Multi-site evaluation of HIV testing algorithms

Multi-site evaluation of HIV testing algorithms

Study Protocol

Final version

MSF International
Epicentre
ITM

August 10, 2011

Updated June 7, 2012

Document Log

Version:		August 10, 2011- ERB (MSF and ITM) approved Updated June 7, 2012	
Study sites	OCA: OCB: OCP: OCG:	Uganda, Democratic Republic of Congo Guinea Kenya, Uganda Cameroon	
Methodology:		Medecins Sans Frontieres and Epicentre	
Collaboration:		Ministry of Health Uganda Ministry of Health Democratic Republic of Congo Ministry of Health Guinea Ministry of Health Kenya Ministry of Health Cameroon	
		Institute of Tropical Medicine, Antwerp, Belgium Epicentre, Paris, France Medecins Sans Frontieres	
Principle investigator:	Cara Kosack, MD, MSc	cara.kosack@amsterdam.msf.org	Medecins Sans Frontieres
Investigators:	Anne-Laure Page, PhD	Anne-Laure.PAGE@epicentre.msf.org	Epicentre
	Leslie Shanks, MD, MPh	Leslie.Shanks@amsterdam.msf.org	Medecins Sans Frontieres
	Pascale Chaillet, Mrs on behalf of MSF Laboratory Working Group	Pascale.Chaillet@brussles.msf.org	Medecins Sans Frontieres
	Greet Beelaert, Mrs	GBeelaert@itg.be	ITM
	Katrien Fransen,	KFransen@itg.be	ITM, Head of Unit HIV/STI Reference Laboratory
	Dr. Tumwesigye T. Benson	btumwesigye12@gmail.com	National Coordinator HIV Counseling and Testing, MoH Uganda
	To be identified		Ministry of Health DRC
	Dr Aboubacar Savane	asavane53@yahoo.fr	Responsable du laboratoire national, Guinea
	Dr Anne Nganga	annie@nascop.or.ke	National AIDS and Sexually Transmitted Infections Control Programme, KENYA
	To be identified		Minitry of Health Cameroon

Development study protocol:	Cara Kosack, MD, MSc Anne-Laure Page, PhD Leslie Shanks, MD, MPh Pascale Chaillet, Mrs Greet Baelert, Mrs Katrien Fransen		Medecins Sans Frontieres Epicentre Medecins Sans Frontieres Medecins Sans Frontieres ITM ITM

Table of Contents

List of Abbreviations

AIDS	Aquired Immune Deficiency Syndrome
ARL	Aids Reference Laboratory
CI	Confidence Intervals
CT	Counselling and testing
CSW	Commercial Sex Workers
DBS	Dried Blood Spot
DPS	Dried Plasma Spot
DR	Democratic Republic of Congo
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immuno Sorbent Assay
ERB	Ethical review Board
HAT	Human African Trypanomiasis
HIV	Human Immunodefiency Virus
ICT	Immunochromatography
ITM	Institute Tropical Medicine
KA	Kala Azar
LIA	Line Immunoassay
MoH	Ministry of Health
MSF	Médecins sans Frontières
NPV	Negative Predictive Value
NGO	Non-governmental Organization
OCA	Operational Centre Amsterdam
OCB	Operational Centre Brussels
OCP	Operational Centre Paris
OIC	Orgenics ImmunoComb® II HIV 1&2 CombFirm
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
PV	Predictive Value
SN	Sensitivity
SP	Specificity
VCT	Voluntary Counselling and Testing
WB	Western Blot
WHO	World Health Organization

1 Introduction and background

1.1 HIV rapid diagnostic tests (HIV RDTs)

RDTs for the detection of HIV antibodies are mostly used in counselling and testing (CT) services, prevention of mother-to-child-transmission (PMTCT) initiatives and nowadays also in mobile units [1]. It is essential to use the most appropriate test in such circumstances for described initiatives to be effective.

RDTs are simple in that they need little or no equipment and fast in that results are mostly available within 15-20 minutes. Most RDTs have very few manipulation steps, can be read visually and be carried out at ambient temperature. Often kits can be stored between 2 °C and 30 °C. These characteristics make RDTs the ideal assay in resource-limited settings where the infrastructure and human resources do not support the use of more complex techniques such as ELISA or confirmation test (i.e. Western Blot and Line Immunoassay).

The development of RDTs that can detect HIV antibodies in whole blood in addition to serum and plasma has allowed the use of these assays in situations where the necessities such as electricity, equipment (e.g. centrifuge) and skilled personnel (e.g. nurses, laboratory technicians, doctors) are lacking. The possibility of error in specimen labelling is also reduced as the original specimen is used and there is no necessity to remove an aliquot of the serum into a new vial [2]. A disadvantage of whole blood specimens is that they deteriorate on storage and therefore cannot be used for further testing at a later date, in which case a serum or plasma specimen would be required [2].

1.2 False positive results in HIV testing strategies

Although HIV RDTs are regularly validated by the WHO with high sensitive and specific results (e.g. Determine®, Uni-Gold® on whole blood specimens with final sensitivity of 100.0% for both tests and a specificity of 99.4 % for Determine® and 100.0 % Uni-Gold®) [2], an unacceptably high frequency of false positive test results has been reported within some MSF missions [3] and by other actors, with both serial and parallel testing strategies [4,8,9,11].

Given the severity and implications of an HIV+ diagnosis, a false positive result is likely to be psychologically traumatic and may result in inappropriate and potentially harmful treatment. Additionally, reporting false positive results, even if due to a test's technical limitations, can damage patient confidence in the CT centre.

Cross reactivity has been reported [5,6,11] and has been postulated to be linked to geography[3] amongst other factors.

In Ethiopia patients with Kala Azar who tested HIV positive by RDT at MSF's CT services were confirmatory tested by PCR and found to be HIV negative. However, the data was not collected systematically and therefore needs further investigation to know if this is a valid correlation or not.

At MSF's Bukavu program in the Democratic Republic of Congo (DRC) a high frequency of false positive results, mainly weak positives were found. A potential factor causing cross-reactions could not be identified in this context [3].

1.3 False negative results in HIV testing strategies

Various studies have demonstrated that HIV immunoassays, when used properly and combined appropriately, are able to perform with a sensitivity close to 100 %. In circumstances such as very

early infection, false negative results can occur due to the absence of sufficient analyte even though the individual is infected [10].

