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HIV Incidence Assay Performance Required for National Incidence Estimates and to Monitor HIV Prevention Intervention Impact

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Conference on Retroviruses and Opportunistic Infections

Boston, MA

February 22-25, 2016

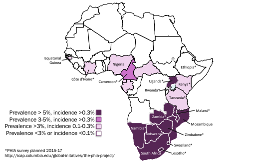
BACKGROUND

- HIV incidence is one of several indicators being measured as part of population-based HIV impact assessments (PHIAs) being implemented by ICAP, with support from PEPFAR, in 13 African countries in 2015-17 (Panel 1) (http://icap.columbia.edu/global-initatives/the-phia-project/).
  - The objectives of these surveys are to "measure the reach and impact of HIV programs in PEPFAR-supported countries and guide policy and funding priorities".
  - National HIV incidence rates will be estimated using a Recent Infection Testing Algorithm (RITA) consisting of the limiting antigen immunoassay (LAg) and viral load (VL).
- DREAMS is a partnership funded by PEPFAR, BMGF and The Girl Effect to reduce HIV infections among adolescent girls and young women in 10 sub-Saharan African countries. The goal of DREAMS is to help girls develop into Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe women.
  - This initiative has set targets of 25% reduction in HIV incidence among adolescent girls and young women (aged 15-24) within the highest burden geographic areas of 10 sub-Saharan African countries by 2016, and a 40% reduction by 2017 (http://www.pepfar.gov/documents/organization/247548.pdf).
- The sample sizes and associated implementation costs required for precise estimation of incidence are driven by the prevalence, incidence, the mean duration of recent infection (MDRI), and the false recent rate (FRR) in addition to the desired precision of the incidence estimate and survey design effects (panel 2)
  - MDRI and FRR for a particular assay vary with HIV subtype, the algorithm used for HIV diagnosis and screening, and ART coverage.
- We sought to evaluate the applicability of a recent infection testing algorithm (RITA) combining LAg and VL to estimate incidence in 2 scenarios:
  - scenario 1: a single time point population survey with a desired precision of the incidence estimate (relative standard error or RSE) of 20% (as recommended by WHO/UNAIDS) or 30%
  - scenario 2: two surveys performed to assess the impact of population-level prevention interventions (PLPI) i.e. to enable detection of a statistically significant reduction of 25% or 40% (power 0.8, alpha 0.05)
- Both scenarios were modeled for national surveys similar to PHIAs and for key populations including adolescent girls and young women (AGYW), female sex workers (FSW) and men who have sex with men (MSM).

METHODS

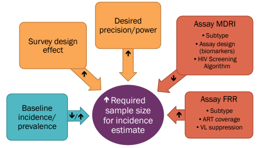
- Sample sizes were calculated using publicly available spreadsheet tools (http://www.incidence-estimation.org) adapted to the R programming environment and implemented online (https://finddx.shinyapps.io/Sample_size_table, https://finddx.shinyapps.io/Sample_size_table_one_survey), for the 2 scenarios described above
- Potential bias due to use of a multi level cluster randomized sampling was accounted for by adding a design effect (DE) correction to the sample size estimates. The adjustments take into account both the proportion of HIV infected individuals (DE value 1.3) and the proportion of "recent" results among subjects testing HIV positive (DE value 1.3). In the absence of specific data for each of the countries or KPs, the same values were used in all these examples.
- MDRI was estimated as outlined below using pooled longitudinal data for LAg and subtype C from CEPHIA seroconverter panels and other sources. We assumed a low FRR (0.25%) because of the inclusion of VL in a RITA (panel 3).
  - VL data for CEPHIA specimens but not from other sources was available and enabled an estimation of the impact on MDRI of a supplemental VL using a threshold of 1000 copies/ml (at a LAg threshold of 1.5). This was estimated as a reduction of 43 days (95% CI: 29-60).
  - The VL effect when using a threshold of 100 copies/ml (at a LAg threshold of 2.0) was estimated at 16 days (8-25).
  - These VL effects were then applied to the MDRIs derived from the pooled data set according to subtype.
  - The MDRIs were further adjusted by 24 days to account for the use of a pooled NAAT screening assay (as opposed to Western Blot; Masciotra et al. (2011) and Owen M. personal communication)
- The combined RSE of the MDRI estimate, 'viral load effect' and diagnostic delay adjustment is between 6 - 11%. We used 7% for all sample size calculations. The error around the MDRI estimate for subtype A is larger.

