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TITLE:

Characterizing persons diagnosed with HIV as either recent or long-term using recent infection surveillance data in Malawi

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SHORT TITLE: Characterizing recent HIV infections in Malawi

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Surveillance, recent infection testing algorithm, RITA, rapid test for recent infection, RTRI, HIV, HIV prevention

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ABSTRACT

Objectives: In Malawi a recent infection testing algorithm (RITA) is used to characterize infections of persons newly diagnosed with HIV as recent or long-term. This paper shares results from recent HIV infection surveillance, with the aim of describing the distribution, and predictors of recent infections among people newly diagnosed with HIV.

Setting: Data from 155 health facilities in 11 districts in Malawi were pooled from September 2019 to March 2020.

Participants: Eligible participants were ≥13 years, and newly diagnosed with HIV. Client's HIV infections were then categorized as a RITA recent infection if the rapid test for recent infection (RTRI) test result was recent and viral load (VL) ≥1,000 copies/mL. RITA recent infections were stratified by age, sex, pregnancy/breastfeeding status, and district. Factors associated with recent infection were modeled using logistic regression.

Results: Of the 14,022 eligible persons approached, 13,838 (98.7%) consented to recent HIV infection surveillance. Of the 12,365 participants included in the analysis, 446 (3.6%) met the definition of RITA recent infection. The highest percentage of infections were classified as recent among newly diagnosed females (4.3%), young people aged 15-24 years-old (5.8%), persons who reported a negative HIV test within the past 12 months (6.1%), and breastfeeding women (7.7%). Factors associated with recent versus long-term infection in multivariate

analysis included aged 15-24 years (Adjusted Odds Ratio [AOR] 1.5; CI 1.2-1.8), having a negative HIV test within the previous 12 months (AOR 3.0; CI 2.3-3.8) and residents of Machinga (AOR 1.9; CI 1.1-3.3) and Mzimba (AOR 2.5; CI 1.3-4.7) districts. Persons 45 and older were less likely to be recent (AOR 0.6; CI 0.4-0.9).

Conclusions: Malawi's recent HIV infection surveillance system demonstrated high uptake and identified sub-populations of new HIV diagnoses with a higher percentage of recent infections. Females and young people may benefit from targeted HIV prevention efforts.

INTRODUCTION:

The East and Southern Africa regions bear the highest burden of the global HIV/AIDS epidemic. In 2018, the number of people living with HIV (PLHIV) in these two regions was approximately 20.6 million, accounting for 47% of HIV infections worldwide [1]. In 2018, the HIV prevalence in Malawi was estimated at 9.2%, with almost one million PLHIV and 38,000 new HIV infections annually [2]. This prevalence is a considerable decline of almost 5% from 2005, when the prevalence was 14.1% and there were 66,000 new infections annually [2].

Malawi has also made great progress in reaching the UNAIDS 95-95-95 goals that were set in 2014. According to the 2020-21 Malawi population-based HIV impact assessment (MPHIA), 88% of PLHIV knew their HIV status, 98% of PLHIV who knew their status were on treatment, and 97% of those on treatment had achieved viral suppression [3]. Expanded access to HIV treatment has resulted in a substantial 55% decrease in AIDS-related deaths, from 29,000 in 2010 to 13,000 in 2018, with more PLHIV living healthy and longer lives on antiretroviral therapy (ART) than ever before [2].

Although prevalence is a basic epidemiological measure in countries with generalized HIV epidemics, it is a poor indicator of changes in the epidemic. Since HIV is a lifelong infection, prevalence measures are cumulative, and do not differentiate between those who have been living with HIV for many years and recent transmission [4]. To better understand the epidemic

and focus appropriate HIV-prevention programs among specific populations and locations, it is important to identify patterns and trends of recent HIV infections [5]. Detecting hotspots of ongoing HIV transmission and describing factors associated with recent infections can provide critical information for targeting HIV prevention strategies and measuring their impact [4,6–9].

Antibody-based rapid tests for recent infection (RTRI) can distinguish recent HIV infection, i.e., an infection that has occurred within the last 12 months, from long-term infection [10-12]. However, their interpretation is challenged by factors that can cause 'false-recent' results such as variable immune responses at the individual-level, variable performance of the assay across diverse HIV-1 subtypes and across populations with naturally low viral loads, or current ARV use and advanced HIV disease [13]. To improve the accuracy of interpretation, recent infection testing algorithms (RITAs) incorporate the RTRI result with other markers of chronic infection, (low viral load, evidence of treatment) [14]. In Malawi, use of RTRI at the point-of-care was first evaluated in a pilot study in 4 districts between November 2017 and June 2018 [15]. Lessons learned from this pilot study were used to establish a plan for a nationwide surveillance system for recent HIV infections starting in April 2019, using RTRI and RITA [16].

This paper shares results from the Malawi recent HIV infection surveillance system, with the aim of describing the distribution, and predictors of recent HIV infections among people newly diagnosed with HIV.

METHODS:

This analysis includes persons enrolled in HIV recent infection surveillance between September 2019 and March 2020 at 155 health facilities in 11 districts of Malawi. Surveillance

was implemented over time, so not all facilities and districts were collecting data during this entire time period. The districts were prioritized for surveillance based on numbers of HIV-positive diagnoses in 2018 [17]. All persons presenting for testing who reported being HIV negative (or never having tested HIV positive) and were aged 13 years and older were eligible for recent infection surveillance. Of the 14,022 eligible persons approached, 13,838 (98.7%) consented to recent HIV infection surveillance. Of the 12,365 participants included in the analysis, 446 (3.6%) met the definition of RITA recent infection. Persons were excluded if they tested HIV-positive using self-testing , or were tested for HIV in the community and accessed the health facility only for HIV confirmatory testing.

All HIV testing was accompanied by pre- and post-test counselling and followed the national testing algorithm [18]. Persons who were reactive on the first HIV rapid test in the national algorithm (Determine®) (i.e., eligible for recency testing), and then consented to RTRI, were subsequently tested with Asanté™ HIV Rapid Recency Assay (Sedia Biosciences, Portland OR, USA) simultaneously with the HIV rapid test UniGold® [19,20]. Both the UniGold® and Asanté™ HIV Rapid Recency Assay results were entered into the study database via a tablet. Persons testing negative with UniGold® were dropped from all analyses. A dried blood spot specimen for VL testing was collected from persons who tested both HIV-positive and recent on the RTRI assay to assess recent infection per the RITA. HIV plasma RNA VL was quantified using Abbott m2000 Real Time HIV Viral Load Assay according to manufacturer instructions [21].

Routinely collected HIV testing data, including demographics, self-reported HIV testing history, and RTRI results were recorded in a surveillance data register at each facility. We abstracted these data using ODK software [22] and sent them to a central data repository. Once

VL results were available from the National HIV Reference Laboratory, these data were merged with the RTRI data using Stata version 13.0 (Stata Corporation, College Station, Texas, USA). In the merged dataset, all persons with recent results on the RTRI assay and VL ≥ 1000 copies/mL were classified as RITA recent infections. All persons with long-term results on the RTRI assay were classified as long-term infections. Persons with a recent result on the RTRI assay and VL <1000 copies/mL were excluded from the analysis since viral suppression likely indicates current or recent ART use and, therefore, not a new diagnosis.

We calculated percentages of people with newly diagnosed HIV infections classified as RITA recent by age, sex, and district of residence. We used Chi-square and the Kruskal-Wallis test to compare proportions and medians (interquartile range [IQR]), respectively. We used logistic regression, with cluster-based robust standard errors [23] to account for clustering of individuals within health facilities, to model factors associated with recent infection. We calculated unadjusted and adjusted odds ratios (ORs and AORs, respectively) with 95% confidence intervals (CIs) to assess the associations between recent infection and demographic factors. Variables associated with recent infection at a significance level of p-value <0.05 in univariable analysis and those known to be risk factors for HIV infection or suspected confounders were included in the multivariable logistic regression model through the stepwise method. In multivariable logistic regression, we thus adjusted for age group and sex, urban/rural location of the participant's residence, district of participant's residence, and HIV testing history (tested HIV-negative ≤ 12 months prior, tested HIV-negative > 12 months prior, never tested). Statistical analyses were performed using Stata.

Patient and Public Involvement

Our study involved the analysis of routinely collected Government of Malawi surveillance data and thus involvement from patient or members of the public in the design, conduct, reporting and dissemination plans for the study was not possible.

RESULTS:

Between September 2019 and March 2020, 14,022 persons aged ≥13 years and reactive on Determine® were offered recent infection testing (Figure 1). A total of 13,838 (98.7%) persons gave consent and were then tested using the RTRI assay. Of these, a total of 1,135 (8.2%) were excluded for the following reasons: an HIV-negative or invalid UniGold® result (n=806), disclosure of a previous HIV-positive diagnosis during post-test counselling (n=184), missing VL results (n=80), missing data on previous HIV test (n=43) or a negative (or invalid) result on the RTRI assay (n=22) (Figure 1). Persons for whom data on HIV testing history were missing were also excluded. A further 338 persons with an RTRI recent result were reclassified as long-term because they were virally suppressed (VL < 1000 copies/mL; Figure 1) and therefore likely are on ART. The final analysis thus included 446 (3.6%) RITA-recent cases.

Characteristics of surveillance participants

Females accounted for 60.5% (n=7,481) of the participants included in the analysis (Table 1), of whom 71.6% were not pregnant (n=5,362), 25.4% were pregnant (n=1,898) and 3.0% were breastfeeding (n=221). The overall median age for all participants was 31 years (IQR: 25–39 years), with the most common age group being 25-34 years old (38.2%, n=4,722). Participants were split almost equally between urban and rural settings. A large proportion of recent infection surveillance participants were from Blantyre district (47.3%, n=5,851), followed

by Zomba district (14.6%, n=1,800), Lilongwe district (7.7%, n=949) and Machinga (7.6%, n=941). The proportion of participants who reported an HIV test within the previous 12 months (40.9%, n=5,055) versus more than 12 months ago (40.7%, n=5,030) were similar, while a lower proportion reported never having tested before (18.4%, n=2,280; Table 1).

Table 1: Characteristics of recent HIV infection surveillance participants in Malawi, from September 2019 to March 2020.

Characteristic	Total % (n)
Sex	
Male	39.5 (4,884)
Female	60.5 (7,481)
Pregnancy & Breastfeeding	
Not pregnant	71.6 (5,362)
Pregnant	25.4 (1,898)
Breastfeeding	3.0 (221)
Age, years	
13-14	0.6 (68)
15-24	22.2 (2,748)
25-34	38.2 (4,722)
35-44	26.0 (3,210)
≥45	13.1 (1,617)
Median (IQR)	31 (25-39)
Residence	
Urban	48.5 (5,992)
Rural	51.5 (6,373)
District of residence	
Balaka	2.3 (283)
Blantyre	47.3 (5,851)
Chikwawa	6.2 (763)
Lilongwe	7.7 (949)
Machinga	7.6 (941)
Mangochi	6.5 (806)
Mzimba	1.7 (214)
Zomba	14.6 (1,800)
Other	6.1 (758)
Previous HIV test	
≤12 months	40.9 (5,055)
>12 months	40.7 (5,030)

Never tested	18.4 (2,280)

Characteristics of persons with recent HIV infection

Using RITA, 446 (3.6%) participants were classified as having a recent HIV infection and included in the analysis and 11,919 (96.4%) were classified as having a long-term infection and were not included in the analysis. The overall proportion of recent HIV infections was 3.6% and was higher in women (4.3%) than men (2.5%; Table 2).

Table 2: Prevalence of RITA recent HIV infections by participant characteristic and factors associated with RITA recent HIV infection in persons with new HIV diagnoses among surveillance participants in Malawi, from September 2019 to March 2020.

Characteristic	Total	Recent infections Number (%)	Crude OR (95% CI)	Adjusted* OR (95% CI)
Total	12,365	446 (3.6)	--	--
Sex				
Male	4,884	122 (2.5)	1	1
Female	7,481	322 (4.3)	1.7 (1.4-2.1)	1.2 (1.0-1.5)
Pregnancy & breastfeeding				
Not pregnant	5,362	230 (4.3)	1.1 (0.8-1.5)	1.4 (1.0-1.8)
Pregnant	1,898	76 (4.0)	1	1
Breastfeeding	221	15 (7.7)	2.0 (1.2-3.3)	1.7 (1.0-2.7)
Age, years				
13-14	68	1 (1.5)	0.4 (0.1-2.7)	0.6 (0.8-4.0)
15-24	2,748	159 (5.8)	1.6 (1.33-1.9)	1.5 (1.2-1.8)
25-34	4,722	175 (3.7)	1	1
35-44	3,210	87 (2.7)	0.7 (0.6-0.9)	0.9 (0.7-1.1)
≥45	1,617	26 (1.6)	0.4 (0.3-0.6)	0.6 (0.4-0.9)
Residence				
Urban	5,992	186 (3.1)	1	1
Rural	6,373	261 (4.1)	1.3 (1.0-1.8)	1.1 (0.7-1.6)
District of residence				
Balaka	283	16 (5.7)	1.7 (0.7-4.1)	1.6 (0.7-3.8)
Blantyre	5,851	152 (2.6)	0.8 (0.5-1.2)	0.8 (0.5-1.3)
Chikwawa	763	40 (5.2)	1.6 (1.0-2.6)	1.4 (0.8-2.4)
Lilongwe	949	52 (5.5)	1.6 (1.0-2.6)	1.7 (1.0-2.9)
Machinga	941	64 (6.8)	2.1 (1.2-3.5)	1.9 (1.1-3.3)
Mangochi	806	28 (3.5)	1.0 (0.6-1.8)	1.0 (0.6-1.7)
Mzimba	214	16 (7.5)	2.3 (1.3-4.0)	2.5 (1.3-4.7)
Zomba	1,800	61 (3.4)	1	1

Other	758	19 (2.5)	0.7 (0.4-1.2)	0.8 (0.4-1.3)
Previous HIV test				
≤12 months	5,055	308 (6.1)	3.3 (2.6-4.2)	3.0 (2.3-3.8)
>12 months	5,030	96 (1.9)	1	1
Never tested	2,280	46 (2.0)	1.1 (0.7-1.5)	1.1 (0.8-1.6)

IQR: interquartile range, OR: odds ratio, CI: confidence interval

*Adjusted for age group and sex, urban/rural location of the participant's residence, district of participant's residence and HIV testing history (tested ≤ 12 months prior, test > 12 months prior, never tested)

Breastfeeding women (7.7%) had the highest proportion of recent infections, which was significantly higher than that of pregnant (4.0%) and non-pregnant women (4.3%; Table 2). By age group, recent HIV infections were highest in females (6.0%) and males (4.7%) aged 15-24 years (Figure 2). A larger percentage of recent infection among people with new HIV diagnoses was found in persons from rural settings (4.1%) compared to urban settings (3.1%). Residents of Mzimba (7.5%), Machinga (6.8%), Balaka (5.7%), Lilongwe (5.5%) and Chikwawa (5.2%) districts had the highest proportions of recent infections. By HIV testing history, the highest percentage of recent infections (6.1%) was among persons who self- reported having had a negative HIV test within the 12 months prior (Table 2).