The new generation rapid test uses “sandwich” technique, employing conjugated antigen instead of an anti-IgG conjugate. This antigen recognizes all Ig classes including IgM antibodies which reduce the window period to approximately 20-25 days.

1.4 Confirmatory testing for HIV diagnosis

The gold standard confirmatory strategy using Western Blot (WB) or Line Immuno Assays (LIA) is not recommended in resource-limited settings. An affordable (USD 5-7/test) and relatively simple method for confirmatory testing in resource-limited settings is an EIA based test, the Orgenics *ImmunoComb® II HIV 1&2 CombFirm* (OIC). This test allows the differentiation of the individual HIV antibodies, increasing the specificity of the test and has been used with positive experience in MSF-OCA.

1.5 QC of HIV RDT

Currently no formal quality control for HIV RDTs is carried out with positive control reagents for RDTs as they are not provided by manufacturers. DPS are easy to collect and once dried, can be stored at room temperature. DPS can be safely transported from the place of collection to the laboratory at ambient temperature via standard postal systems and may therefore be a good alternative tool for quality assurance especially where no laboratory is in place to repeat testing as quality control measure.

2 Study rationale

False positive HIV testing and discordant results have been reported at varying levels according to the context. In the absence of standardized testing and confirmation methods, it is difficult to determine whether these variations are due to cross reactivity or purely methodological reasons. In order to better understand the extent of false positive HIV testing and to find solutions to decrease it, it is important to establish a standardized multi-centric study in which comparable results can be obtained from all sites. In addition, this study will allow identifying the best HIV testing algorithm in different African locations.

Due to the intrinsic limits of rapid tests, and in particular the limited number of antigens used by all rapid tests, simple and rapid confirmatory tests identifying antibodies against several antigens are needed. We will evaluate the accuracy of Orgenics *ImmunoComb® II HIV 1&2 CombFirm* comparable to the classical confirmation tests.

In addition to the improvement of performance of rapid test algorithms, controlling the quality of testing procedures and interpretation is mandatory to improve the quality of HIV testing. A first step for this is to develop standardized quality control and quality assessment procedures and tools. For potential use as quality control procedure of CT centres HIV testing of DPS will be evaluated.

Ultimately, we aim for designing feasible algorithms that minimize the risk of false HIV results in resource-limited settings.

3 Objectives

3.1 Primary objective

- To evaluate the overall and site-specific performance of the diagnostic algorithm performed at 6 MSF African program sites (i.e. using RDT results from the program sites) comparing using the diagnostic algorithm with ELISA, LIA, EIA-Ag and DNA-PCR as gold standard.

3.2 Secondary objectives

- To evaluate the accuracy (sensitivity, specificity and predictive values) of Orgenics *ImmunoComb® II HIV 1&2 Combfirm* as an HIV confirmatory test.
- To model different HIV RDT testing algorithms in order to define acceptable testing algorithm in each study setting (i.e. using RDT results from reference laboratory).
- To determine the inter-user reliability of RDT testing (i.e. program sites vs. reference laboratory)
- To evaluate accuracy of each HIV RDT measured by the sensitivity (SN), specificity (SP) and predictive values based on the prevalence of each testing centre.
- To evaluate the accuracy of HIV testing using DPS samples for quality control purpose in HIV testing.
- To assess whether additional confirmatory testing (i.e. Orgenics *ImmunoComb® II HIV 1&2 Combfirm*) improves the accuracy of the diagnostic algorithm used at the different study sites.
- To perform a descriptive analysis on the differentiation between HIV 1 and 2 of the discriminative RDTs.

4 Methods

All clients participating in the study will be receiving HIV CT services according to the algorithm currently used in the respective program. The study protocol will not change the testing protocol used by the site for routine CT.

However, all participants will have access to the reference results obtained at ITM if they want. If the reference results are different than the results delivered to the patient, then the testing centre and follow-up clinic if applicable will be notified to inform the client.

4.1 Study Design

This is a multi-centric prospective evaluation of the performance of 6 currently used diagnostic algorithms for HIV.

4.2 Sample size

As per WHO recommendations, each study site will submit at least 200 algorithm positive and 200 algorithm negative samples for evaluation [7]. This sample size is calculated based on the assumption that both sensitivity and specificity are 98% and in order to provide a 95 % confidence interval of less than +/- 2 % for both sensitivity and specificity.

From each study site the prevalence of positive results from each CT centre is known.

- If the prevalence is close to 50 % (i.e. between 40 % and 60 %), then all consecutive samples will be collected and the total sample size will be calculated based on the prevalence in order to obtain at least 200 positive and 200 negative samples (i.e. highest of 200/p or 200/(1-p) – maximum sample size 500). In addition, the sample size will be increased by 10 % to account for losses, problems in shipment, etc.
- If the prevalence is below 40 %, then a subsample of positive and negative samples (according to the algorithm in place) will be obtained. As the HIV testing algorithms are expected to be very accurate, we expect very few misclassifications. Assuming a conservative 10 % of misclassification, we will collect a sub-sample of 220 positive and 220 negative samples plus all indeterminate and/or discordant samples according to the algorithm in order to ensure having at least 200 true positive and 200 true negative samples.

For the comparison of results from DPS and from plasma, the kappa coefficient will be used to determine the measure of agreement. Based on the following hypotheses: expected kappa coefficient of 0.8, precision of 5 %, alpha risk 0.05, proportion of positive test of 0.5 by both methods, the required sample size for the kappa test for evaluation of inter-method correlation is 554 for a 5 % precision. From each site 100 samples will be analyzed (i.e. 50 % positive and 50 % negatives) in order to reach the required sample size.

4.3 Study sites and duration

The study will be carried out in 6 HIV CT programs that use RDTs for diagnosis.

