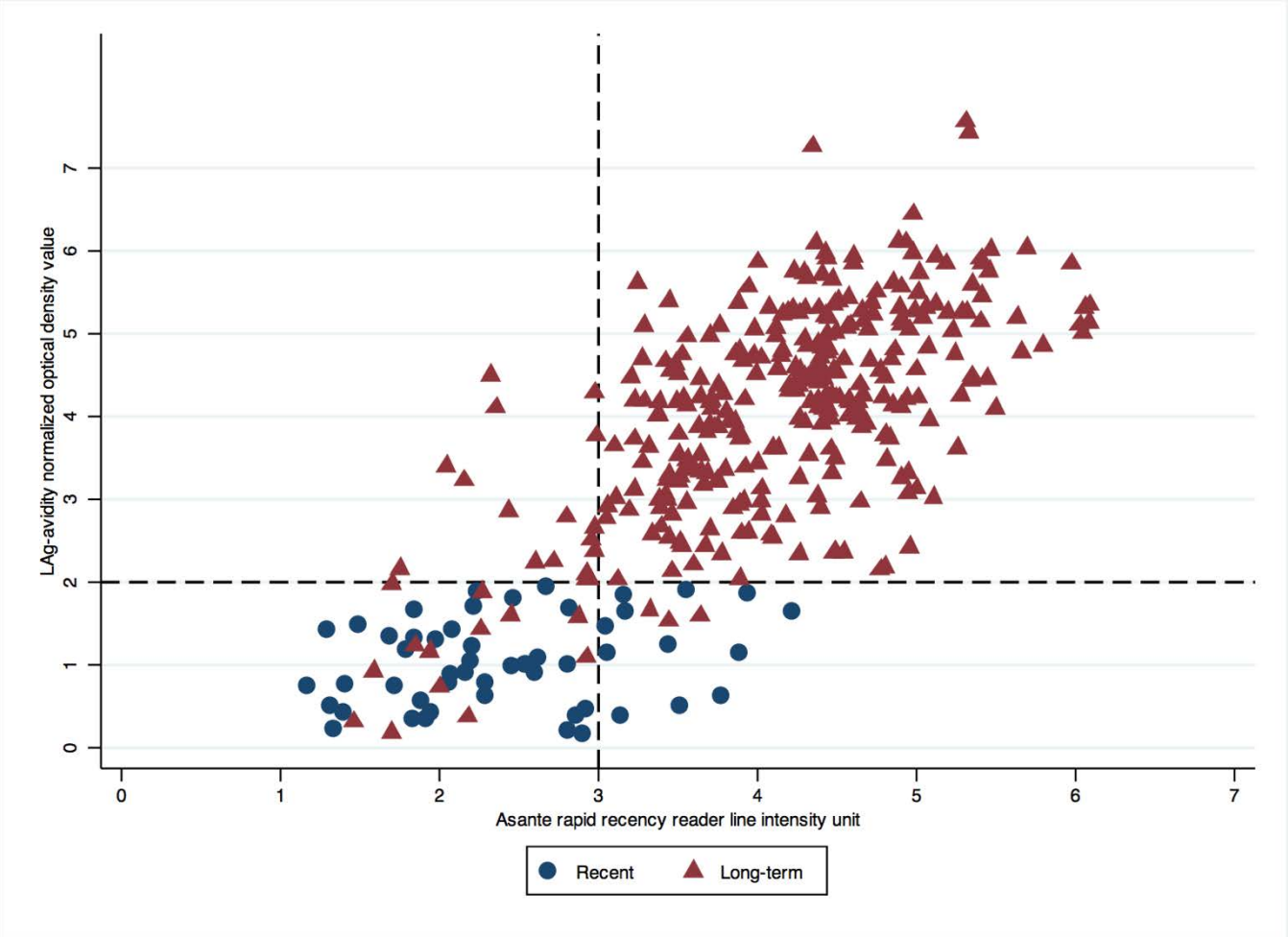


Figure 7: Scatter plot of recency test results from the LAg-avidity EIA and Asante Rapid Recency Assay non-recent line reader, plasma specimens tested at Kamuzu Central Hospital laboratory (n = 133). The cutoff thresholds for recency classification (≤ 2.0 ODn for LAg and <3.0 line intensity units for the Asanté™ reader) are displayed as black dashed lines intersecting the two axes. Blue circles represent specimens classified as "recent" by the LAg-based algorithm (LAg EIA ≤ 2.0 ODn + viral load > 1,000 copies/mL) and blue triangles represent specimens classified as "non-recent" (LAg EIA > 2.0 ODn + viral load ≤ 1,000 copies/mL). The red triangles that appear in the bottom left quadrant of the plot were classified as "potential recent" by LAg, but were reclassified as non-recent by the VL result. Unlike Table 17 and Figure 6, this plot includes the initial test data from the 133 that were later retested at the Zomba Central Hospital laboratory.

