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# PERFORMANCE OF ENZYME IMMUNOASSAYS FOR HIV SEROLOGY IN SURVEYS CONDUCTED BY THE DEMOGRAPHIC AND HEALTH SURVEYS PROGRAM

## DHS COMPARATIVE REPORTS 39



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**Performance of Enzyme Immunoassays for HIV Serology  
in Surveys Conducted by  
The Demographic and Health Surveys Program**

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# Contents

<b>Tables and Figures .....</b>	<b>v</b>
<b>Preface.....</b>	<b>vii</b>
<b>Abstract.....</b>	<b>ix</b>
<b>1. Introduction.....</b>	<b>1</b>
<b>2. Background.....</b>	<b>3</b>
<b>3. Methods .....</b>	<b>5</b>
3.1. Characteristics of the Sample.....	5
3.2. Specimen Collection and Testing Procedures.....	6
3.3. Data Management and Analysis .....	9
<b>4. Results .....</b>	<b>11</b>
4.1. Agreement between EIA 1 and EIA 2 .....	11
4.2. Signal to Cutoff Values.....	13
<b>5. Discussion .....</b>	<b>19</b>
<b>References.....</b>	<b>23</b>
<b>Appendix.....</b>	<b>25</b>



## Tables and Figures

Table 1.	Description of the sample.....	5
Table 2.	HIV assays used and specimen matrix .....	7
Table 3.	Agreement of assays.....	11
Table 4.	Agreement of repeat EIA testing.....	13
Table 5.	Signal to cutoff values.....	14
Table 6.	Final HIV results according to signal to cutoff values on assay 1.....	15
Table 7.	Distribution of final HIV positive specimens by assay results and signal to cutoff values .....	16
Appendix Table 1.1.	Descriptive statistics for signal to cutoff values among weak positive specimens, assay 1.....	25
Appendix Table 1.2.	Descriptive statistics for specimens with weak signal to cutoff values, assay 2 .....	26
Appendix Table 2.	Weak positive signal to cutoff values according to first round test results .....	27
Appendix Table 3.	Internal quality control .....	28
Figure 1.	DHS HIV testing algorithm.....	3



## **Preface**

The Demographic and Health Surveys (DHS) Program is one of the principal sources of international data on fertility, family planning, maternal and child health, nutrition, mortality, environmental health, HIV/AIDS, malaria, and provision of health services.

One of the objectives of The DHS Program is to provide policymakers and program managers in low- and middle-income countries with easily accessible data on levels and trends for a wide range of health and demographic indicators. DHS Comparative Reports provide such information, usually for a large number of countries in each report. These reports are largely descriptive, without multivariate methods, but when possible they include confidence intervals and/or statistical tests.

The topics in the DHS Comparative Reports series are selected by The DHS Program in consultation with the U.S. Agency for International Development.

It is hoped that the DHS Comparative Reports will be useful to researchers, policymakers, and survey specialists, particularly those engaged in work in low- and middle-income countries.

Sunita Kishor  
Director, The DHS Program



## Abstract

Since 2001, The Demographic and Health Surveys Program has conducted HIV testing in surveys to measure HIV prevalence. Recently, concerns have been raised that the testing strategy used in these surveys may result in overestimation of HIV prevalence. This report analyzes existing laboratory data from 20 recent surveys to assess two indicators of the performance of the enzyme immunoassays (EIAs) used in the HIV testing algorithms: agreement between the two EIAs used in the algorithm for each survey, and the distribution of signal-to-cutoff (S/CO) values for each assay. S/CO values are a measure of the strength of the reaction on an assay. Specimens with high S/CO values are more likely to be true positive specimens than those with low S/CO values. However, false positive results can have high S/CO values.

Overall agreement between the two EIAs reached the optimal value of 99 percent or greater in 6 of the 20 surveys. Analysis of the S/CO values shows that the majority of specimens with low S/CO values (less than 5.0) on the first EIA were found negative during later stages of the testing algorithm—reducing, but not eliminating concerns about false positive results. In 4 of 20 surveys, the proportion of final positive specimens found to have low S/CO values was greater than 20 percent.

This analysis can provide general insight into the quality of testing and the potential for false positivity in HIV test results, but it does not include retesting of EIA positive specimens on a more specific assay, which would be required to quantify any over- or underestimation in a survey's HIV prevalence estimate. Nevertheless, the risk of false positive results does appear to vary widely across surveys. In addition, the impact that any testing error would have on the accuracy of the prevalence estimate for a survey must be considered in the context of other errors affecting this estimate, notably sampling error and selection bias. These results suggest the need for more vigilance in the quality of laboratory testing, with even more checks built in to every step of the testing process. Finally, testing strategies that include a confirmatory assay should reduce false positivity and improve the accuracy of HIV prevalence estimates.



# 1. Introduction

From 2001 to March 2016, The Demographic and Health Surveys (DHS) Program conducted HIV testing for 65 surveys in 37 countries. The HIV serology testing for these surveys has relied on centralized laboratory testing based on a WHO-recommended testing strategy for the measurement of HIV prevalence in population-based surveys (UNAIDS/WHO 2005). Specifically, the HIV testing has employed two enzyme immunoassays (EIAs) and a third test, usually a blot assay, to resolve EIA-discordant specimens. Recently, concerns have been raised that the testing strategy recommended in the 2005 UNAIDS/WHO guidelines may result in overestimation of HIV prevalence. In response to these concerns, The DHS Program has undertaken a review of the quality of EIA data from recent DHS and AIS surveys. The review is a secondary analysis of existing laboratory data for past surveys.

This analysis will assess two indicators of the performance of the HIV EIAs: (1) agreement between the two EIAs and (2) the distribution of signal-to-cutoff values, which can provide general insight into the potential for false positivity in HIV test results. The objective of this analysis is not to quantify any under- or overestimation in any specific survey's HIV prevalence estimate. The only way to measure overestimation is to retest positive survey specimens on a more specific confirmatory assay and to compare those results with the published survey estimates. Due to funding constraints and issues with the availability of blood specimens collected one to five years ago, as well as potentially poor storage conditions, retesting specimens for all of these surveys is not feasible.

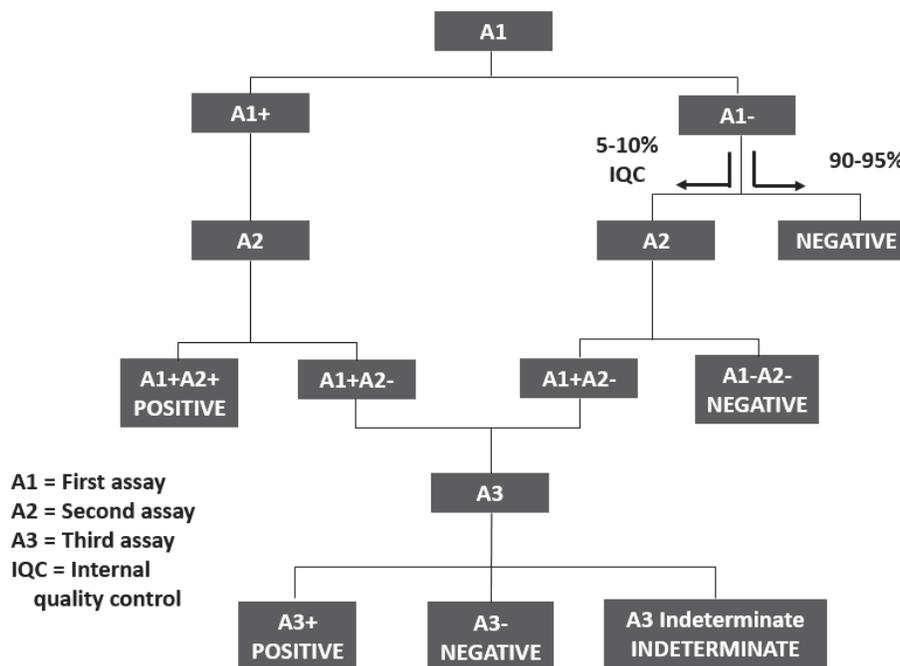


## 2. Background

Generally, the HIV testing strategy followed by The Demographic and Health Surveys Program has consisted of two enzyme immunoassays (EIAs) and a third assay used as a tie-breaker when the two EIAs disagree. The first EIA should be the most sensitive assay available, the second EIA should have higher specificity, and the two assays should use a different antigen preparation to minimize the risk of shared false reactivity (WHO 2015). As shown in Figure 1, if the first EIA result is negative, the specimen is rendered negative. If the first EIA is positive, the specimen is also tested with the second EIA. If the second EIA result is positive, the specimen is rendered HIV positive with no further testing. If the result of the second assay is negative, the specimen is tested on the third assay, and the result of the third assay is considered the definitive result for the specimen. In some surveys specimens with discrepant results on EIA 1 and EIA 2 are retested on both assays in parallel. In these surveys, the specimen is rendered positive if both assays are positive and negative if both assays are negative. If the results of the two assays remain discrepant after retesting, the specimen is tested on the third and definitive assay.

To ensure internal quality control (IQC), The DHS Program routinely retests on EIA 2 a random sample of 5 to 10 percent of specimens that are negative on EIA 1. This testing is conducted as a part of the routine EIA 2 testing, and if any IQC specimen has a positive result, the specimen is put back into the testing algorithm and either (1) retested on EIA 1 and EIA 2 in parallel if repeat testing of the assays was included in the testing algorithm or (2) tested on assay 3 if repeat testing was not included. If the percentage of IQC specimens with positive results on EIA 2 is over 1 percent, the testing procedures should be reviewed and any problems corrected. Note that this testing strategy is no longer fully in line with more recent WHO guidelines for HIV diagnosis and surveillance (WHO 2015) or UNAIDS/WHO guidelines on HIV testing in population-based surveys (UNAIDS/WHO 2015). However, the approach does reflect the UNAIDS/WHO guidelines for HIV testing in population-based surveys that were in place during the time these surveys were conducted (UNAIDS/WHO 2005).