The sites will be selected according to the following criteria:

- Authorization to ship specimen abroad
- Availability international transporter able to ship UN3373 samples in cold chain

- Presence of an MSF laboratory and space and staff available for sample preparation and storage.
- VCT performed by MSF or, if performed by national program, willingness to participate in the study
- Prevalence (1 or 2 sites with prevalence <10%)

Country:	Kenya	Uganda	Guinea	Uganda	Cameroon	DRC
Project:	Homa- Bay	Arua	Guéckédou	Kitgum	Douala ATAR	Baraka
MSF section	OCP	OCP	OCB	OCA	OCG	OCA
Prevalence in country (15-49 years)*	7.1-8.5%	5.0-6.1%	1.3-2.2%	5.0-6.1%	5.1%	1.2-1.5%
CT by MSF or MoH:	MSF	MoH and MSF	MSF	MoH and MSF	MoH	MoH and MSF
Sample:	Fingerprick	Venous sample (plasma)	Fingerprick	Fingerprick by counsellor and venous by lab	Venous sample	Fingerprick by counsellor and venous by lab
No of clients tested per month:	170	1000	200	800	630	140 VCT and 180 as blood donors
Positivity rate of CT in 2009:	49%	11%	37%	7.8%	20%	11%
Discordance rate:	1.4%	0.1%	2.4%	0.5%	1%	unknown
Sex distribution:	52% female 48% male	67% female 37% male	60% female 40% male	65% female 35% male	60% male 40% female	70% female 30% male
Serial or parallel testing:	Serial with tie-breaker	Serial with tie-breaker	Serial	Parallel	Serial	Parallel
Tests in use:	1. Determine 2. Uni-Gold. 3. SD Bioline ***	1.Determine 2.Stat-pack 3. Uni-Gold	1. Determine 2. Immunoflow 3. Genie II	Determine and Uni-Gold	1. Determine 2. Immuno-comb (Orgenics)	Determine and Uni-Gold
Sample size	450 consecutive clients	220 positive** and 220 negative** by local algorithm, (+ all discordant results)	220 positive** and 220 negative** by local algorithm, (+ all discordant results)	220 positive and 220 negative** by local algorithm, (+ all discordant results)	220 positive and 220 negative** by local algorithm, (+ all discordant results)	220 positive and 220 negative** by local algorithm, (+ all discordant results)

*According to Epidemiological Fact Sheets on HIV and AIDS by WHO, UNICEF and UNAIDS 2008. http://apps.who.int/globalatlas/predefinedReports/

** If a client tested initially discordant but is given a final result (either positive or negative), then add sample into positive/negative group until reaching the sample size of 220. In addition, send all discordant results.

*** SD Bioline has been replaced with Stat-Pak in 2012.

Table 1: Characteristics of study sites and routine HIV testing algorithm

4.3.1 Study population

Clients who attend any of the identified CT program will get tested with HIV RDTs according to the local testing algorithm.

4.3.2 Inclusion criteria

Clients who participate in routine CT programmes are offered to participate in the study if all of the following criteria are met:

1. Age ≥ 5 years as it is difficult to obtain venous sample in younger children
2. Written informed consent by the client or legal guardian

4.3.3 Exclusion criteria

The following reasons are considered exclusions:

- Withdrawal of consent
- Inability to obtain a venous blood sample or insufficient blood
- Client taking anti-retroviral treatment (currently or in the past)

4.3.4 Sampling

In CT centres where prevalence is expected below 40%, then a sub-sample of negative samples will be collected.

There are two possible solutions for collecting these samples as follows:

- Collect all samples collected at the beginning until 220
- Select 1 or 2 days a week for collection of all samples during the whole duration of the study

The second possibility will be preferred but the first one can be used if the second is too complex to organize.

4.4 Study procedures

4.4.1 Enrolment and blood sample collection

Clients will be counselled and participate in follow-up procedures according to the CT centre procedures. In particular, all clients will be tested according to the algorithm in place in the CT centre (tests and type of sample).

All clients meeting the inclusion criteria will be referred to the study nurse/lab technician, who will explain the purpose of the study and will ask for written consent (Annex 6.2). 10 ml of venous blood will be collected into two 5 ml EDTA tube by the study nurse/lab technician.

Information recorded will include age, sex and result of HIV RDTs performed on site.

4.4.2 Field laboratory procedures

Preparation DBS

Venous samples will be collected in two 5 ml EDTA tube. Each well of the filter paper (Whatman No 903) will be filled by applying 50 µl of whole blood from the EDTA tube on each well (5 x 50 µl) (Annex 6.4). After being dried, they will be kept at room temperature in a sealed plastic envelope with Silica gel in a tight dark box until shipment.

Preparation DPS

Immediately after, the tube will be centrifuged at 3.000-4.500 rpm for 15 minutes (approximately 1.100 g). As with the preparation of the DBS, 5x50 µl of plasma will then be transferred on to the Whatman No 903 filter paper, before being dried and stored at room temperature.

Preparation Aliquots

The prepared plasma will be transferred into two storage tubes. Both samples will be frozen and kept at -20 °C until shipment. Every other month samples collected, (i.e. one plasma aliquot sample of each client and DBS and DPS) will be shipped to ITM Belgium.

Each DBS, DPS and plasma tube will be labelled with a unique identification number, age and sex of the client.

One serum sample and both DBS and DPS of each client will be shipped to ITM for further analysis. The samples do NOT have to be transported at -20 ºC but in cold chain (+4 ºC). One serum sample will be kept as back-up at each testing site and stored at -20 ºC.

A nurse or laboratory technician should be hired for the duration of the sample collection at each study site. This person will be responsible for carrying out sample collection, storage and shipment.

4.4.3 Shipment of samples to ITM, Antwerp, Belgium

For analysis, all samples will be sent every other month to ITM. The samples will be packed according to IATA regulation following the recommendations for UN3373 samples and be transported in cold chain with ice packs to the following address:

Instituut voor Tropische Geneeskunde
HIV/STI reference laboratory
Attn.: G. Beelaert/L. Casier
Nationalestraat 155
2000 Antwerpen
Belgium

4.4.4 Procedures and analysis at ITM, Antwerp, Belgium

4.4.4.1 HIV RDTs

All samples received will be tested by ITM testing algorithm and an array of 8 RDTs:

- Determine HIV 1/2, Inverness, UK
- Uni-Gold HIV, Trinity Biotech, Ireland
- Genie III, BioRad Laboratories, France
- Vikia HIV 1/2, Biomerieux, France
- SD Bioline HIV 1/2 3.0, Standard Diagnostics Inc., Korea
- ImmunoFlow HIV-1 HIV-2, Core Diagnostics, United Kingdom
- Stat-Pak HIV 1/2, Clearview, USA
- INSTI HIV Antibody test, bioLytical, Canada

Each test will be performed according to the manufacturer's instructions and read by two laboratory technicians who will be blinded from the previous testing result. If the reader disagrees, a third reader will act as tie-breaker. The technicians will record the result of the test.

