MINISTRY OF HEALTH

National Public Health Laboratory Services

NATIONAL HIV TEST KIT EVALUATION GUIDELINE

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FOREWORD

Since HIV/AIDS contributes to high morbidity and mortality there is increased demand for testing services. To meet the ever increasing demand there is high turnaround of new kits which requires evaluation to ascertain their suitability for local use. HIV antibody testing and knowledge of HIV status is crucial for controlling the epidemic because it is the critical entry point into both HIV prevention and care. In an effort to ensure access to HIV testing for large populations and to facilitate access to life saving antiretroviral treatment in the context of the WHO's universal access strategy, radical scaling up of HIV testing and counseling services has been employed.

The need for individuals to know their HIV status has become apparent and fostered the development of rapid diagnostic HIV testing. Despite significant progress, the vast majority of HIV-infected people are unaware of their status, making HIV testing and counseling a pivotal element of HIV prevention, care and treatment services. The use of HIV rapid tests will facilitate this in many settings, particularly in services in which the people most likely to benefit from knowing their HIV status can be reached. To address this, NPHL, with support from other stakeholders, initiated a series of activities to establish a national HIV kit Evaluation guideline.

It is good practice to evaluate the performance of each HIV test kit to determine the suitability of these assays prior for their use in the country. This guideline establishes good practice consistent with Evaluations and evaluations of HIV rapid/EIA tests. Sufficient support should be made available for NHRL to provide a suitable program to select, evaluate, and monitor the consistency of batches (lot-to-lot) in the country.

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EXECUTIVE SUMMARY

Kenya is committed to the achievement of the 90-90-90 UNAIDS global targets stating that 90% of people with HIV know their HIV status. To achieve this goal, targeted HIV testing on populations using rapid testing kits will be employed at various settings including PMTCT and HTC settings and results given to clients the same day.

There are many HIV testing kits on the market. It is vitally important that before these assays are utilized, their performance and suitability for use within the country is verified. Evaluation is critical in ensuring the quality of test results given to the clients and safeguards the integrity of the testing personnel. In addition, there is a need to monitor the consistency of batches (lot-to-lot comparison) that are already in use in the country to inform the decision to revise or replace the approved testing strategy.

This document is intended to act as practical guidance and reference to the laboratory personnel who conduct evaluations of HIV rapid/EIA tests. The guidelines provide recommendations for specimen selection, collection, storage, and testing and for the selection and Evaluation of appropriate HIV testing strategies and technologies to meet the country's objectives. These technical guidelines target laboratory staff and other health professionals involved in HIV testing for surveillance and prevention purposes in Kenya.

The document describes in detail the technical aspects of kit evaluation including the methodology, quality assurance measures, and data management requirements. Since kit evaluation is conducted as part of the project management function, this document highlights issues of planning, timelines, data reporting. Furthermore, the document provides a list of references and quick links to further information relevant to kit evaluation.

List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
CDC	US Centers for Disease Control and Prevention
DBS	Dried Blood Spot
DHIS	District Health Information System
EIA	Enzyme Immuno Assay
ELISA	Enzyme-Linked Immunosorbent Assay
EQA	External Quality Assessment
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HTC	HIV Testing and Counseling
KAIS	Kenya AIDS Indicator Survey
KASF	Kenya Aids Strategic Framework
KEMSA	Kenya Medical Supplies Agency
KMLTTB	Kenya Medical Laboratory Technologist and Technician Board
MOH	Ministry of Health
NASCOP	National AIDS/STI Coordinating Program
NBTC	National Blood Transfusion Centre
NHRL	National HIV Reference Laboratory
NPHL	National Public Health Laboratories
NPV	Negative Predictive Value
OD	Optical Density
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
PMS	Post Market Surveillance
PMTCT	Prevention of Mother-to-Child Transmission of HIV
PITC	Provider Initiated Testing and Counseling
QA	Quality Assurance

QC	Quality Control
TWG	Technical Working Group
UNAIDS	United Nations Program on HIV/AIDS
VCT	Voluntary Counseling and testing
WB	Western Blot
WHO	World Health Organization

DEFINITION OF TERMS

Algorithm – For HIV testing, the sequence in which assays are performed to detect HIV antibody in a body fluid

Confidence Interval - Interval estimate of a population parameter computed so that the statement "the population parameter lies in this interval" will be true at a stated confidence, usually 95%.

Evaluation- Process for determining whether a test system meets defined needs in the potential user's environment.

Evaluation Panel – Set of specimens used during verification evaluation for which the sero-status has been previously defined by the gold standard.

External Quality Assessment (EQA) - A program that allows laboratories or testing sites to assess the quality of their performance by comparison of their results with other laboratories, through analyzing proficiency testing panels, or blinded rechecking. EQA also includes on-site Evaluation of the laboratory to review the quality of test performance and operations.

Gold Standard – this refers to a diagnostic test or benchmark that is regarded as definitive.

Negative predictive value - In HIV testing, the probability that when a test is determined non-reactive, the specimen does not have antibody to HIV

Positive predictive value - In HIV testing, the probability that when a test is determined reactive, the specimen actually contains antibody to HIV

Prevalence - Percentage of persons in a given population with a disease or condition at a given point in time.

Proficiency testing panel - A set of samples, usually 4-8 with known values used to assess the performance capabilities of testing personnel.

Quality Assurance - Planned and systematic activities to provide confidence that requirement for quality testing are met.

Quality Control - Operational techniques and activities that are used to fulfill requirements for quality.

Reference Panel – For HIV, aliquoted stable serum or plasma specimens that have been highly characterized; i.e. known cutoff points, subtype, titer, etc.