**Figure 1. DHS HIV-testing algorithm**



Cause for concern over the accuracy of this testing strategy arises from a recent investigation comparing EIA-based HIV testing strategies with other strategies, including use of rapid diagnostic tests (RDT) or highly specific supplemental assays to confirm dual EIA positive results. These investigations have shown that a potential for false positives exists even among specimens with two positive EIA results (CDC 2014). As a result, the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA, now recommends that all specimens with positive results on two EIAs undergo testing on a third more specific assay, such as Western blot, Geenius, or Multispot (CDC 2014). The current UNAIDS/WHO guidelines for HIV testing in surveys also recommend the use of a third assay on specimens with positive results on assay 1 and assay 2 in settings with prevalence less than 5 percent, and in all settings where assay 1 and assay 2 are EIAs (UNAIDS/WHO 2015).

Enzyme immunoassays for HIV have undergone continual development to detect a broader group of HIV strains. For example, first generation EIAs only detected antibodies against group M strains of HIV, whereas third generation EIAs detect antibodies against groups M and O and against HIV-2 subtype. Both third and fourth generation tests detect HIV infection using synthetic peptides or recombinant protein antigens to bind HIV antibodies. These EIAs can detect both IgG and IgM antibodies. Fourth generation EIAs also include monoclonal antibodies to bind HIV-1 p24 antigen (CDC 2014; WHO 2015). The standard EIA format uses 96-well microtiter plates, with one specimen per well. Upon completion of the assay, development of color in a well indicates the presence of HIV antibody or HIV antigen. This color reaction is measured and converted into an optical density (OD) by a microplate reader. Optical densities are continuous numeric values. The test result is determined by comparing the value of the OD for a specimen to a cutoff value. If the OD is greater than or equal to the cutoff, the specimen is HIV positive; if the OD is less than the cutoff, the specimen is negative. The method for generating the cutoff value is assay-specific, but it is always determined on a plate-by-plate basis, using a formula provided by the kit manufacturer that includes the OD values of control specimens from the test kit that are run on each plate.

EIA results can also be described in terms of a signal-to-cutoff (S/CO) value, calculated by dividing the OD value of the specimen by the cutoff value for the plate. An S/CO value of 1.0 or greater indicates the specimen is reactive, or positive. The higher the S/CO value, the stronger is the reaction on the assay. In general, there appears to be an association between the “strength” of the reaction on the assay and the accuracy of the classification of HIV serostatus, with specimens having lower S/CO values being more likely to be false positive. In an evaluation of six EIAs, Nkengasong and colleagues describe a categorization that has been adopted by others. They describe specimens with S/CO values greater than 5.0 as “highly reactive,” those with S/CO values of 3.0-5.0 as “moderately reactive,” and those with S/CO values of 1.0-3.0 as “weakly reactive” (1999). Specimens rendered positive by an EIA, but with a S/CO value of less than 5.0 have been found to be much more likely than those with S/CO values of 5.0 and above to be false positive (Nkengasong et al. 1999; Fanmi et al. 2013; Urassa et al. 1999). However, S/CO values cannot be used to distinguish between true and false positive results. The ranges of S/CO values for true and false positive results can overlap (Urassa et al. 1999), and false positive results with high S/CO values have been documented in several studies (Fanmi et al. 2012; Hakim et al. 2016; Zhang et al. 2013). Conversely, it is possible for a true positive specimen to produce a weak reaction on an EIA. Specimens drawn from individuals early in the seroconversion process and those infected with divergent HIV strains can also produce weak positive reactions (Kfutwah et al. 2013).

### 3. Methods

#### 3.1. Characteristics of the Sample

We attempted to analyze data from all surveys with HIV testing that were conducted after 2010 as part of The DHS Program and had results published by August 2015. We were unable to analyze 6 of these 26 surveys for various reasons. The 2011 Ethiopia DHS, 2010 Burkina Faso DHS, 2013 Liberia DHS, and 2010-11 Zimbabwe DHS were excluded due to unavailability of the detailed laboratory results. The 2011 Equatorial Guinea DHS survey data are not available for public use, and all HIV test results for the 2011-12 Benin DHS have been destroyed due to the inclusion of a community assets questionnaire in that survey, which introduced a risk that sampled communities and individuals could be identified even after the dataset was anonymized. The list of 20 remaining surveys, including 18 DHS surveys and 2 AIDS Indicator surveys (AIS), is included in Table 1.

**Table 1. Description of the sample**

Weighted HIV prevalence among men and women age 15-49, the unweighted total number of respondents tested for HIV (all ages), and the unweighted total number of HIV positive respondents, by survey

Survey	HIV prevalence among women and men age 15-49 (weighted)		Total number tested (all ages, unweighted)	Total number HIV positive (all ages, unweighted)
	Point estimate	95% confidence interval		
<b>Sub-Saharan Africa</b>				
Burundi DHS 2010	1.4	(1.1, 1.7)	8,588	164
Cameroon DHS 2011	4.3	(3.9, 4.8)	14,202	649
Cote d'Ivoire DHS 2011-12	3.7	(3.1, 4.3)	9,008	336
Democratic Republic of the Congo DHS 2013-14	1.2	(0.8, 1.5)	17,638	177
Gabon DHS 2012	4.1	(3.3, 4.8)	10,992	483
Gambia DHS 2013	1.9	(1.4, 2.4)	7,769	136
Guinea DHS 2012	1.7	(1.4, 2.1)	8,380	164
Malawi DHS 2010	10.6	(9.7, 11.5)	13,910	1,420
Mali DHS 2012-2013	1.1	(0.9, 1.4)	8,861	97
Namibia DHS 2013	14.0	(12.8, 15.3)	8,858	1,233
Niger DHS 2012	0.4	(0.2, 0.5)	8,628	44
Rwanda DHS 2010	3.0	(2.7, 3.4)	13,248	420
Senegal DHS 2010-11	0.7	(0.5, 0.9)	9,917	93
Sierra Leone DHS 2013	1.5	(1.2, 1.8)	14,600	222
Tanzania AIS/MIS 2011-12	5.1	(4.6, 5.6)	17,745	815
Togo DHS 2013-14	2.5	(2, 2.9)	9,172	199
Uganda AIS 2011	7.3	(6.8, 7.9)	21,367	1,495
Zambia DHS 2013-14	13.3	(12.5, 14.1)	29,006	3,901
<b>Latin America/Caribbean</b>				
Dominican Republic DHS 2013	0.8	(0.6, 1)	18,614	193
Haiti DHS 2012	2.2	(1.9, 2.5)	18,531	419

Note: In most of these surveys, the age ranges for interview and HIV testing are 15-49 for women and 15-59 for men. Exceptions include Malawi (women age 15-49 and men age 15-54), Namibia (women and men age 15-64), Tanzania (women and men age 15-49), and Uganda (women and men age 15-59). Women and men from the entire age range are included in this analysis. However, the HIV prevalence estimates in this table are shown for women and men age 15-49 to avoid using an estimate based on different ages for women and men, and for consistency across countries.

The national HIV prevalence among women and men age 15-49 in this group of surveys ranges from 0.4 percent in Niger to 14 percent in Namibia. The HIV prevalence is near or below 2 percent in around half of these surveys. Among all 65 DHS Program surveys that have included HIV testing, the proportion with HIV prevalence below 2 percent is also around half. The sample size for the surveys varies widely from fewer than 8,000 people tested for HIV in Gambia to nearly 30,000 people tested for HIV in Zambia. Table

1 also shows the unweighted number of respondents who tested positive in each survey. This number is quite low, even at the national level, in the lower prevalence countries. For example, in the 2012 Niger DHS, there were only 44 HIV-positive respondents. Many of the analyses in this report are based only on the HIV-positive cases, and small sample sizes limit interpretation of the results.

### **3.2. Specimen Collection and Testing Procedures**

In most surveys conducted with the assistance of The DHS Program, the blood draw is conducted via a finger prick, and drops of blood are collected on a Whatman 903 filter paper card. The blood spots are allowed to dry overnight, and the cards are then individually packed in a low-gas permeable Ziploc bag, with glassine paper protecting the dried spots (DBS), and with one or two sachets of desiccants and a humidity monitor card, which are checked daily and replaced as needed. DBS specimens generally remain in the field for 10-14 days before being transported to the central laboratory, where they are stored at -20°C or colder until testing begins. In most surveys, the field staff who collect these blood specimens have no prior clinical or laboratory training. Training on the collection of these blood specimens usually includes 2-3 days of classroom instruction and 1-2 days of supervised field practice in households. Classroom instruction includes both theory and practice, and covers procedures for informed consent and infection prevention as well as proper techniques for pricking the finger, collecting the blood specimen, and managing the specimens in the field. Further details can be found in The DHS Program standard biomarker training manual (MEASURE DHS 2012).

Laboratory testing of survey specimens is conducted in the host country. A DHS biomarker specialist selects the lab in conjunction with the local implementing agency, ideally after an assessment of at least three potential labs. However, countries often do not have multiple laboratories or, at times, even one laboratory with the pre-existing capacity to carry out the HIV testing algorithm for the survey. In such cases, the DHS Program invests heavily in training laboratory technologists and providing supplies and equipment to enable the laboratory to meet the standards required to ensure high quality testing.

The specific assays used in the HIV testing algorithm are generally determined in coordination with the host country; however, the general testing strategy, as shown in Figure 1, tends to be consistent across countries. Table 2 includes the testing algorithm and the specimen matrix for each of the 20 surveys included in this analysis. HIV testing is typically conducted by three or four laboratory technologists whose time is fully dedicated to testing the survey specimens throughout the duration of the project. Training of the laboratory technologists includes a visit of 2-3 weeks to the laboratory by a DHS Program biomarker specialist. During this visit, the biomarker specialist trains the laboratory technologists to test the survey specimens for HIV in accordance with The DHS Program's standard operating procedures. All of the surveys in Table 2, except for the 2011 Uganda AIS, used dried blood spot (DBS) specimens for HIV testing. Before testing, the blood must be eluted from the filter paper card. Elution is effected by incubating a sample of the blood-soaked filter paper card in a buffer for 18-24 hours at 4°C. Note that none of the EIAs included in the testing algorithms for these surveys have been validated by the manufacturer for DBS, though their use with DBS has been common for HIV surveillance. The elution procedures used by the DHS Program are recommended by the CDC in Atlanta, Georgia.

