RESEARCH ARTICLE

Recent HIV infections among newly diagnosed individuals living with HIV in rural Lesotho: Secondary data from the VIBRA cluster-randomized trial

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Abstract

Background

HIV recency assays are used to distinguish recently acquired infection from long-term infection among individuals newly diagnosed with HIV. Since 2015, the World Health Organisation recommends the use of an algorithm to assess recency of infections which is based on an HIV recency assay and viral load (VL) quantification. We determined the proportion of recent HIV infections among participants of the VIBRA (Village-Based Refill of Antiretroviral therapy) cluster-randomized trial in Lesotho and assessed risk factors for these recent infections.

Methods

The VIBRA trial recruited individuals living with HIV and not taking antiretroviral therapy during a door-to-door HIV testing campaign in two rural districts (Butha-Buthe and Mokhotlong). Samples were collected from participants newly diagnosed and tested for HIV recency using the Asante® HIV-1 Rapid Recency Assay and VL using the Roche Cobas System. Clinical and socio-demographic data were extracted from the trial database. Univariate analysis was conducted to determine factors associated with recent compared to long-term infection.

Results

Participants were recruited from August 2018 to May 2019 and 184 patient-samples included in this study. The majority were female (108 [59%]) with a median age of 36 years (interquartile range 30–50 years). We found 13 (7.0%) recent infections, while 171 (93.0%) were classified as long-term HIV infections. No conclusive evidence for risk factors of recent infection was found.

Conclusions

During door-to-door testing among a general population sample in rural Lesotho, 7% of those who were newly diagnosed had acquired HIV in the preceding 6 months. More efforts and research are needed to curb ongoing transmissions in these rural communities.
Introduction

In the past decades, progress in curbing the HIV/AIDS epidemic has been achieved [1]. However, the Joint United Nations Programme on HIV/AIDS (UNAIDS) indicates that there are still regions and population groups where new infection rates are rising [1]. The identification of newly infected individuals is important to help HIV prevention programs to determine where interventions are most needed [2]. Asante HIV-1 Rapid Recency Assay is a rapid in-vitro immunoassay that is designed to differentiate HIV infections in terms of their recency; whether they are long-term infections or recent infections (within the previous 6 months) [3]. During the early period of HIV infection, antibodies usually have low avidity (binding strength), and their avidity increases as the infection progresses. This assay makes use of this principle [4]. Moreover, this assay incorporates a recombinant protein that contains sequences from all major HIV subtypes, thus is applicable for all HIV subtypes [5]. The World Health Organisation (WHO) recommends using the Asante HIV-1 Rapid Recency Assay in combination with HIV viral load (VL) quantification as a recent infection testing algorithm (RITA). Individuals taking antiretroviral therapy (ART) should not be tested with the Asante HIV-1 Rapid Recency Assay, as a low VL leads to low antibody avidity and thus contributes to misclassification. Hence, the addition of VL in the WHO-recommended RITA [6]. The Asante HIV-1 Rapid Recency Assay has been validated under controlled conditions not only by the manufacturer but also by independent bodies such as the Center for Disease Control [3, 7, 8]. In sub-Saharan Africa, the assay has successfully been applied in various settings such as Nigeria [9], Malawi [10], Zimbabwe [11], and Lesotho [12]. Despite some concerns with its sensitivity [13], the Asante HIV-1 Rapid Recency Assay does reach acceptable criteria when combined with VL, as outlined by the Consortium for the Evaluation and Performance of HIV Incidence Assays [14], and was used by major national surveys in southern Africa [15].

HIV continues to cause a substantial disease burden in Lesotho, which has the second highest prevalence of HIV in the world [16]. Recency data may help to inform prevention measures to curb the epidemic, but such data are scarce.

The Village-Based Refill of Antiretroviral Therapy (VIBRA) trial was a cluster-randomized trial that started with a home-based HIV testing campaign among the general population in rural villages of two districts in Lesotho and was used as a platform for this study [17]. The VIBRA trial investigated village-based ART refill following same-day ART initiation versus clinic-based ART refill among individuals found living with HIV but not on ART during a home-based door-to-door HIV testing campaign and its effect on viral suppression at 1 year [18].

This nested study aimed to determine the proportion of recent HIV infections among newly diagnosed participants of the VIBRA study and to assess risk factors for these recent infections.