Factors associated with recent HIV infection

After adjusting for age group and sex, urban/rural location of the participant's residence, district of participant's residence and HIV testing history (tested < 12 months prior, test > 12 months prior, never tested), factors significantly associated with recent infection among newly diagnosed PLHIV included living in Mzimba (AOR 2.5; CI 1.3-4.7) or Machinga (AOR 1.9; CI 1.1-3.3) districts compared to Zomba district; being aged 15-24 years (AOR 1.5; CI 1.2-1.8) compared to being aged 25-34 years; and having had a previous HIV test within 12

months prior to the study (AOR 3.0; CI 2.3-3.8) compared to having had a previous HIV test more than 12 months ago (Table 2).

DISCUSSION:

Our results demonstrate an association of recency with plausible risks such as younger age (<25 years), sex (women vs men) and last negative test within last 12 months, confirming successful implementation of recency surveillance in Malawi. In addition, recency testing identified breastfeeding women and residents of certain districts as persons at higher risk of ongoing HIV transmission. In multivariate regression, factors that remained associated with recent infection included younger age, previous HIV test within the last 12 months, and district of residence.

The percentage of recent infections among newly diagnosed females aged 15-24 years, or adolescent girls and young women (AGYW), in this study was lower than that found in previous studies in Malawi [24] and in nearby countries [25-27]. The differences in percentages may be attributed to factors such as the case definition of recent infection. For example, in Kim et al. in Kenya the case definition for recent infection included testing recent on LAg and having no evidence of antiretroviral therapy use [25]. This case definition for recent infection is different from what is used in this analysis, which used a point-of-care test and did not use antiretroviral therapy use as an eligibility criterion.

Although women and young persons had the highest percentage of recent infections, in multivariable analysis being female was not associated with recent infection, especially after adjusting for history of HIV testing. This may be because in multivariable analysis the result was adjusted for age, among both women and men in Malawi, and younger persons are less likely

to have had a history of HIV testing [28]. Higher percentages of recent infection in women can also be partly explained by data showing higher HIV testing rates in women compared to men [28,29]. The 2015-16 Malawi Population–based HIV Impact Assessment (MPHIA) survey estimated that 5.1% of women had never tested for HIV or received an HIV test result compared to 12.4% of men [30]. This is especially true in antenatal care (ANC) settings, which are accessed by 95% of pregnant women in Malawi [31], compared to studies that show that as few as 35% of the male partners of pregnant women are tested for HIV [32,33]. Women may be offered HIV testing both during maternal health visits (ANC, labour and delivery, and postnatal) and during paediatric services for their children. Therefore, women may have multiple opportunities for routine HIV testing, increasing the likelihood of being diagnosed with HIV early [34].

The higher proportion of women currently breastfeeding who tested recent for HIV may similarly be explained by HIV testing practices in Malawi for pregnant and breastfeeding women. Women who test positive during breastfeeding may have tested negative during pregnancy but seroconverted either later in the pregnancy or postpartum after becoming sexually exposed to HIV. Others might not have had their HIV status ascertained during pregnancy but tested positive during the breastfeeding period [35]. The smaller number of women breastfeeding compared to women who were not pregnant or breastfeeding, or were currently pregnant, may have influenced the results and explain why this variable did not remain strongly significant in the multivariable model, though it does border on significance. Chagomerana et al. conclude in their ANC cohort study in Malawi that mother-to-child-transmission (MTCT) occurred disproportionately among women with a last positive HIV test

during breastfeeding. This means that testing delayed until the postpartum period may lead to higher MTCT and that PMTCT programmes should focus on early ART initiation and providing targeted testing, prevention, treatment, and support to breastfeeding women [35]. Because breastfeeding women do not routinely attend health facilities after the first set of infant vaccinations, the Malawi 2022 HIV testing guidelines has added HIV testing of mothers at the measles vaccination visit (9-12 months) to increase the chances of finding incident infected mothers [36].

There is a clear association in our survey between a history of HIV testing within the last 12 months and recent HIV infection. This finding is consistent with other studies that demonstrate a higher rate of HIV testing history among newly HIV-positive individuals, with as much as 70% of newly diagnosed individuals reporting previously testing either positive (meaning they were not truly newly diagnosed) or negative [37]. Since recent infection assays are designed to detect HIV infections that have occurred during the previous 12 months, it seems reasonable that persons who tested positive with a recent infection during our surveillance were more likely to have perceived themselves at risk and sought HIV testing sometime during the previous 12 months when compared to those persons who had not, or who reported never having been tested for HIV. Also, people newly diagnosed with HIV and with a recent history of testing negative for HIV are likely to have seroconverted since their last test and thus are more likely to be recently infected. More needs to be done to enhance the quality of HIV prevention counseling, such as initiation of pre-exposure prophylaxis PrEP and a thorough assessment of factors that may have influenced the person to seek a test.

There are several implications from our findings. The high percentage of long-term infections among newly diagnosed PLHIV found in the surveillance is alarming given that these late diagnoses mean that a significant proportion of the population is unaware of their HIV status and likely transmitting infection [38,39]. In addition, when these persons with long-term infections are then linked to treatment programs, they are more likely to experience poor health outcomes, including in the younger age groups [40-42]. This finding underscores the continued need for expanding HIV testing, as well as testing strategies that are narrowly focused on specific populations. Still, many of the long-term infections identified may be due to persons who reported testing negative on their last test but in truth were previously diagnosed with HIV and possibly had a history of ART use [43,44]. Given the high rate of retesting and re-diagnosis in Malawi [45] more research is needed to better understand stigma and misconceptions associated with revealing a history of testing positive, reasons why those who test positive will often retest even after starting treatment and how a person's retesting history, including the length of time between tests, may influence HIV outcomes [36]. This is increasingly important given that across sub-Saharan Africa, approximately 84% of people have knowledge of their HIV status [44].

AGYW continue to face the highest risk of HIV in Malawi. More needs to be done to expand HIV testing among AGYW in Malawi beyond traditional facility-based testing to modalities that are preferred by adolescents [46,47]. In a recent HIV testing study in Kenya, most AGYW participants (77.5%) chose staff-aided testing either at home or at a mobile event; (22.4%) chose self-testing; and only 2 (.2%) chose facility referral [48]. Even with the elevated risk that AGYW face, young men aged 15-24 in our surveillance also had high rates of new

infections, and in multivariate analysis only young age remained significant, clearly indicating a need for renewed focus on HIV prevention in all youth aged 15-24 in Malawi.

The prevalence of recent infection was highest in four districts: Mzimba (in the northern region), Machinga and Chikwawa (in the southern region), and Lilongwe (in the central region). These findings provide new information to complement prevalence data from MPHIA studies that have found that southern districts had higher HIV prevalence compared to central and northern districts (even though the MPHIA was not powered to provide district-level prevalence estimates) [3]. Since recent HIV surveillance can generate a disaggregated summary of where recent HIV infections occur at more granular geographic sub-units, such as district and health facilities [16], the rapid identification of such HIV transmission clusters is the next important step in utilizing the recent infection surveillance data in Malawi. This in fact has begun with a geospatial transmission hotspot analysis using Malawi recency data [49]. Continuing such analyses, and following them up with local facility-based investigations, may help explain our district-level recent infection findings more fully and can provide the basis for using recent infection surveillance to identify gaps in HIV prevention and care services [4-5,50,51].

The strength of this study includes the large sample of persons with new HIV diagnoses from districts in Malawi with high HIV prevalence. Since RITA was integrated into the national HIV testing services (HTS) model with high acceptability, these data are likely a good representation of the general characteristics of persons newly diagnosed with HIV seeking healthcare from health facilities in Malawi and similar settings. The study had some limitations. First, since the initial phase of the recent HIV infection surveillance system was focused on

integrating recent HIV infection testing into routine HTS, additional data were not collected that may have helped identify factors associated with recent infection, such as marital status, cultural beliefs and socio-economic status. Future surveillance may benefit from linking information generated from recent HIV infection testing data with other sociodemographic factors and triangulating additional factors such as clinical history that may be related to recent HIV infection.

Participation in this investigation relied on self-reported history of HIV testing among eligible persons. Hence, as noted above, it is possible that participants were reluctant to disclose a previous HIV-positive diagnosis and were inadvertently included as a new HIV diagnosis. Indeed, UNAIDS/WHO estimates that approximately up to 50% of people testing positive in Malawi are re-diagnoses [45]. This would result in an underestimation of the proportion of recent infections. Finally, some validation studies of recency assays indicate a tendency to produce false-recent results, particularly for those individuals on antiretroviral therapy [10-12], which would result in an overestimation of the proportion of recent infections. However, efforts have been made to reduce false-recent results through the addition of recent infection testing algorithms (RITAs) [13] such as was used in this study.

CONCLUSION:

Recent HIV infection surveillance can help to identify socio-demographic, clinical and geographic factors associated with recent HIV infection. Given that recent infection surveillance in Malawi confirms the high risk of HIV faced by AGYW, youth-focused programs that aim to limit HIV acquisition and transmission among young people, especially young women, should remain a priority and be strengthened to sustain the gains made towards HIV epidemic control

in Malawi. More data derived from triangulation and modelling with other data sources, as well as recent infection cluster analyses, are needed to allow for the targeting of HIV interventions at the district level in the country. The higher percentage of long-term infections than recent infections among newly diagnosed PLHIV underscores the continuing need for innovative ways to expand targeted HIV testing to ensure early diagnosis and treatment, especially among hard-to-reach populations.

ETHICS APPROVAL STATEMENT

The protocol was approved by the National Health Sciences Research Committee in Malawi (# 18/11/2190) and by the U.S. Centers for Disease Control and Prevention (# 2019-13). All eligible clients provided verbal informed consent prior to being tested for recent HIV infection. Recent infection results were not returned to the client.

CONTRIBUTORSHIP STATEMENT

MTM supported implementation, paper conceptualization, data analysis and led writing of the final manuscript. EWM and STG co-wrote the final manuscript. JT, IN, FB, CLB, DP, NWK, ANK, EK, AA, YB and GB led implementation in Malawi and contributed to writing and review of the final manuscript. GOM served as Principal Investigator at UW and contributed to writing and review of the final manuscript. KGC, MA, TD, and VS at CDC Atlanta supported technical implementation and contributed to review of the final manuscript. KN and RN supported implementation in Malawi and contributed to review of the final manuscript.

COMPETING INTERESTS

None of the authors have a competing interest to disclose.

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DATA SHARING AGREEMENT

This paper is based on public health surveillance data collected on an ongoing basis by the Ministry of Health and Social Services in Malawi. To request data please contact author Rose Nyirenda (rnyirenda@hivmw.org).