4.4.4.2 Gold standard algorithm (ELISA, LIA, Ag-EIA and HIV DNA-PCR)

For the gold standard algorithm all samples received will be tested by ELISA (Vironostika® HIV Uni-Form II Ag/Ab, Biomerieux) and all positive samples will be confirmed by a Line-Immunoassay (LIA, i.e. INNO-LIA™ HIV I/II Score).

INNO-LIA™ HIV I/II Score will be used to confirm the presence of antibodies against the human immunodeficiency virus type 1 (HIV-1), including group O, and type 2 (HIV-2). The INNO-LIA™ HIV I/II Score detects antibodies against gp120, gp41, p31, p24, p17, gp105, and gp36. The test will be interpreted according to manufacturer's instructions.

If the INNO-LIA™ HIV I/II Score is negative or indeterminate (5 % indeterminate results by HIV RDT/ELISA and LIA are expected) the samples will be tested with an Antigen-Enzym-Immunoassay (Ag-EIA, i.e. INNOTEST HIV Antigen mAb).

If both the LIA and Ag-EIA are negative the final result will be HIV negative. If the LIA is indeterminate and Ag-EIA is negative, the final result will be indeterminate. If the LIA is negative or indeterminate and the Ag-EIA is positive (confirmed by neutralization), it may be a potential seroconversion. For these two scenarios a HIV DNA-PCR will be performed according to the ITM in-house method.

4.4.4.3 Evaluation of the Orgenics *ImmunoComb® II HIV 1&2 CombFirm*

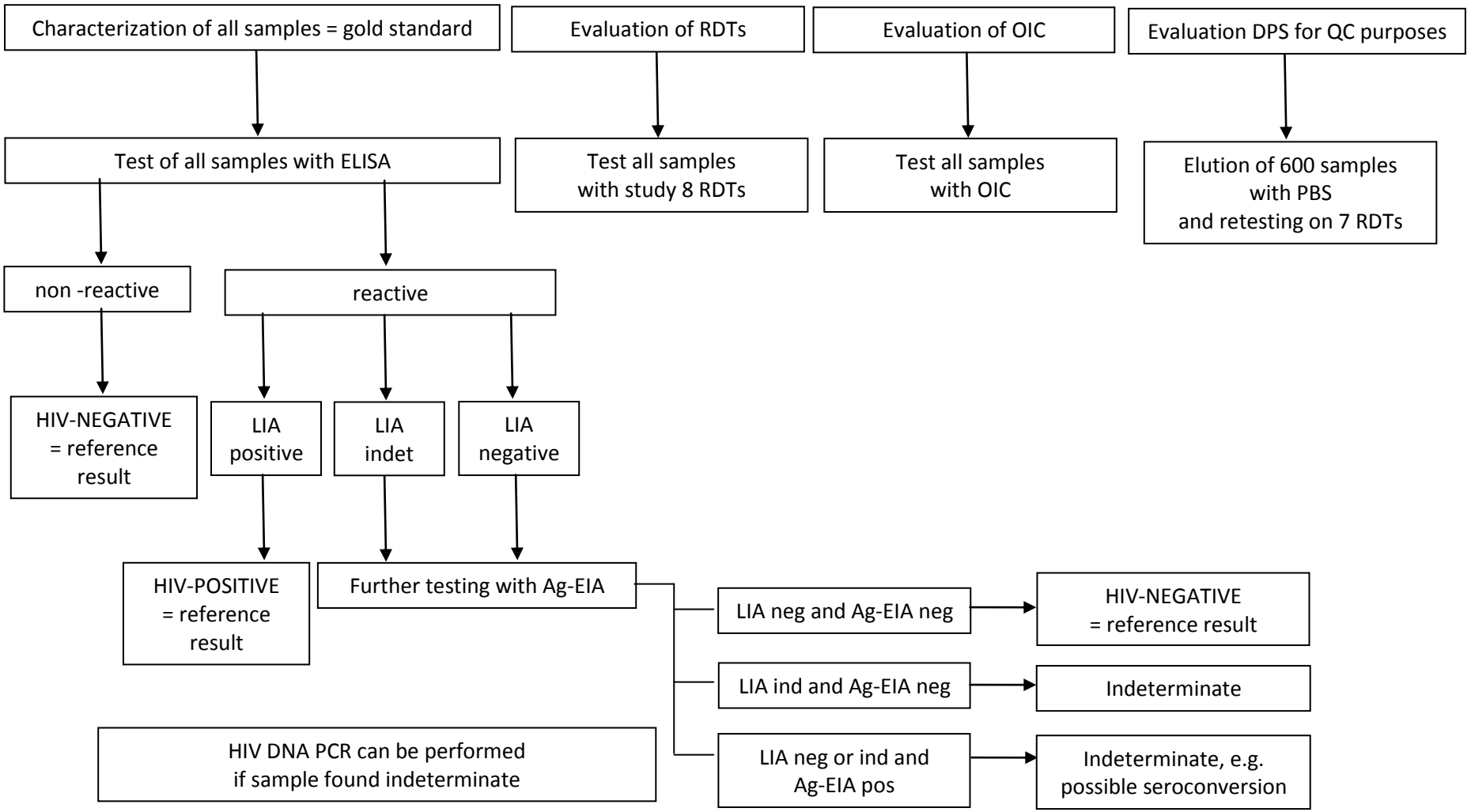
The *ImmunoComb® II HIV 1&2 CombFirm* will be carried out on all samples. The *ImmunoComb® II HIV 1&2 CombFirm* detects antibodies against gp120, gp41, gp36, p24 and p31. The results of each band will be recorded and the test will be interpreted according to the manufacturer's instructions. A descriptive analysis will in addition be carried out on an alternative interpretation of the *ImmunoComb® II HIV 1&2 CombFirm* as suggested by OCA will be performed in addition (see Annex 6.10).

4.4.4.4 DPS for HIV RDT quality control testing

DPS will be prepared at each study site following instruction described in Annex 6.4. At ITM's laboratory three 6 mm of 600 patient samples discs will be punched out from the card and eluted with 375 µl phosphate buffered saline (PBS) into a 2 ml tube. The tube will then be placed on

a shaker for one hour at room temperature. The elute will then be used to retest all study RDTs and the ITM's gold standard testing algorithm.