Methods

Study design and participants

This laboratory based cross-sectional study is embedded in VIBRA trial, a cluster-randomized trial that was conducted in two rural districts (Butha-Buthe and Mokhotlong) of Lesotho from 16 August 2018 to 28 May 2019 [18]. Eligible village-clusters were rural, were confined to the catchment area of the 20 health facilities, had a consenting village chief, and had at least 1 registered village health worker who agreed to participate and passed a skill assessment. All community members with a confirmed positive HIV test result (either known HIV-positive or newly tested on the day of the campaign) and not taking ART, i.e., never taken ART (ART-
naive) or had stopped ART more than 30 days prior (ART defaulter), were screened by the
study nurses for eligibility. VIBRA trial excluded individuals who planned to get care outside
the two study districts (e.g., in neighbouring South Africa), were physically, mentally, and
emotionally not able to participate according to the study nurse, were younger than 10 years,
had less than 35kg body weight, or were already in care for another chronic disease, because
VIBRA trial assessed a village-based ART refill model of care with by that time standard efavir-
enz-based first-line antiretroviral therapy.

The samples included in this nested study are from consenting and eligible VIBRA trial par-
ticipants. In addition, we restricted the sample to only those being newly diagnosed on the day
of the testing campaign. New diagnosis was self-reported and double-checked by the study
staff at the nearby health facility and their HIV testing registry.

**Data collection**

If eligible for VIBRA trial, a number of data and laboratory assessments were conducted. After
obtaining written informed consent, a venous blood draw as well as a finger prick were done.
The finger prick was conducted to perform several point-of-care tests (CD4 cell count, serum
creatinine, haemoglobin) to assess parameters for treatment initiation on the day of diagnosis
as described in the VIBRA study protocol [17]. In addition, venous blood was drawn by the
study nurse for storage of plasma in the -80˚C biobank.

Additionally, a questionnaire was administered to assess clinical, socio-demographic data,
which included age, sex, gender, district, employment, schooling and education, regular sexual
partner, as well as alcohol and cannabis use among others. Alcohol use was assessed with the
CAGE questionnaire which includes four questions that have been proven to assess alcohol
dependency [19]. The data were stored in the password protected VIBRA cloud database and
could only be accessed through regulated user profiles.

**Laboratory analyses**

During door-to-door HIV testing campaign, screening for HIV was performed by using
point-of-care Determine HIV1&2 and every reactive test was confirmed with Unigold
HIV1&2 [17]. Whole blood samples from new diagnoses were transported in cooler boxes
containing ice packs to the nearest hospital laboratory within 30 hours (Mokhotlong hospital
laboratory, Butha-Buthe hospital laboratory or Seboche hospital laboratory). On arrival at the
laboratories, the Ethylenediamine tetraacetic acid (EDTA) whole blood samples were centri-
fuged to obtain plasma and were divided into aliquots. At Mokhotlong hospital and Seboche
hospital, the plasma was then stored in a -20 degrees celsius freezer and shipped once a week
to biobank in the main study laboratory at Butha-Buthe Government Hospital Laboratory. In
Butha-Buthe Government Hospital Laboratory the samples were stored in a -80 degrees celsius
biobank freezer.

VL quantification was conducted on all the samples collected not only to confirm HIV-1
positive status but to use the VL for the RITA used in this study. This was done at Butha-Buthe
Government Hospital Laboratory on the Cobas 4800 system (Roche Molecular Diagnostics
[20]). HIV recency testing was conducted using the Asanté HIV-1 Rapid Recency Assay, a
point of care test [21]. For this, plasma was pipetted into a separate tube containing sample
buffer. The assay test strip was then inserted into the tube containing the sample-buffer mixture.
The mixture was absorbed into the absorbent pad of the test strip. Results were read visu-
ally after 20 minutes. The final classification of recent versus long-term infection was based on
the RITA recommended by the manufacturer and WHO, i.e. incorporating HIV VL [6, 21].
Samples that tested recent with the Asanté HIV-1 Rapid Recency Assay and had a VL greater
than 1,000 copies/ml were classified as true recent infection. Samples than were tested as recent on the Asanté HIV-1 Rapid Recency Assay but had a VL that was equal or less than 1,000 copies/ml were re-classified as long-term infections [6, 21].