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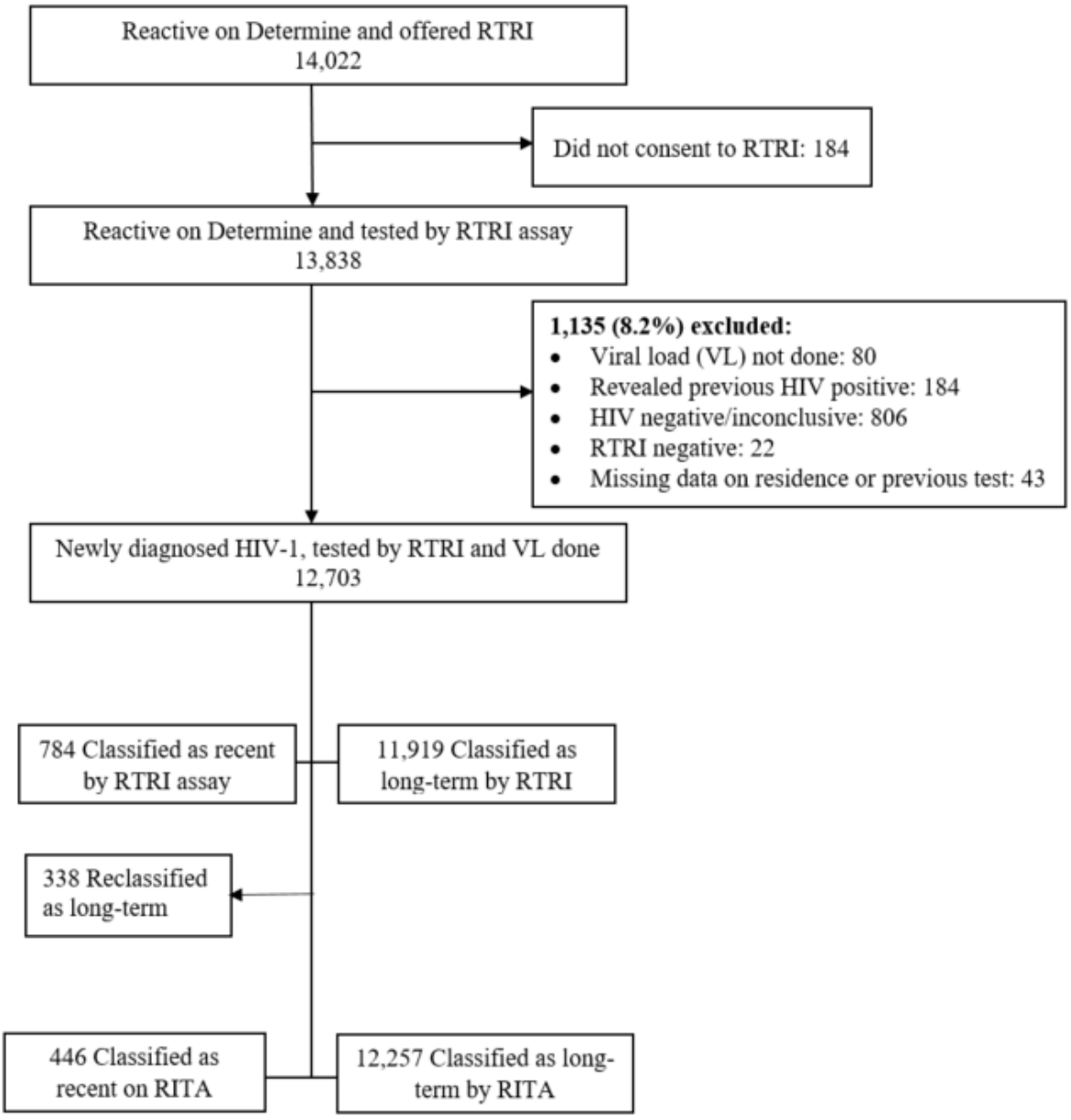
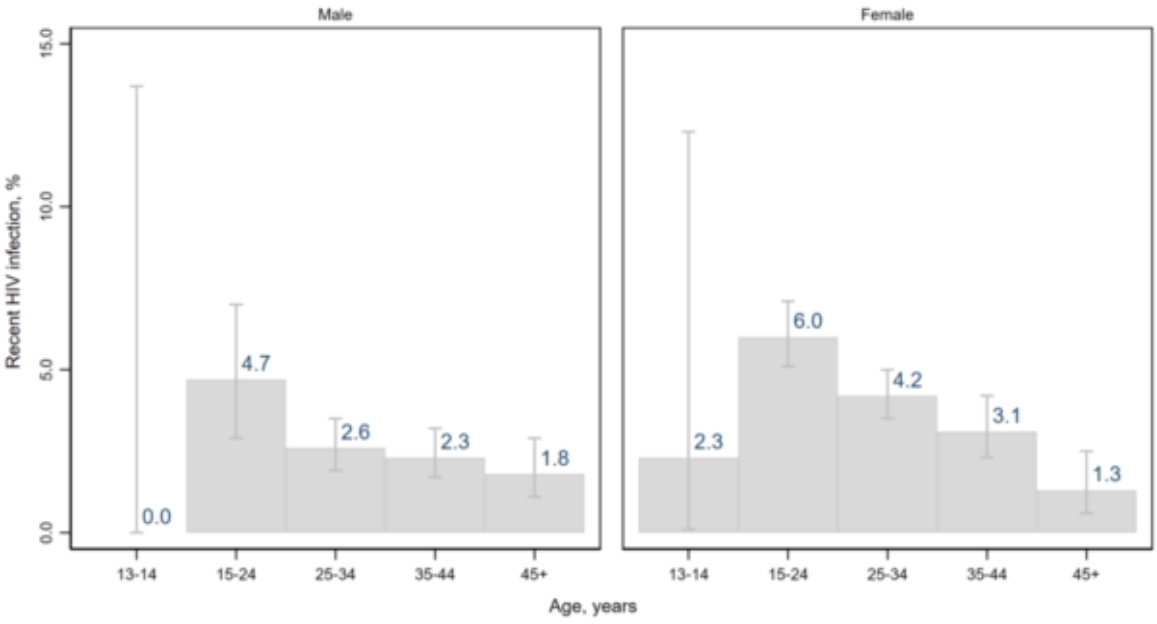


Figure 1: Flowchart of persons included in the analysis of HIV recency surveillance data in Malawi, from September 2019 to March 2020.

RTRI: rapid test for recent infection, RITA: recent infection testing algorithm, VL: viral load
Reclassification: RTRI recent assays classified as recent but with controlled viral load (VL < 1000 copies/mL) are not considered to be RITA recent and are reclassified



# RITA-recent	0	22	43	41	18	1	137	130	46	8

Figure 2: Number and percentage of recent HIV infections, per RITA, by age group among men and women included in HIV recency surveillance in Malawi, from September 2019 to March 2020.

RITA: recent infection testing algorithm

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

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

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 5-6
		(*b*) Report category boundaries when continuous variables were categorized	Pages 5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Pages 5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pages 5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pages 7-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 7-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 7-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 7-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Title Page

*Give information separately for exposed and unexposed groups.

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

BMJ Open

Characterizing persons diagnosed with HIV as either recent or long-term using a cross-sectional analysis of recent infection surveillance data collected in Malawi from September 2019 to March 2020

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TITLE:

Characterizing persons diagnosed with HIV as either recent or long-term using a cross-sectional analysis of recent infection surveillance data collected in Malawi from September 2019 to March 2020

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SHORT TITLE: Characterizing recent HIV infections in Malawi

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

KEY WORDS:

Surveillance, recent infection testing algorithm, RITA, rapid test for recent infection, RTRI, HIV, HIV prevention

Word Count (not including title page, references, figures and tables): 4574

ABSTRACT

Objectives: In Malawi a recent infection testing algorithm (RITA) is used to characterize infections of persons newly diagnosed with HIV as recent or long-term. This paper shares results from recent HIV infection surveillance and describes distribution and predictors.

Setting: Data from 155 health facilities in 11 districts in Malawi were pooled from September 2019 to March 2020.

Participants: Eligible participants were ≥13 years, and newly diagnosed with HIV. Clients had RITA recent infections if the rapid test for recent infection (RTRI) test result was recent and viral load (VL) ≥1,000 copies/mL; if VL was <1000 copies/mL the RTRI result was reclassified as long-term. Results were stratified by age, sex, pregnancy/breastfeeding status, and district.

Results: 13,838 persons consented to RTRI testing and 12,703 had valid RTRI test results and VL results after excluding clients not newly HIV-positive, RTRI negative or missing data (n=1135). A total of 12,365 of the 12,703 were included in the analysis after excluding those whose RTRI results were reclassified as long term (n=338/784 or 43.1%). The remainder, 446/12,703 or 3.5%, met the definition of RITA recent infection. The highest percentage of recent infections was among breastfeeding women (7.7%; 95% CI 4.8-12.0), young people aged 15-24 years-old (5.8%; 95% CI 5.0-6.7) and persons who reported a negative HIV test within the past 12 months

(6.1%; 95% CI 5.4-6.7). Factors associated with recent infection in multivariable analysis included being a non-pregnant female (Adjusted Odds Ratio [AOR] 1.4; 95% CI 1.2-1.8), a breastfeeding female (AOR 2.2; 95% CI 1.4-3.5), aged 15-24 years (AOR 1.6; 95% CI 1.3-1.9) and residents of Machinga (AOR 2.0; 95% CI 1.2-3.5) and Mzimba (AOR 2.4; 95% CI 1.3-4.5) districts.

Conclusions: Malawi's recent HIV infection surveillance system demonstrated high uptake and identified sub-populations of new HIV diagnoses with a higher percentage of recent infections.

STRENGTHS AND LIMITATIONS OF THE STUDY

- This paper reports on HIV recent infections that are verified by viral load (VL); if the rapid test for recent infection (RTRI) test result is recent and VL ≥1,000 copies/mL the RTRI recent result is considered valid and if VL is <1000 copies/mL the RTRI recent test result is reclassified as a long-term infection.
- HIV recent infection surveillance in Malawi is integrated into HIV testing services so that all eligible persons who test HIV-positive, and provide consent, are tested with an RTRI.
- When implementing HIV recent infection surveillance in Malawi it is not possible to deduplicate HIV positive persons retesting for HIV because there are no unique national IDs at the moment that would allow deduplication.
- Data reported in this paper include varying levels of implementation of HIV recent infection surveillance by district in Malawi and these differences in coverage could influence district-level HIV recent infection rates reported and the statistical significance of those findings.

INTRODUCTION:

The East and Southern Africa regions bear the highest burden of the global HIV/AIDS epidemic. In 2018, the number of people living with HIV (PLHIV) in these two regions was approximately 20.6 million, accounting for 47% of HIV infections worldwide [1]. In 2018, the HIV prevalence in Malawi was estimated at 9.2%, with almost one million PLHIV and 38,000 new HIV infections annually [2]. This prevalence is a considerable decline of almost 5% from 2005, when the prevalence was 14.1% and there were 66,000 new infections annually [2].

Malawi has also made great progress in reaching the UNAIDS 95-95-95 goals that were set in 2014. According to the 2020-21 Malawi population-based HIV impact assessment (MPHIA), 88% of PLHIV knew their HIV status, 98% of PLHIV who knew their status were on treatment, and 97% of those on treatment had achieved viral suppression [3]. Expanded access to HIV treatment has resulted in a substantial 55% decrease in AIDS-related deaths, from 29,000 in 2010 to 13,000 in 2018, with more PLHIV living healthy and longer lives on antiretroviral therapy (ART) than ever before [2].

Although prevalence is a basic epidemiological measure in countries with generalized HIV epidemics, it is a poor indicator of changes in the epidemic. Since HIV is a lifelong infection, prevalence measures are cumulative, and do not differentiate between those who have been living with HIV for many years and recent transmission [4]. To better understand the epidemic and focus appropriate HIV-prevention programs among specific populations and locations, it is important to identify patterns and trends of recent HIV infections [5]. Detecting locales with high amounts of ongoing HIV transmission and describing factors associated with recent

infections can provide critical information for targeting HIV prevention strategies and measuring their impact [4,6–9].

Antibody-based rapid tests for recent infection (RTRI) can distinguish recent HIV infection, i.e., an infection that has likely occurred within the last 12 months, from long-term infection [10-12]. However, their interpretation is challenged by factors that can cause 'false-recent' results such as variable immune responses at the individual-level, variable performance of the assay across diverse HIV-1 subtypes and across populations with naturally low viral loads, or current ARV use and advanced HIV disease [13]. To improve the accuracy of interpretation, recent infection testing algorithms (RITAs) incorporate the RTRI result with other markers of chronic infection (low viral load, evidence of treatment) [14]. In Malawi, use of RTRI at the point-of-care was first evaluated in a pilot study in 4 districts between November 2017 and June 2018 [15]. Lessons learned from this pilot study were used to establish a plan for a nationwide surveillance system for recent HIV infections starting in April 2019, using RTRI and RITA [16].

This paper shares results from the Malawi recent HIV infection surveillance system, with the aim of describing the distribution and predictors of recent HIV infections among people newly diagnosed with HIV.

METHODS:

This analysis includes persons enrolled in HIV recent infection surveillance between September 2019 and March 2020 at 155 health facilities in 11 districts of Malawi. Data from April 2019 to August 2019 were excluded from the analysis due to variability in facility data collection during the 5-month startup phase of the surveillance system. Surveillance was

implemented over time, so not all facilities and districts were collecting data during this entire time period; for the time period reported on, recency surveillance had expanded to 11 of 28 districts and to 155 of an envisioned 251 facilities. The districts were prioritized for surveillance based on numbers of HIV-positive diagnoses in 2018 [17]. All persons presenting for testing who reported being HIV negative or unknown (or never having tested HIV positive) and were aged 13 years and older were eligible for recent infection surveillance. Persons were excluded from the analysis if they tested HIV-positive using self-testing, or were otherwise screened for HIV in the community and accessed the health facility only for HIV confirmatory testing.

All HIV testing was accompanied by pre- and post-test counselling and followed the national testing algorithm [18]. Only persons who were 1) reactive on the first HIV rapid test in the national algorithm (Determine®) and 2) had provided verbal consent (i.e., eligible for recency testing) were subsequently tested with Asanté™ HIV Rapid Recency Assay (Sedia Biosciences, Portland OR, USA) simultaneously with the HIV rapid test UniGold® [19,20]. Verbal consent was the preferred consent format due to the low-risk nature of the surveillance and to better integrate recency testing into Government of Malawi-approved HIV testing guidelines. Persons under the age of 18 provided verbal consent to participate in recency testing and assent was not used. Even though the Asanté assay is currently validated for persons 15 years and older [19], ethics committees approved verbal consent for persons 13 years and older because this age is also allowed to consent to HIV testing per Malawi Government HIV testing services (HTS) guidelines.

Both the UniGold® and Asanté™ HIV Rapid Recency Assay results were entered into the study database via a tablet. Persons testing negative with UniGold® were dropped from all

analyses. A dried blood spot specimen for VL testing was collected from persons who tested both HIV-positive and recent on the RTRI assay to assess recent infection per the RITA. HIV plasma RNA VL was quantified using Abbott m2000 Real Time HIV Viral Load Assay according to manufacturer instructions [21].

Routinely collected HIV testing data, including demographics, self-reported HIV testing history, and RTRI results were recorded in a surveillance data register at each facility. We abstracted these data using ODK software [22] and sent them to a central data repository. Once VL results were available from the National HIV Reference Laboratory, these data were merged with the RTRI data using Stata version 13.0 (Stata Corporation, College Station, Texas, USA). In the merged dataset, all persons with recent results on the RTRI assay and VL ≥ 1000 copies/mL were classified as RITA recent infections. All persons with long-term results on the RTRI assay were classified as long-term infections. Persons with an RTRI result indicating a recent infection and VL <1000 copies/mL were excluded from the analysis since viral suppression likely indicates current or recent ART use and, therefore, not a new diagnosis. The RTRI and RITA results were not returned to the clients (clients were aware when they provided consent) or clinicians but only used for surveillance. We were unable to identify people retesting for HIV or already on ART because the current system for HTS and ART does not use a unique identifier (eg national identification number) that would allow deduplication.