**Table 2. HIV assays used and specimen matrix**

Name, manufacturer, and generation of assays used in the HIV testing algorithm, and specimen matrix, by survey

Survey	Assay 1	Assay 1 generation	Assay 2	Assay 2 generation	Assays 1 and 2 repeated if discrepant	Assay 3	Specimen matrix
<b>Sub-Saharan Africa</b>							
Burundi DHS 2010	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	No	Inno-Lia HIV I/II Score line immunoassay (Innogenetics)	DBS
Cameroon DHS 2011	Murex HIV Ag/Ab (DiaSorin)	4	Genscreen ULTRA HIV Ag/Ab (Bio-Rad)	4	No	New Lav Blot (Bio-Rad)	DBS
Cote d'Ivoire DHS 2011-12	Enzygnost Integral II (Siemens)	4	Vironostika HIV Ag/Ab (Biomérieux)	4	Yes	No third assay <sup>1</sup>	DBS
Democratic Republic of the Congo DHS 2013-14	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	Yes	HIV 2.2 Blot (DiaSorin)	DBS
Gabon DHS 2012	Genscreen Plus HIV Ag/Ab 5PL (Bio-Rad)	4	Enzygnost Integral II (Siemens)	4	No	HIV 2.2 Blot (DiaSorin)	DBS
Gambia DHS 2013	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	No	HIV 2.2 Blot (DiaSorin)	DBS
Guinea DHS 2012	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	Yes	No third assay <sup>1</sup>	DBS
Malawi DHS 2010	Vironostika HIV Uni-Form II Plus O (Biomerieux)	3	Enzygnost Anti-HIV 1/2 Plus (Seimens)	3	Yes	Western Blot 2.2 (Abbott Labs)	DBS
Mali DHS 2012-13	Vironostika HIV Uni-Form II Plus O (Biomerieux)	3	Enzygnost Integral II (Siemens)	4	No	HIV 2.2 Blot (DiaSorin)	DBS
Namibia DHS 2013	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	No	Inno-Lia HIV I/II Score line immunoassay (Innogenetics)	DBS
Niger DHS 2012	Vironostika HIV Uni-Form II Plus O (Biomérieux)	3	Enzygnost Anti-HIV 1/2 Plus (Seimens)	3	No	Inno-Lia HIV I/II Score line immunoassay (Innogenetics)	DBS
Rwanda DHS 2010	Vironostika HIV Ag/Ab (Biomérieux)	4	Murex HIV Ag/Ab (DiaSorin)	4	No	HIV 2.2 Blot (DiaSorin)	DBS
Senegal DHS 2010-11	Vironostika HIV Uni-Form II Plus O (Biomérieux)	3	Enzygnost Anti-HIV 1/2 Plus (Seimens)	3	No	Inno-Lia HIV I/II Score line immunoassay (Innogenetics)	DBS
Sierra Leone DHS 2013	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	No	Inno-Lia HIV I/II Score line immunoassay (Innogenetics)	DBS
Tanzania AIS/MIS 2011-12	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	Yes	HIV 2.2 Blot (DiaSorin)	DBS
Togo DHS 2013-14	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	yes	Inno-Lia HIV I/II Score line immunoassay (Innogenetics)	DBS
Uganda AIS 2011	Murex HIV 1.2.0 (Abbott Labs)	3	Vironostika HIV Uni-Form II Plus O (Biomérieux)	3	No	Ani Labsystems HIVEIA	Plasma <sup>2</sup>
Zambia DHS 2013-2014	Vironostika HIV Ag/Ab (Biomerieux)	4	Enzygnost Integral II (Siemens)	4	Yes	Western Blot 2.2 (Abbott Labs)	DBS
<b>Latin America/Caribbean</b>							
Dominican Republic DHS 2013	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	Yes	HIV 2.2 Blot (DiaSorin)	DBS
Haiti DHS 2012	Vironostika HIV Ag/Ab (Biomérieux)	4	Enzygnost Integral II (Siemens)	4	No	HIV 2.2 Blot (DiaSorin)	DBS

DBS = Dried blood spot

<sup>1</sup> The testing algorithms for the 2012 Guinea DHS and the 2013 Cote d'Ivoire DHS did not include a third assay. The few specimens that remained discordant on assay 1 and assay 2 were treated as negative in the HIV prevalence calculation.<sup>2</sup> Respondents were allowed to provide a DBS specimen from a finger prick if they refused venipuncture. Specimens tested for HIV in the 2011 Uganda AIS therefore include a mix of plasma and DBS. Less than 2 percent of specimens were DBS.

Before testing survey blood specimens, it is critical to confirm that the HIV test system—microplate reader and washer, DBS control materials, elution procedures, assay method, and data management system—is working correctly. To do this, the laboratory prepares at least 40 paired DBS and plasma specimens (i.e., DBS and plasma specimens using the blood of the same volunteer) to test the HIV algorithm, equipment, and data management system. Each technologist is required to test all specimens on EIA 1 and EIA 2. To qualify to test the survey specimens on an assay, each technologist must achieve a minimum expected level of agreement between the DBS and plasma results for each matched pair. Specifically, each technician is allowed to remove one discrepant pair from their results (if the results for any pair were discrepant), and the agreement between the DBS and plasma results on the remaining pairs must be 99 percent or greater. This phase is also used to train the technologists to program the plate reader and plate washer for each of the two EIAs and to use the data management system.

The DHS Program uses a data management tool called the CPro HIV Test Tracking System, or CHTTS, to manage HIV test results in the laboratory. This system generates plate maps of specimens to be tested on each microplate and captures the optical density (OD) values for each specimen from the plate reader. It calculates a cutoff for each plate from the OD values for the kit controls run on each plate using the manufacturer guidelines for the particular assay, and assigns each specimen a result of reactive or non-reactive on the assay. Specimens are considered to be HIV negative if their OD value is below the plate cutoff and HIV positive if the OD is equal to or greater than the plate cutoff. CHTTS tracks the results of each specimen on each assay to ensure that each specimen receives exactly the tests needed to render a final HIV status according to the logic of the testing algorithm. Each plate is given a unique identifier which can be linked to the technologist who ran the plate. As the results of each plate are read, the technologist must review the results and decide whether to save or cancel the plate. If the technologist cancels the results for a plate of specimens, the specimens must be retested on that assay. The saved results of each plate must then undergo a second step, referred to as validation, before the results for the plate are considered final. The laboratory supervisor in charge of testing for the survey is responsible for reviewing, and validating or rejecting, the results for each plate. CHTTS is also programmed to produce error messages if the OD values of the negative controls do not fall within the range specified by the manufacturer or if the percentage of specimens on the plate that are positive exceed a limit set by the DHS for that survey.

The DHS Program recommends that each technologist process two microplates—roughly 180 specimens, depending on the assay—each working day. However, during the first week after training, technologists are encouraged to test only one microplate of specimens each day to allow them time to increase their confidence and develop proficiency. During the first 2-3 weeks after training, all technologists test specimens on EIA 1 in order to accumulate a sufficient number of specimens that need to be tested on EIA 2. Because fewer specimens are tested on EIA 2, sometimes only one technologist is assigned this task. In countries where the HIV testing algorithm includes repeating EIA 1 and EIA 2 in parallel when the results are discordant, the repeat testing is also often performed by just one technologist.

The DHS Program employs multiple strategies for quality assurance throughout the HIV testing process. High positive, low positive, and negative dried blood spot control materials provided by the CDC in Atlanta are included on each microtiter plate. A subsample of 5-10 percent of EIA 1 negative specimens is randomly selected for testing on EIA 2 to check for false negative results on EIA 1. DHS biomarker staff monitor testing remotely by reviewing the results of a sample of the plates processed each week and provide feedback to the laboratory staff accordingly. In addition, DHS biomarker staff review photographs taken of the banding pattern of specimens tested on blot assays such as Western blot or INNO-LIA to confirm the final result for the assay.

### 3.3. Data Management and Analysis

An extensive amount of data management was required for this analysis. The DHS Program has not formatted or archived the detailed laboratory results files needed for this work for public release. The data management system, CHTTS, produces several raw data files, two of which contain data relevant to this analysis: a test log file and a plate file. The test log file includes specimens as the unit of analysis. It includes the identification information for each specimen, and information used to guide the laboratory technicians through the HIV testing algorithm, including the optical density and HIV test result for each EIA. It retains data from runs that fail and are not used in the final results and clearly identifies those data as canceled. However, this produces a challenge when attempting to use the database analytically because the final results for each stage of the testing algorithm can be found in different variables of the data file for different specimens. In addition, this file has not included the cutoff values for ascribing seropositivity for each specimen for each assay, making the calculation of a signal-to-cutoff value impossible. Cutoff values, as well as the complete results for each microplate are included in a second raw data file, which uses the microplate as the unit of analysis.

An application was written in CPro (1) to restructure the data in the test log file so that the final assay results for each stage of the testing algorithm appear in the same variable for each specimen, (2) to merge in the cutoff values for each assay for each specimen from the plate file, and (3) to delete specimens that could not be linked to a de facto, interviewed individual in the survey. This third step standardized the universe of specimens for each survey to those that were included in the HIV prevalence estimate. Each survey file was then imported and analyzed using Stata v13. Each file was checked for internal consistency and completeness and merged with the questionnaire data to allow linkage with the sex and age of the respondent. Analysis consists of simple frequencies and bivariate tabulations.