**Statistical analysis**

Appropriate descriptive statistics were used, i.e. absolute and relative frequencies for categorical data and medians and interquartile ranges for continuous variables. Inferential statistical testing was performed to assess associated factors between clinical and socio-demographic characteristics and recent HIV diagnoses. For this risk factor analysis, we used a logistic regression model. We assessed sex, age, district, schooling, information about sexual partner frequency, substance abuse, CD4 cell count and viral load information in univariate models and in multivariate models as a second step if any associations shown. The choice of these variables was based on known or plausible setting-specific and clinical associations with recent or long-term infection [22]. Results are presented as odds ratios with 95% confidence intervals. All analyses were performed using Stata (version 14, Stata Corporation, Austin/Texas, USA). For all tests, we used complete case analysis, and two-sided p-values with alpha 0.05 level of significance.

**Ethics statement**

The VIBRA trial with its nested sub-studies has been approved by the National Health Research and Ethics Committee of the Ministry of Health of Lesotho (ID06–2018) and the ethics committee in Switzerland (Ethikkommission Nordwest- und Zentralschweiz; ID2018–00283). After initial approval, four minor amendments to the study protocol were submitted and approved by the National Health Research and Ethics Committee of the Ministry of Health of Lesotho, whereby the last amendment was submitted specifically for approval of this nested study (Protocol version 9). The study nurse obtained written informed consent in Sesotho, the local language. Illiterate participants provided a thumbprint, and a witness older than 21 years, chosen by the participant, co-signed the consent form. For participants aged < 18 years, a literate caregiver older than 21 years provided consent. All samples were assigned unique identification numbers to anonymize the data and protect confidentiality. At no point during the study the original source of the samples, i.e. participant’s names, were revealed.

**Results**

During the door-to-door HIV testing campaign, 11,291 were screened for VIBRA trial and 292 (2.6%) were found living with HIV (already known or newly diagnosed) (Fig 1). Of the 292 individuals living with HIV, 257 (88.0%) individuals were eligible for and consented to VIBRA trial.

For this nested study, we had to exclude another 73 (28.4%) participant-samples, 59 (23.0%) because they were not newly diagnosed and 14 (5.4%) because of insufficient sample quality, leaving a total of 184 (71.6%) participant-samples included in this nested study from the overall VIBRA trial participants.

**Study population characteristics**

The demographic, socio-demographic and clinical characteristics of the 184 study participants are shown in Table 1 below. The majority of the participants were female (108/184 [598%]) with a median age of 36 years (interquartile range [IQR] 30–50 years). New infections occurred not only among people below the age of 40 years, but considerable numbers were
11,291 household members who were screened for VIBRA trial during the door-to-door HIV testing campaign

292 HIV positive

10'977 HIV negative

35 ineligible for VIBRA trial:
25 wished to receive care outside of the study districts,
4 were physically, mentally or emotionally not able to participate according to the study nurse,
3 had body weights <35 kg,
3 were already in care for another chronic disease

257 eligible and consenting HIV-positive individuals enrolled in VIBRA trial

59 individuals: Already knew their HIV status

14 individuals: Insufficient sample quality

184 individuals and their samples included in this nested study

Fig 1. Study sample flow.

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Table 1. Demographic and clinical characteristics of study participants, by recency outcome.

