Guidelines for Appropriate Evaluations of HIV Testing Technologies in Africa

World Health Organization
Regional Office for Africa

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention

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Acknowledgements

The Centers for Disease Control and Prevention (CDC) and the African Regional Office of the World Health Organization (WHO/AFRO) wishes to express their gratitude to individuals who contributed their time and expertise by participating in a workgroup meeting November 28–December 1, 2001 in Harare, Zimbabwe, to develop these guidelines.

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Appreciation is also given to Dr. El hadj Belabbes (Algeria), Dr. Robert Downing (Uganda), Dr. Chantal Maurice (Cote d'Ivoire), Professeur Souleymane Mboup (Senegal), and Dr. John Nkegasong (Cote d'Ivoire) for their technical contributions.

Abbreviations

CDC	Centers for Disease Control and Prevention
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunoassay
EQA	External Quality Assessment
HIV	Human Immunodeficiency Virus
PMTCT	Prevention of Mother to Child Transmission
NAP	National AIDS Program
NPV	Negative Predictive Value
NRL	National Reference Laboratory
POS	Point of Service
PPV	Positive Predictive Value
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
Se	Sensitivity
Sp	Specificity
SOP	Standard Operating Procedure
VCT	Voluntary Counseling and Testing
WB	Western Blot
WHO	World Health Organization
WHO/AFRO	World Health Organization — Regional Office for Africa

Definition of Terms

Algorithm — The sequence in which assays are performed to detect HIV antibody in a body fluid.

Confidence Interval — An 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 — A process for determining whether a test system meets defined needs in the potential user's environment.

Evaluation Panel — Specimens that are used during the evaluation for which the serostatus 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 panels, or blind rechecking. EQA also includes on—site evaluation of the laboratory to review the quality of test performance and operations.

Gold Standard — A country defined algorithm for determining a sample's true serostatus.

National Reference Laboratory — A nationally recognized laboratory with appropriate testing capabilities and facilities for performing or providing access to confirmatory HIV testing sufficient to determine HIV status.

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

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

Prevalence — The percentage of persons in a given population with a disease or condition at a given point in time.

Proficiency testing panel — A set of approximately 3-5 samples with known values used to assess the performance capabilities of testing personnel.

Quality Assurance — Planned and systematic activities to provide adequate confidence that requirements for quality will be met.

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

Reference Panel — Aliquotted, stable serum or plasma specimens that have been highly characterized; known cutoff points, subtype, titer, etc.

Sensitivity of a test — A measure of the probability for correctly identifying an HIV-infected person.

Serum Library — A source of serum specimens from which a panel is drawn for evaluation purposes.

Specificity of a test — A measure of the probability of correctly identifying an HIV-uninfected person.

Testing strategy — The 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.

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

Executive Summary

Ensuring the quality of HIV testing in support of prevention and care efforts has been identified as a priority by the U.S. Centers for Disease Control and Prevention (CDC) and the World Health Organization/African Regional Office (WHO/AFRO). Rapid/simple HIV tests are marketed widely, and promoted for use by a variety of HIV/AIDS prevention strategies such as voluntary counseling and testing (VCT) and prevention of mother to child transmission (MTCT). It is vitally important that before these and other HIV assays are utilized, countries evaluate the performance of each assay to determine its performance characteristics and suitability for use within a given country setting. This evaluation is considered a critical aspect of assuring the quality of test results, and all countries must make this a priority.

This document is intended to provide those involved with planning or conducting any aspect of test evaluations practical guidance for developing country-specific protocols for conducting evaluations of HIV EIA and rapid/simple test methods. As test evaluations require both time and resources, specific guidance is given on the rationale and justification for evaluating new tests, issues to consider when planning an evaluation, and projected timeline for an evaluation. Detailed descriptions of phases of the evaluation quality assurance, evaluation materials, e.g., specimens, and laboratory safety precautions are also presented in this document.

1.0 Background

1.1 Serodiagnosis of HIV

Africa is the continent most affected with human immunodeficiency virus (HIV) epidemic: of the estimated 40 million persons infected with HIV in the world by the year 2001, 28 million live in Africa [1]. HIV antibody testing is critical for controlling the epidemic because it is the critical entry point for both prevention and care efforts for HIV/AIDS. For instance, short-course regimen of antiviral therapeutics administered to HIV-infected pregnant women reduces rates of transmission of HIV-1 from infected mothers to infants by 38% to 50% [2,3, 4, 5, 6]. Also, cotrimoxazole administered together with standard tuberculosis therapy reduces mortality and morbidity by 40 - 45% among HIV- infected tuberculosis patients [7]. For HIV-infected persons to benefit from such therapies, they must be diagnosed appropriately. Serologic 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 enzyme immunosorbent assays (EIAs) or simple/rapid immuno-diagnostics are selected for their high sensitivity of detecting antibodies to HIV. Supplemental or confirmatory tests, such as Western blot (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.