We calculated percentages of people with newly diagnosed HIV infections classified as RITA recent by age, sex, and district of residence. We used Chi-square and the Kruskal-Wallis test to compare proportions and medians (interquartile range [IQR]), respectively. We used logistic regression, with cluster-based robust standard errors [23] to account for clustering of

individuals within health facilities, to model factors associated with recent infection. We calculated unadjusted and adjusted odds ratios (ORs and AORs, respectively) with 95% confidence intervals (CIs) to assess the associations between recent infection and demographic factors. The a priori variables chosen for inclusion in multivariable analysis were age and sex. Variables associated with recent infection at a significance level of p-value <0.05 in univariable analysis and those known to be risk factors for HIV infection or suspected confounders were included in the multivariable logistic regression model through the stepwise method. In multivariable logistic regression, we thus adjusted for age group and sex, urban/rural location of the participant's residence, and district of participant's residence. HIV testing history was not adjusted for in the multivariable analysis due to its clear role in mediating recent HIV infection. Statistical analyses were performed using Stata.

The protocol was approved by the National Health Sciences Research Committee in Malawi (# 18/11/2190) and by the U.S. Centers for Disease Control and Prevention (# 2019-13).

Patient and Public Involvement

Our study involved the analysis of routinely collected Government of Malawi surveillance data and thus involvement from patient or members of the public in the design, conduct, reporting and dissemination plans for the study was not possible. Starting in late 2021, investigations of locales with higher rates of recent infections, and initiation of public health interventions based on the results, have included more patient and public involvement.

RESULTS:

Between September 2019 and March 2020, 14,022 eligible persons aged ≥13 were asked to participate in recency testing. Of these, 13,838/14,022 (98.7%) consented to recent HIV infection surveillance and were then tested using the RTRI assay (Figure 1). Of these, a total of 1,135/13,838 (8.2%) were excluded for the following reasons: an HIV-negative or invalid UniGold® result (n=806), disclosure of a previous HIV-positive diagnosis during post-test counselling (n=184), missing VL results (n=80), missing data on previous HIV test (n=43) or a negative (or invalid) result on the RTRI assay (n=22) (Figure 1). Of the 12,703 participants included in the analysis after these exclusions, 784/12,703 were RTRI recent (6.2%) and 11,919/12,703 were RTRI long-term (93.8%). Of the 784 RTRI recent participants, 446/784 (56.9%) met the definition of RITA recent infection and 338/784 (43.1%) met the definition of RITA long-term infection. This corresponds to an overall RITA-recent rate of 3.5% (446/12,703) and a RITA long-term rate of 96.5% (12,257/12,704). The final analysis thus included 446 RITA-recent cases and 11,919 long-term infection cases (as classified by RTRI) or a total of 12,365 participants. The final analysis did not include RITA long-term cases (338) (Figure 1).

Characteristics of surveillance participants

Females accounted for 60.5% (n=7,481) of the participants included in the analysis (Table 1) and, among those of reproductive age, 70.2% were not pregnant (n=4,974), 26.7% were pregnant (n=1,893) and 3.1% were breastfeeding (n=220). The overall median age for all participants was 31 years (IQR: 25–39 years), with the most common age group being 25-34 years old (38.2%, n=4,722). Participants were split almost equally between urban and rural settings. A large proportion of recent infection surveillance participants were from Blantyre district (47.3%, n=5,851), followed by Zomba district (14.6%, n=1,800), Lilongwe district (7.7%,

n=949) and Machinga (7.6%, n=941). The proportion of participants who reported an HIV test within the previous 12 months (40.9%, n=5,055) versus more than 12 months ago (40.7%, n=5,030) were similar, while a lower proportion reported never having tested before (18.4%, n=2,280; Table 1).

Table 1: Characteristics of recent HIV infection surveillance among Determine reactive participants in Malawi, from September 2019 to March 2020 (n = 12,365).

Characteristic	Total % (n)
Sex	
Male	39.5 (4,884)
Female	60.5 (7,481)
[a]Pregnancy & Breastfeeding	
Not pregnant	70.2 (4,974)
Pregnant	26.7 (1,893)
Breastfeeding	3.1 (220)
Age, years	
13-14	0.6 (68)
15-24	22.2 (2,748)
25-34	38.2 (4,722)
35-44	26.0 (3,210)
45-49	6.2 (770)
≥50	6.9 (847)
Median (IQR)	31 (25-39)
Residence	
Urban	48.5 (5,992)
Rural	51.5 (7,373)
District of residence	
Balaka	2.3 (283)
Blantyre	47.3 (5,851)
Chikwawa	6.2 (763)
Lilongwe	7.7 (949)
Machinga	7.6 (941)
Mangochi	6.5 (806)
Mzimba	1.7 (214)
Zomba	14.6 (1,800)
Other	6.1 (758)
[b]Previous HIV test	
≤12 months	40.9 (5,055)
>12 months	40.7 (5,030)

Never tested	18.4 (2,280)

[a]Data for all females in the reproductive age group (15-49 years)

[b]≤12 months and >12 months represent clients last self-reported HIV-negative test

Characteristics of persons with recent HIV infection

Breastfeeding women (7.7%; 95% CI 4.8-12.1) had the highest proportion of recent infections, which was significantly higher than that of pregnant (4.0%; 95% CI 3.2-4.9) and non-pregnant women (4.3%; 95% CI 3.8-4.9) (Table 2). By age group, recent HIV infections were highest in females (6.0%) and males (4.7%) aged 15-24 years (Figure 2) and highest in the 15-24 age group overall (5.8%; 95% CI 5.0-6.7) (Table 2). A larger percentage of recent infection among people with new HIV diagnoses was found in persons from rural settings (4.1%; 95% CI 3.6-4.6) compared to urban settings (3.1%; 95% CI 2.7-3.6). Residents of Mzimba (7.5%; 95% CI 4.6-11.9), Machinga (6.8%; 95% CI 5.4-8.6), Balaka (5.7%; 95% CI 3.5-9.0), Lilongwe (5.5%; 95% CI 4.2-7.1) and Chikwawa (5.2%; 95% CI 3.9-7.1) districts had the highest proportions of recent infections. By HIV testing history, the highest percentage of recent infections (6.1%; 95% CI 5.4-6.7) was among persons who self-reported having had a negative HIV test within the 12 months prior (Table 2). For a description of persons with long-term infections (n = 338), please see additional table in Appendix.

Table 2: Prevalence of RITA recent HIV infections by participant characteristic and factors associated with RITA recent HIV infection in persons with new HIV diagnoses among surveillance participants in Malawi, from September 2019 to March 2020 (n = 12,365).

	Total	**Recent infections**	**Crude**	**Adjusted***
Characteristic		**% (95%CI)**	**OR (95% CI)**	**OR (95% CI)**

Total	12,365	3.6 (3.3-4.0)	--	--
Sex				
Male	4,884	2.5 (2.1-3.0)	1	1
Female not pregnant	5,362	4.3 (3.8-4.9)	1.7 (1.4-2.1)	1.4 (1.2-1.8)
Female pregnant	1,898	4.0 (3.2-4.9)	1.6 (1.2-2.1)	1.1 (0.8-1.5)
Female breastfeeding	221	7.7 (4.8-12.0)	3.2 (2.0-5.0)	2.2 (1.4-3.5)
Age, years				
13-14	68	1.5 (0.2-9.7)	0.4 (0.1-2.7)	0.4 (0.1-2.6)
15-24	2,748	5.8 (5.0-6.7)	1.6 (1.33-1.9)	1.6 (1.3-1.9)
25-34	4,722	3.7 (3.2-4.2)	1	1
35-44	3,210	2.7 (2.2-3.3)	0.7 (0.6-0.9)	0.8 (0.6-0.9)
45-49	770	1.7 (1.0-2.9)	0.5 (0.3-0.8)	0.5 (0.3-0.9)
≥50	847	1.5 (0.9-2.6)	0.4 (0.2-0.7)	0.4 (0.2-0.7)
Residence				
Urban	5,992	3.1 (2.7-3.6)	1	1
Rural	6,373	4.1 (3.6-4.6)	1.3 (1.0-1.8)	1.0 (0.7-1.5)
District of residence				
Balaka	283	5.7 (3.5-9.0)	1.7 (0.7-4.1)	1.7 (0.7-4.0)
Blantyre	5,851	2.6 (2.2-3.0)	0.8 (0.5-1.2)	0.8 (0.5-1.2)
Chikwawa	763	5.2 (3.9-7.1)	1.6 (1.0-2.6)	1.5 (0.9-2.4)
Lilongwe	949	5.5 (4.2-7.1)	1.6 (1.0-2.6)	1.7 (1.0-2.8)
Machinga	941	6.8 (5.4-8.6)	2.1 (1.2-3.5)	2.0 (1.2-3.5)
Mangochi	806	3.5 (2.4-5.0)	1.0 (0.6-1.8)	1.0 (0.6-1.8)
Mzimba	214	7.5 (4.6-11.9)	2.3 (1.3-4.0)	2.4 (1.3-4.5)
Zomba	1,800	3.4 (2.6-4.3)	1	1
Other	758	2.5 (1.6-3.9)	0.7 (0.4-1.2)	0.7 (0.4-1.2)
[b]Previous HIV test				
≤12 months	5,055	6.1 (5.4-6.7)	3.3 (2.6-4.2)	
>12 months	5,030	1.9 (1.5-2.3)	1	
Never tested	2,280	2.0 (1.5-2.6)	1.1 (0.7-1.5)	

IQR: interquartile range, OR: odds ratio, CI: confidence interval

*Adjusted for age group, sex, urban/rural location of the participant's residence and district of participant's residence

[b]≤12 months and >12 months represent clients last self-reported HIV-negative test

Factors associated with recent HIV infection

After adjusting for age group and sex, urban/rural location of the participant's residence, and district of participant's residence, factors significantly associated with recent infection among newly diagnosed PLHIV included living in Mzimba (AOR 2.4; 95% CI 1.3-4.5) or Machinga (AOR 2.0; 95% CI 1.2-3.5) districts compared to Zomba district and being aged 15-24

years (AOR 1.6; 95% CI 1.3-1.9) compared to being aged 25-34 years. Women who were not pregnant (AOR 1.4; 95% CI 1.2-1.8) and women who were breastfeeding (AOR 2.2; 95% CI 1.4-3.5) were also at higher risk of recent infection (Table 2).

DISCUSSION:

Our results demonstrate an association of recency with plausible risks such as younger age (<25 years) and sex (women vs men), confirming successful implementation of recency surveillance in Malawi. In addition, recency testing identified breastfeeding women and residents of certain districts as persons at higher risk of ongoing HIV transmission. In multivariable regression, factors that remained associated with recent infection included younger age and district of residence.

The proportion of recent infections found in this study, using RITA, was 3.5%. There have been no other national-level recent infection studies in Malawi to compare these findings to. A study in Kenya using similar methods, though with a much smaller sample size and using a laboratory-based recency test, reported a recent infection percent of 8.6% in Nairobi [24] and another recency study in Zimbabwe reported a recent infection percent of 10.5% among female sex workers [25]. Our study likely has a comparatively lower recent infection rate given the large, national sample that constitutes the recent infection surveillance system in Malawi. For example, a larger surveillance study recently completed in Cambodia found a RITA recent rate of 5.0% and surveillance of 27,792 newly HIV-diagnosed individuals in Nigeria found a RITA recent rate of 2.4% [26,27]. Furthermore, recency surveillance in Eswatini found an overall RITA

recent rate of 3.1% and surveillance in DRC found an overall RITA recent rate of 5.0% [28,29]. All of these surveillance studies were aided by the use of clinical information such as viral load, history of prior HIV diagnosis, and antiretroviral therapy-exposure to confirm that a recent infection was truly recently acquired and not a long-term infection. The validity of our recent infection rate in Malawi is also aided by the inclusion of viral load testing to rule out long-term infections—without the viral load testing the percent HIV recent would have been 6.2%.

The percentage of recent infections among newly diagnosed females aged 15-24 years, or adolescent girls and young women (AGYW), in this study was lower than that found in previous studies in Malawi [30] and in nearby countries [31-33]. The differences in percentages may be attributed to factors such as the case definition of recent infection. For example, in Kim et al. in Kenya the case definition for recent infection included testing recent on LAg and having no evidence of antiretroviral therapy use [31]. This case definition for recent infection is different from what is used in this analysis, which used a point-of-care test and did not use antiretroviral therapy use as an eligibility criterion.

The multivariable analysis indicating a risk of recent HIV infection among younger persons may be partly because older persons are less likely to have had a history of HIV testing [34]. Higher percentages of recent infection in women can also be partly explained by data showing higher HIV testing rates in women compared to men [34,35]. The 2015-16 Malawi Population–based HIV Impact Assessment (MPHIA) survey estimated that 5.1% of women had never tested for HIV or received an HIV test result compared to 12.4% of men [36]. This is especially true in antenatal care (ANC) settings, which are accessed by 95% of pregnant women in Malawi [37], compared to studies that show that as few as 35% of the male partners of

pregnant women are tested for HIV [38,39]. Women may be offered HIV testing both during maternal health visits (ANC, labour and delivery, and postnatal) and during paediatric services for their children. Therefore, women may have multiple opportunities for routine HIV testing, increasing the likelihood of being diagnosed with HIV early [40].