1. High Incidence/Prevalence African Countries (2014 UNAIDS Estimates, Adults Age 15-49)



*PHIA survey planned 2015-17
http://icap.columbia.edu/global-initatives/the-phia-project/

2. Factors Influencing Survey Sample Size

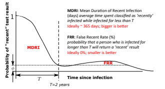


Arrows (↑ or ↓) indicate whether an increase or decrease in the factor leads to an increased sample size requirement

3. MDRI Estimates for Sample Size Calculations

Assay/RITA (cutoffs)	Adjusted MDRI (days)	Specimen requirements	HIV screening requirements	Feasibility	Availability
LAg (1.5) + VL (1000)	130	DBS or plasma	Ab only	High	Current
LAg (1.5) + VL (1000)	154	DBS or plasma	NAAT*+Ab	Moderate	Current
LAg (1.5) + VL (100)	184	plasma	NAAT*+Ab	Moderate	Current
LAg (2.0) + VL (100)	222	plasma	NAAT*+Ab	Moderate	Current
New assay	300	TBD	TBD	TBD	Future

*NAAT: nucleic acid amplification testing; 50% of specimens yielded positive results on the Genprobe Aptima qualitative RNA assay 26 days before Western Blot seroconversion. An adjustment of MDRI by 24 days was used to account for the loss of sensitivity associated with pooled NAAT(Masciotra et al. J Clin Virol 2011)



MDRI: Mean Duration of Recent Infection (days) *average time spent classified as 'recently' infected while infected for less than T*
Ideally ~ 365 days; bigger is better

FRR: False Recent Rate (%) *probability that a person who is infected for longer than T will return a 'recent' result*
Ideally 0%; smaller is better

NOTES
- All MDRI estimates are for subtype C
- The standard ODn threshold for LAg is 1.5
- VL thresholds lower than 1000 copies/ml are not feasible with DBS specimens
- FRR assumed to be low (0.25%) due to use of VL in the RITA

4. Prevalence and Incidence Estimates for Sample Size Calculations (National)

Country	Prevalence (%)	Incidence (% per yr)	Predominant Subtype[1]
Botswana	25.2	1.40	C
Cameroon	4.8	0.38	A, CRF02_AG[3]
Equatorial Guinea	6.2	0.29	A, CRF02_AG[3]
Kenya	5.3	0.23	A, D[2]
Lesotho	23.4	2.01	C
Malawi	10.0	0.45	C
Mozambique	10.6	0.74	C
Namibia	16.0	0.91	C
South Africa	18.9	1.27	C
Swaziland	27.7	1.89	C
Tanzania	5.3	0.26	A, C, D[2]
Uganda	7.3	0.60	A, D[2]
Zambia	12.4	0.75	C
Zimbabwe	16.7	0.92	C

[1]*Based on sequence data from Los Alamos HIV Sequence Database; may not be representative of HIV-infected population*
[2]*subtype D has elevated FRR and longer MDRI*
[3]*FRR and MDRI unknown for CRF02_AG*

Data from 2014 UNAIDS estimates for adults age 15-49; http://www.aidsinfoonline.org

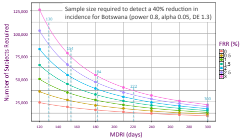
5. Prevalence and Incidence Estimates for Sample Size Calculations (Key Populations)