The analysis focuses on two variables that can help to characterize the quality of the EIA testing. The first indicator is the agreement between EIA 1 and EIA 2. Given the high published sensitivity and specificity of EIAs, both EIAs should find concordant results for nearly all specimens. Poor agreement between the first and second EIA indicates quality issues in the testing process. The analysis looks at the agreement between EIA 1 and EIA 2 in the first round of testing, and after the second round of testing in those surveys where specimens with discrepant results on EIA 1 and EIA 2 were tested again on both EIAs. Overall agreement of 99 percent or greater is optimal.

The second indicator is the percentage of positive cases with an S/CO value less than 5.0. The choice of 5.0 is somewhat arbitrary, but this value has been used conventionally to identify specimens that may be more likely to be false positive than those with higher S/CO values. For simplicity, specimens are divided into just two categories—those with an S/CO value of less than 5.0, and those with an S/CO value of 5.0 or greater—rather than using the three categories defined in Nkengasong et al. (1999)—1.0-3.0, >3.0-5.0, and greater than 5.0. For this analysis, all positive specimens with an S/CO of less than 5.0 are described as “weak” positives. As discussed, S/CO values are difficult to use as an indication of false positivity for an individual specimen because it is possible for a true positive specimen to have a weak reaction and for a false positive specimen to have a strong reaction. Nonetheless, the distribution of S/CO values for an assay across a population can be evaluated as more or less plausible. There is no single optimal value for the percentage of positive specimens with an S/CO value of less than 5.0—this will depend on the population tested and the specific properties of the assay used. However, in general, the proportion of the HIV positive population with weak reactions on an EIA is expected to be relatively low.



## 4. Results

### 4.1. Agreement between EIA 1 and EIA 2

The first indicator of quality in the EIA testing, agreement between the two assays, is explored in Tables 3 and 4. In the HIV testing algorithm used by the DHS Program in these surveys, all samples that test positive on the first EIA are retested on a second EIA. The first column in Table 3 shows the percentage of specimens with positive results on EIA 1 that are also positive on EIA 2. This percentage varies widely across surveys, from 31 percent in Niger to 97 percent in Cote d'Ivoire. Cote d'Ivoire was the only country to use Enzygnost as the first assay in the algorithm. The percentage of EIA 1 positive specimens that are also positive on EIA 2 is also quite low in Guinea, the Democratic Republic of the Congo (DRC), Cameroon, and Uganda (44-52 percent). Third generation assays were used as EIA 1 in Niger (Vironostika) and Uganda (Murex), while fourth generation assays were used as EIA 1 in Cameroon (Murex), DRC, and Guinea (Vironostika for both).

**Table 3. Agreement of assays**

Among specimens positive on assay 1, percentage positive on assay 2; among specimens negative on assay 1 and retested on assay 2 for internal quality control, percentage positive on assay 2; among specimens tested on both assay 1 and assay 2, percentage concordant after the first round of testing, and percentage concordant after repeat testing on both assays, by survey [Unweighted]

Survey	Assay 1 positive specimens, first round of testing		Assay 1 negative specimens retested for internal quality control <sup>1</sup>		Overall agreement between assay 1 and assay 2		
	Percentage that are positive on assay 2	Number of specimens positive on assay 1	Percentage that are positive on assay 2	Number of specimens negative on assay 1	After first round of testing	After repeat testing	Number of samples tested on assay 1 and assay 2
<b>Sub-Saharan Africa</b>							
Burundi DHS 2010	60.6	259	1.3	830	89.6	na	1,089
Cameroon DHS 2011	51.4	1,259	0.6	663	68.0	na	1,922
Cote d'Ivoire DHS 2011-12	97.1	344	0.2	865	99.0	99.7	1,209
Democratic Republic of the Congo DHS 2013-14	45.4	388	1.5	863	82.0	99.7	1,251
Gabon DHS 2012	84.1	561	0.2	523	91.7	na	1,084
Gambia DHS 2013	87.6	153	0.3	749	97.7	na	902
Guinea DHS 2012	43.8	372	1.2	413	72.7	99.2	785
Malawi DHS 2010	81.0	1,718	1.0	1,238	88.6	99.0	2,956
Mali DHS 2012-13	84.2	114	0.6	875	97.7	na	989
Namibia DHS 2013	87.7	1,387	2.9	421	89.9	na	1,808
Niger DHS 2012	31.0	100	0.2	863	92.6	na	963
Rwanda DHS 2010	94.8	442	0.0	1,283	98.7	na	1,725
Senegal DHS 2010-11	78.2	119	0.0	976	97.6	na	1,095
Sierra Leone DHS 2013	70.6	269	6.3	1,440	90.1	na	1,709
Tanzania AIS/MIS 2011-12	84.9	952	3.8	1,678	92.1	99.7	2,630
Togo DHS 2013-14	82.5	240	1.2	893	95.3	99.1	1,133
Uganda AIS 2011	51.8	2,629	na	na	51.8	na	2,629
Zambia DHS 2013-2014	78.3	4,741	12.7	2,423	81.3	95.5	7,164
<b>Latin America/Caribbean</b>							
Dominican Republic DHS 2013	70.1	271	0.1	1,847	96.1	99.9	2,118
Haiti DHS 2012	74.6	559	0.9	901	89.7	na	1,460

<sup>1</sup> A random subsample of specimens that are negative on assay 1 in the first round of testing are routinely retested on assay 2 for internal quality control. The 2011 Uganda AIS did not include retesting of assay 1 negative samples for internal quality control.

The percentage of the subsample of EIA 1 negative specimens retested for IQC that are positive on EIA 2 is shown in column 3 of Table 3. In most countries this percentage is below 1 percent. The percentage of IQC specimens found positive is especially high in Sierra Leone (6 percent) and Zambia (13 percent).

Finally, Table 3 shows the overall agreement between EIA 1 and EIA 2 after the first round of testing on each assay, and after the second round of testing on EIA 1 and EIA 2 in surveys where EIA 1 and EIA 2 were repeated if the results were discrepant in the first round of testing. The denominators for columns 5 and 6 in Table 3 are shown in column 7 and include all specimens that were positive on EIA 1 as well as the EIA 1 negative specimens that were selected for IQC. All of these specimens were retested on EIA 2. Agreement is defined as having either a positive result on both EIAs or a negative result on both EIAs. Overall agreement of the two EIAs after the first round of testing ranges from 52 percent in Uganda to 99 percent in Cote d'Ivoire. Because there was no IQC testing in Uganda, this percentage is equivalent to the percentage of EIA 1 positive specimens that are positive on EIA 2, shown in the first column. Agreement after the first round of testing reaches 99 percent in only one of the 20 surveys.

In 8 of the 20 surveys included in this analysis, specimens with discrepant results on EIA 1 and EIA 2 were tested again on both EIAs. The agreement following the repeat round of testing is calculated as follows: if the specimen was retested on EIA 1 and EIA 2, the repeat test results were included; however, if the specimens were concordant on EIA 1 and EIA 2 in the first round of testing, then these first round results were used. The step of repeating EIA 1 and EIA 2 on specimens with discrepant results improved agreement in all eight surveys. Agreement reaches or exceeds the benchmark of 99 percent among all surveys with repeat testing on the two EIAs, except for Zambia where agreement is 96 percent. Taking into account the results of repeat testing, where conducted, final assay agreement reaches 99 percent in 7 of the 20 surveys.

Table 4 shows a distribution of those specimens that were discrepant in the first round of testing by the results of the repeated tests. These results are broken down by the results of the first round of testing on EIA 1 and EIA 2: A1+A2- or A1-A2+ (the IQC specimens). Overall, the vast majority of these discrepant samples resolved to negative-negative. In Cote d'Ivoire and Zambia, specimens were less likely than in the other countries to resolve to negative-negative. In Cote d'Ivoire, there was a very high level of agreement observed between the first and second EIAs—97 percent of specimens that were positive on the first EIA were also positive on the second, as shown in Table 3. There were only 12 specimens that were discordant on the two EIAs and proceeded to repeat testing. Though it is not clear if this is related to either the high level of agreement between the EIAs on initial testing or the lower likelihood of resolving to negative-negative during repeat testing, it should be noted that in Cote d'Ivoire the usual order of EIA 1 and EIA 2 was reversed, with Enzygnost (fourth generation) serving as the first EIA and Vironostika (fourth generation) as the second. By contrast, in Zambia, 22 percent of samples that were found positive on the first EIA were found negative on the second EIA. This level of disagreement is comparable to that observed during the first round of testing in the Dominican Republic, Malawi, Tanzania, and Togo, and is lower than that observed in DRC and Guinea; however, the discrepant specimens in all of those countries were more likely to resolve to negative-negative in repeat testing than in Zambia.

In each survey, the IQC specimens make up a minority of the total number of discrepant specimens, but this percentage does vary across surveys from 2 percent in Guinea and the Dominican Republic to 30 percent in Tanzania. In most of these surveys, the IQC specimens were roughly equally likely or slightly more likely to resolve to negative-negative than the A1+A2- specimens. In Togo and Zambia, the IQC specimens were more likely than A1+A2- specimens to remain discrepant after repeat testing. It is interesting to note that the IQC specimens in Cote d'Ivoire and the Dominican Republic all resolved to positive-positive; however, each survey had only two IQC specimens with positive results, limiting the interpretation of this finding.