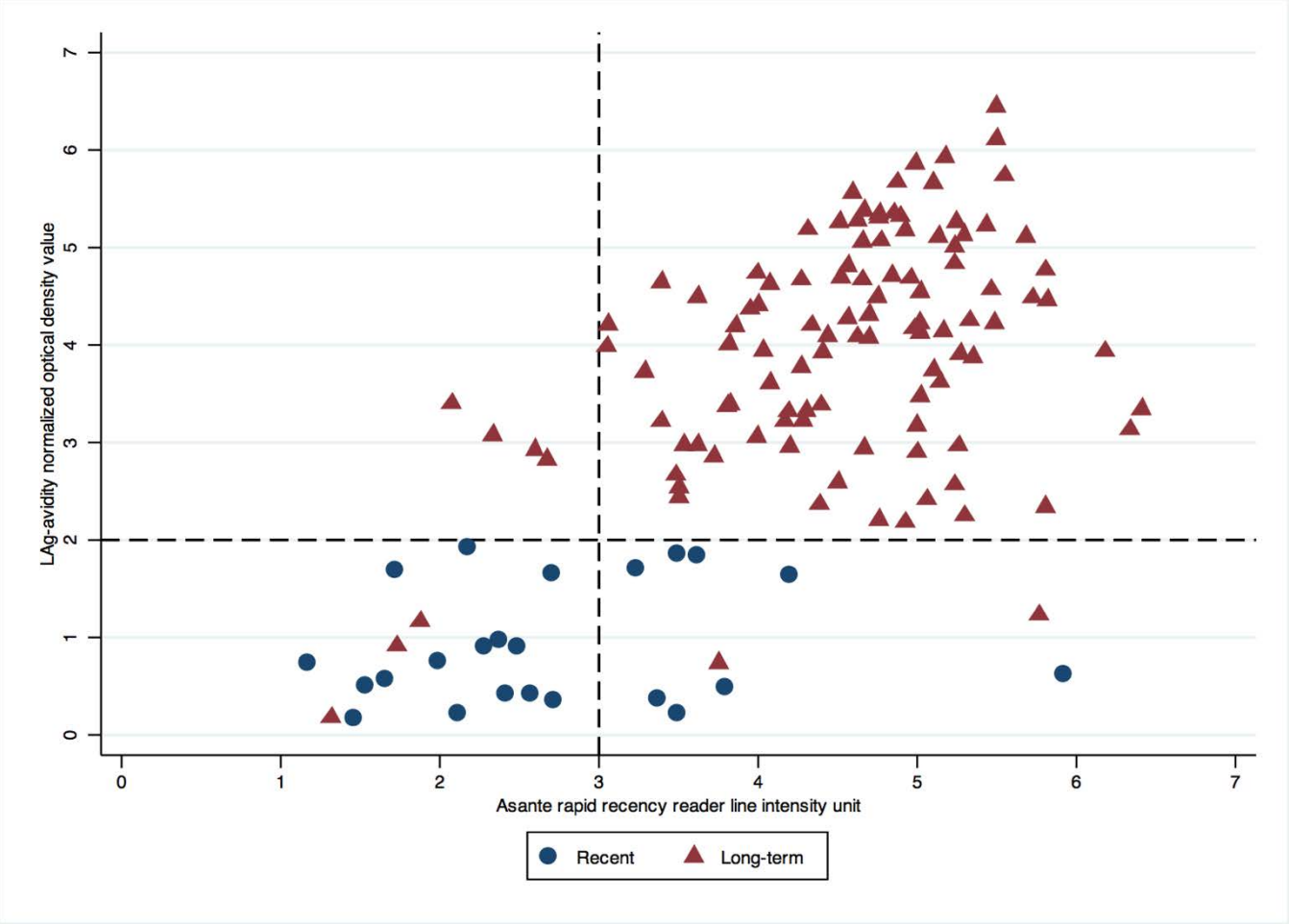