|                                    | Total (n = 184) | Longterm (n = 171) | Recent (n = 13) |
|------------------------------------|-----------------|--------------------|---------------|
| **Sex**                            |                 |                    |               |
| Female                             | 108 (59%)       | 97 (57%)           | 11 (85%)      |
| Male                               | 76 (41%)        | 74 (43%)           | 2 (15%)       |
| **Age in years, median (IQR)**     |                 |                    |               |
| 16–29 years                        | 45 (24%)        | 40 (23%)           | 5 (38%)       |
| 30–39 years                        | 60 (33%)        | 58 (34%)           | 2 (15%)       |
| 40–49 years                        | 33 (18%)        | 31 (18%)           | 2 (15%)       |
| 50–81 years                        | 46 (25%)        | 42 (25%)           | 4 (31%)       |
| **District**                       |                 |                    |               |
| Butha-Buthe                         | 16 (9%)         | 15 (9%)            | 1 (8%)        |
| Mokhotlong                         | 168 (91%)       | 156 (91%)          | 12 (92%)      |
| **Schooling in years, median (IQR)** |               |                    |               |
| 7 years or more of schooling       |                 |                    |               |
| No                                 | 108 (59%)       | 103 (60%)          | 5 (38%)       |
| Yes                                | 75 (41%)        | 67 (39%)           | 8 (62%)       |
| Missing                            | 1 (1%)          | 1 (1%)             | 0 (0%)        |
| **Education**                      |                 |                    |               |
| Secondary or higher education      | 40 (22%)        | 35 (20%)           | 5 (38%)       |
| No school or only primary          | 143 (78%)       | 135 (79%)          | 8 (62%)       |
| Missing                            | 1 (1%)          | 1 (1%)             | 0 (0%)        |
| **Employment**                     |                 |                    |               |
| No employment and no regular income| 155 (84%)       | 142 (83%)          | 13 (100%)     |
| (Self-)Employment with regular income | 29 (16%)    | 29 (17%)           | 0 (0%)        |
| **Alcohol abuse**                  |                 |                    |               |
| No                                 | 44 (24%)        | 43 (25%)           | 1 (8%)        |
| Yes                                | 15 (8%)         | 15 (9%)            | 0 (0%)        |
| Missing                            | 125 (68%)       | 113 (66%)          | 12 (92%)      |
| **History of local cannabis use**  |                 |                    |               |
| No cannabis use                    | 160 (87%)       | 147 (86%)          | 13 (100%)     |
| Cannabis use                       | 24 (13%)        | 24 (14%)           | 0 (0%)        |
| **Regular sexual partner**         |                 |                    |               |
| Yes, one                           | 90 (49%)        | 83 (49%)           | 7 (54%)       |
| Yes, several                       | 16 (9%)         | 16 (9%)            | 0 (0%)        |
| No                                 | 72 (39%)        | 68 (40%)           | 4 (31%)       |
| Refused to answer or not applicable | 6 (3%)        | 4 (2%)             | 2 (15%)       |
| **CD4 count (cells/μl), median (IQR)** |         |                    |               |
| CD4 count                          |                 |                    |               |
| <200                                | 20 (11%)        | 20 (12%)           | 0 (0%)        |
| 200–499                             | 81 (44%)        | 73 (43%)           | 8 (62%)       |
| >500                                | 47 (26%)        | 43 (25%)           | 4 (31%)       |
| Missing                             | 36 (20%)        | 35 (20%)           | 1 (8%)        |
| **Viral load (copies/mL), log-transformed, median (IQR)** | | | |
| Virus load above 30,000 copies/mL  |                 |                    |               |
| No                                 | 83 (45%)        | 78 (46%)           | 5 (38%)       |
| Yes                                | 101 (55%)       | 93 (54%)           | 8 (62%)       |

Abbreviations: IQR (interquartile range)
Numbers are presented as N (%), unless otherwise stated

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also found among people above 50 years. Majority of the participants were from Mokhotlong district (168/184 [91%]).

Lack of employment with no regular income was high (155/184 [84%]). Overall, 143/184 (78%) participants completed primary school only or had no schooling at all, and 108/184 (59%) participants had less than 7 years of schooling. Only 40/184 (22%) participants had secondary or higher education. A few participants reported to have several regular sexual partners (16/184 [9%]), 15/184 (8%) participants screened positive for alcohol dependency and 24/184 (13%) participants had a history of regular cannabis use. Median CD4 cell count was 414 cells/μL (IQR 264–534 cells/μL). All participants had a viral load available, median viral load was 36,650 copies/mL (IQR 8,565–115,500 copies/mL).

**Reency outcomes**

Fig 2 outlines the outcomes along the RITA based on the Asante recency test and HIV VL. Of the 184 included participant-samples, 19 (10.3%) samples were defined as preliminary recent, 6 of those samples (32.6%) were then re-classified as final long-term because their viral load was below 1,000 copies/mL. Overall, among the 184 samples included in this study, we found 13/184 (7.0%) recent infections, i.e., acquired within the previous 6 months, while 171/184 (93.0%) were classified as long-term HIV infections.

**Association of study participants’ characteristics and recency**

Table 2 displays the univariate logistic regression that displays the associations between participants’ characteristics and recency of HIV infection. We were not able to include alcohol nor cannabis use due to low number of events, i.e., recent infection. Across the univariate logistic
regression analyses, we were not able to identify significant associations and, thus, no multivariate logistic regression models were built.