Sensitivity of a test - Measure of the probability of correctly identifying an HIV-infected person

Specificity of a test - Measure of the probability of correctly identifying an HIV-uninfected person

Testing strategy - Use of an appropriate HIV test or combination of HIV tests for identifying positive specimens. The choice of testing strategy used is based on the objective of the test, the sensitivity and specificity of the test, and HIV prevalence in the population being tested

Verification – of an HIV test, is the production of documented evidence that the test, when operated within established parameters, can perform effectively and reproducibly to produce the specified result

Window period - The period of time following exposure and infection with HIV and the generation of detectable antibodies by the infected person

Chapter 1

Introduction

1.1 Overview of HIV in Kenya

In 2013, an estimated 1.65 million Kenyans were living with HIV, including 1.4 million adults and over 191,000 children, with a national HIV prevalence of 6% among people aged 15-64 years (Kenya HIV estimates, June 2014). HIV/AIDS remains a major challenge in Kenya and the country continually strives to halt and reverse its spread. HIV antibody testing and knowledge of HIV status is critical for controlling the epidemic, because it is the critical entry point into both HIV prevention and care efforts. Knowledge of HIV status among HIV-infected persons tripled from 16.3% in 2007 to 46.9% in 2012.

Currently, testing and counseling services are offered at 6,050 health facilities in the country through various settings such as PITC, PMTCT, and VCT. From July 2014 to June 2015, about 8.5M people were tested at various HIV HTC DHIS. Current UNAIDS global targets aim at having 90% of all people living with HIV know their HIV status by year 2020.

Serological diagnosis of HIV infection is based on a multi-test algorithm for detecting antibodies to HIV. Screening tests provide presumptive identification of specimens that contain antibody to HIV. These EIAs or simple/rapid immuno-diagnostics are selected for their high sensitivity of detecting antibodies to HIV. Supplemental or confirmatory tests, such as WB; can be used to confirm infection in samples that are initially reactive on conventional EIAs. Alternatively, repetitive testing incorporating EIAs or rapid tests selected for their specificity may be used to confirm whether specimens found to be reactive for HIV antibodies with a particular screening test are specific to HIV. For practical purposes, resource-poor settings depend heavily on EIA and rapid tests for screening and confirmation.

In the majority of these settings, a combination of carefully selected and approved HIV rapid tests are used and results are given to the clients on the spot. To assure the quality of HIV screening and confirmatory testing within all testing settings, it has been recommended that all test kits used in Kenya undergo careful in-country selection and evaluation, as outlined in the National Guidelines for HIV Testing and Counseling in Kenya (2008).

1.2. Rationale of Test Kit Evaluation

There are many HIV testing kits in the market. It is vital that before these assays are utilized, the country need to evaluate their performance and suitability for use within the country. Test kit Evaluation is critical in ensuring the quality of test results given to the clients and safeguards the integrity of the testing personnel. Verifications of HIV tests are performed to determine an algorithm of simple rapid tests that can be used at the point-of-service for HTC services and surveillance.

If a country had previously conducted Evaluations and has selected an algorithm of rapid tests that performs adequately, then there must be compelling reasons for considering evaluating additional tests. There is often much demand from manufacturers or donors to evaluate specific tests for use within a country. Due to the number of kits appearing on the market, a preliminary review of available performance data cannot be over emphasized.

Data are often available regionally that permit a presumptive determination of the assay's sensitivity and specificity, reducing the need to verify numerous tests. As a consequence of available data, the decision may be made to tailor an Evaluation to focus solely on the potential implications of integrating the product into an existing algorithm. An Evaluation of testing algorithms requires time and resources, and each country must determine the potential advantages of a test(s) before deciding to perform a formal Evaluation.

The questions to be answered while making decisions on whether to evaluate a new kit include:

- Is there evidence from published studies that indicate the test has greatly improved performance characteristics.
- Is the test(s) much simpler to perform.
- Is the test(s) more stable to ship and store.
- Is there a significantly reduced cost with evidence that the proposed cost will not increase significantly after implementation.

In many cases there may be no demonstrable improvement gained in a full scale Evaluation of a new product, either because evidence is already sufficient to determine its efficacy or there is no demonstrable need. For tests that will be evaluated in-country, every effort should be made to allow manufacturers or marketers to bear the costs of evaluating new tests, as Evaluations consume a considerable amount of time and precious resources. Adopting new tests without adequate Evaluation should NOT be considered an option. Doing so will compromise the integrity of the testing facility, personnel, and quality of reported results to the patient and/or client.

Chapter 2

HIV Test Kit Evaluation

2.1 Introduction

Evaluation is the process for determining whether a test system meets defined needs in the potential user's environment. Kit Evaluation is laboratory-based and provides preliminary results on test performance characteristics of sensitivity, ease of performance and specificity using a standard characterized panel of test samples. After initial evaluation, test kits undergo periodic evaluation and batch testing prior to distribution to the field. A regular assessment of HIV test kits used in HIV HTC service delivery points is conducted according to an established protocol to ensure that test kits used in the field are of the highest quality.

This work is coordinated and performed by NHRL. Evaluation shall be conducted by either re-testing a proportion of specimens already tested for HIV using a gold standard method, or by using highly characterized proficiency panels. The current national HTC guidelines (2008) recommend both methods, where the setting and resources permit. The objective of Evaluation is to determine the sensitivity, specificity, predictive values, and performance characteristics of commercially produced HIV test kits availed to the country, validate the performance of different testing algorithms based on the results of the sensitivity and specificity of the tests evaluated, and monitor lot-to-lot variation of test kits as they are deployed in country.

2.2 Guidelines for Evaluation of HIV test kits

The HTC Guidelines (2008), requires test kit used for HIV screening, diagnosis, and surveillance to be evaluated and approved. About 100 test kits have been approved in the country (Appendix 2). Currently, new HIV testing kits, reagents, and equipment are first registered with KMLTTB as mandated by the CAP 253A.