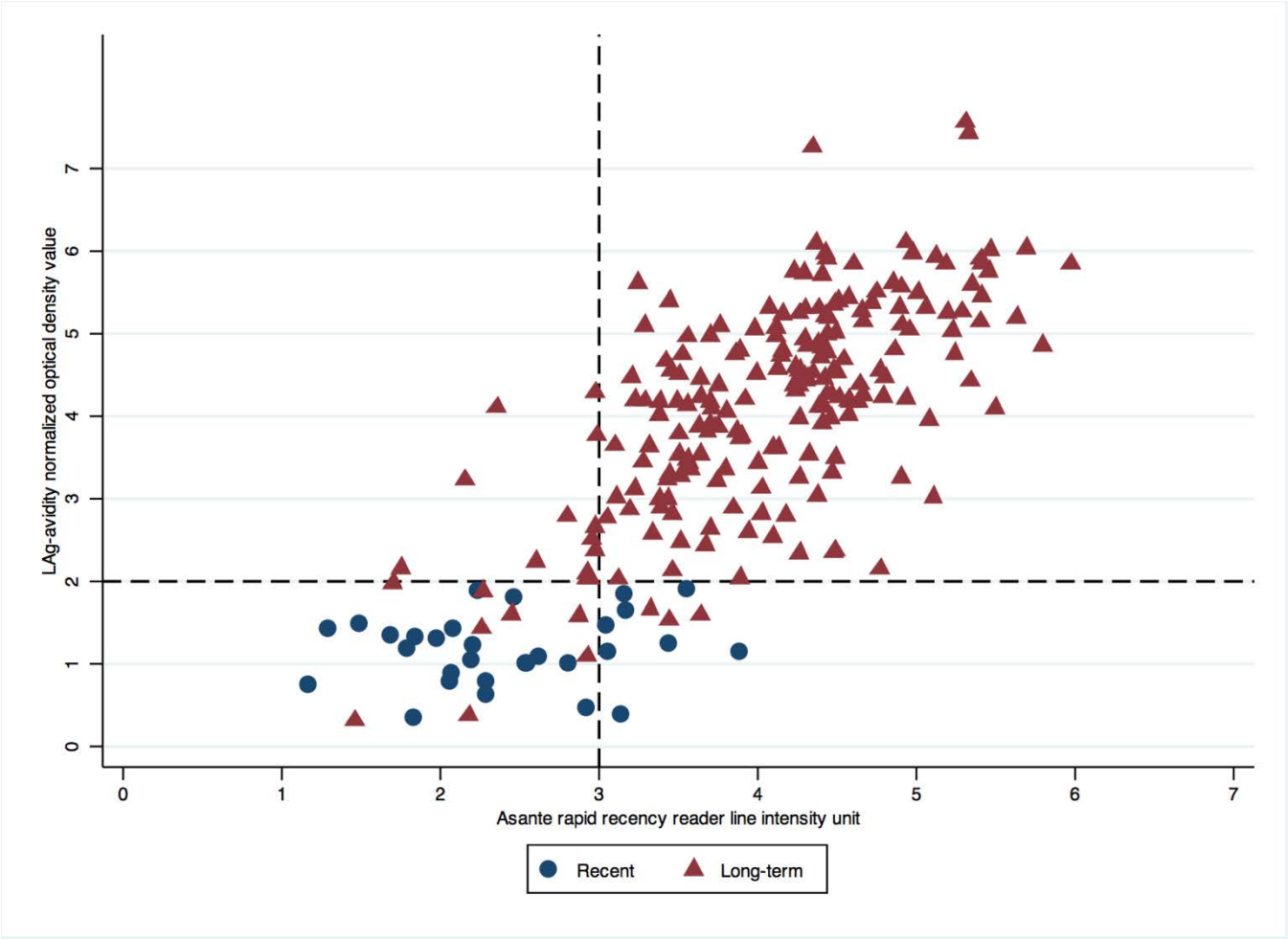