Key Population	Prevalence (%)	Incidence (% per yr)	Predominant Subtype
KZN AGYW 14-17 y	27.6%	4.7%	C
KZN AGYW ≥18 y	52.0%	6.9%	C
RWA FSW ≥18 y	24.0%	2.8%	A[1]
THA MSM 18-21 y	15.9%	8.8%	CRF01_AE[2]
THA MSM 22-29 y	22.6%	6.4%	CRF01_AE[2]
THA MSM over 30 y	22.4%	3.7%	CRF01_AE[2]

- KZN AGYW: adolescent girls and young women in KwaZulu Natal, South Africa *[Karim et al. AIDS Behav. 2012 16(7): 1870–1876]*
- RWA FSW: Female sex workers in Kigali, Rwanda *[Braunstein et al. The Open AIDS J 2012 6 (Suppl 1:M8) 112-121]*
- THA MSM: Men who have sex with men in Bangkok, Thailand *[Van Grievensen et al. Lancet HIV. 2015 Feb;2(2):e64-70]*

[1] *Rusine et al. PLoS ONE 2012 7(8): e42557. doi:10.1371/journal.pone.0042557; Mutagoma et al. Antivir Ther 2015 doi: 10.3851/IMP2999. MDRI for subtype A ~39 days longer than subtype C*
[2] *Leelawiwat et al. AIDS Res Hum Retroviruses. 2015 Apr;31(4):393-400. MDRI for subtype CRF01_AE ~50 days shorter than subtype C*

6. Longer MDRI Lowers Sample Size Requirements and Impact of FRR



https://internal.shinyapps.io/finddx/Sample_size_calculator

7. National Surveys, Scenario 1 (Incidence Point Estimate for 15-49y)

Sample Size Required for Survey of Target Population for Indicated RSE and MDRI

Country	RSE 30%, MDRI (days): 130	154	184	222	300	RSE 20%, MDRI (days): 130	154	184	222	300
Botswana	5,007	4,091	3,330	2,696	1,941	12,654	10,213	8,240	6,630	4,743
Cameroon	13,236	10,975	9,043	7,395	5,384	32,486	26,847	22,068	18,013	13,088
Equatorial Guinea	19,004	15,545	12,663	10,255	7,377	47,811	38,690	31,272	25,182	18,014
Kenya	24,042	19,618	15,949	12,894	9,256	60,824	49,016	39,489	31,718	22,623
Lesotho	3,174	2,628	2,163	1,769	1,289	7,818	6,444	5,288	4,314	3,136
Malawi	12,978	10,586	8,603	6,954	4,992	32,878	26,473	21,316	17,114	12,205
Mozambique	7,404	6,118	5,027	4,102	2,978	18,273	15,024	12,300	10,008	7,248
Namibia	6,671	5,475	4,473	3,632	2,622	16,681	13,568	11,012	8,900	6,396
South Africa	4,853	3,999	3,278	2,670	1,935	12,046	9,858	8,042	6,527	4,714
Swaziland	3,723	3,060	2,504	2,037	1,475	9,290	7,573	6,159	4,988	3,596
Tanzania	20,772	17,023	13,889	11,263	8,115	52,037	42,245	34,230	27,619	19,801
Uganda	8,606	7,138	5,884	4,813	3,506	21,116	17,457	14,355	11,723	8,524
Zambia	7,628	6,278	5,141	4,183	3,027	18,960	15,494	12,622	10,230	7,375
Zimbabwe	6,702	5,493	4,483	3,637	2,623	16,804	13,639	11,052	8,920	6,401
N < 20,000*	12	14	14	14	14	8	9	9	11	13
N < 10,000	9	9	11	11	14	2	3	4	6	9

*the number of countries (of 14) with survey sizes below 20,000 or 10,000; for the 15-49 year old group, this corresponds roughly to total sample size 30,000 or 15,000 in a PHIA

MIN | >20,000 | >50,000

8. National Surveys, Scenario 2 (Impact Assessment for 15-49y)

Sample Size Required for Survey of Target Population for Indicated Reduction and MDRI