The higher proportion of women currently breastfeeding who tested recent for HIV may similarly be explained by HIV testing practices in Malawi for pregnant and breastfeeding women. Women who test positive during breastfeeding may have tested negative during pregnancy but seroconverted either later in the pregnancy or postpartum after becoming sexually exposed to HIV. Others might not have had their HIV status ascertained during pregnancy but tested positive during the breastfeeding period [41]. The smaller number of women breastfeeding compared to women who were not pregnant or breastfeeding, or were currently pregnant, may have influenced the results and explain why this variable did not remain strongly significant in the multivariable model, though it does border on significance. Chagomerana et al. conclude in their ANC cohort study in Malawi that mother-to-child-transmission (MTCT) occurred disproportionately among women with a last positive HIV test during breastfeeding. This means that testing delayed until the postpartum period may lead to higher MTCT and that PMTCT programmes should focus on early ART initiation and providing targeted testing, prevention, treatment, and support to breastfeeding women [41]. Because breastfeeding women do not routinely attend health facilities after the first set of infant vaccinations, the Malawi 2022 HIV testing guidelines has added HIV testing of mothers at the measles vaccination visit (9-12 months) to increase the chances of finding incident infected mothers [42].

There is a clear association in our survey between a history of HIV testing within the last 12 months and recent HIV infection. This finding is consistent with other studies that demonstrate a higher rate of HIV testing history among newly HIV-positive individuals, with as much as 70% of newly diagnosed individuals reporting previously testing either positive (meaning they were not truly newly diagnosed) or negative [43]. Since recent infection assays are designed to detect HIV infections that have occurred during the previous 12 months, it seems reasonable that persons who tested positive with a recent infection during our surveillance were more likely to have perceived themselves at risk and sought HIV testing sometime during the previous 12 months when compared to those persons who had not, or who reported never having been tested for HIV. Also, people newly diagnosed with HIV and with a recent history of testing negative for HIV are likely to have seroconverted since their last test and thus are more likely to be recently infected. More needs to be done to enhance the quality of HIV prevention counseling, such as initiation of pre-exposure prophylaxis PrEP and a thorough assessment of factors that may have influenced the person to seek a test.

There are several implications from our findings. The high percentage of long-term infections among newly diagnosed PLHIV found in the surveillance is alarming given that these late diagnoses mean that a significant proportion of the population is unaware of their HIV status and likely transmitting infection [44,45]. In addition, when these persons with long-term infections are then linked to treatment programs, they are more likely to experience poor health outcomes, including in the younger age groups [46-48]. This finding underscores the continued need for expanding HIV testing, as well as testing strategies that are narrowly focused on specific populations. Still, many of the long-term infections identified may be due to

persons who reported testing negative on their last test but in truth were previously diagnosed with HIV and possibly had a history of ART use, as shown by the many people who tested RTRI recent but were subsequently reclassified as long-term when their viral load test indicated they had a controlled viral load [49,50]. Given the high rate of retesting and re-diagnosis in Malawi [51] more research is needed to better understand stigma and misconceptions associated with revealing a history of testing positive, reasons why those who test positive will often retest even after starting treatment and how a person's retesting history, including the length of time between tests, may influence HIV outcomes [42]. Our study also points to the need for a unique identifier in Malawi that can be used during HTS to quickly identify and quantify HIV-positive retesters. This is increasingly important given that across sub-Saharan Africa, approximately 84% of people have knowledge of their HIV status [50].

AGYW continue to face the highest risk of HIV in Malawi. More needs to be done to expand HIV testing among AGYW in Malawi beyond traditional facility-based testing to modalities that are preferred by adolescents [52,53]. In a recent HIV testing study in Kenya, most AGYW participants (77.5%) chose staff-aided testing either at home or at a mobile event; (22.4%) chose self-testing; and only 2 (.2%) chose facility referral [54]. Even with the elevated risk that AGYW face, young men aged 15-24 in our surveillance also had high rates of new infections, and in multivariable analysis only young age remained significant, clearly indicating a need for renewed focus on HIV prevention in all youth aged 15-24 in Malawi.

The prevalence of recent infection was highest in four districts: Mzimba (in the northern region), Machinga and Chikwawa (in the southern region), and Lilongwe (in the central region). These findings provide new information to complement prevalence data from MPHIA studies

that have found that southern districts had higher HIV prevalence compared to central and northern districts (even though the MPHIA was not powered to provide district-level prevalence estimates) [3]. Since recent HIV surveillance can generate a disaggregated summary of where recent HIV infections occur at more granular geographic sub-units, such as district and health facilities [16], the rapid identification of such HIV transmission clusters is the next important step in utilizing the recent infection surveillance data in Malawi. This in fact has begun with a geospatial transmission "hotspot" analysis using Malawi recency data [55]. Continuing such analyses, and following them up with local facility-based investigations, may help explain our district-level recent infection findings more fully and can provide the basis for using recent infection surveillance to identify gaps in HIV prevention and care services [4-5,56,57].

The strength of this study includes the large sample of persons with new HIV diagnoses from districts in Malawi with high HIV prevalence. Since RITA was integrated into the national HTS model with high acceptability, these data are likely a good representation of the general characteristics of persons newly diagnosed with HIV seeking healthcare from health facilities in Malawi and similar settings. The study had some limitations. First, since the initial phase of the recent HIV infection surveillance system was focused on integrating recent HIV infection testing into routine HTS, additional data were not collected that may have helped identify factors associated with recent infection, such as marital status, cultural beliefs and socio-economic status. Future surveillance may benefit from linking information generated from recent HIV infection testing data with other sociodemographic factors and triangulating additional factors such as clinical history that may be related to recent HIV infection.

Participation in this investigation relied on self-reported history of HIV testing among eligible persons. Hence, as noted above, it is possible that participants were reluctant to disclose a previous HIV-positive diagnosis and were inadvertently included as a new HIV diagnosis. Indeed, UNAIDS/WHO estimates that approximately up to 50% of people testing positive in Malawi are re-diagnoses [51]. This would result in an underestimation of the proportion of recent infections. An overestimation of the proportion of recent infections would result from our exclusion from the analysis of persons screening HIV-positive with a self-test or visiting the facility for confirmatory testing (and testing HIV-positive). Another limitation is that the included districts, and therefore participants, are likely not representative of all of Malawi especially since districts were prioritized for HIV recency surveillance based on the number of newly reported HIV cases the year before. Additionally, during the time period reported additional districts and facilities were continuing to be added to the surveillance system and so these results cannot be generalized to all of Malawi. Finally, some validation studies of recency assays indicate a tendency to produce false-recent results, particularly for those individuals on antiretroviral therapy [10-12], which would result in an overestimation of the proportion of recent infections. However, efforts have been made to reduce false-recent results through the addition of recent infection testing algorithms (RITAs) [13] such as was used in this study.

CONCLUSION:

Recent HIV infection surveillance can help to identify socio-demographic, clinical and geographic factors associated with recent HIV infection. Given that recent infection surveillance in Malawi confirms the high risk of HIV faced by AGYW, youth-focused programs that aim to limit HIV acquisition and transmission among young people, especially young women, should

remain a priority and be strengthened to sustain the gains made towards HIV epidemic control in Malawi. More data derived from triangulation and modelling with other data sources, as well as recent infection cluster analyses, are needed to allow for the targeting of HIV interventions at the district level in the country. The higher percentage of long-term infections than recent infections among newly diagnosed PLHIV underscores the continuing need for innovative ways to expand targeted HIV testing to ensure early diagnosis and treatment, especially among hard-to-reach populations.

ETHICS APPROVAL STATEMENT

The protocol was approved by the National Health Sciences Research Committee in Malawi (# 18/11/2190) and by the U.S. Centers for Disease Control and Prevention (# 2019-13). All eligible clients provided verbal informed consent prior to being tested for recent HIV infection and verbal consent was documented. Recent infection results were not returned to the client. Results of recency testing (RTRI) were available to clinicians if needed, although these results were not intended to be used for clinical care. RITA results are not available to clinicians.

CONTRIBUTORSHIP STATEMENT

MTM supported implementation, paper conceptualization, data analysis and led writing of the final manuscript. EWM and STG co-wrote the final manuscript. JT, IN, FB, CLB, DP, NWK, ANK, EK, AA, YB and GB led implementation in Malawi and contributed to writing and review of the final manuscript. GOM served as Principal Investigator at UW and contributed to writing and review of the final manuscript. KGC, MA, TD, and VS at CDC Atlanta supported technical

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COMPETING INTERESTS

None of the authors have a competing interest to disclose.

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DATA SHARING AGREEMENT

This paper is based on public health surveillance data collected on an ongoing basis by the Ministry of Health and Social Services in Malawi. To request data please contact author Rose Nyirenda (rnyirenda@hivmw.org).

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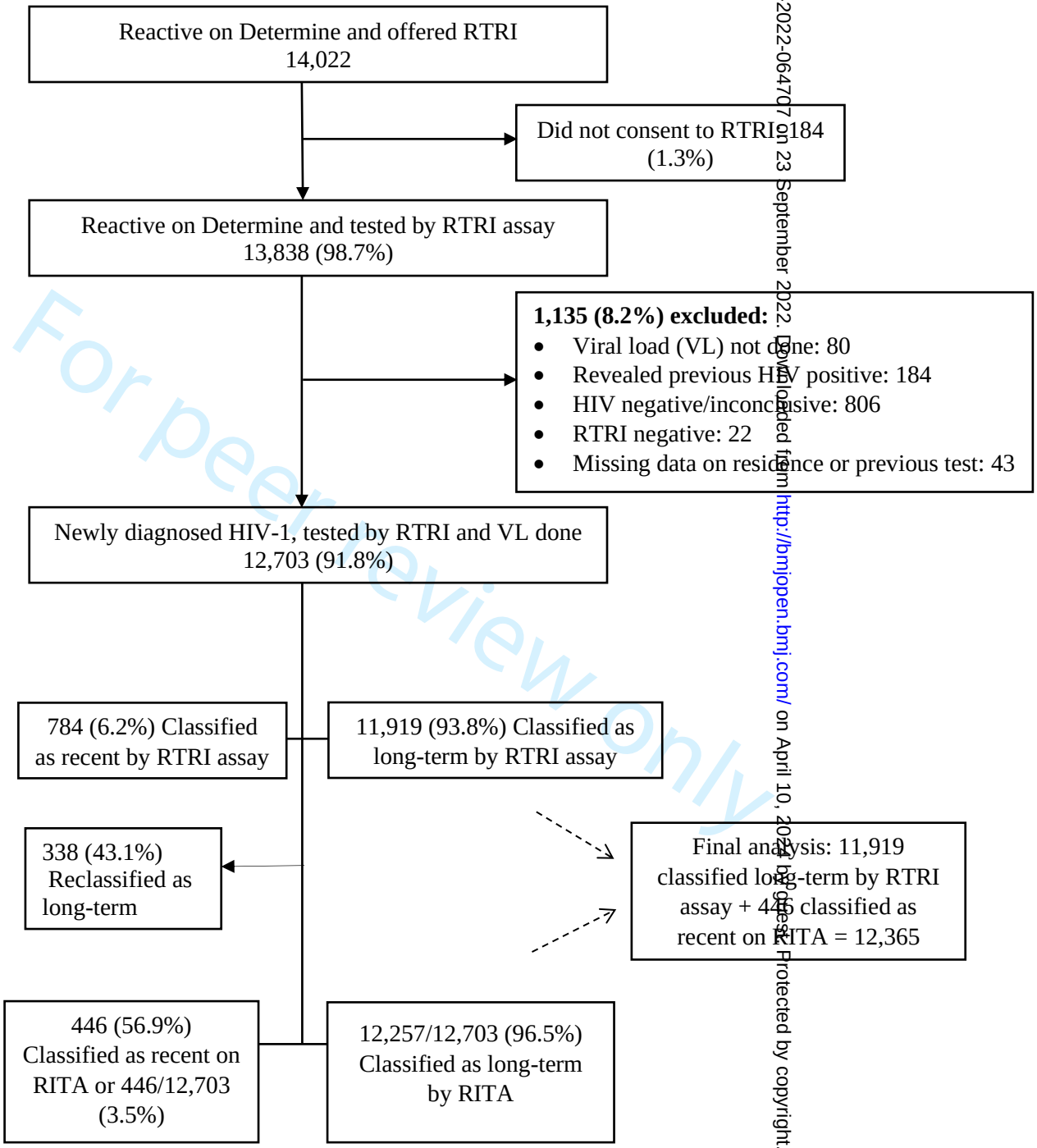
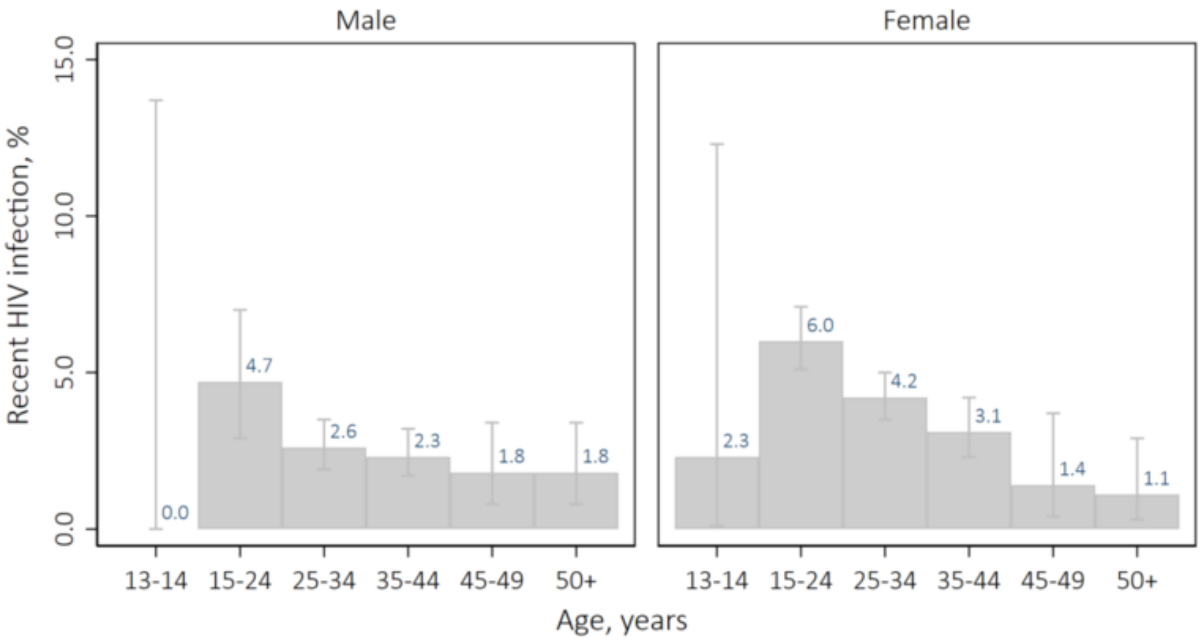
FIGURE LEGENDS/CAPTION

Figure 1: Participant eligibility flowchart for participants included in HIV recency surveillance in Malawi, from September 2019 to March 2020.