HIV testing – flow diagram at ITM



4.5 Confirmatory results

Clients at each CT will be informed and counselled according to local guidelines used at the CT. Each client who tested HIV positive will be informed that additional confirmatory testing will be carried out on their blood sample. Each testing centre will receive a list of the results of the ARL. Study participants will be offered a follow up appointment to receive confirmatory results. In addition, discordant results will be made available to clinicians associated with the CT.

4.6 Outcomes, data entry and analysis

Data will be double-entered using EpiData 3.1 software (EpiData, Odense, Denmark) at each study site. Analysis will be carried out using STATA version 10 (StataCorp, College Station, Texas, USA).

Only the samples collected for the main sample size (i.e. all samples for Mathare and Chamanculo, and 220 positive + 220 negative samples in other sites) will be considered for the main analysis.

The gold standard algorithm with ELISA, LIA, EIA-Ag and DNA-PCR will be used to calculate:

- Sensitivity, specificity and predictive values of field-specific algorithms
- Sensitivity, specificity, and predictive values of each RDT performed at ITM, for each site and globally
- Sensitivity, specificity, and predictive values of *ImmunoComb® II HIV 1&2 CombFirm* (i.e. Mathare samples)
- Sensitivity, specificity of individual RDT performed on site
- Sensitivity, specificity of modelled algorithms using RDT results from ITM

In a separate analysis, the reference standard result of all discordant samples (including the ones including in and out of the main sample size) will be described.

To measure the inter-user reliability, results of RDT in the field and at ITM will be displayed on a 2x2 contingency table for each RDT used in the field, and each study site separately. The McNemar test will be applied. A value of $p=0.05$ will be considered as a threshold of statistical significance, hence the methods will be considered non concordant if $p>0.05$.

If the users are found to be concordant, the level of concordance between the users will be evaluated using the kappa coefficient. A Kappa coefficient greater than 0.8 will be considered as a measure of very good agreement

Similarly to measure the agreement between results of RDTs using DPS with the results from plasma, the results will be displayed on a 2x2 contingency table. The McNemar test will be applied to assess whether there are statistical differences between the 2 methods and the kappa coefficient will be used to evaluate the level of concordance.

4.7 Formal and ethical approval

The study has been approved by the MSF Ethical Review Board. It will be implemented after approval by ITM ethical review as well as local ethics committees.

The study will be carried out in accordance with the Declaration of Helsinki concerning medical research in humans. All enrolled clients will sign or fingerprint the informed consent. Minors below

the legal age of consent in the study country will only be included if their legal guardian consents to the study. Participation will be voluntary.

In case discordant test results (i.e. between the test result obtained at the center and the reference test result obtained at ITM) are found every participant will be informed during the follow-up appointment. All discordant results will be made available to the clinicians associated with the center and they will inform the participant about the discrepancy. All possible effort will be made to trace participants with discordant test results.

Risks A risk compared to normal procedures is associated with the additional venous blood puncture where most current test protocols require only a finger prick. Venous blood collection is a very common procedure, which may cause minor pain.

Benefits Personal benefits: Study participants will have the opportunity to know their results of the reference standard testing which otherwise would not be available to them. Each testing centre will receive the repeated testing results from the ARL and in addition of the confirmatory tests.

Communal benefits: MSF operations will use the results to improve the accuracy of the testing procedures by changing testing protocols, implementing quality control measures and/or changing algorithms and/or introducing confirmatory testing. Further the information will be used to discuss the Ministry of Health and other testing facilities to improve accuracy based on the study outcome. Participation in this study will inform each program about the accuracy of their HIV testing results.

4.8 Local collaboration

The local partner will be the Ministry of Health counterpart. In most of the study sites, MSF staff work alongside Ministry of Health staff and the protocol will be implemented in conjunction with them. The Ministry of Health at local and national level will be consulted prior to implementation of the study, and the research findings will be discussed with relevant Ministry of Health bodies on completion of the study.

4.9 Community involvement

The community of people living with HIV/AIDS (PLWHA) will be informed of the study and its rationale. This will be done through open meetings with the community and PLWHAs specifically. Where appropriate, written materials in the local language will be made available. The results will be shared with the community upon completion, again through meetings with the community, PLWHAs and local health professionals. The study is expected to directly benefit the study community by contributing to the knowledge base on determining appropriate algorithms for HIV testing and confirmation. As the accuracy of the HIV testing algorithm is subject to regional variation, it is expected that this information will be of great benefit to the community. As stated in section 4.7, the information will be used to change MSF practice to improve the quality of testing delivered as well as lobby MoH and other local testing centres.

4.10 Financing the study

The study has been granted funding by MSF's Innovation Fund which will pay for all material and personnel required in the field and for additional tests and costs at ITM.

5 References

1) Mbopi-Keou FX, Ongolo-Zogo P, Angwafo F, Ndumbe PM, Belec L (2007) High impact of mobile units for mass HIV testing in Africa, *AIDS*, Vol 21:14

2) WHO/UNAIDS: HIV simple/rapid assays: operational characteristics (Phase I), report 12, whole blood specimens, January 2002

3) Klarkowski DB, Wazome JM, Lokuge KM, Shanks L, Mills CF, O'Brien DP (2009) The evaluation of a rapid in situ HIV confirmation test in a programme with a high failure rate of the WHO HIV Two-test diagnostic algorithm, *PLoS ONE*, Vol 4:2

4) RH Gray, F Makumbi, D Serwadda, T Lutalo, F Nalugoda, P Opendi, G Kigozi, SJ Reynolds, NK Sewankambo, MJ Wawer (2007) Limitations of rapid HIV-1 tests during screening for trials in Uganda: diagnostic test accuracy study *BMJ* 28:335(7612): 188

5) Watt et al. (2000) Human Immunodeficiency Virus Type 1 Test Results in Patients with Malaria and Dengue Infections, *Clinical Infectious Diseases,* 30:819

6) Meles et al. (2002) Indeterminate Human Immunodeficiency Virus Western Blot Profiles in Ethiopians with Discordant Screening-Assay Results, *Clinical and Diagnostic Laboratory Immunology*

7) Guidelines for Appropriate evaluations of HIV testing technologies in Africa. Department of Health and Human Services, Center for Disease Control and Prevention and the African Regional Office of the World Health Organization

8) Singer DE, Kiwanuka N, Serwadda D, et al. Use of stored serum from Uganda for development and evaluation of a human immunodeficiency virus type 1 testing algorithm involving multiple rapid immunoassays. *J Clin Microbiol* 2005; 43(10):5312-5