**Table 4. Agreement of repeat EIA testing**

Percent distribution of specimens retested on both EIAs according to the results of the repeated tests, by survey [Unweighted]					
Survey	Resolve to negative-negative	Resolve to positive-positive	Remain discordant	Total	Number
<b>Sub-Saharan Africa</b>					
<b>Cote d'Ivoire DHS 2011-12</b>					
A1+A2-	60.0	0.0	40.0	100.0	10
A1-A2+ (IQC)	0.0	100.0	0.0	100.0	2
All round 1 discrepant specimens	50.0	16.7	33.3	100.0	12
<b>Democratic Republic of the Congo DHS 2013-14</b>					
A1+A2-	98.1	0.0	1.9	100.0	212
A1-A2+ (IQC)	100.0	0.0	0.0	100.0	13
All round 1 discrepant specimens	98.2	0.0	1.8	100.0	225
<b>Guinea DHS 2012</b>					
A1+A2-	96.7	0.5	2.9	100.0	209
A1-A2+ (IQC)	100.0	0.0	0.0	100.0	5
All round 1 discrepant specimens	96.7	0.5	2.8	100.0	214
<b>Malawi DHS 2010</b>					
A1+A2-	84.7	6.4	8.9	100.0	326
A1-A2+ (IQC)	100.0	0.0	0.0	100.0	12
All round 1 discrepant specimens	85.2	6.2	8.6	100.0	338
<b>Tanzania AIS/MIS 2011-12</b>					
A1+A2-	93.1	2.1	4.9	100.0	144
A1-A2+ (IQC)	96.8	0.0	3.2	100.0	63
All round 1 discrepant specimens	94.2	1.4	4.3	100.0	207
<b>Togo DHS 2013-14</b>					
A1+A2-	90.5	2.4	7.1	100.0	42
A1-A2+ (IQC)	36.4	0.0	63.6	100.0	11
All round 1 discrepant specimens	79.2	1.9	18.9	100.0	53
<b>Zambia DHS 2013-2014</b>					
A1+A2-	67.3	14.0	18.7	100.0	1029
A1-A2+ (IQC)	44.3	12.7	43.0	100.0	307
All round 1 discrepant specimens	62.1	13.7	24.3	100.0	1336
<b>Latin America/Caribbean</b>					
<b>Dominican Republic DHS 2013</b>					
A1+A2-	97.5	1.3	1.3	100.0	79
A1-A2+ (IQC)	0.0	100.0	0.0	0.0	2
All round 1 discrepant specimens	95.1	3.7	1.2	100.0	81

Note: Only 8 of the 20 surveys included in this analysis included repeat testing on both EIAs for samples that had discrepant results on EIA 1 and EIA 2.

#### 4.2. Signal to Cutoff Values

Table 5 examines the S/CO values of specimens that were found positive on the first round of testing on EIA 1 and EIA 2. The percent of EIA 1 positive specimens that have an S/CO value of less than 5.0 ranges from 3 percent in Cote d'Ivoire to 58 percent in Guinea. The percentage of EIA 1 positive specimens with an S/CO value below 5.0 exceeds 20 percent in roughly half of the surveys included in this analysis (9 out of 20). There are some differences noted by assay type. The percentage of EIA 1 positive specimens with an S/CO value of less than 5.0 is over 20 percent in 1 of 4 surveys using a third generation Vironostika as EIA 1, compared with 6 of 12 surveys using a fourth generation Vironostika as EIA 1. The percentage of EIA 1 positive specimens with a S/CO value of less than 5.0 is over 20 percent in both surveys using Murex as EIA 1, with this percentage being higher in Gabon, which used a fourth generation Murex, than in Uganda, which used a third generation Murex. It is important to note, however, that the percentage of EIA 1 positive specimens with an S/CO value of less than 5.0 in surveys using a fourth generation assay as EIA 1 is not high across the board. For example, this percentage was only 3 percent in Cote d'Ivoire, which used fourth generation Enzygnost, and 5 percent and 8 percent in Rwanda and Gambia, respectively, both of which used fourth generation Vironostika.

**Table 5. Signal to cutoff values**

Among specimens positive on assay 1 in the first round of testing, percentage with a signal to cutoff (S/CO) value of less than 5.0; among specimens positive on assay 2 in the first round of testing, percentage with an S/CO of less than 5.0, by survey [Unweighted]

Survey	Assay 1 positive specimens		Assay 2 positive specimens	
	Percentage with S/CO <5.0	Number	Percentage with S/CO <5.0	Number
<b>Sub-Saharan Africa</b>				
Burundi DHS 2010	28.2	259	7.7	168
Cameroon DHS 2011	49.5	1,259	2.0	651
Cote d'Ivoire DHS 2011-12	2.9	344	0.9	336
Democratic Republic of the Congo DHS 2013-14	51.8	388	12.2	189
Gabon DHS 2012	12.1	561	4.2	473
Gambia DHS 2013	7.8	153	5.9	136
Guinea DHS 2012	57.5	372	7.1	168
Malawi DHS 2010	15.7	1,718	12.1	1,404
Mali DHS 2012-13	10.5	114	9.9	101
Namibia DHS 2013	10.5	1,387	1.9	1,229
Niger DHS 2012	46.0	100	78.8	33
Rwanda DHS 2010	5.4	442	2.4	419
Senegal DHS 2010-11	11.8	119	21.5	93
Sierra Leone DHS 2013	13.8	269	28.8	281
Tanzania AIS/MIS 2011-12	13.3	952	12.7	871
Togo DHS 2013-14	16.7	240	6.2	209
Uganda AIS 2011 <sup>1</sup>	36.9	2,629	3.6	1,360
Zambia DHS 2013-2014	25.7	4,741	24.3	4,018
<b>Latin America/Caribbean</b>				
Dominican Republic DHS 2013	21.8	271	0.0	192
Haiti DHS 2012	24.9	559	3.1	425

<sup>1</sup> The 2011 Uganda AIS includes one sample with a missing signal-to-cutoff value for assay 2.

Descriptive statistics on the distribution of weak positive specimens by S/CO value indicate that in many surveys there is a clustering of these specimens at lower S/CO values, close to the cutoff for seropositivity (Appendix Table 1). The percentage of positive specimens with S/CO values less than 5.0 is also lower among specimens with positive results on both EIA 1 and EIA 2 than among specimens that are EIA 1 positive and EIA 2 negative (Appendix Table 2).

Table 5 also shows the percentage of EIA 2 positive specimens with an S/CO value less than 5.0. As a reminder, specimens tested on EIA 2 in the first round of testing include all specimens that were positive on EIA 1 and a portion of those that tested negative on EIA 1. The proportion of EIA 2 positive specimens that comes from the IQC process varies by survey from 0 percent in Rwanda and Senegal to 32 percent in Sierra Leone (see Appendix Table 2).<sup>1</sup> The proportion of EIA 2 positive specimens with an S/CO value less than 5.0 is lower in most surveys than the proportion of EIA 1 positive specimens. In the majority of surveys, this percentage is less than 10 percent. The highest percentage of EIA 2 positive specimens with an S/CO value less than 5.0 is observed in Niger (79 percent). Senegal (22 percent), Sierra Leone (29 percent), and Zambia (24 percent) also had exceptionally high percentages of EIA 2 positive samples with weak S/CO values.

Assessing differences by assay is difficult because 14 of the 20 surveys used the same assay as EIA 2: fourth generation Enzygnost. Among surveys using this assay as EIA 2, the proportion of EIA 2 positive

<sup>1</sup> The percentage of EIA 2 positive specimens that come from IQC in Table 5 should not be confused with the percentage of specimens with discrepant results on EIA 1 and EIA 2 that come from IQC, as discussed in Table 4. In Table 4, the percentage of specimens from IQC is equivalent to  $A1-A2+/(A1+A2- \text{ or } A1-A2+)$ . By contrast, in Table 5, the percentage of specimens from IQC is equivalent to  $A1-A2+/(A1+A2+ \text{ or } A1-A2+)$ .

specimens with an S/CO value less than 5.0 exceeds 20 percent in only two. The percentage of EIA 2 positive specimens with an S/CO value less than 5.0 exceeds 20 percent in two of the three surveys using third generation Enzygnost as EIA 2. Among the two surveys that used Vironostika as EIA 2, the percentage of EIA 2 positive specimens with an S/CO value less than 5.0 is 4 percent in Uganda, which used the third generation assay, compared with 1 percent in Cote d'Ivoire, which used the fourth generation assay.

In general, the percentage of EIA 2 positive specimens with an S/CO value less than 5.0 is higher among IQC specimens than specimens with positive results on both EIA 1 and EIA 2 (see Appendix Table 2). However, it does not appear that the portion of EIA 2 positive specimens that come from IQC explains much of the variation across surveys in the proportion of EIA 2 positive specimens with weak reactions. On the one hand, the proportion of EIA 2 positive specimens with S/CO values less than 5.0 (29 percent) and the proportion of EIA 2 positive specimens that come from IQC (32 percent) are both high in Sierra Leone. On the other hand, in Senegal, where 22 percent of the EIA 2 positive specimens have S/CO values less than 5.0, none of the EIA 2 positive specimens were from IQC.

Given the high percentage of HIV positive specimens with an S/CO value less than 5.0 in the first round of testing, and the potential for false positive results among them, Table 6 presents the proportion of EIA 1 positive specimens that are assigned a final result of HIV positive after the completion of the testing algorithm, according to S/CO value. EIA 1 positive specimens are much more likely to be assigned a final result of HIV positive if they have an S/CO value of 5.0 or greater. The percentage of EIA 1 positive specimens with S/CO values of 5.0 or greater that are rendered positive after completion of the HIV testing algorithm ranges from 78 percent in Niger to 99 percent in Cameroon. On the other hand, the majority of specimens with S/CO values less than 5.0 on the first round of testing on EIA 1 are rendered negative through later stages of the HIV testing algorithm. The percentage of specimens with weak positive results on the first round of EIA 1 testing that remained positive after completion of the testing algorithm ranges from less than 1 percent in DRC to 38 percent in Zambia. In 13 of the 20 surveys, the percentage of EIA 1 weak positives that are ultimately rendered positive is less than 10 percent.