Figure 2: Number and percentage of recent HIV infections, per RITA, by age group among men and women included in HIV recency surveillance in Malawi, from September 2019 to March 2020.

RITA: recent infection testing algorithm





# RITA-recent	0	22	43	41	9	9	1	137	130	46	4	4
# of clients	0	473	1651	1746	493	496	43	2275	3071	1464	277	351

Figure 2: Number and percentage of recent HIV infections, per RITA, by age group among men and women included in HIV recency surveillance in Malawi, from September 2019 to March 2020.

RITA: recent infection testing algorithm

Appendix Table:

Characteristics of RTRI recent cases reclassified as long-term after Viral Load test (n = 338)

Characteristic	RTRI recent reclassified LT after VL testing, n(%)
Number of clients	338
Sex	
Male	70(20.7)
Female not pregnant	201(59.5)
Female pregnant	59(17.5)
Female breastfeeding	8(2.4)
Age (years)	
13-14	1(0.3)
15-24	108(32.0)
25-34	141(41.7)
35-44	71(21.0)
45-49	9(2.7)
50+	8(2.4)
Residence	
Urban	164(48.5)
Rural	174(51.5)
Previous HIV test	
≤12 months	189(55.9)
>12 months	112(33.1)
Never tested	37(10.9)

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

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

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 5-6
		(*b*) Report category boundaries when continuous variables were categorized	Pages 5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Pages 5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pages 5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pages 7-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 7-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 7-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 7-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Title Page

*Give information separately for exposed and unexposed groups.

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

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Characterizing persons diagnosed with HIV as either recent or long-term using a cross-sectional analysis of recent infection surveillance data collected in Malawi from September 2019 to March 2020

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TITLE:

Characterizing persons diagnosed with HIV as either recent or long-term using a cross-sectional analysis of recent infection surveillance data collected in Malawi from September 2019 to March 2020

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SHORT TITLE: Characterizing recent HIV infections in Malawi

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KEY WORDS:

Surveillance, recent infection testing algorithm, RITA, rapid test for recent infection, RTRI, HIV, HIV prevention

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ABSTRACT

Objectives: In Malawi a recent infection testing algorithm (RITA) is used to characterize infections of persons newly diagnosed with HIV as recent or long-term. This paper shares results from recent HIV infection surveillance and describes distribution and predictors.

Setting: Data from 155 health facilities in 11 districts in Malawi were pooled from September 2019 to March 2020.

Participants: Eligible participants were ≥13 years, and newly diagnosed with HIV. Clients had RITA recent infections if the rapid test for recent infection (RTRI) test result was recent and viral load (VL) ≥1,000 copies/mL; if VL was <1000 copies/mL the RTRI result was reclassified as long-term. Results were stratified by age, sex, pregnancy/breastfeeding status, and district.

Results: 13,838 persons consented to RTRI testing and 12,703 had valid RTRI test results and VL results after excluding clients not newly HIV-positive, RTRI negative or missing data (n=1135). A total of 12,365 of the 12,703 were included in the analysis after excluding those whose RTRI results were reclassified as long term (n=338/784 or 43.1%). The remainder, 446/12,703 or 3.5%, met the definition of RITA recent infection. The highest percentage of recent infections was among breastfeeding women (Crude Odds Ratio [COR] 3.2; 95% CI 2.0-5.0), young people aged 15-24 years-old (COR 1.6; 95% CI 1.3-1.9) and persons who reported a negative HIV test within the past 12 months (COR 3.3; 95% CI 2.6-4.2). Factors associated with recent infection in multivariable analysis included being a non-pregnant female (Adjusted Odds Ratio [AOR] 1.4; 95% CI 1.2-1.8), a breastfeeding female (AOR 2.2; 95% CI 1.4-3.5), aged 15-24 years (AOR 1.6; 95% CI 1.3-1.9) and residents of Machinga (AOR 2.0; 95% CI 1.2-3.5) and Mzimba (AOR 2.4; 95% CI 1.3-4.5) districts.

Conclusions: Malawi's recent HIV infection surveillance system demonstrated high uptake and identified sub-populations of new HIV diagnoses with a higher percentage of recent infections.

STRENGTHS AND LIMITATIONS OF THE STUDY

- This paper reports on HIV recent infections that are verified by viral load (VL); if the rapid test for recent infection (RTRI) test result is recent and VL ≥1,000 copies/mL the RTRI recent result is considered valid and if VL is <1000 copies/mL the RTRI recent test result is reclassified as a long-term infection.
- HIV recent infection surveillance in Malawi is integrated into HIV testing services so that all eligible persons who test HIV-positive, and provide consent, are tested with an RTRI.
- When implementing HIV recent infection surveillance in Malawi it is not possible to deduplicate HIV positive persons retesting for HIV because there are no unique national IDs at the moment that would allow deduplication.
- Data reported in this paper include varying levels of implementation of HIV recent infection surveillance by district in Malawi and these differences in coverage could influence district-level HIV recent infection rates reported and the statistical significance of those findings.

INTRODUCTION:

The East and Southern Africa regions bear the highest burden of the global HIV/AIDS epidemic. In 2018, the number of people living with HIV (PLHIV) in these two regions was

approximately 20.6 million, accounting for 47% of HIV infections worldwide [1]. In 2018, the HIV prevalence in Malawi was estimated at 9.2%, with almost one million PLHIV and 38,000 new HIV infections annually [2]. This prevalence is a considerable decline of almost 5% from 2005, when the prevalence was 14.1% and there were 66,000 new infections annually [2].

Malawi has also made great progress in reaching the UNAIDS 95-95-95 goals that were set in 2014. According to the 2020-21 Malawi population-based HIV impact assessment (MPHIA), 88% of PLHIV knew their HIV status, 98% of PLHIV who knew their status were on treatment, and 97% of those on treatment had achieved viral suppression [3]. Expanded access to HIV treatment has resulted in a substantial 55% decrease in AIDS-related deaths, from 29,000 in 2010 to 13,000 in 2018, with more PLHIV living healthy and longer lives on antiretroviral therapy (ART) than ever before [2].

Although prevalence is a basic epidemiological measure in countries with generalized HIV epidemics, it is a poor indicator of changes in the epidemic. Since HIV is a lifelong infection, prevalence measures are cumulative, and do not differentiate between those who have been living with HIV for many years and recent transmission [4]. To better understand the epidemic and focus appropriate HIV-prevention programs among specific populations and locations, it is important to identify patterns and trends of recent HIV infections [5]. Detecting locales with high amounts of ongoing HIV transmission and describing factors associated with recent infections can provide critical information for targeting HIV prevention strategies and measuring their impact [4,6–9].

Antibody-based rapid tests for recent infection (RTRI) can distinguish recent HIV infection, i.e., an infection that has likely occurred within the last 12 months, from long-term

infection [10-12]. However, their interpretation is challenged by factors that can cause 'false-recent' results such as variable immune responses at the individual-level, variable performance of the assay across diverse HIV-1 subtypes and across populations with naturally low viral loads, or current ARV use and advanced HIV disease [13]. To improve the accuracy of interpretation, recent infection testing algorithms (RITAs) incorporate the RTRI result with other markers of chronic infection (low viral load, evidence of treatment) [14]. In Malawi, use of RTRI at the point-of-care was first evaluated in a pilot study in 4 districts between November 2017 and June 2018 [15]. Lessons learned from this pilot study were used to establish a plan for a nationwide surveillance system for recent HIV infections starting in April 2019, using RTRI and RITA [16].

This paper shares results from the Malawi recent HIV infection surveillance system, with the aim of describing the distribution and predictors of recent HIV infections among people newly diagnosed with HIV.

METHODS:

This analysis includes persons enrolled in HIV recent infection surveillance between September 2019 and March 2020 at 155 health facilities in 11 districts of Malawi. Data from April 2019 to August 2019 were excluded from the analysis due to variability in facility data collection during the 5-month startup phase of the surveillance system. Surveillance was implemented over time, so not all facilities and districts were collecting data during this entire time period; for the time period reported on, recency surveillance had expanded to 11 of 28 districts and to 155 of an envisioned 251 facilities. The districts were prioritized for surveillance based on numbers of HIV-positive diagnoses in 2018 [17]. All persons presenting for testing

who reported being HIV negative or unknown (or having never tested HIV positive) and who were aged 13 years and older were eligible to consent to recent infection surveillance. Among those who provided consent, only those who were subsequently reactive for HIV using a rapid test were enrolled in recent infection surveillance. Persons were excluded from the analysis if they tested HIV-positive using self-testing, or were otherwise screened for HIV in the community and accessed the health facility only for HIV confirmatory testing.

All HIV testing was accompanied by pre- and post-test counselling and followed the national testing algorithm [18]. As noted, only persons who were 1) reactive on the first HIV rapid test in the national algorithm (Determine®) and 2) had provided verbal consent (i.e., eligible for recency testing) were subsequently tested with Asanté™ HIV Rapid Recency Assay (Sedia Biosciences, Portland OR, USA) simultaneously with the HIV rapid test UniGold® [19,20]. Verbal consent was the preferred consent format due to the low-risk nature of the surveillance and to better integrate recency testing into Government of Malawi-approved HIV testing guidelines. Persons under the age of 18 provided verbal consent to participate in recency testing and assent was not used. Even though the Asanté assay is currently validated for persons 15 years and older [19], ethics committees approved verbal consent for persons 13 years and older because this age is also allowed to consent to HIV testing per Malawi Government HIV testing services (HTS) guidelines. The protocol was approved by the National Health Sciences Research Committee in Malawi and by a U.S. Centers for Disease Control and Prevention institutional review board (IRB).

Both the UniGold® and Asanté™ HIV Rapid Recency Assay results were entered into the study database via a tablet. Persons testing negative with UniGold® were dropped from all

analyses. A dried blood spot specimen for VL testing was collected from persons who tested both HIV-positive and recent on the RTRI assay to assess recent infection per the RITA. HIV plasma RNA VL was quantified using Abbott m2000 Real Time HIV Viral Load Assay according to manufacturer instructions [21].

Routinely collected HIV testing data, including demographics, self-reported HIV testing history, and RTRI results were recorded in a surveillance data register at each facility. We abstracted these data using ODK software [22] and sent them to a central data repository. Once VL results were available from the National HIV Reference Laboratory, these data were merged with the RTRI data using Stata version 13.0 (Stata Corporation, College Station, Texas, USA). In the merged dataset, all persons with recent results on the RTRI assay and VL ≥ 1000 copies/mL were classified as RITA recent infections. All persons with long-term results on the RTRI assay were classified as long-term infections. Persons with an RTRI result indicating a recent infection and VL <1000 copies/mL were excluded from the analysis since viral suppression likely indicates current or recent ART use and, therefore, not a new diagnosis. The RTRI and RITA results were not returned to the clients (clients were aware when they provided consent) or clinicians but only used for surveillance. We were unable to identify people retesting for HIV or already on ART because the current system for HTS and ART does not use a unique identifier (eg national identification number) that would allow deduplication.

We calculated percentages of people with newly diagnosed HIV infections classified as RITA recent by age, sex, and district of residence. We used Chi-square and the Kruskal-Wallis test to compare proportions and medians (interquartile range [IQR]), respectively. We used logistic regression, with cluster-based robust standard errors [23] to account for clustering of

individuals within health facilities, to model factors associated with recent infection. We calculated unadjusted and adjusted odds ratios (ORs and AORs, respectively) with 95% confidence intervals (CIs) to assess the associations between recent infection and demographic factors. The a priori variables chosen for inclusion in multivariable analysis were age and sex. Variables associated with recent infection at a significance level of p-value <0.05 in univariable analysis and those known to be risk factors for HIV infection or suspected confounders were included in the multivariable logistic regression model through the stepwise method. In multivariable logistic regression, we thus adjusted for age group and sex, urban/rural location of the participant's residence, and district of participant's residence. HIV testing history was not adjusted for in the multivariable analysis due to its clear role in mediating recent HIV infection. Statistical analyses were performed using Stata.

The protocol was approved by the National Health Sciences Research Committee in Malawi (# 18/11/2190) and by the U.S. Centers for Disease Control and Prevention (# 2019-13).

Patient and Public Involvement

Our study involved the analysis of routinely collected Government of Malawi surveillance data and thus involvement from patient or members of the public in the design, conduct, reporting and dissemination plans for the study was not possible. Starting in late 2021, investigations of locales with higher rates of recent infections, and initiation of public health interventions based on the results, have included more patient and public involvement.