Figure 8: Scatter plot of recency test results from the LAg-avidity EIA and Asante Rapid Recency Assay non-recent line reader, plasma specimens tested at Zomba Central Hospital laboratory (n = 246). The cutoff thresholds for recency classification (≤ 2.0 ODn for LAg and <3.0 line intensity units for the Asanté™ reader) are displayed as black dashed lines intersecting the two axes. Blue circles represent specimens classified as "recent" by the LAg-based algorithm (LAg EIA ≤ 2.0 ODn + viral load > 1,000 copies/mL) and blue triangles represent specimens classified as "non-recent" (LAg EIA > 2.0 ODn + viral load ≤ 1,000 copies/mL). The red triangles that appear in the bottom left quadrant of the plot were classified as "potential recent" by LAg, but were reclassified as non-recent by the VL result. Four specimens in which the line intensity units were < 2.8 by the Asante reader (which indicates an HIV negative test) were excluded from the comparison.



9.0 Discussion

9.1 Summary of Key Findings

The Malawi Recency Study met each of its primary objectives: producing estimates of HIV incidence, describing the prevalence and potential correlates of recent infection and providing data about the feasibility of using POC tests for recent infection among AGYW in high-prevalence districts of Malawi. In doing so, the study has demonstrated a framework for surveillance of recent HIV infection that can be used in ANC and other settings where routine HTS services are provided, in Malawi and elsewhere. Results from the Malawi Recency Study also provide important and timely information that can be used to inform HIV testing, prevention and treatment interventions among AGYW and their male partners.

Estimates of HIV incidence

The annualized rate of HIV incidence among pregnant AGYW across the four districts was 0.59 (95% CI: 0.42-0.75), which equates to 59 new infections per 10,000 uninfected pregnant AGYW each year. This estimate can be used as a benchmark against which programs designed to reduce new infections among AGYW by 70% by 2020 can be measured in the coming years [2]. HIV incidence in the Malawi Recency Study was slightly higher than the estimate from the nationally representative 2015-16 MPHIA, which estimated national incidence to be 0.37 per annum (95% CI: 0.20-0.54) among all adults aged 15-64 years and 0.38 per annum (95% CI: 0.02-0.74) among AGYW [1]. These results provide further evidence that AGYW – particularly those who are pregnant – are an important sub-population who contribute to the overall rate of new infections nationwide. Relatively high HIV incidence observed among pregnant AGYW in the Malawi Recency Study is likely attributable to the fact that all AGYW who contributed data were – in becoming pregnant – sexually active within the past 9 months, whereas MPHIA included AGYW that were sexually active and not sexually active. It is also possible that the AGYW in the four study districts were not representative of AGYW in other districts in Malawi. Therefore, a definitive comparison of the incidence estimates generated by the Malawi Recency Study and MPHIA is discouraged, as the target population for the former was unlikely to have been representative of all AGYW in Malawi. This is discussed in greater detail in Section 9.2.

Differences in HIV incidence by geography and age that were detected by the Malawi Recency Study point to where additional prevention resources may have the greatest impact. By district, incidence among pregnant AGYW was significantly higher in Blantyre (1.11, CI: 0.74-1.48) compared to Lilongwe (0.57, CI: 0.32-0.83, P=0.02), Machinga (0.32, CI: 0.00-0.60, P <0.001) and Zomba (0.23, CI: 0.01-0.40, P <0.001) districts. Additionally, the Recency Study results align with estimated geographic variations in viral load suppression (VLS) among all people living with HIV in Malawi. The MPHIA found that VLS is lowest in Blantyre (59.9%) and Lilongwe (64.9%), highest in the south-East (71%) – which includes Zomba and Machinga districts – and low among males across the country (60.9%) [1]. The likelihood of onward transmission of HIV is higher when the VLS is lower [15, 16, 17]. Therefore, interventions aimed at reducing new infections among AGYW must also include strategies to increase HIV serostatus awareness and initiation of and adherence to ART and VLS among men. These interventions may include targeted index-case testing, self-testing, work-place testing and extended clinic hours for testing and treatment at facility and community sites.

The Malawi Recency Study also found that HIV incidence was significantly higher among AGYW aged 20-24 years relative to those aged 15-19 years. This difference may indicate that AGYW may be at particularly high risk for acquiring HIV in the years that mark their transition into adulthood. This finding is important in that it highlights

the evolving context of risk and the need to tailor age-appropriate interventions to AGYW across the 10-year period from adolescence into early adulthood. For example, school-based prevention interventions may be an efficient means of reaching younger AGYW but could fail to reach older AGYW who have passed their school-going years. Ability to make decisions regarding marriage and reproduction – and subsequently the context of risk – may also vary between younger and older AGYW. Although it was not possible to assess behavioral correlates of incidence in this study due to the small sample size, the higher HIV incidence among older AGYW may be attributable to patterns of coupling with older male partners. Older study participants were more likely to have male partners who were older by 5-10 years or more, whereas younger participants were more likely to have partners who were the same or closer in age. This characteristic of older study participants – which has been associated with increased HIV acquisition in other settings [18, 19, 20] – is likely to reflect the broader population characteristics of AGYW and may contribute to the relatively high rate of incidence among older AGYW across the four districts. More information about factors contributing to the relative risks for HIV infection between older and younger AGYW is needed.

Characterization of recent and long-term HIV infection.