For evaluation of HIV test kits, the following shall apply:

1. Application by the manufacturer or vendor to the MOH through KMLTTB for licensure.
2. Appointment and engagement of validating institution by KMLTTB to carry out evaluation.
3. Prepare of Evaluation protocol by the appointed institutions after notification by KMLTTB.
4. The protocol shall contain the Evaluation requirements and cost for Laboratory and /or field Evaluation.
5. Payment of Evaluation costs by the manufacturer/vendor.
6. The vendors or manufacturers will submit agreed numbers of test kits as per the protocol to KMLTTB who will then distribute the test kits to the evaluating laboratory.
7. Conducting the Evaluation, data analysis and report writing by the appointed institution.
8. The evaluation reports are sent to the regulatory institution to inform decision making.

2.3 Field Test Kit Evaluation

The field test kit Evaluation involves a prospective Evaluation of the selected kit/algorithm at a few representative regional laboratories and hospitals in the country. This ensures that test kits are evaluated in the field thus allowing for comparing kit performance against demographic information collected at the particular site. Testing and interpretation of results may be done by non-laboratory clinic staff. Field Evaluation, using routine clinical specimens, will be conducted for test kits that require fresh specimens.. The objective is to demonstrate that under the selected sites/settings and conditions, each test kit perform satisfactorily and evaluate the performance of the test algorithm in the point of service setting. The evaluation report shall contain:

- Test performance.
- Kits sensitivity.
- Kits specificity.
- Ease of performance.
- Conclusion of Evaluation and recommendations.
- Other information as per the protocol.

Kit evaluation shall be conducted every time a new kit is available in the country.

2.4 Guidelines for Evaluation of HIV test kits that are in country

The steps followed during Evaluation of kits already in use in the country shall include:

1. Sampling of test kits in accordance with the MOH kit Evaluation sampling protocol by the distributing agency or warehouse (KEMSA).
2. NHRL shall receive new kits, batch to batch, lot to lot or post market surveillance.
3. Conduct kit Evaluation as per in country HIV kits Evaluation protocol.
4. The Evaluation protocol shall include and not limited to; packaging, storage condition, ease of performance, and need for additional requirements.
5. Kits assessment for technical performance (readings of positive and negative controls, sensitivity, and specificity, positive and negative predictive values).
6. A report of results and recommendations will be prepared and submitted to TWG.

The report shall contain:

- Test performance.
- Storage requirements.
- Kits sensitivity.
- Kits specificity.
- Ease of performance.
- Conclusion of Evaluation and recommendations.
- And other information as per the protocol.

2.5 Batch to batch / Lot to lot

The goal of batch evaluation is to provide assurance that new batches of kits entering the country are fit for use. This is done by assessing samples from each new batch released by the manufacturer. This safeguards consumers and public health interests against declining manufacturing processes and poor business practices.

The purpose of lot to lot Evaluation is to ensure that initial and subsequent test kits used in the country conform to required standards, the quality of test kits throughout the distribution chain is monitored, ensuring that, in spite of varying environmental conditions, there are no clinical significant differences in the results obtained when different lot numbers of reagents are used, guiding immediate corrective action in case of identified problems and to monitor lot-to-lot variation of test kits as they are deployed in country for use at the different service delivery points.

2.6 Post market surveillance

Post Market surveillance (PMS) is the continuous watchfulness through use of scientific methods that provide thorough scrutiny, supervision, and inspection of kits being used to ascertain conformance to quality. The primary objective is to assess test kit performance and reliability under point of service conditions, while comparing kit performance against demographic information collected at each particular site. It provides regular information on the quality of kits being used in the country.

The specific objectives to be met under post-market surveillance activities include:

(i). To demonstrate in selected sites/settings and conditions that each test complies with prequalification requirements, once marketed.

(ii). To evaluate the performance of the test algorithm in the point of service setting

(iii). To determine the quality of the test kits in the field and institute a product recall mechanisms whenever product elements of quality are compromised.

(iv). Provides a continuous feedback about the product.

(v). To evaluate transport and storage conditions (temperature, humidity, exposure to sunlight) that may affect rapid test performance.

Chapter 3

Laboratory Quality Assurance

3.0 QUALITY ASSUARANCE IN VALIDATING LABORATORIES

3.1 Importance of quality assurance

Laboratory quality assurance is planned and systematic activities to provide adequate confidence that requirement for quality will be met. All laboratories conducting kit evaluationevaluation or Evaluation testing shall have a QA/QC program to monitor and evaluate laboratory functions and services throughout the pre-analytical, analytical, and post-analytical phases of laboratory testing.

Specific activities at each QA phase shall include the following:

1. Pre analytical phase
    - Test request.
    - Test selection.
    - Trained testing personnel.
    - Patient/client preparation.
    - Specimen collection, labeling and transport.
2. Analytical phase
    - Specimen processing and storage.
    - Reagent preparation.
    - Preventive maintenance/equipment checks.
    - Quality control.
    - Test performance.
    - Proficiency testing/External quality assessment.
    - Specimen storage.
    - Post analytical phase
    - Reviewing quality control.
    - Transcribing results.
    - Reporting results.
    - Interpreting results.
    - Maintaining records.

A successful QA program will need the support of NHRL and requirements shall be rigorously complied with to ensure the accuracy of the results from the Evaluation and all other assays.

3.2 Quality control

QC refers to those measures that are taken to monitor the quality of the assay itself. QC shall include the assay of samples/materials with known test results to verify the procedure itself is working properly. When QC materials analyzed daily produce acceptable results, and all other testing conditions have been met, then the results of the samples being analyzed may be considered acceptable.