### Discussion

This secondary analysis of baseline samples from the VIBRA trial assessed the proportion of recently acquired HIV infections among individuals newly diagnosed with HIV during a door-to-door HIV testing campaign among the general population in 2018/2019 in two rural districts of Lesotho. We found that 7% of new diagnoses were recent infections, i.e., acquired within the 6 months before the testing campaign. Most of the new and recent infections were among women in Mokhotlong district.

No clear evidence for variables associated with recent infections were found. Other similar studies from Eastern Africa and Southeast Asia identified female sex, being married, higher number of sex partners, history of sexually transmitted diseases, younger age and lack of male circumcision as important risk factors, while education and substance use showed no association [22–24]. Due to the low numbers in our sample we lacked statistical power to reach conclusive evidence. Nevertheless, our findings identify ongoing recent transmission of HIV infections, especially in the district of Mokhotlong, with high VL levels of a median of 37,100 copies/mL (IQR 16,700–179,000). All of these new infections occurred among a general population in rural areas that was newly diagnosed during a home-based testing campaign. In a country like Lesotho where HIV prevention, testing and care is a major pillar of the Ministry of Health programme and receives large funding, it is surprising to still find so many new and recent diagnoses during a home-based testing campaign. On the other hand, the remoteness of the study area, especially Mokhotlong district (one of the two mountainous districts in the country with weak infrastructure), may be an important contributor.

A recency study, conducted on 1,025 stored samples of individuals living with HIV from a population survey in 2007 in Kenya, found 6.2% recent infections [23]. However, their denominator included all HIV-positive participant-samples including those with known HIV diagnosis and those on ART and therefore cannot be directly compared to our findings. Another study from Kenya and Zimbabwe, in 2018, was conducted in HIV routine service settings and thus only included new diagnoses. The investigators assessed recency data among female sex workers (FSW), women attending antenatal care (ANC) and general HIV testing facilities. They found similar recency rates among the general testing clients, but lower rates among the...
ANC clients and higher rates among the FSW [25]. During a household HIV testing survey in 2017 in Nigeria, 2.9% (11/370) were identified as recent using the same recency algorithm as we did [9]. Again, they included all HIV-positive participant-samples, not only new diagnoses, which is reflected by nearly 20% of the 370 participants being virally suppressed, and thus resulting in an overall lower recency rate. Higher rates of recency were reported in a study conducted in Malawi in 2017/18 among adolescent women aged 15–24 years old [26]. Among the 589 study participants, 11.5% had a recent infection. Young women in sub-Sahara Africa are the population group with the highest HIV incidence [27] and thus higher recency rates than in our general population sample may be expected.

These figures provide important insights into ongoing transmission patterns in the region. However, they should be viewed critically since there is still a lack of proper field validation of these rapid recency assays [28] and especially misleading when not combined with VL testing [29]. More research is needed to validate such assays in the field.

Our study has several limitations. First, a point-of-care rapid recency test and not a laboratory-based recency test was used. Second, this study was conducted in only two districts of Lesotho (Butha-Buthe and Mokhotlong). As a result, the findings of this study may not reflect the characteristics of other districts in Lesotho. Third, the sample size was small. This prevented us to conclude on the risk factor analyses. Fourth, we were not able to get a reliable incidence estimation, as we would have needed a larger sample and preferably from a large-scale cross-section survey, not from a trial. Lastly, we did not measure the presence of antiretrovirals to exclude participants on ART from the recency sample. However, all samples were tested for VL, that improves the false positive recent rate [29], and the VIBRA trial team verified every new diagnosis they found in the field with the surrounding health facilities, thus minimizing the risk of including persons who were already taking ART or knew their status.

Our study found that 7% of new HIV diagnoses found during door-to-door testing in two rural districts of Northern Lesotho were acquired only during the past 6 months. Despite substantial activities of the local HIV programme, recent new infections are still occurring. This indicates ongoing transmission within the general population in these areas in Lesotho. More resources and research is needed in these rural districts to understand the pattern of new transmissions and to tackle them adequately.

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References

1. UNAIDS_FactSheet_en.pdf [Internet]. [cited 2021 Dec 10]. Available from: https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf

2. Kim AA, Behel S, Northbrook S, Parekh BS. Tracking with recency assays to control the epidemic: real-time HIV surveillance and public health response. AIDS. 2019 Jul 15; 33(9):1527–9. https://doi.org/10.1097/QAD.0000000000002239 PMID: 31021850

3. Parekh B, Detorio M, Shanmugam V, Yufenyuy E, Dobbs T, Kim A, et al. Performance Evaluation of AsanteTM Rapid Recency Assay for HIV Diagnosis and Detection of Recent Infection: Potential for Surveillance and Prevention. 9th IAS Conference on HIV Science, Paris, France 2017 Jul. In p. 1.