RESULTS:

Between September 2019 and March 2020, 14,022 eligible persons aged ≥13 were asked to participate in recency testing. Of these, 13,838/14,022 (98.7%) consented to recent HIV infection surveillance and were then tested using the RTRI assay (Figure 1). Of these, a total of 1,135/13,838 (8.2%) were excluded for the following reasons: an HIV-negative or invalid UniGold® result (n=806), disclosure of a previous HIV-positive diagnosis during post-test counselling (n=184), missing VL results (n=80), missing data on previous HIV test (n=43) or a negative (or invalid) result on the RTRI assay (n=22) (Figure 1). Of the 12,703 participants included in the analysis after these exclusions, 784/12,703 were RTRI recent (6.2%) and 11,919/12,703 were RTRI long-term (93.8%). Of the 784 RTRI recent participants, 446/784 (56.9%) met the definition of RITA recent infection and 338/784 (43.1%) met the definition of RITA long-term infection. This corresponds to an overall RITA-recent rate of 3.5% (446/12,703) and a RITA long-term rate of 96.5% (12,257/12,704). The final analysis thus included 446 RITA-recent cases and 11,919 long-term infection cases (as classified by RTRI) or a total of 12,365 participants. The final analysis did not include RITA long-term cases since technically these cases could not be reclassified as RTRI long-term (338) (Figure 1).

Characteristics of surveillance participants

Females accounted for 60.5% (n=7,481) of the participants included in the analysis (Table 1) and, among those of reproductive age, 70.2% were not pregnant (n=4,974), 26.7% were pregnant (n=1,893) and 3.1% were breastfeeding (n=220). The overall median age for all participants was 31 years (IQR: 25–39 years), with the most common age group being 25-34 years old (38.2%, n=4,722). Participants were split almost equally between urban and rural

settings. A large proportion of recent infection surveillance participants were from Blantyre district (47.3%, n=5,851), followed by Zomba district (14.6%, n=1,800), Lilongwe district (7.7%, n=949) and Machinga (7.6%, n=941). The proportion of participants who reported an HIV test within the previous 12 months (40.9%, n=5,055) versus more than 12 months ago (40.7%, n=5,030) were similar, while a lower proportion reported never having tested before (18.4%, n=2,280; Table 1).

Table 1: Characteristics of recent HIV infection surveillance among Determine reactive participants in Malawi, from September 2019 to March 2020 (n = 12,365).

Characteristic	Total % (n)
Sex	
Male	39.5 (4,884)
Female	60.5 (7,481)
[a]Pregnancy & Breastfeeding	
Not pregnant	70.2 (4,974)
Pregnant	26.7 (1,893)
Breastfeeding	3.1 (220)
Age, years	
13-14	0.6 (68)
15-24	22.2 (2,748)
25-34	38.2 (4,722)
35-44	26.0 (3,210)
45-49	6.2 (770)
≥50	6.9 (847)
Median (IQR)	31 (25-39)
Residence	
Urban	48.5 (5,992)
Rural	51.5 (7,373)
District of residence	
Balaka	2.3 (283)
Blantyre	47.3 (5,851)
Chikwawa	6.2 (763)
Lilongwe	7.7 (949)
Machinga	7.6 (941)
Mangochi	6.5 (806)
Mzimba	1.7 (214)
Zomba	14.6 (1,800)

Other	6.1 (758)
[b]Previous HIV test	
≤12 months	40.9 (5,055)
>12 months	40.7 (5,030)
Never tested	18.4 (2,280)

[a]Data for all females in the reproductive age group (15-49 years)

[b]≤12 months and >12 months represent clients last self-reported HIV-negative test

Characteristics of persons with recent HIV infection

Breastfeeding women (COR 3.2; 95% CI 2.0-5.0) had the highest proportion of recent infections, which was significantly higher than that of pregnant (COR 1.6; 95% CI 1.2-2.1) and non-pregnant women (COR 1.7; 95% CI 1.4-2.1) (Table 2). By age group, recent HIV infections were highest in females (6.0%) and males (4.7%) aged 15-24 years (Figure 2) and highest in the 15-24 age group overall (COR 1.6; 95% CI 1.3-1.9) (Table 2). A larger percentage of recent infection among people with new HIV diagnoses was found in persons from rural settings (COR 1.3; 95% CI 1.0-1.8) compared to urban settings, although this was not statistically significant. Residents of Mzimba (COR 2.3; 95% CI 1.3-4.0), Machinga (COR 2.1; 95% CI 1.2-3.5), Balaka (COR 1.7; 95% CI 0.7-4.1), Lilongwe (COR 1.6; 95% CI 1.0-2.6) and Chikwawa (COR 1.6; 95% CI 1.0-2.6) districts had the highest proportions of recent infections, although not statistically significant in Balaka, Lilongwe and Chikwawa. By HIV testing history, the highest percentage of recent infections (COR 3.3; 95% CI 2.6-4.2) was among persons who self-reported having had a negative HIV test within the 12 months prior (Table 2). For a description of persons with long-term infections (n = 338), please see additional table in Appendix.

Table 2: Prevalence of RITA recent HIV infections by participant characteristic and factors associated with RITA recent HIV infection in persons with new HIV diagnoses among surveillance participants in Malawi, from September 2019 to March 2020 (n = 12,365).

	Total	Recent infections	Crude	Adjusted*
Characteristic		**%**	**OR (95% CI)**	**OR (95% CI)**
Total	12,365	3.6	--	--
Sex				
Male	4,884	2.5	1	1
Female not pregnant	5,362	4.3	1.7 (1.4-2.1)	1.4 (1.2-1.8)
Female pregnant	1,898	4.0	1.6 (1.2-2.1)	1.1 (0.8-1.5)
Female breastfeeding	221	7.7	3.2 (2.0-5.0)	2.2 (1.4-3.5)
Age, years				
13-14	68	1.5	0.4 (0.1-2.7)	0.4 (0.1-2.6)
15-24	2,748	5.8	1.6 (1.3-1.9)	1.6 (1.3-1.9)
25-34	4,722	3.7	1	1
35-44	3,210	2.7	0.7 (0.6-0.9)	0.8 (0.6-0.9)
45-49	770	1.7	0.5 (0.3-0.8)	0.5 (0.3-0.9)
≥50	847	1.5	0.4 (0.2-0.7)	0.4 (0.2-0.7)
Residence				
Urban	5,992	3.1	1	1
Rural	6,373	4.1	1.3 (1.0-1.8)	1.0 (0.7-1.5)
District of residence				
Balaka	283	5.7	1.7 (0.7-4.1)	1.7 (0.7-4.0)
Blantyre	5,851	2.6	0.8 (0.5-1.2)	0.8 (0.5-1.2)
Chikwawa	763	5.2	1.6 (1.0-2.6)	1.5 (0.9-2.4)
Lilongwe	949	5.5	1.6 (1.0-2.6)	1.7 (1.0-2.8)
Machinga	941	6.8	2.1 (1.2-3.5)	2.0 (1.2-3.5)
Mangochi	806	3.5	1.0 (0.6-1.8)	1.0 (0.6-1.8)
Mzimba	214	7.5	2.3 (1.3-4.0)	2.4 (1.3-4.5)
Zomba	1,800	3.4	1	1
Other	758	2.5	0.7 (0.4-1.2)	0.7 (0.4-1.2)
[b]Previous HIV test				
≤12 months	5,055	6.1	3.3 (2.6-4.2)	
>12 months	5,030	1.9	1	
Never tested	2,280	2.0	1.1 (0.7-1.5)	

IQR: interquartile range, OR: odds ratio, CI: confidence interval

*Adjusted for age group, sex, urban/rural location of the participant's residence and district of participant's residence

[b]≤12 months and >12 months represent clients last self-reported HIV-negative test

Factors associated with recent HIV infection

After adjusting for age group and sex, urban/rural location of the participant's residence, and district of participant's residence, factors significantly associated with recent infection among newly diagnosed PLHIV included living in Mzimba (AOR 2.4; 95% CI 1.3-4.5) or Machinga (AOR 2.0; 95% CI 1.2-3.5) districts compared to Zomba district and being aged 15-24 years (AOR 1.6; 95% CI 1.3-1.9) compared to being aged 25-34 years. Women who were not pregnant (AOR 1.4; 95% CI 1.2-1.8) and women who were breastfeeding (AOR 2.2; 95% CI 1.4-3.5) compared to males were also at higher risk of recent infection (Table 2).

DISCUSSION:

Our results demonstrate an association of recency with plausible risks such as younger age (<25 years) and sex (women vs men), confirming successful implementation of recency surveillance in Malawi. In addition, recency testing identified breastfeeding women and residents of certain districts as persons at higher risk of ongoing HIV transmission. In multivariable regression, factors that remained associated with recent infection included younger age and district of residence.

The proportion of recent infections found in this study, using RITA, was 3.5%. There have been no other national-level recent infection studies in Malawi to compare these findings to. A study in Kenya using similar methods, though with a much smaller sample size and using a laboratory-based recency test, reported a recent infection percent of 8.6% in Nairobi [24] and another recency study in Zimbabwe reported a recent infection percent of 10.5% among female sex workers [25]. Our study likely has a comparatively lower recent infection rate given the

large, national sample that constitutes the recent infection surveillance system in Malawi. For example, a larger surveillance study recently completed in Cambodia found a RITA recent rate of 5.0% and surveillance of 27,792 newly HIV-diagnosed individuals in Nigeria found a RITA recent rate of 2.4% [26,27]. Furthermore, recency surveillance in Eswatini found an overall RITA recent rate of 3.1% and surveillance in DRC found an overall RITA recent rate of 5.0% [28,29]. All of these surveillance studies were aided by the use of clinical information such as viral load, history of prior HIV diagnosis, and antiretroviral therapy-exposure to confirm that a recent infection was truly recently acquired and not a long-term infection. The validity of our recent infection rate in Malawi is also aided by the inclusion of viral load testing to rule out long-term infections—without the viral load testing the percent HIV recent would have been 6.2%.

The percentage of recent infections among newly diagnosed females aged 15-24 years, or adolescent girls and young women (AGYW), in this study was lower than that found in previous studies in Malawi [30] and in nearby countries [31-33]. The differences in percentages may be attributed to factors such as the case definition of recent infection. For example, in Kim et al. in Kenya the case definition for recent infection included testing recent on LAg and having no evidence of antiretroviral therapy use [31]. This case definition for recent infection is different from what is used in this analysis, which used a point-of-care test and did not use antiretroviral therapy use as an eligibility criterion.

The multivariable analysis indicating a risk of recent HIV infection among younger persons may be partly because older persons are less likely to have had a history of HIV testing [34]. Higher percentages of recent infection in women can also be partly explained by data showing higher HIV testing rates in women compared to men [34,35]. The 2015-16 Malawi

Population–based HIV Impact Assessment (MPHIA) survey estimated that 5.1% of women had never tested for HIV or received an HIV test result compared to 12.4% of men [36]. This is especially true in antenatal care (ANC) settings, which are accessed by 95% of pregnant women in Malawi [37], compared to studies that show that as few as 35% of the male partners of pregnant women are tested for HIV [38,39]. Women may be offered HIV testing both during maternal health visits (ANC, labour and delivery, and postnatal) and during paediatric services for their children. Therefore, women may have multiple opportunities for routine HIV testing, increasing the likelihood of being diagnosed with HIV early [40].

The higher proportion of women currently breastfeeding who tested recent for HIV may similarly be explained by HIV testing practices in Malawi for pregnant and breastfeeding women. Women who test positive during breastfeeding may have tested negative during pregnancy but seroconverted either later in the pregnancy or postpartum after becoming sexually exposed to HIV. Others might not have had their HIV status ascertained during pregnancy but tested positive during the breastfeeding period [41]. The smaller number of women breastfeeding compared to women who were not pregnant or breastfeeding, or were currently pregnant, may have influenced the results and explain why this variable did not remain strongly significant in the multivariable model, though it does border on significance. Chagomerana et al. conclude in their ANC cohort study in Malawi that mother-to-child-transmission (MTCT) occurred disproportionately among women with a last positive HIV test during breastfeeding. This means that testing delayed until the postpartum period may lead to higher MTCT and that PMTCT programmes should focus on early ART initiation and providing targeted testing, prevention, treatment, and support to breastfeeding women [41]. Because

breastfeeding women do not routinely attend health facilities after the first set of infant vaccinations, the Malawi 2022 HIV testing guidelines has added HIV testing of mothers at the measles vaccination visit (9-12 months) to increase the chances of finding incident infected mothers [42].

There is a clear association in our survey between a history of HIV testing within the last 12 months and recent HIV infection. This finding is consistent with other studies that demonstrate a higher rate of HIV testing history among newly HIV-positive individuals, with as much as 70% of newly diagnosed individuals reporting previously testing either positive (meaning they were not truly newly diagnosed) or negative [43]. Since recent infection assays are designed to detect HIV infections that have occurred during the previous 12 months, it seems reasonable that persons who tested positive with a recent infection during our surveillance were more likely to have perceived themselves at risk and sought HIV testing sometime during the previous 12 months when compared to those persons who had not, or who reported never having been tested for HIV. Also, people newly diagnosed with HIV and with a recent history of testing negative for HIV are likely to have seroconverted since their last test and thus are more likely to be recently infected. More needs to be done to enhance the quality of HIV prevention counseling, such as initiation of pre-exposure prophylaxis PrEP and a thorough assessment of factors that may have influenced the person to seek a test.

There are several implications from our findings. The high percentage of long-term infections among newly diagnosed PLHIV found in the surveillance is alarming given that these late diagnoses mean that a significant proportion of the population is unaware of their HIV status and likely transmitting infection [44,45]. In addition, when these persons with long-term

infections are then linked to treatment programs, they are more likely to experience poor health outcomes, including in the younger age groups [46-48]. This finding underscores the continued need for expanding HIV testing, as well as testing strategies that are narrowly focused on specific populations. Still, many of the long-term infections identified may be due to persons who reported testing negative on their last test but in truth were previously diagnosed with HIV and possibly had a history of ART use, as shown by the many people who tested RTRI recent but were subsequently reclassified as long-term when their viral load test indicated they had a controlled viral load [49,50]. Given the high rate of retesting and re-diagnosis in Malawi [51] more research is needed to better understand stigma and misconceptions associated with revealing a history of testing positive, reasons why those who test positive will often retest even after starting treatment and how a person's retesting history, including the length of time between tests, may influence HIV outcomes [42]. Our study also points to the need for a unique identifier in Malawi that can be used during HTS to quickly identify and quantify HIV-positive retesters. This is increasingly important given that across sub-Saharan Africa, approximately 84% of people have knowledge of their HIV status [50].