**Table 6. Final HIV results according to signal to cutoff values on assay 1**

Among specimens that are positive on assay 1 in the first round of testing, percentage that remain positive after all HIV testing, according to the signal to cutoff (S/CO) value on assay 1, by survey [Unweighted]

Survey	Percentage HIV positive after all testing	Number of assay 1 positive samples with S/CO <5.0	Percentage HIV positive after all testing	Number of assay 1 positive samples with S/CO ≥5.0
<b>Sub-Saharan Africa</b>				
Burundi DHS 2010	2.7	73	86.6	186
Cameroon DHS 2011	2.7	623	99.4	636
Cote d'Ivoire DHS 2011-12	20.0	10	97.6	334
Democratic Republic of the Congo DHS 2013-14	0.5	201	94.1	187
Gabon DHS 2012	2.9	68	97.6	493
Gambia DHS 2013	8.3	12	95.0	141
Guinea DHS 2012	3.7	214	98.7	158
Malawi DHS 2010	3.7	269	97.3	1,449
Mali DHS 2012-13	8.3	12	94.1	102
Namibia DHS 2013	8.9	146	97.9	1,241
Niger DHS 2012	2.2	46	77.8	54
Rwanda DHS 2010	29.2	24	98.8	418
Senegal DHS 2010-11	14.3	14	86.7	105
Sierra Leone DHS 2013	21.6	37	92.2	232
Tanzania AIS/MIS 2011-12	11.0	127	97.1	825
Togo DHS 2013-14	2.5	40	99.0	200
Uganda AIS 2011	14.1	970	81.9	1,659
Zambia DHS 2013-2014	37.8	1,217	96.5	3,524
<b>Latin America/Caribbean</b>				
Dominican Republic DHS 2013	1.7	59	89.6	212
Haiti DHS 2012	5.8	139	97.9	420

Table 7 shows the distribution of all specimens that were rendered positive after the completion of the HIV testing algorithm according to EIA agreement and S/CO value.<sup>2</sup> The S/CO values of EIA 1 and EIA 2 are taken from the first round of testing unless the specimens were retested on EIA 1 and EIA 2, in which case the S/CO values from EIA 1 and EIA 2 from the repeat round replaced the S/CO values from EIA 1 and EIA 2 from the first round. As a reminder, EIA 1 and EIA 2 were repeated only if (1) the results on the two assays were discrepant in the first round of testing and (2) repeat testing of the EIAs was included in the survey's testing algorithm (as noted in Table 2). Over 85 percent of positive specimens within each country have S/CO values of 5.0 or higher on both EIAs in all surveys except Niger (16 percent), Senegal (77 percent), Sierra Leone (71 percent), and Zambia (78 percent). The proportion of positive specimens with a high S/CO value on one EIA and a low S/CO value on the other EIA is less than 5 percent in most countries. Higher percentages (9-25 percent) were observed in Burundi, Malawi, Senegal, Sierra Leone, and Zambia. The especially high percentage observed in Niger (55 percent) is related to the high proportion of weak positive specimens on the second EIA, which was Enzygnost (79 percent, as shown in Table 5). This pattern was not observed in the other two surveys that used the third generation Enzygnost assay as EIA 2. The percentage of positive specimens with S/CO values less than 5.0 on both EIAs is 1 percent or less in most surveys. Guinea, Sierra Leone, and Uganda have slightly higher percentages, 2-3 percent. However, Zambia, with 12 percent, is a notable outlier.

**Table 7. Distribution of final HIV positive specimens by assay results and signal to cutoff values**

Among specimens rendered positive after the completion of all HIV testing, percent distribution by EIA results and signal to cutoff (S/CO) values, by survey [Unweighted]

Survey	High S/CO on both EIA	S/CO high on one EIA, low on the other EIA	Low S/CO on both EIA	High S/CO on one EIA, negative on the other EIA	Low S/CO on one EIA, negative on the other EIA	Total	Number final positive specimens
<b>Sub-Saharan Africa</b>							
Burundi DHS 2010	86.6	9.1	0.0	4.3	0.0	100.0	164
Cameroon DHS 2011	96.8	1.7	1.1	0.5	0.0	100.0	649
Cote d'Ivoire DHS 2011-12	96.4	3.0	0.6	0.0	0.0	100.0	336
Democratic Republic of the Congo DHS 2013-14	93.8	5.1	0.6	0.6	0.0	100.0	177
Gabon DHS 2012	93.6	4.1	0.0	2.1	0.2	100.0	483
Gambia DHS 2013	93.4	4.4	0.7	1.5	0.0	100.0	136
Guinea DHS 2012	93.3	4.3	2.4	0.0	0.0	100.0	164
Malawi DHS 2010	87.7	11.4	0.4	0.4	0.1	100.0	1,420
Mali DHS 2012-13	93.8	5.2	0.0	0.0	1.0	100.0	97
Namibia DHS 2013	96.0	2.5	0.2	1.3	0.0	100.0	1,233
Niger DHS 2012	15.9	54.5	0.0	25.0	4.5	100.0	44
Rwanda DHS 2010	96.9	1.9	1.0	0.0	0.2	100.0	420
Senegal DHS 2010-11	77.4	21.5	1.1	0.0	0.0	100.0	93
Sierra Leone DHS 2013	70.7	12.2	2.7	14.4	0.0	100.0	222
Tanzania AIS/MIS 2011-12	92.8	5.9	0.9	0.2	0.2	100.0	815
Togo DHS 2013-14	98.0	1.5	0.5	0.0	0.0	100.0	199
Uganda AIS 2011 <sup>1</sup>	87.1	1.7	2.2	2.7	6.3	100.0	1,494
Zambia DHS 2013-2014	77.9	9.8	12.1	0.2	0.0	100.0	3,901
<b>Latin America/Caribbean</b>							
Dominican Republic DHS 2013	99.5	0.0	0.5	0.0	0.0	100.0	193
Haiti DHS 2012	96.9	1.7	1.0	0.5	0.0	100.0	419

Notes: High S/CO defined as  $\geq 5.000$ . Low S/CO defined as 1.000-4.999. The assay results and S/CO reported in this table are taken from the repeat round of EIA testing if a specimen was subjected to repeat testing, and from the first round of testing if the specimen was tested on EIA 1 and 2 only once.

<sup>2</sup> Table 7 includes specimens from the IQC process to the extent that they were rendered positive after the completion of all HIV testing. Across all surveys, the number of specimens that re-enter the testing algorithm due to a positive result on the IQC test and that are assigned a final status of HIV positive is few, if any (Appendix Table 3).

The last two columns in the distribution include specimens that were positive on one EIA and negative on the other. These specimens were rendered positive by virtue of a positive result on the third assay in the testing algorithm. In most countries this assay was a Western blot or a line immunoassay, such as INNO-LIA. Uganda is the only survey that used a third EIA as the tie-breaker assay. The percentage of positive specimens with a high positive result on one EIA and a negative result on the other ranges from 0 to 4 percent in most countries, but reaches 14 percent in Sierra Leone and 25 percent in Niger. The percentage of positive specimens with a weak positive result on one EIA and a negative result on the other is 1 percent or less in every survey except Niger (5 percent) and Uganda (6 percent).

This analysis also examined the test results of specimens selected for IQC to assess the potential for false negative classification of specimens in this group of surveys. The percentage of IQC specimens (i.e., the subsample of specimens with negative results on EIA 1 that were selected for retesting on EIA 2) that were found to be positive on EIA 2 is shown in the third column of Table 3. This percentage is very low in most surveys. Specimens found to be positive through IQC were either retested on EIA 1 and EIA 2 (if these assays are repeated when discrepant in the survey's algorithm) or immediately tested on a third assay to break the tie between EIA 1 and EIA 2. An analysis of the final serostatus of the specimens found to be positive on EIA 2 during the IQC process shows that the vast majority—and, in many cases, all—of these specimens are rendered negative by the third assay (Appendix Table 3).



## 5. Discussion

The results of this analysis cannot say definitively if there has been any over- or underestimation of HIV prevalence in these surveys, or how great any error may be. However, it appears likely that testing error associated with false positivity is present to some degree in the surveys included in this analysis. With regard to the two quality indicators measured—agreement between EIA tests and proportion of positive specimens with an S/CO value less than 5.0—the analysis shows that they vary widely across surveys.

Upon completion of the testing algorithm, agreement between the first and second EIA was high in most surveys, but only 7 of the 20 surveys reached the desired benchmark of at least 99 percent. Cameroon (68 percent) and Uganda (52 percent) had the lowest levels of agreement on EIA 1 and EIA 2. Agreement in the remaining 11 surveys ranged from just under 89 percent to just under 99 percent. It is important to examine possible reasons why some of the surveys in this analysis have lower than desired levels of agreement.

A principal factor contributing to lower levels of agreement is a low percentage of EIA 1 positive specimens being found positive on EIA 2. The percentage of EIA 1 positive specimens that are found positive on EIA 2 after the first round of testing varies by survey, but is below 80 percent in 11 of the 20 countries and below 90 percent in all but two surveys. Published literature indicates that EIAs are highly sensitive. In several studies, EIAs have been found to detect every antibody-positive infection, resulting in no false negative results (Fanmi et al. 2012; Hakim et al. 2016; Urassa et al. 1999; Zhang et al. 2013). In the 20-country analysis presented in this report, EIA 1 positive specimens found negative on EIA 2 tended to have weak reactions on EIA 1. This finding supports evidence from the published literature that where the percentage of EIA 1 positive specimens that are positive on EIA 2 is low, much if not all of the disagreement is likely due to false positive results on EIA 1.

This analysis shows that repeat testing of discrepant specimens on EIA 1 and EIA 2 improved the level of agreement between the two assays. The improvement in agreement is achieved primarily through resolution of the discrepant results to negative-negative. In each of the eight surveys with repeat testing most, though not all, of the discrepant results resolve to negative-negative. Further, the benefit of repeat testing seems to be stronger where the first round test results are weaker. For example, Guinea improved from 73 percent agreement after the first round of testing to over 99 percent agreement after the second round of testing—on par with Cote d'Ivoire, in which assay agreement was 99 percent after the first round of testing. Though the reasons for this improvement are not clear, two observations can be made. First, in the sequence of steps in the laboratory testing for these surveys, the repeat testing on specimens with discrepant results on EIA 1 and EIA 2 is usually conducted after completing the first round of testing on EIA 1 and EIA 2 for all specimens. By this point, technologists may have become more proficient in their techniques on these assays and in working with the DBS specimens. Second, the repeat testing is often conducted by fewer, more experienced technologists.