3.3 External Quality Assessment/Proficiency Testing

Every validating facility shall demonstrate and document its competence in performing HIV serology that is carried out as part of its routine services. EQA is one component of a laboratory QA program. The focus of EQA is on identifying laboratories or testing sites and technicians exhibiting poor performance.

There are three methods that can be used as part evaluating laboratory performance:

- On-site Evaluation.
- Proficiency Testing.
- Blinded Rechecking.

The choices for which type of EQA program to implement shall depend on both the available resources and the ability to obtain additional resources as needed, to support the EQA program. Evaluation lab shall be deemed competent if it meets the following criteria:

1. Accredited.
2. Participate and pass all external quality assessment programs.
3. Assessed and deemed that all the testing officers are certified as competent.

3.4 Safety Precautions

Each laboratory or testing site must follow Universal (Standard) Precautions designed to prevent transmission of HIV, HBV, and other blood borne pathogens. When laboratories adhere to universal precautions, blood and certain body fluids of all patients are considered potentially infectious for HIV, HBV and other blood borne pathogens.

Chapter 4

Materials & Methods

4.1. Sample collection and Panel preparation

4.1.1. Laboratory Evaluation

NHRL shall obtain specimens from two general sources: Blood transfusion centers and blood banks from where large volumes of blood will be collected from discarded and expired units. These samples will be used either as whole blood or plasma/serum. Whole blood will also be used to prepare DBS. These specimens will constitute the bulk of the Evaluation panels. These panels will also include a number of challenging or unusual specimens (from infected or uninfected persons with unusual screening results or interference substances, (for example from lipemic, high bilirubin, seroconversion and haemolysed samples) to provide a unique challenge to the tests being evaluated. NHRL will prepare a panel of 400 samples of the relevant panel required and send 125 aliquots to each of the national evaluating laboratories. After evaluating the kits, each evaluating laboratory will complete the standard reporting forms and forward these to the laboratory manager for dispatch to NHRL.

4.1.2. Field Evaluation

Whole blood, oral fluids and urine shall be obtained from clinical settings; NHRL will provide guidance on specific requirements to the collecting sites to ensure representative samples for the country are collected.

4.2 Sample size

A minimum of 200 known positive and 200 known negative specimens shall be used for test Evaluation. This shall provide 95% confidence intervals of less than +/-2% for both the estimated sensitivity and specificity.

4.3 Sample preparation

Unique laboratory specimen IDs shall be assigned to each specimen collected. A dedicated freezer with reliable electricity supply will be identified for long term storage, and an archiving and retrieving system will be developed. To ensure sample integrity, and to avoid loss of antibody titer and formation of serum flocculates, the number of freeze-thaw cycles will be kept to a minimum. Thus, several aliquots of the same sample will be prepared.

1. Laboratory-based Evaluation panel: this will comprise of well characterized samples stored for long term use. Sample characterization will be done using a specified algorithm (characterization gold standard). The characterized Evaluation panel will contain both positive and negative samples and will be aliquoted and stored at the NHRL and subsequently distributed to the four national evaluating laboratories together with the test kits to be evaluated.

- Plasma: Centrifuged anti-coagulated whole blood will be separated and the plasma aliquoted into assigned labeled cryo-tubes.
- Serum: Plasma will be converted to serum, and the whole blood centrifuged and separated as above.

2. DBS: these will be prepared from whole blood.
3. Field Evaluation: some field Evaluation studies may require using fresh specimens. These include saliva, urine and other body fluid samples. These will be prepared according to the relevant SOPs.
4. Control sample sets; large pools of positive and negative control samples will be prepared by NHRL or evaluating laboratory to be used regularly with every test run

4.4 Transfer and storage of specimens

The NHRL SOP for sample management on kit Evaluation shall be followed.

4.5 Laboratory kit analysis (Testing)

Evaluation of test kits shall be conducted under the same conditions where the test will be performed. The testing for test kit to be validated shall be done in duplicate and test performances will be compared against the current testing algorithm ("gold" standard). Quality measures including in-house control samples, second reader, etc. will be put in place. After evaluating a test kit, data forms are completed and forwarded to the laboratory in-charge, for re-checking and forwarding to NHRL for data analysis.

4.6. Evaluating superior kits

There are situations where a kit which is more superior and more technologically advanced than the gold standard kits in use, may require an Evaluation. To avoid bias, which may likely be attributed to the use of inferior gold standard kits, all discrepant results shall be confirmed by PCR, WB, or both assays.