AGYW continue to face the highest risk of HIV in Malawi. More needs to be done to expand HIV testing among AGYW in Malawi beyond traditional facility-based testing to modalities that are preferred by adolescents [52,53]. In a recent HIV testing study in Kenya, most AGYW participants (77.5%) chose staff-aided testing either at home or at a mobile event; (22.4%) chose self-testing; and only 2 (.2%) chose facility referral [54]. Even with the elevated risk that AGYW face, young men aged 15-24 in our surveillance also had high rates of new

infections, and in multivariable analysis only young age remained significant, clearly indicating a need for renewed focus on HIV prevention in all youth aged 15-24 in Malawi.

The prevalence of recent infection was highest in four districts: Mzimba (in the northern region), Machinga and Chikwawa (in the southern region), and Lilongwe (in the central region). These findings provide new information to complement prevalence data from MPHIA studies that have found that southern districts had higher HIV prevalence compared to central and northern districts (even though the MPHIA was not powered to provide district-level prevalence estimates) [3]. Since recent HIV surveillance can generate a disaggregated summary of where recent HIV infections occur at more granular geographic sub-units, such as district and health facilities [16], the rapid identification of such HIV transmission clusters is the next important step in utilizing the recent infection surveillance data in Malawi. This in fact has begun with a geospatial transmission "hotspot" analysis using Malawi recency data [55]. Continuing such analyses, and following them up with local facility-based investigations, may help explain our district-level recent infection findings more fully and can provide the basis for using recent infection surveillance to identify gaps in HIV prevention and care services [4-5,56,57].

The strength of this study includes the large sample of persons with new HIV diagnoses from districts in Malawi with high HIV prevalence. Since RITA was integrated into the national HTS model with high acceptability, these data are likely a good representation of the general characteristics of persons newly diagnosed with HIV seeking healthcare from health facilities in Malawi and similar settings. The study had some limitations. First, since the initial phase of the recent HIV infection surveillance system was focused on integrating recent HIV infection testing

into routine HTS, additional data were not collected that may have helped identify factors associated with recent infection, such as marital status, cultural beliefs and socio-economic status. Future surveillance may benefit from linking information generated from recent HIV infection testing data with other sociodemographic factors and triangulating additional factors such as clinical history that may be related to recent HIV infection.

Participation in this investigation relied on self-reported history of HIV testing among eligible persons. Hence, as noted above, it is possible that participants were reluctant to disclose a previous HIV-positive diagnosis and were inadvertently included as a new HIV diagnosis. Indeed, UNAIDS/WHO estimates that approximately up to 50% of people testing positive in Malawi are re-diagnoses [51]. This would result in an underestimation of the proportion of recent infections. An overestimation of the proportion of recent infections would result from our exclusion from the analysis of persons screening HIV-positive with a self-test or visiting the facility for confirmatory testing (and testing HIV-positive). Another limitation is that the included districts, and therefore participants, are likely not representative of all of Malawi especially since districts were prioritized for HIV recency surveillance based on the number of newly reported HIV cases the year before. Additionally, during the time period reported additional districts and facilities were continuing to be added to the surveillance system and so these results cannot be generalized to all of Malawi. Finally, some validation studies of recency assays indicate a tendency to produce false-recent results, particularly for those individuals on antiretroviral therapy [10-12], which would result in an overestimation of the proportion of recent infections. However, efforts have been made to reduce false-recent results through the addition of recent infection testing algorithms (RITAs) [13] such as was used in this study.

CONCLUSION:

Recent HIV infection surveillance can help to identify socio-demographic, clinical and geographic factors associated with recent HIV infection. Given that recent infection surveillance in Malawi confirms the high risk of HIV faced by AGYW, youth-focused programs that aim to limit HIV acquisition and transmission among young people, especially young women, should remain a priority and be strengthened to sustain the gains made towards HIV epidemic control in Malawi. More data derived from triangulation and modelling with other data sources, as well as recent infection cluster analyses, are needed to allow for the targeting of HIV interventions at the district level in the country. The higher percentage of long-term infections than recent infections among newly diagnosed PLHIV underscores the continuing need for innovative ways to expand targeted HIV testing to ensure early diagnosis and treatment, especially among hard-to-reach populations.

ETHICS APPROVAL STATEMENT

The protocol was approved by the National Health Sciences Research Committee in Malawi (# 18/11/2190) and by the U.S. Centers for Disease Control and Prevention (# 2019-13). All eligible clients provided verbal informed consent prior to being tested for recent HIV infection and verbal consent was documented. Recent infection results were not returned to the client. Results of recency testing (RTRI) were available to clinicians if needed, although these results were not intended to be used for clinical care. RITA results are not available to clinicians.

CONTRIBUTORSHIP STATEMENT

MTM supported implementation, paper conceptualization, data analysis and led writing of the final manuscript. EWM and STG co-wrote the final manuscript. JT, IN, FB, CLB, DP, NWK, ANK, EK, AA, YB and GB led implementation in Malawi and contributed to writing and review of the final manuscript. GOM served as Principal Investigator at UW and contributed to writing and review of the final manuscript. KGC, MA, TD, and VS at CDC Atlanta supported technical implementation and contributed to review of the final manuscript. KN and RN supported implementation in Malawi and contributed to review of the final manuscript.

COMPETING INTERESTS

None of the authors have a competing interest to disclose.

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DATA SHARING AGREEMENT

This paper is based on public health surveillance data collected on an ongoing basis by the Ministry of Health and Social Services in Malawi. To request data please contact author Rose Nyirenda (rnyirenda@hivmw.org).

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Figure Legends/Captions:

Figure 1:

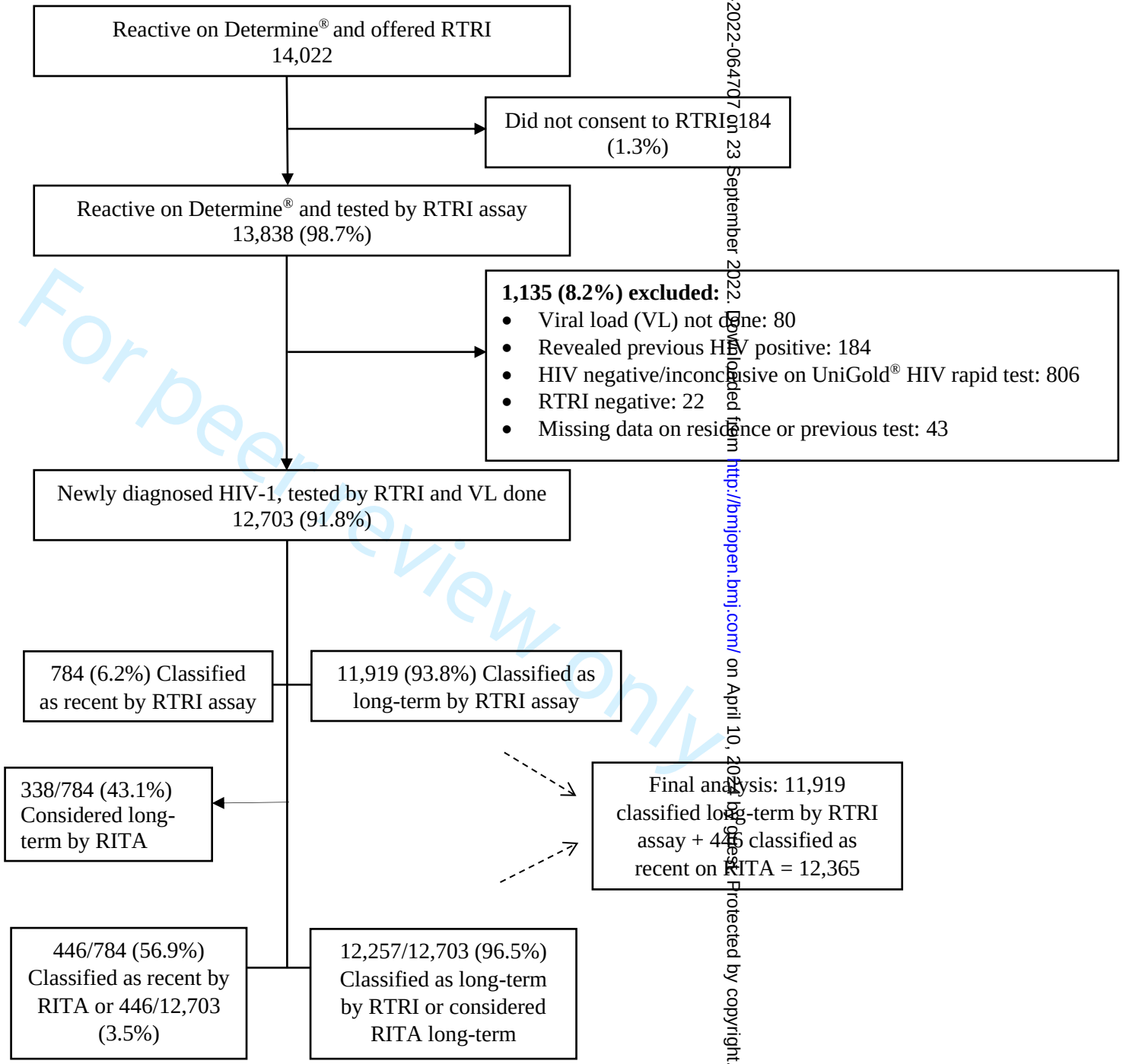
Flowchart of persons included in the analysis of HIV recency surveillance data in Malawi, from September 2019 to March 2020.

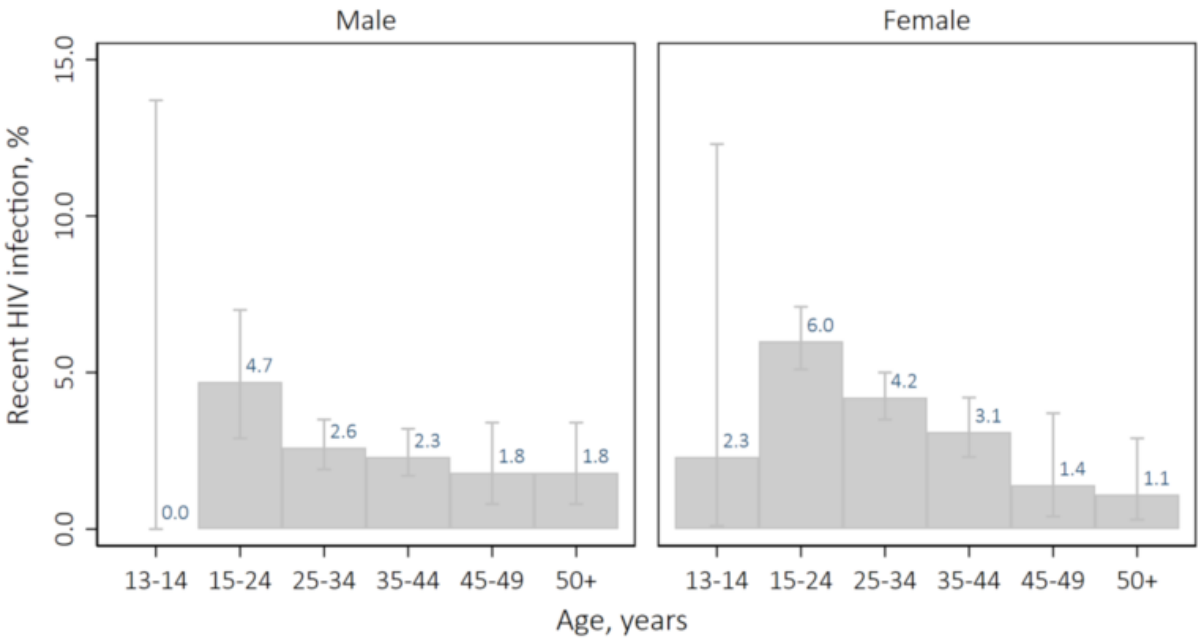
RTRI: rapid test for recent infection, RITA: recent infection testing algorithm, VL: viral load

Figure 2:

Number and percentage of recent HIV infections, per RITA, by age group among men and women included in HIV recency surveillance in Malawi, from September 2019 to March 2020.

RITA: recent infection testing algorithm





# RITA-recent	0	22	43	41	9	9	1	137	130	46	4	4
# of clients	0	473	1651	1746	493	496	43	2275	3071	1464	277	351

Figure 2: Number and percentage of recent HIV infections, per RITA, by age group among men and women included in HIV recency surveillance in Malawi, from September 2019 to March 2020.

RITA: recent infection testing algorithm

Appendix Table:

Characteristics of RTRI recent cases reclassified as long-term after Viral Load test (n = 338)

Characteristic	RTRI recent reclassified LT after VL testing, n(%)
Number of clients	338
Sex	
Male	70(20.7)
Female not pregnant	201(59.5)
Female pregnant	59(17.5)
Female breastfeeding	8(2.4)
Age (years)	
13-14	1(0.3)
15-24	108(32.0)
25-34	141(41.7)
35-44	71(21.0)
45-49	9(2.7)
50+	8(2.4)
Residence	
Urban	164(48.5)
Rural	174(51.5)
Previous HIV test	
≤12 months	189(55.9)
>12 months	112(33.1)
Never tested	37(10.9)

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

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

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 5-6
		(*b*) Report category boundaries when continuous variables were categorized	Pages 5-6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Pages 5-6
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pages 5-6
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pages 7-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 7-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 7-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 7-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Title Page

*Give information separately for exposed and unexposed groups.

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