9) Urassa W, Nozohoor S, Jaffer S, Karama K, Mhalu F, Biberfeld G. Evaluation of an alternative confirmatory strategy for the diagnosis of HIV infection in Dar Es Salaam, Tanzania, based on simple rapid assays. *J Virol Methods* 2002; 100(1-2)**:**115-20

10) Chapel RJ, KM Wilson, EM Dax. Immunoassays for the diagnosis of HIV: meeting future needs by enhancing the quality of testing. Future Microbiology 2009; 4(8):963-982

11) V Lejon, D Mumba Ngoyi, M Ilunga, G Beelaert, I Maes, P Büscher, K Fransen. Low specificities of HIV diagnostic tests caused by *Trypanosoma brucei gambiense* sleeping sickness. *JCM* 00456-10

6 Annexes

6.1 Characteristics of HIV RDTs used in the study

	Determine HIV-1/2	**Uni-Gold HIV**	**Genie III HIV-1/HIV-2 Assay**	**MultiSpot HIV-/HIV-2 Rapid Test**	**Vikia HIV 1/2**	**SD Bioline HIV 1/2 3.0**	**ImmunoFlow HIV-1/HIV-2**	**HIV 1/2 Stat-Pak**	**INSTI HIV Antibody test**
Manufacturer:	Inverness, UK or Abbott Laboratories, Dainabot Co. Ltd., Tokyo, Japan	Trintity Biotech PLC, Bray, Ireland	Bio Rad Laboratories, France	Bio Rad Laboratories, France	bioMérieux, France	Standard Diagnostics Inc, Korea	Core Diagnostics, UK	Clearview, USA (owned by Inverness)	bioLytical, Canada
Catalogue number (number of tests/box):	7D2347 (100 tests)	1206502 (20 tests)	72328 (50 tests)	25228 (50 tests)	31 112 (25 tests)	03FK10 (30 tests)	HIV-110022 (25 tests)	92110 (20 test)	90-1021 (48 tests)
Testing principle:	ICT	ICT	ICT	EIA (immuno-concentration)	ICT	ICT	ICT	ICT	ICT
Format:	strip	cassette	cassette	cassette	cassette	cassette	cassette	cassette	cassette
Antigen type:	HIV1: gp41, gp120 HIV2: gp36	HIV1: gp41, gp120 HIV2: gp36	HIV 1: gp120, gp41, p24 HIV 2: gp36		HIV1: gp41 HIV2: gp36	HIV1: gp41, p24 HIV2: gp36	HIV1: gp41, gp120, 'O' fusion polypeptide (O subtype) HIV2: gp36	HIV1: gp41, gp120 HIV2: gp36	HIV1: gp41 HIV2: gp36
Sample type:	WB, S, P	WB, S, P	WB, S, P	S, P	WB, S, P	WB, S, P	WB, S	WB, S, P	WB, S, P
Shelf life (at °C):	18 months (2-30)	20 months (2-30)	18 months (2-30)	3 months when stores at 20-30, longer if 2-8	18 months (4-30)	18 months (1-30)	24 months (4-30)	24 months (8-30)	>12months (to be identified)
Controls				Yes, come with each pack					

Table 2: Characteristics of HIV RDTs used in the study

6.2 Informed Consent (English)

INFORMED CONSENT FOR PATIENTS: MULTI-SITE EVALUATION OF HIV TESTING ALGORITHMS

To be translated by local staff and back-translated by someone external from the study and read to the patient in the local language. Patients will then be asked for consent by signature or fingerprint. A record of their consent will be kept.

HIV testing relies on the use of rapid tests. This study aims to check the performance of the current HIV testing procedure followed. MSF and the local Ministry of Health will compare the obtained results from the VCT sites with the results obtained in a central reference Laboratory in Antwerp (Belgium).

Risks

Therefore we would like your permission to take some extra blood (10 ml). The drawing of the blood for the test is slightly painful for a short moment but has no serious side effects.

Privacy and confidentiality

The blood used for this study will not be labeled with your name so that your privacy will be guaranteed. The blood will be exclusively used for this study.
Also, study records will contain only ID codes but will NOT show individual names. This will be ensured for the counselling and testing process as well as for all analysis linked to the study.

Voluntary participation

Your participation to this study is voluntary. If you do not wish to participate, you do not have to, and you do not have to give a reason. You can also withdraw your consent at any time during the counselling and testing procedure. Your decision to participate or not has no influence to your further treatment. Your HIV status will be defined with the current algorithm used in this center. There will not be any financial expenses for you to participate in the study. You will also not be paid to participate.

Benefits

If the test result that you receive today is different from the test result found at the reference laboratory you can agree to be traced to receive this information by phone, at your home or at another address.
You can make a new appointment to receive the test results obtained at the reference laboratory or it can be revealed to you at your next follow-up appointment.

If you agree to participate in the study, we will ask you to fill in and sign the form below in 2 copies. One copy will be kept by us and one copy is for you.

For additional information, you can ask a doctor of this center.

The investigator team

CONSENT PAGE

MULTI-SITE EVALUATION OF HIV TESTING ALGORITHMS

Principal Investigator: Cara Kosack, MSF International, Amsterdam, Netherlands

Email: cara.kosack@amsterdam.msf.org

Local contact: xxx

Phone number: xxx

I have read or listened and understand the above information. I have been advised of the goals and procedures of the study, and the benefits and risks of participating in the study.

I hereby give my consent to participating in the study.

_____ _____

Name of client Date Signature

Witness

I certify that the person named above has been given an opportunity to understand the above given information and ask questions, that he or she understands the issues discussed, that his or her decision to participate in the study is an informed and voluntary one, and that I have witnessed his or her signature.

_____ _____

Name of witness Date Signature

_____ _____

Name of study nurse/doctor Date Signature

CONSENT PAGE

TRACING CONSENT - MULTI-SITE EVALUATION OF HIV TESTING ALGORITHMS

Principal Investigator: Cara Kosack, MSF International, Amsterdam, Netherlands

Email: cara.kosack@amsterdam.msf.org

Local contact: xxx

Phone number: xxx

I have read or listened and understand the above information. I have been advised of the goals and procedures of the study, and the benefits and risks of participating in the study.