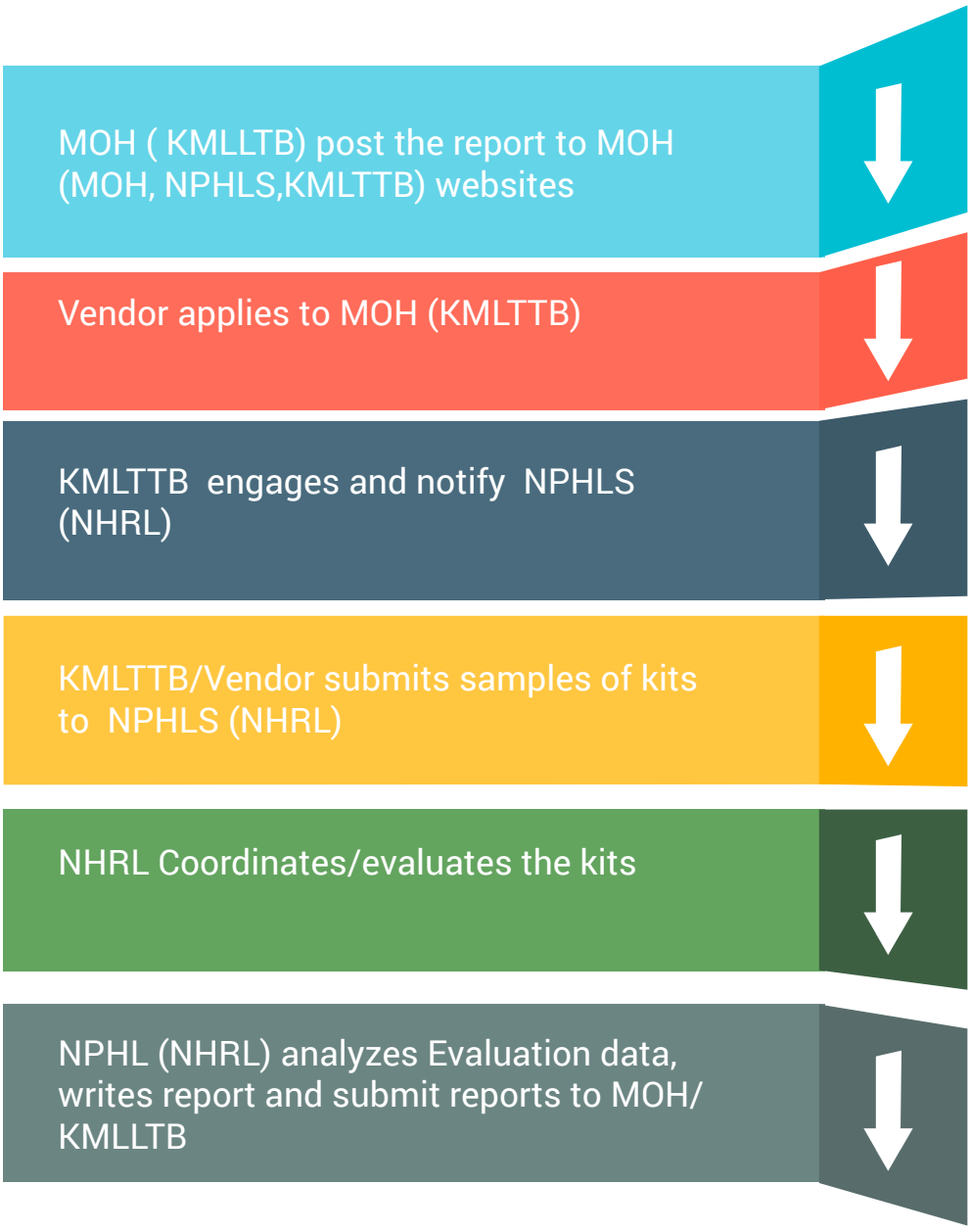
4.6.1. Testing protocol

As described for kit evaluation and Evaluation sections, all test samples shall be treated as real samples and assayed accordingly. All reference samples will be tested in duplicate. A mean result is determined for each reference sample and checked against range. If all the values are within the range, the test kits are deemed to have passed. If the results are out of range, the tests will be repeated in duplicate.

4.7. Ethical clearance

When patient samples are required, ethical clearance will be obtained from the relevant authorities. Individual patient consent will also be obtained and samples will be delinked. Test results will not be provided to patients, but other data such as geographical origin, will be maintained.

Flow Chart for New Kit evaluation



Chapter 5

Roles and Responsibilities

5.1 Kenya Medical Laboratory Technicians and Technologist Board

KMLTTB will:

- Identification of laboratories for validating the test kits using predetermined criteria.
- Receive new HIV test kits and forward to the validating laboratories.
- Receives evaluation report, approves and registers the suitable kits for use in the country.
- Publish the list of the approved HIV test kits through the office of DMS

5.2 National HIV Reference Laboratory

The role of NHRL is several-fold:

- Provide technical coordination of the test kits evaluation.
- Assess the competence of the validating laboratories. (EQA for evaluation competence).
- Coordinate development of standard procedures for test kit evaluation.
- Compiling and analyzing evaluation data and submitting recommendations to KMLTTB and NHRL.

5.3 Evaluating Laboratories

Evaluating Laboratories will be responsible for testing the evaluating panels prepared and characterized by NHRL using test kits to be evaluated and will provide feedback to the NHRL which will inform the authorized body. The national evaluating laboratories will also be involved in collecting samples for panel preparation. The KMLTTB appoint/select Evaluation laboratories based on geographical representation and level of the laboratories. The labs shall be of acceptable standards and responsible for the following:

- Validate kits
- Generate reports and submit to KMLTTB and NHRL
- Meet and maintain minimum set criteria for test kits validating laboratories.

Implementing Partners (Refer to chapter 5)

- Provide technical assistance in lot to lot evaluation and post market surveillance. This may include sampling of test kits, training of laboratory personnel at facility level, data analysis to inform quality improvement.
- Linkage with the county health management team and the MOH at national level.

KMLTTB will utilize the evaluation report for approval of new test kit for in-county use. NHRL will utilize the evaluation report for quality monitoring and improvement in HIV testing programming.

5.4 Manufacturers/vendors

Vendors shall apply for Evaluation services for their products to MOH through the KMLTTB. They shall provide sufficient test kits to be validated and the gold standard.

5.5 KEMSA

The roles of KEMSA in the context of batch-to-batch monitoring of test kits include:

1. Quantification and monitoring.
2. Request from manufacturers the performance index of new lots to be shipped.
3. Inform vendors and manufacturers about the upcoming monitoring systems.
4. Replace, substitute or dispose of faulty test kits.