4. Constantine NT, Zink H. HIV testing technologies after two decades of evolution. INDIAN J MED RES. 2005;20. PMID: 15817961

5. Wei X, Liu X, Dobbs T, Kuehl D, Nkengasong JN, Hu DJ, et al. Development of two avidity-based assays to detect recent HIV type 1 seroconversion using a multisubtype gp41 recombinant protein. AIDS Res Hum Retroviruses. 2010 Jan; 26(1):61–71. https://doi.org/10.1089/aid.2009.0133 PMID: 20063992

6. World Health Organization. When and how to use assays for recent infection to estimate HIV incidence at a population level. Circonstances et modalités d’utilisation des tests d’infection récente pour estimer l’incidence de l’infection à VIH au niveau d’une population [Internet]. 2011 [cited 2021 Dec 9]; Available from: https://apps.who.int/iris/handle/10665/44612

7. Yufenyuy EL, Detorio M, Dobbs T, Patel HK, Jackson K, Vedapuri S, et al. Performance evaluation of the Asante Rapid Recency Assay for verification of HIV diagnosis and detection of recent HIV-1 infections: Implications for epidemic control. PLOS Global Public Health. 2022 Mar 5; 2(5):e000316.

8. Parekh B, Detorio M, Shanmugam V, Yufenyuy E, Dobbs T, Kim A, et al. Performance Evaluation of AsanteTM Rapid Recency Assay for HIV Diagnosis and Detection of Recent Infection: Potential for Surveillance and Prevention. CDC Atlanta, GA, USA.: 1.

9. Negedu-Momoh OR, Balogun O, Dafa I, Etuk A, Oladele EA, Adedokun O, et al. Estimating HIV incidence in the Akwa Ibom AIDS indicator survey (AKAIS), Nigeria using the limiting antigen avidity recency assay. J Intern AIDS Soc [Internet]. 2021 Feb [cited 2021 Dec 5]; 24(2). Available from: https://onlinelibrary.wiley.com/doi/10.1002/jia2.25669

10. Agyemang EA, Kim AA, Dobbs T, Zungu I, Payne D, Maher AD, et al. Performance of a novel rapid test for recent HIV infection among newly-diagnosed pregnant adolescent girls and young women in four high-HIV-prevalence districts-Malawi, 2017–2018. PLoS One. 2022; 17(2):e0262071. https://doi.org/10.1371/journal.pone.0262071 PMID: 35148312

11. Goniwango RM, Ssuuna C, Kaleebu P, Kigozi G, Kagaayi J, Nakigozi G, et al. Short Communication: Validation of the Asante HIV-1 Rapid Recency Assay for Detection of Recent HIV-1 Infections in Uganda. AIDS Res Hum Retroviruses. 2021 Dec; 37(12):893–6. https://doi.org/10.1089/AID.2020.0279 PMID: 33499732

12. Grebe E, Facente SN, Hampton D, Cheng C, Owen R, Keating SM, et al. AsanteTM HIV-1 Rapid Recency® Assay Evaluation Report. The CEPHIA Consortium. 2019, [Internet]. Zenodo; 2019 Oct [cited 2022 Sep 5]. Available from: https://zenodo.org/record/3509834
15. Low A, Teasdale C, Brown K, Barradas DT, Mugurungi O, Sachathep K, et al. Human Immunodeficiency Virus Infection in Adolescents and Mode of Transmission in Southern Africa: A Multinational Analysis of Population-Based Survey Data. Clinical Infectious Diseases. 2021 Aug 16; 73(4):594–604. https://doi.org/10.1093/cid/ciab031 PMID: 33912973

16. Global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2017, and forecasts to 2030, for 195 countries and territories: a systematic analysis for the Global Burden of Diseases, Injuries, and Risk Factors Study 2017. Lancet HIV. 2019 Aug 19; 6(12):e831–59. https://doi.org/10.1016/S2352-3018(19)30196-1 PMID: 31439004