The Malawi Recency Study is the first to estimate the prevalence of recent HIV infection and describe the characteristics of recent infection among newly diagnosed pregnant AGYW in a public ANC setting. Therefore, estimates that are comparable in person, place and method are scarce. Perhaps the most important finding was that 11.7% of HIV-infected AGYW who participated in the Malawi Recency Study had an infection that was acquired within the past 12 months. Conversely, almost 90% had a long-term infection that was acquired more than 12 months ago. These findings have several implications. Firstly, routine HTS services are not reaching AGYW with sufficient frequency to identify new infections within a sufficiently short period of time after they are acquired. The vast majority of AGYW in the Malawi Recency Study had an undiagnosed, unsuppressed and transmissible virus for at least 12 months prior to their ANC first visit. This means that most AGYW acquired their HIV infection before their current pregnancy and reported having at least one sexual partner (i.e., the impregnating partner) since becoming infected. It was not possible to determine from whom AGYW acquired their HIV infection. Nonetheless, these findings highlight the potential for onward transmission from AGYW to any HIV-uninfected partners that they may have had since becoming infected [21, 22]. Further research on barriers to service access and utilization is needed to improve early diagnosis and health outcomes among these AGYW. In the meantime, alternative models of testing, such as community-based testing, self-testing and index partner tracing among males newly diagnosed with HIV may be needed to improve HIV diagnosis among AGYW.

Evaluation of the Asanté™ rapid recency assay

The agreement between the Asanté™ assay and the LAg EIA-based RITA was robust in the central laboratories, with and without the inclusion of VL testing. However, agreement between the LAg-based RITA and the Asanté™ assay when it was performed at the ANC site by nurses was much lower. These data indicate that standardized guidelines, training and supervisory support structures, and consideration of other trained health cadres to conduct testing would need to be developed and assessed prior to expanded use of rapid recency assays. Enhanced monitoring and continuous quality improvement plans will also be needed to ensure that recency results are as accurate and reliable at the site level as they are in high quality laboratory settings. An efficient approach could be to incorporate recency testing into national HIV testing guidelines, trainings and supervisory infrastructure of routine HTS services.

Importantly, we did observe better performance of the Asanté™ assay when VL testing was included to confirm probable recent results. Consistent with previous studies [11], inclusion of the VL reduced the proportion of false recent results, likely due to participants on ART misreporting that they had not been previously diagnosed. This has important implications for the potential use of rapid recency testing in clinical settings. Assuming recency testing is performed at the point of patient care, systems for incorporation of laboratory-based VL tests to confirm probable rapid-recency test results should be considered. For instance, informing patients of their probable recent infection test results and requesting that they provide additional blood specimens for VL testing could improve the performance of the algorithm. Systems for VL testing in the context of recency testing could perhaps be incorporated into established specimen referral networks for VL scale-up and monitoring.

9.2 Limitations

Several aspects of the study methodology and implementation may have affected the interpretation of the results:

- The Malawi Recency Study was only conducted in Blantyre, Lilongwe, Machinga and Zomba districts. Therefore, the results from the study may not reflect the characteristics of AGYW in other districts of Malawi.

- Pregnant AGYW seeking ANC are not representative of all AGYW. Pregnant AGYW – who are by definition sexually active and have not used condoms consistently within the past 9 months – may have higher incidence than the broader population of AGYW, who may not be sexually inactive or using condoms consistently.

- Enrollment in the Malawi Recency Study was lower than anticipated. Low enrollment was primarily due to non-availability or suboptimal engagement of trained study staff. Low levels of participation in surveys may limit the generalizability of findings and bias results if the characteristics of those who did not participate were different from those who did participate. We observed differences in enrollment rates by age group and district. Consequently, we applied post-stratification weights to limit potential bias from non-participation. Future surveys that plan to use existing ANC staff should consider strategies for improving staff engagement and availability so that all eligible persons are given the opportunity to participate.

- The demographic and behavioral characteristics of AGYW – and their potential correlation with recent infection – were based on the self-reported responses of participants, which may be prone to some response bias. For example, AGYW may have underreported their number of recent sexual partners or over-reported condom use given the social desirability of those behaviors. It is also possible that participants did not understand all questions asked of them. However, ANC staff were trained to make participants feel comfortable in order to reduce social desirability bias and to ask probing questions to clarify seemingly inconsistent or incorrect responses.