Country	40% Reduction, MDRI (days): 130	154	184	222	300	25% Reduction, MDRI (days): 130	154	184	222	300
Botswana	34,374	28,164	22,952	18,582	13,354	94,810	77,844	63,563	51,553	37,140
Cameroon	91,388	75,697	62,297	50,874	36,954	253,577	210,316	173,296	141,673	103,054
Equatorial Guinea	130,737	107,187	87,388	70,755	50,820	360,774	296,375	242,074	196,330	141,330
Kenya	165,116	135,162	110,036	88,972	63,789	455,268	373,453	304,614	246,740	177,316
Lesotho	21,897	18,114	14,895	12,159	8,838	60,702	50,292	41,412	33,850	24,646
Malawi	89,081	72,904	59,341	47,977	34,397	245,583	201,409	164,259	133,041	95,613
Mozambique	51,119	42,220	34,657	28,240	20,459	141,615	117,142	96,296	78,565	57,014
Namibia	45,954	37,760	30,852	25,034	18,039	126,954	104,514	85,543	69,521	50,202
South Africa	33,476	27,587	22,601	18,386	13,296	92,620	76,457	62,740	51,114	37,036
Swaziland	25,643	21,096	17,259	14,024	10,131	70,879	58,421	47,878	38,966	28,209
Tanzania	143,069	117,445	95,862	77,699	55,884	395,073	324,935	265,689	215,695	155,466
Uganda	59,407	49,221	40,520	33,101	24,058	164,859	136,772	112,731	92,190	67,099
Zambia	52,619	43,326	35,465	28,824	20,813	145,530	120,036	98,417	80,106	57,955
Zimbabwe	46,134	37,875	30,921	25,071	18,048	127,390	104,787	85,702	69,603	50,217
N < 20,000*	0	1	2	4	6	0	0	0	0	0
N < 10,000	0	0	0	0	1	0	0	0	0	0

*the number of countries (of 14) with survey sizes below 20,000 or 10,000; for the 15-49 year old group, this corresponds roughly to total sample size 30,000 or 15,000 in a PHIA

MIN | >20,000 | >50,000

9. Key Population Surveys

Scenario 1 (Incidence Point Estimate)

Sample Size Required for Survey of Target Population for Indicated RSE and MDRI

Key Population	RSE 30%, MDRI (days): 130	154	184	222	300	RSE 20%, MDRI (days): 130	154	184	222	300
KZN AGYW 0-17	1,373	1,149	956	789	583	3,346	2,797	2,324	1,916	1,415
KZN AGYW 18-35	1,096	918	763	631	468	2,676	2,235	1,858	1,534	1,136
RWA-FSW	2,234	1,860	1,539	1,264	927	5,465	4,539	3,749	3,076	2,253
THA MSM 18-21	614	519	435	363	272	1,491	1,259	1,057	880	660
THA MSM 22-29	925	778	650	539	402	2,248	1,890	1,579	1,309	974

MIN | >5,000 | >20,000

Scenario 2 (Impact Assessment)

Sample Size Required for Survey of Target Population for Indicated Reduction and MDRI

Country	40% Reduction, MDRI (days): 130	154	184	222	300	25% Reduction, MDRI (days): 130	154	184	222	300
KZN AGYW 0-17	9,429	7,877	6,538	5,386	3,968	26,267	21,965	18,248	15,046	11,098
KZN AGYW 18-35	7,526	6,285	5,218	4,302	3,178	20,954	17,520	14,561	12,019	8,893
RWA-FSW	15,390	12,795	10,570	8,666	6,338	42,773	35,603	29,444	24,167	17,699
THA MSM 18-21	4,172	3,520	2,950	2,454	1,834	11,678	9,858	8,266	6,878	5,147
THA MSM 22-29	6,319	5,307	4,428	3,666	2,721	17,649	14,835	12,386	10,261	7,624

- KZN AGYW: adolescent girls and young women in KwaZulu Natal, South Africa
- RWA FSW: Female sex workers in Kigali, Rwanda; . *MDRI for subtype A ~39 days longer than subtype C*
- THA MSM: Men who have sex with men in Bangkok, Thailand; *MDRI for subtype CRF01_AE ~50 days shorter than subtype C*

METHODS (CONT.)