Chapter 6

Data Management

6.1. Data collection

Data collection tools, i.e. a basic questionnaire and tracking record will be used to track records for specimen management. These records will include: number, date, and site of draw. Limited demographic information such as age, sex, profession, and home district may also be captured. In addition, an inventory of specimens being shipped, their origin, destination, and time and date of collection will be captured. Specimen-associated variables such as unique specimen identification number, relevant tracking information, the name of tests used, test results (positive or negative), optical density values, optical density ratios (OD ratio), any additional confirmatory information, will also be recorded. Only negative and positive specimens using the reference method will be used in the calculation of sensitivity and specificity. Results for further testing will be listed in the Evaluation summary to provide further information on the performance of the test used in the Evaluation. If the results of the test being evaluated differ from the results of the reference standard (Kit), the new kit is deemed to have failed.

6.2 Data management activities at different levels of the Evaluation cycle

Sample sourcing:	details of sample from the source e.g. origin, status, ID, date of collection from site and actual sample collection from client
Sample reception:	details of sample received against the sample tracking form, entry of sample (type) and client details into lab log book, using a checklist, rejecting or accepting the sample and noting in the log
Characterization:.	sample status using the respective characterization algorithm and recording results in the lab book/spreadsheet
Aliquoting:	labeling the tubes using the appropriate coding system such that the origin of the sample can be traced back to the site, noting the position and details of each sample
Storage:	recording the number of freeze thaw cycles both on the tube and the sample storage data (only use samples with <2 freeze thaw cycles), maintaining of environmental fridge and freezer temperature monitoring records
Transportation:	filling in the sample tracking form, filing a copy and notifying the Evaluation centers about the sample dispatch
Evaluated kits:	NHRL keeps a copy of all the kits supplied for Evaluation from NASCOP and Evaluation outcome decisions. The NHRL also keeps a database of trainings, personnel, supervision, site performance trends, codes of all relevant Evaluation SOPs
EQAs:	This will keep records of an EQA facility for NHRL and Evaluation sites, equipment type, maintenance, evaluation, calibration, QA charts.

6.2. Resolving discrepant results

In kit Evaluation, there are two types of discrepant results: i) samples that do not meet the criteria of positive or negative using the gold standard method/definition, and ii) the result of the tests being evaluated differs from the result of the gold standard. Before the Evaluation, the laboratory will determine the gold standard for positives and negatives. The main cause of type i ediscrepance is sample mix-up or transcription. These errors should be checked before deciding to perform additional testing to resolve the discordance. In the case of type ii discordance, only specimens that are positive or negative by the gold standard method should be used in calculating the sensitivity and specificity of test performance. The results of further testing may be listed in the Evaluation summary to provide further information on the performance of tests used in the Evaluation. As in type i discrepant, the evaluating laboratory is encouraged to perform additional tests to provide further information on the patient specimen. However, these results should not be included in calculating the sensitivity and specificity.

6.3. Statistical analyses

6.3.1 Sensitivity and Specificity

Key statistical parameters evaluated for each kit include sensitivity, specificity, PPV, and NPV. The sensitivity and specificity of each assay are calculated using the gold standard and are expressed as percentages. Sensitivity is defined as the ability of an assay being evaluated to correctly detect specimens containing antibody to HIV. In other words, sensitivity is the percentage of true positive HIV specimens identified by the assay under Evaluation as positive (A), divided by the number of specimens identified by the reference assays as positive (A+C). Specificity is defined as the ability of an assay being evaluated to correctly detect specimens that do not contain antibody to HIV. In other words, specificity is the percentage of true negative specimens identified by the assay being evaluated as negative (D), divided by the number of specimens identified by the reference assays as negative (B+D).

6.3.2 PPV

Positive Predictive Value is the probability that when the test is reactive, the specimen actually contains antibody to HIV. PPV is calculated as follows: A/(A+B).

PPV can also be calculated as follows:

$$\textbf{PPV} = \frac{(\text{prevalence})\,(\text{sensitivity})}{(\text{prevalence})(\text{sensitivity}) + (1-\text{prevalence})(1-\text{specificity})}$$

6.3.3 NPV

NPV is the probability that when a test is negative, a specimen does not have antibody to HIV. NPV is calculated as follows: D/(C+D) or as:

$$\textbf{NPV} = \frac{(1\text{-prevalence})(\text{specificity})}{(1\text{-prevalence})(\text{specificity}) + (\text{prevalence})(1\text{-sensitivity})}$$

The proportion of false positives and false negatives varies with the prevalence of HIV infection in various segments of the population. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected, i.e., the greater the PPV. Thus, with increasing prevalence, the proportion of false-positive results decreases. Conversely, the likelihood that a person having a negative test result is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false-negative.

Example:Table below shows the format used for calculating sensitivity and specificity parameters against gold standard readings:

		Gold Standard results		
		+	**-**	**Total**
Kit Evaluation Results	**+**	**A** True-positives	**B** False-positives	**A+B**
	-	**C** False-positives	**D** True-negatives	**C+D**
		A+C	**B+D**	**A+B+C+D**

Sensitivity= A/(A+C)x 100
Specificity= D/(B+D)x 100
Positive Predictive value= A/(A+B)x100
Negative Predictive value= D/(C+D)x100

WHO recommends Sensitivity of >99% and Specificity>99% for kit Evaluation, in resource-limited setting where QA measures may not be sufficient. On the other hand, CDC (based on US FDA) recommends sensitivity of > 99% and specificity of 98.5% for settings where all QA measures are in place. Kenya's kit acceptability criteria should not be below the above recommendations.

6.4 Developing an algorithm, reporting results, conclusions, recommendations

6.4.1 Developing an algorithm

Kit Evaluation data will be analyzed to determine the performance of individual tests and the combination of tests used in a proposed algorithm. In kit Evaluation, this will involve determining the performance of various test combinations in addition to individual test performance. As most of the testing algorithms in Kenya are serial, the use of the 2nd test will be dependent on a reactive result in the first test.