- The study's definition of recent infection using the LAg Avidity EIA is based on a cutoff of ODn ≤ 2.0 and VL > 1 000 copies/mL and also assuming an MDRI of 161 days. These cut-offs are summary values and may not always give a representative incidence estimation for pregnant AGYW attending ANC, all pregnant AGYW or all AGYW in all health districts in Malawi. Furthermore, the RITA is an antibody-based

algorithm and will miss some AGYW with acute HIV infection. VL testing was included to exclude those who are on ART, but some false recent infections may still have been missed [18].

- We assumed that all ANC attendees who were classified as previously known positive were "long-term" infections. It is possible that some unknown proportion of self-reported known positives were infected within the past 161 days, thus underestimating the number of recent infections in this population. Consequently, some bias would be introduced to the study's incidence estimates. More research is needed to understand the potential for bias and analytic optimization of incidence estimates that incorporate data from persons with previously diagnosed HIV infection who are not subject to recency testing.

- Although the LAg-Avidity EIA was used as a reference standard against which the Asanté™ assay was validated, there is no "gold standard" laboratory assay for detecting recent infection. Some specimens identified as "recent" or "long-term" by either assay used in the Malawi Recency Study may have been mischaracterized.

- The study did not collect data about participants' experiences after the return of recency results, including partner disclosure or subsequent events. There is a possibility that participants who chose to disclose their recency tests to their partners may have experienced adverse consequences, including intimate partner violence. More data are needed to understand how the potential risks of returning recency test results to participants and disclosure of results to partners can be minimized.

10.0 Conclusions and Recommendations

Results from the Malawi Recency Study may be used to inform future applications of recent HIV infection surveillance and to improve HIV testing, prevention and treatment interventions among AGYW and their male partners. The incidence estimate of 0.59 per 100 AGYW per annum provides a baseline benchmark against which prevention programs may be measured in the coming years for the four participating districts. Prevention and early diagnosis of HIV infections among AGYW and their partners appear to be among the most critical challenges that MOH and its partners may need to address.

In order to expand the use of recency testing and surveillance to more clinical and community-based settings where HTS services are provided, several technical and logistical challenges may be considered. Further development of robust training and monitoring tools, including use of QC specimens and proficiency testing, are needed to ensure high test accuracy outside of a laboratory setting. Finally, incorporation of standardized guidelines, training curricula and supervisory support structures for recency testing into existing routine HTS will help to facilitate the expansion of this new and potentially impactful method for tracking and responding to emergent patterns in the HIV epidemic.

11.0 References

1. Ministry of Health, Malawi. Malawi Population-based HIV Impact Assessment (MPHIA) 2015-16: First Report. Lilongwe, Ministry of Health. November 2017.

2. Ministry of Health, Malawi. National HIV Prevention Revised Strategy, 2018: Lilongwe, Ministry of Health. December 2018 [Unpublished].

3. UNAIDS, The Gap Report 2014 - Adolescent Girls and Young Women. 2014.

4. DREAMS - Working Together for an AIDS-free Future for Girls and Women. The United States President's Emergency Plan For AIDS Relief.

5. Kassanjee R, McWalter TA, Bärnighausen T, Welte A. A new general biomarker-based incidence estimator. Epidemiology. 2012; 23(5): 721-728.

6. Justman J, et al. Real progress in the HIV epidemic: PHIA findings from Zimbabwe, Malawi, and Zambia. Conference on Retroviruses and Opportunistic Infections, Seattle, Washington, February 13-17, 2017; Abstract 114LB.

7. Aghaizu, A., et al., Recent infection testing algorithm (RITA) applied to new HIV diagnoses in England, Wales and Northern Ireland, 2009 to 2011. Euro Surveill, 2014. 19 (2).

8. Granade, T, et al. Development of a novel rapid HIV test for simultaneous detection of recent of long-term HIV type 1 infection using a single testing device. AIDS Res Hum Retroviruses. 2013; 29(1):61-7.

9. Smith, PR, et al. Characterization of a rapid HIV-1 incidence assay based on a CDC-developed multisubtype antigen in a commercial HIV lateral flow assay format, 2014 AIDS Conference, Durban, South Africa; Abstract WEPE105.

10. Kassanjee, R, et al. Independent assessment of candidate HIV incidence assays on specimens in the CEPHIA repository. AIDS, 2014. 28:2439-2449.

11. Duong YT, Kassanjee R, Welte A, Morgan M, De A, Dobbs T, et al. (2015) Recalibration of the Limiting Antigen Avidity EIA to Determine Mean Duration of Recent Infection in Divergent HIV-1 Subtypes. PLoS ONE 10(2): e0114947. https://doi.org/10.1371/journal.pone.0114947

12. EK Rottinghaus, T, et al. Performance of the Maxim Limiting Antigen Avidity EIA for use with Dried Blood Spot Specimens, Conference on Retroviruses and Opportunistic Infections, Boston, MA, March 3-6, 2014.