17. Amstutz A, Lejone TI, Khesa L, Muhairwe J, Nsakala BL, Tlali K, et al. VIBRA trial—Effect of village-based refill of ART following home-based same-day ART initiation vs clinic-based ART refill on viral suppression among individuals living with HIV: protocol of a cluster-randomized clinical trial in rural Lesotho. Trials. 2019 Dec; 20(1):522. https://doi.org/10.1186/s13063-019-3510-5 PMID: 31439004

18. Amstutz A, Lejone TI, Khesa L, Kopo M, Kao M, Muhairwe J, et al. Offering ART refill through community health workers versus clinic-based follow-up after home-based same-day ART initiation in rural Lesotho: The VIBRA cluster-randomized clinical trial. PLOS Medicine. 2021 Oct 21; 18(10):e1003839. https://doi.org/10.1371/journal.pmed.1003839 PMID: 34673765

19. Ewing JA. Detecting alcoholism. The CAGE questionnaire. JAMA. 1984 Oct 12; 252(14):1905–7. https://doi.org/10.1001/jama.252.14.1905 PMID: 6471323

20. Adams P, Vancutsaem E, Nicolaizeau C, Servais JY, Pierard D, François JH, et al. Multicenter evaluation of the cobas® HIV-1 quantitative nucleic acid test for use on the cobas® 4800 system for the quantification of HIV-1 plasma viral load. J Clin Virol. 2019 May; 114:43–9. https://doi.org/10.1016/j.jcv.2019.03.008 PMID: 30991164

21. LN-6122-05-Product-Insert-Asante-HIV-1-Rapid-Recency-Assay.pdf [Internet]. [cited 2021 Dec 9]. Available from: https://www.sediabio.com/wp-content/uploads/2021/06/LN-6122-05-Product-Insert-Asante-HIV-1-Rapid-Recency-Assay.pdf

22. Mermin J, Musinguzi J, Opio A, Kirungi W, Ekwaru JP, Hiadik W, et al. Risk Factors for Recent HIV Infection in Uganda. JAMA. 2008 Aug 6; 300(5):540–9. https://doi.org/10.1001/jama.300.5.540 PMID: 18677026

23. Kim AA, Parekh BS, Umuro M, Galgalo T, Bunnell R, Makokha E, et al. Identifying Risk Factors for Recent HIV Infection in Kenya Using a Recent Infection Testing Algorithm: Results from a Nationally Representative Population-Based Survey. Dezzutti CS, editor. PLoS ONE. 2016 May 19; 11(5):e0155498. https://doi.org/10.1371/journal.pone.0155498 PMID: 27195800

24. Ang LW, Low C, Wong CS, Boudville IC, Toh MPHS, Archuleta S, et al. Epidemiological factors associated with recent HIV infection among newly-diagnosed cases in Singapore, 2013–2017. BMC Public Health. 2021 Mar 2; 21(1):430. https://doi.org/10.1186/s12889-021-10478-5 PMID: 33653290

25. Rice BD, de Wit M, Welfy S, Risher K, Cowan FM, Murphy G, et al. Can HIV recent infection surveillance help us better understand where primary prevention efforts should be targeted? Results of three pilots integrating a recent infection testing algorithm into routine programme activities in Kenya and Zimbabwe. J Int AIDS Soc [Internet]. 2020 Jun 30 [cited 2021 Mar 7]; 23(Suppl 3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7325505/ https://doi.org/10.1002/jia2.25513 PMID: 32602625

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

27. Karim SSA, Baxter C. HIV incidence rates in adolescent girls and young women in sub-Saharan Africa. The Lancet Global Health. 2019 Nov 1; 7(11):e1470–1. https://doi.org/10.1016/S2214-109X(19)30404-8 PMID: 31607449

28. Facente SN, Grebe E, Maher AD, Fox D, Scheer S, Mahy M, et al. Use of HIV Recency Assays for HIV Incidence Estimation and Other Surveillance Use Cases: Systematic Review. JMIR Public Health and Surveillance. 2022 Mar 11; 8(3):e34410. https://doi.org/10.2196/34410 PMID: 35275085

29. Parmley LE, Harris TG, Hakim AJ, Musuka G, Chingombo I, Mugurungi O, et al. Recent HIV Infection Among Men Who Have Sex with Men, Transgender Women, and Genderqueer Individuals with Newly Diagnosed HIV Infection in Zimbabwe: Results from a Respondent-Driven Sampling Survey. AIDS Res Hum Retroviruses. 2022 Sep 1;