I hereby give my consent to be traced

O by phone (phone number): _____

O at my house (provide address): _____

O or another location (please provide details):

if my testing shows a discordant result.

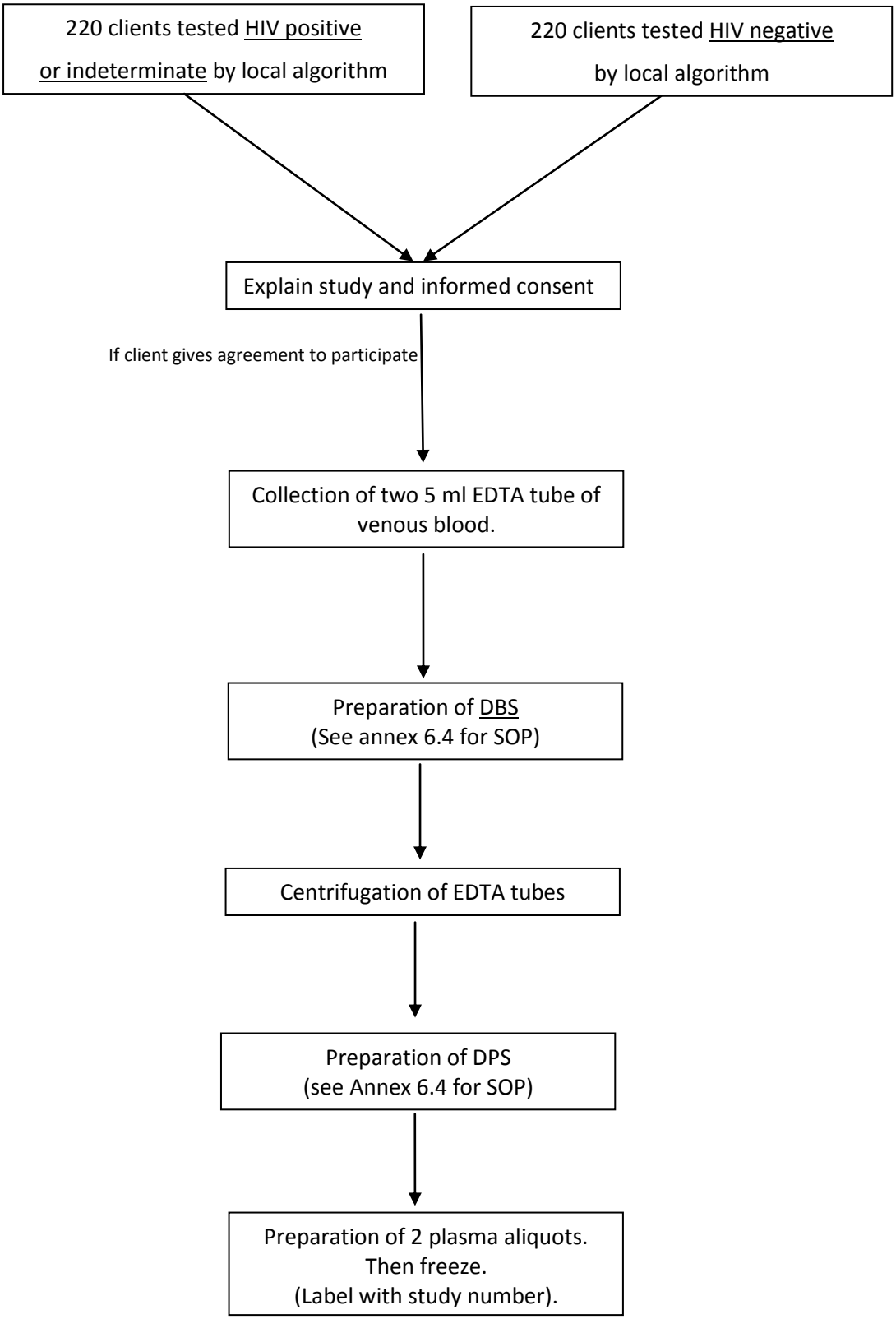
_____ _____

Name of client Date Signature

_____ _____

Name of study nurse/doctor Date Signature

6.3 Workflow at each study site and storage of samples



6.4 SOP Collection, storage and transportation of DBS and DPS

Equipment

- Sample collection card: Protein Saver™ 903® Card Whatman
- Drying rack
- Low gas permeable zip-lock bag
- Desiccant bags
- Humidity card: Tropack Indicator B/1
- Microtube, flat bottom, non-sterile, screw cap 2 ml
- Container for transport – infectious good

Method: collection and storage of DBS and DPS

a) Label card with appropriate identification number and date.
b) Apply 50 µl serum/plasma to each circle (5x50 µl).
c) Let the card dry for 2-3 hours.
d) Place the card in a low gas permeable zip-lock bag with desiccant and humidity card. Press as much as possible the air out of the bag and seal it shut.
e) Keep packed DBS/DPS (in sealed plastic bags) at dry at room temperature until transportation to the reference laboratory.
f) Check the humidity cards regularly weekly: if the card is pink at 30 % exchange the desiccant and store with excessive number of desiccant in the sealable bag.

Method: collection and storage of serum aliquots

g) Label 1 reference tube with appropriate identification number.
h) Centrifuge EDTA tube at 3.000-4.500 rpm for 15 minutes (approximately 1.100g) and transfer at least 1.500 µl plasma in both tubes.
i) Store tubes in a freezer at -20°C. (Maximum storage time in a refrigerator is one week).
j) Place the frozen plasma samples in cold chain with ice for transportation to ITM.

Transportation from project site to transport organization in cold chain

The UN3373 packing instructions will be followed. These instructions will be emailed to each study site.

IMPORTANT: Transport all samples together BUT pack them separately i.e. ensure that DBS and DPS cards are packed in separate envelopes!