13. Kassanjee, R., et al., Viral load criteria and threshold optimization to improve HIV incidence assay characteristics. AIDS, 2016. 30(15): p. 2361-71.

14. R package created by Welte, A, Grebe, E, et. al, South African Centre for Epidemiological Modelling and Analysis, Stellenbosch University. Available for download at http://www.incidence-estimation.org/page/inctools

15. Tanser, F, et al. Effect of population viral load on prospective HIV incidence in a hyperendemic rural African community. Sci Transl Med. 2017 Dec 13; 9(420).

16. Jain, V, et. al. Population levels and geographical distribution of HIV RNA in rural Ugandan and Kenyan communities, including serodiscordant couples: a cross-sectional analysis. Lancet HIV. 2017 Mar; 4(3):e122-e133.

17. Solomon, S. The holy grail of HIV programme effectiveness, Lancet. 2015; 4(3): p. 96-98

18. Schaeffer R et al. *Age-disparate relationships and HIV incidence in adolescent girls and young women: evidence from a general-population cohort in Zimbabwe*. AIDS, online edition, 2017. DOI: 10.1097/QAD.0000000000001506

19. de Oliveira T, Kharsany AB, Gräf T, Cawood C, et. al. Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study. Lancet HIV. 2017 Jan;4(1):e41-e50. doi: 10.1016/S2352-3018(16)30186-2.

20. Maughan-Brown B, Kenyon C, Lurie MN. Partner age differences and concurrency in South Africa: Implications for HIV-infection risk among young women. AIDS Behav. 2014;18(12):2469-76.

21. Pilcher, C.D., et al., Brief but efficient: acute HIV infection and the sexual transmission of HIV. J Infect Dis, 2004. 189(10): p. 1785-92.

22. Pilcher, C.D., et al., Sexual transmission during the incubation period of primary HIV infection. JAMA, 2001. 286(14): p. 1713-4.

23. Performance of a novel point-of-care HIV recency test among newly diagnosed pregnant adolescent girls and young women — Malawi, 2017. E. Agyemang, T. Dobbs, I. Zungu, A. Kim, A. Adhikari, E. Kim, H. Kwalira, H. Limula, A. Auld, J. Kandulu, R. Nyirenda, K. Curran, B. Parekh. 22nd International AIDS conference, Amsterdam, 23-27 July 2018. Abstract # THPEC200.

Appendix 1a. Calculation of post-stratification non-response weights for estimating prevalence and correlates of recent infection among *participating AGYW who completed recency testing, applied to results shown in Tables 9-14.*

Population distribution *(new positives from census of ANC data)*		
Strata	**#**	**%**
Blantyre 15-19	108	0.093
Blantyre 20-24	322	0.278
Lilongwe 15-19	109	0.094
Lilongwe 20-24	356	0.307
Machinga 15-19	25	0.022
Machinga 20-24	94	0.081
Zomba 15-19	48	0.041
Zomba 20-24	97	0.084
TOTAL	**1,159**	**1.00**

Sample distribution *(new positive survey participants who completed recency testing)*		
Strata	**#**	**%**
Blantyre 15-19	43	0.073
Blantyre age 20-24	141	0.239
Lilongwe 15-19	66	0.112
Lilongwe age 20-24	158	0.268
Machinga 15-19	19	0.032
Machinga age 20-24	64	0.109
Zomba 15-19	35	0.059
Zomba age 20-24	63	0.107
TOTAL	**589**	**1.00**

Post stratification weights *(% pop. / % sample)*	
Strata	**weight**
Blantyre 15-19	1.276
Blantyre 20-24	1.161
Lilongwe 15-19	0.839
Lilongwe 20-24	1.145
Machinga 15-19	0.669
Machinga 20-24	0.746
Zomba 15-19	0.697
Zomba 20-24	0.782

Appendix 1b: Calculation of post-stratification non-response weights for estimating the prevalecence of recent infection and annualized incidence among all AGYW attending ANC, *Table 17*.