6.4.2 Reporting results

Immediately following an Evaluation, data analysis and Evaluation table will be completed and reported to the NHRL and other relevant partners. As shown below, the Evaluation report typically includes the data presented in a tabular format that itemizes the test methods, and the Sensitivity, Specificity, PPV and NPV for each method and combination of methods evaluated. Field and batch Evaluations will, in addition to on-site performance data, include subjective input of the client and client flow. All the reports will have details covering both technical and non-technical characteristics of the kit under Evaluation as shown below:

Typical Evaluation report table

Characteristic	**Score**
Operational characteristics (technical)	
Equipment the test uses	
Number of steps involved	
Run time	
Type of specimen	
Specimen volume	
Ready to use reagents	
Ease of interpretation	
Overall ease of use	
Condition for storage	
Amount of waste generated	
Sensitivity >99%	
Specificity >99%	
Predictive values >99%	
No. of specimens used	
Equipment and maintenance requirement	
Positive and negative kit control	
Use of electricity	

Environmental conditions	
Principle of test used	
General/Non-operational (non-technical)	**Details**
Name of test kit	
Lot number	
Kit insert	
Number of test per kit	
Manufacturer	
Manufacture date	
Expiry date	
Training needs	
Shelf life	
Kit box size	
Individual test packaging	

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Appendices

Appendix 1: Logistical requirements for kit Evaluation

The following are supplies required for kit Evaluation at all levels:

Consumables	**Reagents**
15ml / 50ml tubes	Characterization kits
Transfer pipettes	Calcium chloride
Serum vials	(used to convert plasma into serum)
Cryovials	Disinfectants
Cryoboxes	
Cotton wool	**Stationary**
Gauze	File boxes, pens, ruler, printing papers etc
Vacutainer systems for blood collection	
Urine containers	Permanent markers
Other sample collecting devices(depends on the type of kit)	Lab log books
	Flash disks and CDs(data back up and transfer devices)
Surveittes	
Tube racks	Reference materials i.e. WHO reference manuals
Filter papers	
Pipette tips(250 and 1000uL)	
Bench liners	**Others**
Gloves/lab. Coats	Airtime
Glycine papers	Transport
Ziploc bag	Finances
Desiccants	
Humidity indicator cards	
Equipment	
Centrifuge	
Fridge (short term storage)	
Freezer (long term storage)	
Barcode reader	
Cool boxes	
Ice packs	

Appendix 2a: Updated list of Approved Rapid Test Kit

Note: This is the list as at September 2009. This will be adjusted over time as new kits are evaluated and approved.

1. Capillus HIV I and II test kit.
2. Instant check TM HIV I and 2 test kit.
3. Distint HIV I and 2 test kit.
4. Easi dot HIV I and II test kit.
5. Bioelisa (Biokit) HIV I and 2test kit
6. Dot HIV I and 2 test kit.
7. Serostrip HIV I and II test kit.
8. DetermineHIV1and2test kit(2).
9. Bioline SD (BDI) HIV 1and 2test kit
10. Innotest strip HIV I and II test kit.
11. Hexagon HIV I and 2 test kit.
12. BEST HIV I and 2 test kit.
13. Bionor HIV I and 2 test kit.
14. HIV I and 2 one step test kit.
15. HIV I and 2 double check 11test kits
16. Orascreen saliva HIV I and II test kit
17. Immuno comb HIV I and 2test kit
18. Insti HIV I and 2 test kit.
19. Hemastrip I and 2 test kit.
20. KEMRI particle agglutination HIV test kit.
21. Instant screen HIV I and2 test kit
22. Acon one step HIV I and 2 test kit.
23. Comb AIDS-RS immuno Dot HIV 1and2 test kit
24. Serodia HIV I and 2 test kit.
25. Trinity Biotech card HIV 1and 2test kit
26. Effoora HIV I and 2 test kit.
27. Stat pak HIV I and 2 immuno assay test kit
28. Unigold HIV I and 2 test kit.
29. Morwell rapid HIV I and 2 test kit
30. HIV spot HIV I and 2 test kit(2).
31. Oraquick HIV ½ test kit (Blood)
32. OnestepHIV ½ (advanced quality) test kit.
33. Accurate rapid HIV ½ one step card test kit

34. Euro check HIV ½ test kit
35. First response HIV ½ test kit.
36. Boitracer HIV ½ test kit.
37. Genedia HIV ½ test kit.
38. Retrocheck HIV ½ test kit.
39. HIV 1 and 2 stat pak dipstick test kit
40. HIV 1 and 2 stat pak test kit.
41. Calypte Aware BSP Rapid HIV ½ test kit
42. Calypte Aware OMT HIV ½ test kit for oral fluid
43. EZ-5 HIV 1 and 2 test kit.
44. Smartest HIV 1and 2 Rapid test kit

Vikia HIV 1 AND 2 Rapid Test kit.

46. Triline Cassette HIV 1and 2 test kit.
47 Tri-Dot HIV 1 and 2 Test kit.
48. Oraquick HIV 1 and 2 test kit Oral fluids).
49. Kemcom HIV1 and 2 Rapid kit
50. Hema Strip Rapid Kit.
51. Hema Express Rapid Kit.
52. HIV 1&2 Rapid (Colloidal Gold) test kit.

Appendix 2b: Long ELISA HIV Test Kits

Note: This is the list as at September 2009. This will be adjusted over time as new kits are evaluated and approved.