According to the testing strategy followed in these surveys, specimens with discrepant EIA results were rendered positive if the tie-breaker assay was positive. However, according to the HIV testing strategies currently recommended by CDC (2014) and WHO (2015), all of these specimens would be treated as negatives for the purpose of prevalence calculation. Specimens with discrepant EIA results make up less than 1 percent of positive specimens in 12 of the 20 surveys in this analysis, 1-5 percent in five surveys, 9

percent in Uganda, 14 percent in Sierra Leone, and 30 percent in Niger.<sup>3</sup> It should also be noted that in most of these surveys, only a small percentage of all EIA discrepant specimens are positive on the third assay (data not shown). Therefore the specimens with discrepant EIA results that are included among the final positive specimens are a small percentage of all specimens with discrepant EIA results.

Though agreement of around 99 percent between two EIAs is expected when testing is of high quality, these high levels of agreement do not rule out the possibility of false positive results—it is still possible for dual EIA positive specimens to be false positive. The percent of dual EIA positive specimens that are confirmed positive, or the positive predictive value (PPV), varies in the published literature, but low values have been observed. While the PPV of two positive EIAs was found to be 100 percent in Urassa et al. (1999) and 99.9 percent in Nkengasong et al. (1999), a PPV of only 90.8 percent was observed by Hakim et al. (2016), and 84.0 percent by Zhang et al. (2013). It is important to note that the prevalence in the study by Zhang et al. was only 0.03 percent and that PPV is expected to be lower where prevalence is lower.

Although false positives do occur at high S/CO values, the PPV of EIAs generally increases with S/CO value. Therefore, this analysis also examined the distribution of S/CO values on the two EIAs. The percentage of specimens with an S/CO value less than 5.0 on EIA 1 exceeds 20 percent in 9 of the 20 surveys. As noted in the methods section, it is difficult to know what an optimal distribution of S/CO values would look like for these populations; however, it is highly unlikely that the percent of positive specimens with true weak positive reactions would be as high as 20 percent. This finding indicates that there is a risk of a notable number of false positive specimens in the first round of testing on EIA 1.

Combining the S/CO values of the two EIAs, dual EIA positive specimens were categorized into three groups: specimens with low S/CO values (<5.0) on both EIAs, specimens with a low S/CO value on one EIA and a high S/CO value on the other, and specimens with high S/CO values on both EIAs. The proportion of positive specimens with low S/CO values on both EIAs in this 20-survey analysis is below 1 percent in most surveys, compared with a high of 12 percent in Zambia. Evidence from other studies indicates that the positive specimens with low S/CO values on both EIAs are the least likely of the dual EIA positive specimens to be true positives. In an investigation of six EIAs, Nkengasong et al. (1999) found that only around half of specimens with S/CO <3.0 and 73 percent of specimens with S/CO <5.0 on individual EIAs were confirmed positive. Only 4 percent of specimens with S/CO <5.0 were confirmed positive on Enzygnost Integral II in a study by Fanmi et al. (2012). Zhang et al. (2013) and Urassa et al. (1999) found no true positive specimens among those with S/CO values on both EIAs of <5.0 and <3.0, respectively.

The percentage of positive specimens with a low S/CO value on one EIA and a high S/CO value on the other varies across the 20 surveys in this analysis. The percentage of positive specimens with “mixed” S/CO values is below 5 percent in 11 surveys, 5-10 percent in five surveys, and above 10 percent in the remaining four surveys. In the published literature, specimens with a low S/CO value on one EIA and a high S/CO value on the other appear to be uncommon, and there is little evidence of their PPV. There were no specimens with an S/CO value <5.0 on one EIA and  $\geq 5.0$  on the other in the study by Zhang et al. (2013). In the study by Nkengasong et al. (1999), 9 of 12 specimens that had S/CO values <3.0 on one or both EIAs were confirmed positive, for a PPV of 75 percent. All dual EIA positive specimens were confirmed positive

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<sup>3</sup> If all discrepant specimens with a positive result on the third assay were to be considered negative rather than positive, the HIV prevalence estimates would remain unchanged in 15 of the 20 surveys, and would decrease by 0.1-0.2 percentage points in four surveys and by 0.6 percentage points in Uganda (data not shown).

in the study by Urassa et al. (1999), but it is unclear how many of these, if any, may have had mixed S/CO values.

The percentage of final positive specimens with S/CO values of 5.0 or greater on both EIA 1 and EIA 2 is over 85 percent for 16 of the 20 surveys in this analysis, and is over 95 percent in 7 surveys. Though it cannot be assumed that all are positive, some studies do show that the group of specimens with S/CO values greater than 5.0 have much higher PPV than do all positives as a whole. For example, the PPV of Enzygnost Integral II increased from 84.3 percent among all positive specimens to 98.9 percent among specimens with S/CO >5.0 (Fanmi et al., 2012). No false positives were found among specimens with S/CO >3.0 on both EIAs in Nkengasong et al. (1999). On the other hand, in the study by Zhang et al. (2013), the group of positive specimens with S/CO >5.0 had a PPV of 90.8 percent, a relatively small improvement over the PPV of 84.0 percent for all positives. Again, the low HIV prevalence in the study by Zhang et al. and its association with lower PPV should be noted.

The results of this 20-survey analysis also show that the vast majority of specimens with discrepant results on the two EIAs, as well as specimens with low S/CO values on EIA 1, are rendered negative in the later stages of the testing algorithm. However, the literature on the specificity of EIA tests provides evidence that some false positives are likely to remain among dual EIA positive specimens and across all S/CO values.

A few factors that further limit the applicability of results from published studies to the surveys included in this analysis should be noted. The EIAs used in most of these studies were third generation tests. In this analysis, on average, surveys that used a fourth generation Vironostika assay as EIA 1 have a higher percentage of positive specimens with an S/CO value of less than 5.0 than do surveys that used a third generation Vironostika assay. Though this finding is inconclusive, it may be associated with a lower PPV for the fourth generation tests. In addition, with the exception of the study by Hakim et al. (2016), all other studies cited were performed on serum or plasma specimens. By contrast, the specimen matrix was DBS in 19 of the 20 surveys included in this analysis. In at least one published study, assays run on DBS specimens have been found to have slightly lower PPV, and thus a higher risk of false positive results, compared with assays run on serum specimens from the same individual—99.5 percent vs. 100 percent (de Castro, et al. 2008). Finally, variation in the two quality indicators across surveys does not appear to be strongly associated with differences in either the level of HIV prevalence or the specific assays used. This finding suggests that the most likely explanation for differences across surveys may be related to operator performance. This finding, as well as the variation in the PPV of EIAs observed in the published literature, make it difficult to make specific estimates about the percentage of specimens in the 20 surveys in this analysis that may be false positive. Further testing of specimens on a more specific assay would be necessary to measure the magnitude of false positive classification that may have occurred in these surveys.

Though no conclusions as to magnitude can be drawn from this analysis, it appears that the amount of testing error could vary notably by survey. Taking into account the lower percentages of final positive specimens with S/CO values of 5.0 or greater on both EIA 1 and EIA 2, relative errors are likely to be greatest in Niger, Sierra Leone, Senegal, and Zambia. Taking into consideration both relative error and the magnitude of the HIV prevalence, absolute errors are likely to be most notable in Malawi, Uganda, and Zambia.

To understand in context the impact that any error introduced through inaccurate test results may have on the validity of the survey HIV prevalence estimates, it is helpful to consider other sources of error for the surveys. These sources include both sampling and non-sampling errors. Sampling error, driven exclusively by sample size and design, determines the precision of the HIV prevalence estimate. The precision of an

estimate is often described by the relative standard error (RSE), which measures the statistical uncertainty in the estimate as a proportion of the value of the estimate. Across this group of surveys, the RSE for the estimated HIV prevalence ranges from 3 to 20 percent, and tends to be higher where the HIV prevalence is lower. For the sake of comparison, HIV prevalence would have to be overestimated by at least a relative 6 percent to result in a revised HIV prevalence estimate falling outside the confidence interval on any of these surveys. For most of these surveys, relative overestimation of HIV prevalence would need to exceed 15 percent to fall outside the confidence interval.

Additionally, in about half of the surveys included in this analysis, the HIV prevalence is below 2 percent. Even a relative 10 percent overestimation of HIV prevalence, for example, would result in an absolute overestimation of just 0.1 to 0.2 percentage points. Thus, in very low prevalence countries testing error is likely to be small in absolute terms and relative to the precision afforded by the sample design. However, the consequences of overestimation are of greater concern in surveys in the high prevalence countries, because the absolute magnitude of any error would be greater and more important relative to sampling error.

Misclassification of specimens due to testing error is one of many types of non-sampling errors that can affect the validity of HIV prevalence estimates in population-based surveys. Selection bias, that is, differential participation in the survey HIV test by survey respondents according to their likelihood of having HIV, may be an important non-sampling error in HIV prevalence estimates in population-based surveys. The Heckman-selection method for removing selection bias has been applied to DHS surveys, and the results show that HIV prevalence may be higher among respondents who do not participate in the survey HIV test than among respondents who do, creating a potential for underestimation of HIV prevalence (McGovern, et al. 2015). In assessing the risk of under- or overestimation of HIV prevalence in population-based surveys, it is important to take into account the direction and magnitude of all potentially meaningful sources of error.

These results suggest the need for more vigilance in the quality of laboratory testing, with even more checks built into every step of the testing process. In addition, use of a testing strategy, including repeat testing of two EIAs when the results are discrepant, improves assay agreement and appears to improve EIA specificity to some degree. Finally, the HIV testing strategies currently recommended by both UNAIDS/WHO (2015) and CDC (2014) include a more specific assay to confirm all EIA positive results, regardless of the level of HIV prevalence. Testing strategies, including a confirmatory assay such as Western blot, Geenius, or INNO-LIA, should reduce false positivity and improve the accuracy of HIV prevalence estimates.