Transport of specimens via DHL, World Courier or similar to ARL:

- 1 DBS Card (room temperature)
- 1 DPS Card (room temperature)
- 1 Aliquot of plasma with minimum 1.500 µl in **COLD CHAIN** (1 Aliquot will stay in the field). (The frozen samples should be transported with ice packs around the plastic container. The samples may defrost during transport but should not reach more than 8˚C)

Instituut voor Tropische Geneeskunde

Attn.: G. Beelaert/L. Casier

Nationalestraat 155

2000 Antwerpen

Belgium

6.5 Data collection and entry

Information on study participants

Fill out EpiData file containing following variables:

a) Study identification number
b) Name project and country
c) Age
d) Sex
e) Date of testing
f) Name of HIV test 1
g) Result HIV test 1
h) Name of HIV test 2
i) Result of HIV test 2
j) Name of HIV test 3
k) Result of HIV test 3
l) Result given to patient
m) Co-morbidities
n) Nationality (for Arua site only)

Email data file to:
Cara.Kosack@amsterdam.msf.org & Anne-Laure.Page@epicentre.msf.org

6.6 Order list of items for study sites

Each study site has to order the following items:

	Item	Manufacturer	Cat number manufacturer	Cat number MSF	Amount
1	Sample collection card Protein Saver™ 903® Card Whatman	Whatman	For EU orders: 10531018	Need to create Z-code	1000
2	Drying rack	Not specified	N/A	ELAERACK2D	3
3	Low gas permeable zip-lock bag	Not specified	N/A	ELAEBAGP1S	1000
4	Desiccant bags	Not specified	N/A	SLASDESS1S	2000
5	Humidity card	Tropack	MS20003-2	Need to create Z-code	1200
6	Microtube,flat bottom, non-sterile, screw cap, 2 ml	Not specified	N/A	ELAETUBE2--	1500
7	Box with stand for 81 cryotubes	Not specified	N/A	ELAETUMI1B	20
8a	Class 6.2 packaging for Category A and B, BioPack-2. Fibreboard box with container with dimension Ø 150 x 190 mm., incl. absorption material	Care Pack Holland*	Code 500	Not specified yet	6
8b	Specimen holder 50 x 7,5 mm-hole, E.P.E. spacer	Care Pack Holland*	Code 484	Not specified yet	12
8c	Overpack, fibreboard box, for max. 4x BioPack-2	Care Pack Holland*	Code CAS24	Not specified yet	6
9	Tube vacuum, EDTA	Becton Dickinson	N/A	ELAEBSVT5E	1500
10	Tube holder with needle injector	Becton Dickinson	N/A	ELAEBSVV1H-	500
11	Needle for blood sampling system 21G	Becton Dickinson	N/A	ELAEBSVV21N	500
12	Freezer, 111 litres (Vestfrost MF 114)	Vestfrost	N/A	PCOLFREE1E-	1

6.7 Order list of items for ITM by MSF

ITM will receive the following items by MSF:

	Item	Manufacturer	Cat number	Amount (based on 6 sites and elution of DPS)
1	Determine HIV-1/2	Inverness	7D2347 (100 tests/box) DDGTHIVD1T	6x400=2400 plus elution 600 = total 3000 tests
2	Uni-Gold HIV	Trintity Biotech PLC, Bray, Ireland	1206502 (20 tests/box) DDGTHIVU20T	6x400=2400 plus elution 600 = total 3000 tests
3	Genie III HIV-1/HIV-2 Assay	Bio Rad Laboratories, France	72328	6x400=2400 plus elution 600 = total 3000 tests
4	Vikia HIV 1/2	bioMérieux, France	31 112 (25 tests/box)	6x400=2400 plus elution 600 = total 3000 tests
5	SD Bioline HIV 1/2 3.0	Standard Diagnostics Inc, Korea	03FK10 (30 tests)	6x400=2400 plus elution 600 = total 3000 tests
6	ImmunoFlow HIV-1/HIV-2	Core Diagnostics, UK	HIV-110022 (25 tests/box)	6x400=2400 plus elution 600 = total 3000 tests
7	HIV 1/2 Stat-Pak	Chembio, USA	HIV 102 (20 tests/box)	6x400=2400 plus elution 600 = total 3000 tests
8	INSTI HIV Antibody test	bioLytical, Canada	90-1021 (48 tests/box)	6x400=2400 plus elution 600 = total 3000 tests
9	ImmunoComb® II HIV 1&2 CombFirm	Orgenics	60434002 (18 tests/box)	6x400=2400 (no elution)

6.8 Order list of items for ITM by ITM

	Item	Manufacturer	Cat number	Amount estimated
1	ELISA Vironostika® HIV Ag/Ab	Biomerieux	259851 [Packing 192 tests per kit]	2400
2	INNO-LIA™ HIV I/II Score	Innogenetics	80540 (20T CE)]	1200
3	EIA-Ag: INNOTEST HIV Antigen mAb	Innogenetics	80563	100
4	Reagents for DNA-PCR	ITM (in-house method)	-	50

Note: Test kits and reagents for ELISA, LIA and DNA-PCR will be ordered by ITM and MSF will reimburse costs.

6.9 Workflow at ITM laboratory

All samples will be examined using with ITM's testing algorithm (ELISA, LIA, Ag-EIA and PCR if needed). The result will be HIV positive (HIV-1, HIV-2 or HIV-1&-2), HIV negative or indeterminate. If seroconverters are identified (LIA negative and Ag-EIA positive) they will not be included in the study.

All samples will be analyzed using all 7 RDTs and the OIC. Results will be positive or negative and will be used as such for the calculation of sensitivity, specificity and PVs. In addition, it will be differentiated between HIV-1 and HIV-2. Tests at ITM will be performed blinded to CT results from the field.

600 samples will be eluted in PBS and retested on the 7 RDTs in order to investigate whether DPS elution can be used as quality control method.

6.10 Interpretation of ImmunoComb® II HIV 1&2 CombFirm

6.10.1 Interpretation of ImmunoComb® II HIV 1&2 CombFirm by manufacturer

Pattern of HIV spots present on		Interpretation
Left tooth	**Right tooth**	**Positive for**
p24 and/or p31	gp41	HIV-1
No spot	gp41 and gp120	
p24 and/or p31	gp36 only or with gp41	HIV-2
p24 and/or p31	gp36 and gp41 and gp120	HIV-2 only, or coinfection with HIV-1
p24 and/or p31	No spot or gp120	Indeterminate
No spot	gp41 or gp120 and/or gp36	
No spot	No spot	Negative

6.10.2 Alternative interpretation of *ImmunoComb® II HIV 1&2 CombFirm* by OCA

For HIV-1 only: gp120, gp41, p31, p24. Do NOT include gp36!

Pattern of HIV spots present on	Interpretation
3-4 reactions on gp120, gp41, p31 and/or p24. (Do NOT include gp36!)	HIV-1
0 reactions.	HIV negative
1-2 reactions	Indeterminate