- Surveys were considered "feasible" if sample sizes were < 20,000 for adults aged 15-49 years in national surveys (this roughly corresponds to a total sample size of <30,000), or < 5,000 for key population surveys
- National prevalence and incidence data for adults aged 15-49 in 2014 as reported by UNAIDS were used (panel 4) (http://www.aidsinfoonline.org).
- Prevalence and incidence in 3 important key populations were derived from published reports (panel 5)

RESULTS

- The relationship between MDRI, FRR and sample sizes for scenario 2 is depicted in panel 6
  - At longer MDRI, the relative impact of elevated FRR is lower
- Target sample sizes for African countries with prevalence >5% or incidence >0.3% for national level point estimates (scenario 1) and PLPI impact assessment (scenario 2) are shown in Panels 7 and 8
  - With LAg(1.5)+VL(1000) (MDRI 130-154 days):
    - National level incidence estimates with RSE 30% are feasible in nearly all countries, but in only 8-9 of the 14 countries when an RSE of 20% is targeted (panel 7)
    - PLPI impact assessment surveys to detect a reduction in incidence of 25-40% were not feasible except in Lesotho and possibly Swaziland (panel 8)
  - If the MDRI could be increased to 300 days, sample sizes would be reduced by approximately 50%, and surveys with RSE 20% would be feasible in 4-5 additional countries
- Target sample sizes for selected key population surveys are shown in Panel 9
  - Single point estimates of incidence with RSE 20-30% are feasible in most key populations with current tools; sample sizes would be significantly reduced with an improved assay with longer MDRI
  - PLPI impact surveys in adolescent girls and young women in South Africa and most age groups of Thai MSM were only feasible with MDRI of ≥~200 days and only for a reduction of 40%
  - PLPI impact surveys in female sex workers in Rwanda, or when a reduction of only 25% needs to be detected, required sample sizes that are probably not feasible, even for an assay with MDRI 300 days.

LIMITATIONS

- In countries with non-C subtypes, such as Cameroon, Kenya, Tanzania and Uganda, sample sizes may be underestimated due to FRR>0.25% in subtype D or CRF02_AG, but the impact of elevated FRR could be dampened at longer MDRI.
- Because many indicators are sometimes measured as part of national surveys, sample sizes may be driven by requirements not related to incidence estimation that could lessen the benefit of longer MDRI incidence assays
- FRRs used here are approximated, and will be affected by differences in numbers of false recent results from viremic specimens when using RITA with VL (e.g. ARV failures due to drug resistance)
- Design effects were estimated based on assumptions that may not be correct in some contexts. Tools are currently under development that would allow for the use of standard methods in accounting for uncertainty resulting from clustered sampling during incidence estimation. This would allow for more flexible analyses than design effects, which are hard to estimate accurately.

CONCLUSIONS

- The currently available RITA of LAg+VL:
  - Is a feasible approach for generating incidence estimates with reasonable precision (20-30% RSE) in most single point-in-time national surveys and some key populations
    - Sample sizes could be minimized by raising the LAg ODn threshold to 2.0, lowering the VL threshold to 100 copies/ml, and including NAAT in the HIV screening algorithm to capture acutely infected persons
  - Has limited utility for national or key population prevention impact surveys unless sample sizes larger than 30,000 (5000 for key populations) are considered; outside of high incidence settings it may generate imprecise estimates that are not able to detect reductions in incidence.
- Development of new incidence assays with longer MDRI (≥ 300 days) and low FRR are needed to enable broader and more cost-effective use in national and key population surveys, especially for assessment of population-level prevention intervention impact.

ACKNOWLEDGEMENTS

- Bill and Melinda Gates Foundation