Population distribution. *n = number of known or new positive AGYW using census of data abstracted from ANC registers.*

Strata	n	Pop. % (n/ N Total)
Blantyre 15-19, known pos	79	0.034
Blantyre 15-19, new pos	108	0.047
Blantyre 20-24, known pos	277	0.120
Blantyre 20-24, new pos	322	0.140
Lilongwe 15-19, known pos	53	0.023
Lilongwe 15-19, new pos	109	0.047
Lilongwe 20-24, known pos	242	0.105
Lilongwe 20-24, new pos	356	0.154
Machinga 15-19, known pos	65	0.028
Machinga 15-19, new pos	25	0.011
Machinga 20-24, known pos	175	0.076
Machinga 20-24, new pos	94	0.041
Zomba 15-19, known pos	70	0.030
Zomba 15-19, new pos	48	0.021
Zomba 20-24, known pos	187	0.081
Zomba 20-24, new pos	97	0.042
N total	**2307**	**1.00**
known positive	*1148*	
new positive total	*1159*	

Sample distribution. *n = number of known positive AGYW using census of data abstracted from ANC registers [a] or number new positive AGYW who were enrolled in the study and completed recency testing[b]*

Strata	n	Samp. % (n/N total)
Blantyre 15-19, known pos [a]	79	0.045
Blantyre 15-19, new pos [b]	46	0.026
Blantyre 20-24, known pos [a]	277	0.158
Blantyre 20-24, new pos [b]	146	0.083
Lilongwe 15-19, known pos [a]	53	0.030
Lilongwe 15-19, new pos [b]	67	0.038
Lilongwe 20-24, known pos [a]	242	0.138
Lilongwe 20-24, new pos [b]	164	0.094
Machinga 15-19, known pos [a]	65	0.037
Machinga 15-19, new pos [b]	20	0.011
Machinga 20-24, known pos [a]	175	0.100
Machinga 20-24, new pos [b]	66	0.038
Zomba 15-19, known pos [a]	70	0.040
Zomba 15-19, new pos [b]	34	0.019
Zomba 20-24, known pos [a]	187	0.107
Zomba 20-24, new pos [b]	63	0.036
N total	**1754**	**1.00**
known positive	*1148*	
New positive total	*606*	

Post-stratification weights *(Pop. % / Samp.%)*

Strata	weight
Blantyre 15-19, known pos	0.760
Blantyre 15-19, new pos	1.785
Blantyre 20-24, known pos	0.760
Blantyre 20-24, new pos	1.677
Lilongwe 15-19, known pos	0.760
Lilongwe 15-19, new pos	1.237
Lilongwe 20-24, known pos	0.760
Lilongwe 20-24, new pos	1.650
Machinga 15-19, known pos	0.760
Machinga 15-19, new pos	0.950
Machinga 20-24, known pos	0.760
Machinga 20-24, new pos	1.083
Zomba 15-19, known pos	0.760
Zomba 15-19, new pos	1.073
Zomba 20-24, known pos	0.760
Zomba 20-24, new pos	1.171

Appendix 2: Estimates of HIV incidence among pregnant adolescent girls and young women in Blantyre, Lilongwe, Machinga and Zomba districts, 2017-18; *Using adjusted false recency rate of 0.01*

	Prevalence of HIV	Relative standard error (RSE)	Prevelence of recent infection	RSE	Annulized Incidence	95 % CI low	95 % CI high	*P*-val.
Overall	4.29	7.83	5.77	10.52	0.51	0.34	0.67	
By age								
15-24 years	2.54	10.20	7.00	21.51	0.37	0.17	0.57	Ref.
20-24 years	5.51	7.37	5.38	11.18	0.61	0.41	0.81	0.11
By district								
Blantyre	7.08	7.15	6.41	14.45	0.98	0.60	1.36	Ref.
Lilongwe	2.96	18.27	8.30	11.09	0.53	0.29	0.77	0.04
Machinga	3.97	20.27	3.45	37.20	0.24	0.00	0.51	0.002
Zomba	5.11	12.42	1.90	35.32	0.12	0.00	0.29	<0.001

Incidence calculations were performed in R software version 1.1.442 (2009-2018 RStudio, Inc) using -inctools-, which is based on the published work by Kassanjee et al (2012). The following input parameters were used: LAg ODn cutoff < 1.5 or < 2.0 normalized optical density for specimens tested with Maxim and Sedia LAg kits, respectively; 2-year post-infection cutoff time; 161-day mean duration of recent infection; ***0.01 false recency rate,*** *and 1.06 design effect. HIV prevalence was calculated based on a census of data from all ANC attendees age 15-24 years at all sites during the study period and was therefore not weighted. Although they were not subject to recency testing as part of the study, all ANC attendees who were classified as previously diagnosed positive were assumed to be non-recent infections. Estimates are adjusted for non-response by age, district and HIV diagnosis (new or previously diagnosed) and clustering when estimating HIV incidence.*