1. Abbott Murex HIV 1and 2 Ag/Ab test kit
2. Vironostika HIV Uniform 11 Ag/Ab test kit.
3. DRG HIV 1 and 2 test kit.
4. Innotest HIV 1 and II test kit.
5. Vironostika organon HIV
6. Abbott HIV 1 and 2 G.O.E.I.A test kit
7. Genscreen HIV 1 and 2 test kit.
8. Genedia HIV 1 and 2 test kit.
9. Enzygnost (Behring) anti- HIV ½ test kit.
10. Abbott 3rd generation HIV 1 And 2 test kit ½ test kit.
11. IMX system (abbott) HIV 1 and.
12. Murex IEC HIV 1 and 2 test kit II test kits
13. Lab system HIV 1 and 2 test kit.

14. Calypte HIV 1 urine assay test kit
15. Dynarmed HIV ½ (EIA) test kit.
16. Genedia HIV ½ Ag/Ab test kit.
17. Enzaid HIV ½ test kit.
18. Biotech HIV 1+O/2 test kit.
19. Immunolisa HIV 1 and 2 test kit.
20. ANI- Labsystem HIV I and II test kit
21. Axsym Ag/Ab KOMBO HIV
22. Human HIV 1 and 2 test kit.
23. Qualisa HIV1 and 2 ELISAkit
24. PishTaz HIV 1and 2 ELISA kit0

Validated for DBS Technology – No Additives Needed;

Note: This is the list as at September 2009. This will be adjusted over time as new kits are evaluated and approved.

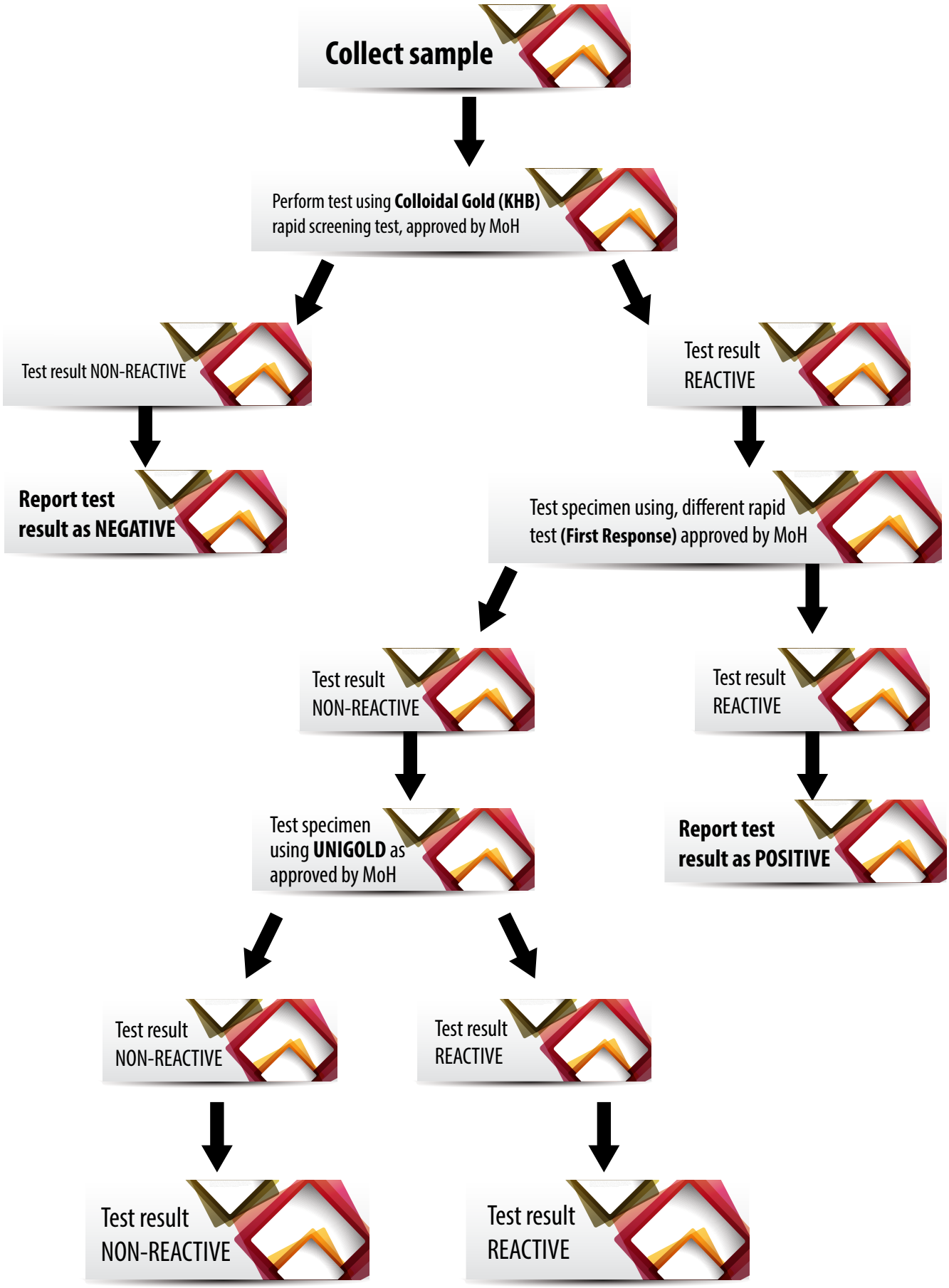
25. Vironostika Ag/Ab kit
26. Vironostika Ab. kit
27. Enzygnost Plus O
28. Enzygnost Integral II

Validated for DBS Technology- Use with tween 20 & lab. skimmed milk or nestle or safariland skimmed milk;

Note: This is the list as at September 2009. This will be adjusted over time as new kits are evaluated and approved.

29. Genedia Ag/Ab kit.
30. Murex Ag/Ab kit.
31. Murex Ag/Ab kit.

Appendix 3: Algorithm for HIV Testing in Kenya, September 2013



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