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# Appendix

**Appendix Table 1.1. Descriptive statistics for signal to cutoff values among weak positive specimens, assay 1**

Median, 25th and 75th percentiles, mean, and standard deviation of the S/CO values for specimens that are positive on assay 1 with signal to cutoff values of 1.000-4.999 on the first round of testing, by assay type and generation and by survey [Unweighted]

Survey	Median	25th percentile	75th percentile	Mean	Standard deviation	Number
<b>Enzygnost 4</b>						
Cote d'Ivoire DHS 2011-12	2.8	2.5	3.3	3.1	1.0	10
<b>Genscreen 4</b>						
Gabon DHS 2012	1.8	1.3	3.5	2.4	1.2	68
<b>Murex 3</b>						
Uganda AIS 2011	1.6	1.2	2.5	2.0	1.0	970
<b>Murex 4</b>						
Cameroon DHS 2011	1.2	1.1	1.3	1.3	0.5	623
<b>Vironostika 3</b>						
Malawi DHS 2010	1.4	1.1	2.0	1.8	0.9	269
Mali DHS 2012-13	1.5	1.1	2.0	1.7	0.8	12
Niger DHS 2012	1.6	1.2	2.3	1.9	0.8	46
Senegal DHS 2010-11	2.4	1.4	3.5	2.5	1.3	14
<b>Vironostika 4</b>						
Burundi DHS 2010	1.5	1.1	2.1	1.8	1.0	73
Democratic Republic of the Congo DHS 2013-14	1.3	1.1	1.8	1.6	0.8	201
Dominican Republic DHS 2013	1.6	1.3	2.2	1.9	0.9	59
Gambia DHS 2013	3.6	2.5	3.8	3.2	1.2	12
Guinea DHS 2012	1.2	1.1	1.4	1.4	0.5	214
Haiti DHS 2012	1.1	1.0	1.3	1.4	0.8	139
Namibia DHS 2013	1.3	1.1	1.9	1.6	0.8	146
Rwanda DHS 2010	2.0	1.3	3.2	2.3	1.1	24
Sierra Leone DHS 2013	1.2	1.1	2.0	1.8	1.2	37
Tanzania AIS/MIS 2011-12	1.2	1.1	1.9	1.7	0.9	127
Togo DHS 2013-14	1.5	1.2	2.0	1.7	0.8	40
Zambia DHS 2013-2014	1.6	1.2	2.3	1.8	0.8	1,217

**Appendix Table 1.2. Descriptive statistics for specimens with weak signal to cutoff values, assay 2**

Median, 25th and 75th percentiles, mean, and standard deviation of the S/CO values for specimens that are positive on assay 2 with signal to cutoff values of 1.000-4.999 on the first round of testing, by assay type and generation and by survey [Unweighted]

Survey	Median	25th percentile	75th percentile	Mean	Standard deviation	Number
<b>Enzygnost 3</b>						
Malawi DHS 2010	4.0	2.7	4.5	3.6	1.2	170
Niger DHS 2012	2.3	1.9	3.5	2.7	1.2	26
Senegal DHS 2010-11	3.5	3.2	4.1	3.5	0.9	20
<b>Enzygnost 4</b>						
Burundi DHS 2010	4.3	4.0	4.8	4.2	0.8	13
Democratic Republic of the Congo DHS 2013-14	1.5	1.1	2.9	2.1	1.3	23
Dominican Republic DHS 2013	na	na	na	na	na	0
Gabon DHS 2012	4.1	3.0	4.5	3.6	1.3	20
Gambia DHS 2013	3.2	2.3	3.9	3.1	1.3	8
Guinea DHS 2012	1.4	1.1	1.6	1.6	1.1	12
Haiti DHS 2012	1.3	1.2	1.5	1.6	1.0	13
Mali DHS 2012-13	2.1	1.4	3.4	2.3	1.1	10
Namibia DHS 2013	3.5	2.5	4.0	3.2	1.1	23
Rwanda DHS 2010	4.0	3.2	4.8	3.8	1.1	10
Sierra Leone DHS 2013	2.8	1.3	4.2	2.8	1.4	81
Tanzania AIS/MIS 2011-12	1.3	1.1	2.5	1.9	1.2	111
Togo DHS 2013-14	1.4	1.1	2.4	1.8	0.9	13
Zambia DHS 2013-2014	1.6	1.2	2.5	2.0	1.0	978
<b>Genscreen 4</b>						
Cameroon DHS 2011	2.1	1.1	4.0	2.6	1.5	13
<b>Vironostika 3</b>						
Uganda AIS 2011	1.7	1.4	2.7	2.0	0.9	49
<b>Vironostika 4</b>						
Cote d'Ivoire DHS 2011-12	3.3	2.4	4.0	3.2	0.8	3

na = Not applicable

**Appendix Table 2. Weak positive signal to cutoff values according to first round test results**

Among specimens positive on assay 1 in the first round of testing, percentage with an S/CO value of less than 5.0 according to the first round results of assays 1 and 2; and among specimens positive on assay 2 in the first round of testing, percentage with an S/CO value of less than 5.0 according to first round results of assays 1 and 2, and percentage that are negative on assay 1, by survey [Unweighted]

Survey	Assay 1 positive specimens				Assay 2 positive specimens				
	A1+A2+		A1+A2-		A1+A2+		A1-A2+ (IQC)		Percentage of A2+ that are A1- (IQC)
	Percent- age with S/CO <5.0	Number							
<b>Sub-Saharan Africa</b>									
Burundi DHS 2010	1.3	157	69.6	102	8.3	157	0.0	11	6.5
Cameroon DHS 2011	2.8	647	98.9	612	1.4	647	100.0	4	0.6
Cote d'Ivoire DHS 2011-12	2.4	334	20.0	10	0.9	334	0.0	2	0.6
Democratic Republic of the Congo DHS 2013-14	0.6	176	94.3	212	5.7	176	100.0	13	6.9
Gabon DHS 2012	0.2	472	75.3	89	4.0	472	0.0	1	0.2
Gambia DHS 2013	0.7	134	57.9	19	5.2	134	50.0	2	1.5
Guinea DHS 2012	4.9	163	98.6	209	4.3	163	100.0	5	3.0
Malawi DHS 2010	0.6	1,392	80.1	326	11.8	1,392	50.0	12	0.9
Mali DHS 2012-13	0.0	96	66.7	18	5.2	96	100.0	5	5.0
Namibia DHS 2013	1.1	1,217	78.2	170	1.8	1,217	8.3	12	1.0
Niger DHS 2012	0.0	31	66.7	69	77.4	31	100.0	2	6.1
Rwanda DHS 2010	1.4	419	78.3	23	2.4	419	na	0	0.0
Senegal DHS 2010-11	2.2	93	46.2	26	21.5	93	na	0	0.0
Sierra Leone DHS 2013	4.2	190	36.7	79	16.3	190	54.9	91	32.4
Tanzania AIS/MIS 2011-12	1.5	808	79.9	144	6.1	808	98.4	63	7.2
Togo DHS 2013-14	0.5	198	92.9	42	2.0	198	81.8	11	5.3
Uganda AIS 2011 <sup>1</sup>	3.2	43	73.1	927	3.6	1,360	na	na	0.0
Zambia DHS 2013-2014	10.7	3,711	79.7	1,030	19.4	3,711	84.4	307	7.6
<b>Latin America/Caribbean</b>									
Dominican Republic DHS 2013	0.0	190	72.8	81	0.0	190	0.0	2	1.0
Haiti DHS 2012	1.9	417	92.3	142	1.7	417	75.0	8	1.9

Note: The 2011 Uganda AIS did not include retesting of assay 1 negative samples for internal quality control.

A1 = assay 1

A2 = assay 2

<sup>1</sup> The 2011 Uganda AIS includes one sample with a missing S/CO value for assay 2.

### Appendix Table 3. Internal quality control

Number of specimens tested for HIV, number found HIV positive after completion of the HIV testing algorithm, number of specimens tested for internal quality control (IQC), number of IQC specimens found positive on assay 2, and the number of IQC specimens that remain positive after completion of the HIV testing algorithm, by survey [Unweighted]

Survey	All specimens tested		Specimens included in IQC testing		
	Number of specimens tested for HIV	Number of final positive specimens	Number of specimens tested for IQC	Number of IQC specimens found positive on assay 2	Number of IQC specimens that remain positive after all testing
<b>Sub-Saharan Africa</b>					
Burundi DHS 2010	8,588	164	830	11	1
Cameroon DHS 2011	14,202	649	663	4	0
Cote d'Ivoire DHS 2011-12	9,008	336	865	2	2
Democratic Republic of the Congo DHS 2013-14	17,638	177	863	13	0
Gabon DHS 2012	10,992	483	523	1	0
Gambia DHS 2013	7,769	136	749	2	1
Guinea DHS 2012	8,380	164	413	5	0
Malawi DHS 2010	13,910	1,420	1,238	12	0
Mali DHS 2012-13	8,861	97	875	5	0
Namibia DHS 2013	8,858	1,233	421	12	5
Niger DHS 2012	8,628	44	863	2	1
Rwanda DHS 2010	13,248	420	1,283	0	na
Senegal DHS 2010-11	9,917	93	976	0	na
Sierra Leone DHS 2013	14,600	222	1,440	91	0
Tanzania AIS/MIS 2011-12	17,745	815	1,678	63	0
Togo DHS 2013-14	9,172	199	893	11	0
Zambia DHS 2013-2014	29,006	3,901	2,423	307	39
<b>Latin America/Caribbean</b>					
Dominican Republic DHS 2013	18,614	193	1,847	2	2
Haiti DHS 2012	18,531	419	901	8	0

Note: The 2011 Uganda AIS did not include retesting of negative specimens for IQC.