1.2 EIAs

EIAs are the most widely used screening tests because of their suitability for analyzing large numbers of specimens, particularly in blood screening centers. Since 1985, EIAs have progressed considerably from first to fourth generation assays: first generation assays were based on purified HIV whole viral lysates, however, sensitivity and specificity of these assays were poor; second generation assays used HIV-recombinant proteins and/or synthetic peptides, which enabled the production of assays capable of detecting HIV-1 and HIV-2. The assays had improved specificity, although their overall sensitivity was similar to that of first-generation assays. Third-generation assays used the solid phase coated with recombinant antigens and /or peptides and similar recombinant antigens and peptides conjugated to a detection enzyme or hapten that could detect HIV-specific antibodies bound to a solid phase. These assays could detect immunoglobulin M, early antibodies to HIV, in addition to IgG, thus resulting in a reduction of the seroconversion window. Fourth generation assays are very similar to third-generations tests but have the ability to detect simultaneously HIV antibodies and antigens. Typical fourth-generation EIAs incorporate cocktails of HIV-1 group M (HIV-1 p24, HIV-1 gp160), HIV-1 group O, and HIV-2 antigens (HIV-2 env peptide). Furthermore, third and fourth-generation assays are able to detect IgM and IgG antibodies to both HIV-1 and HIV-2. These assays may reduce the 2-4 week time period or “window period” of detecting HIV antibodies.

1.3 Rapid/Simple assays

Simple, instrument-free assays are also available and are now widely used in Africa. They include agglutination, immunofiltration, and immunochromatographic assays. The appearance of a colored dot or line, or an agglutination pattern indicates a positive result. Most of these tests

can be performed in less than 20 minutes, and are therefore called simple/rapid assays. Some simple tests, such as agglutination assays, are less rapid and may require about 30 minutes to 2 hours to be completed. In general, these rapid/simple tests are most suitable for use in settings that have limited facilities and process fewer than 100 samples per day.

1.4 Importance of rapid/simple assays

Although EIA–based serodiagnostic algorithms are highly cost effective, their application in resource-poor settings is limited by several factors. They require well-trained personnel, need a consistent supply of electricity, and maintenance and cost of most equipment. Rapid assays have high sensitivity and specificity and perform as well as EIAs on specimens from persons seroconverting for non-B HIV-1 subtypes [8]. Rapid enzyme assays circumvent the issue of low rates of return for serologic results associated with EIA-based testing algorithms because results can be delivered on the same day. In addition, their performance has improved considerably, and some do not require reconstitution of reagents or refrigeration; thus, making them very suitable for use in resource limited settings and hard to reach populations. Practical applications for the use of simple/rapid assays are in settings such as Voluntary Counseling and Testing (VCT) and Prevention of Mother to Child Transmission (PMTCT) programs. Studies have shown that using rapid assay testing algorithms result in remarkable increase in the number of HIV-positive women identified as eligible to receive the short-course therapy that reduces mother-to-child transmission of HIV [9].

1.5 Synopsis of HIV Testing

A testing algorithm for serologic diagnosis of HIV-infection is the sequence in which assays are performed to detect HIV antibody in a body fluid. The most common referenced testing algorithm employs an EIA to screen specimens with those found to be positive then confirmed by WB testing. This so-called conventional algorithm has several limitations:

- WB is expensive and requires technical expertise
- WB often yields indeterminate results with certain types of specimens with uncertain diagnostic significance, e.g., hyperimmunoglobulinemia specimens
- Both ELISA and WB are time consuming and require a well-equipped laboratory infrastructure

Several alternative testing algorithms exist for the serologic diagnosis of HIV infection that are based on a combination of screening assays, without using WB. In a parallel testing algorithm, sera are simultaneously tested by two assays. In the serial algorithm all specimens are tested by a first test that is highly sensitive. Specimens are considered as true negative if they react negatively in the first test. Specimens that are reactive in this assay are retested by a second EIA that has a high specificity. Parallel testing algorithms are often used in the clinic setting, such as with rapid assays using whole blood fingerstick specimens, to avoid requesting a second specimen from the client when the first test is HIV reactive. Serial algorithms may be more cost effective and convenient when sufficient specimen, such as with a venipuncture, is available to perform additional tests when the initial test is HIV reactive.

These algorithms maintain accuracy and minimize cost. Most of these algorithms have been evaluated in field conditions in Africa and found to be highly effective. Regardless of the testing algorithm (Appendix A), the first test must be highly sensitive and the second should be highly specific.

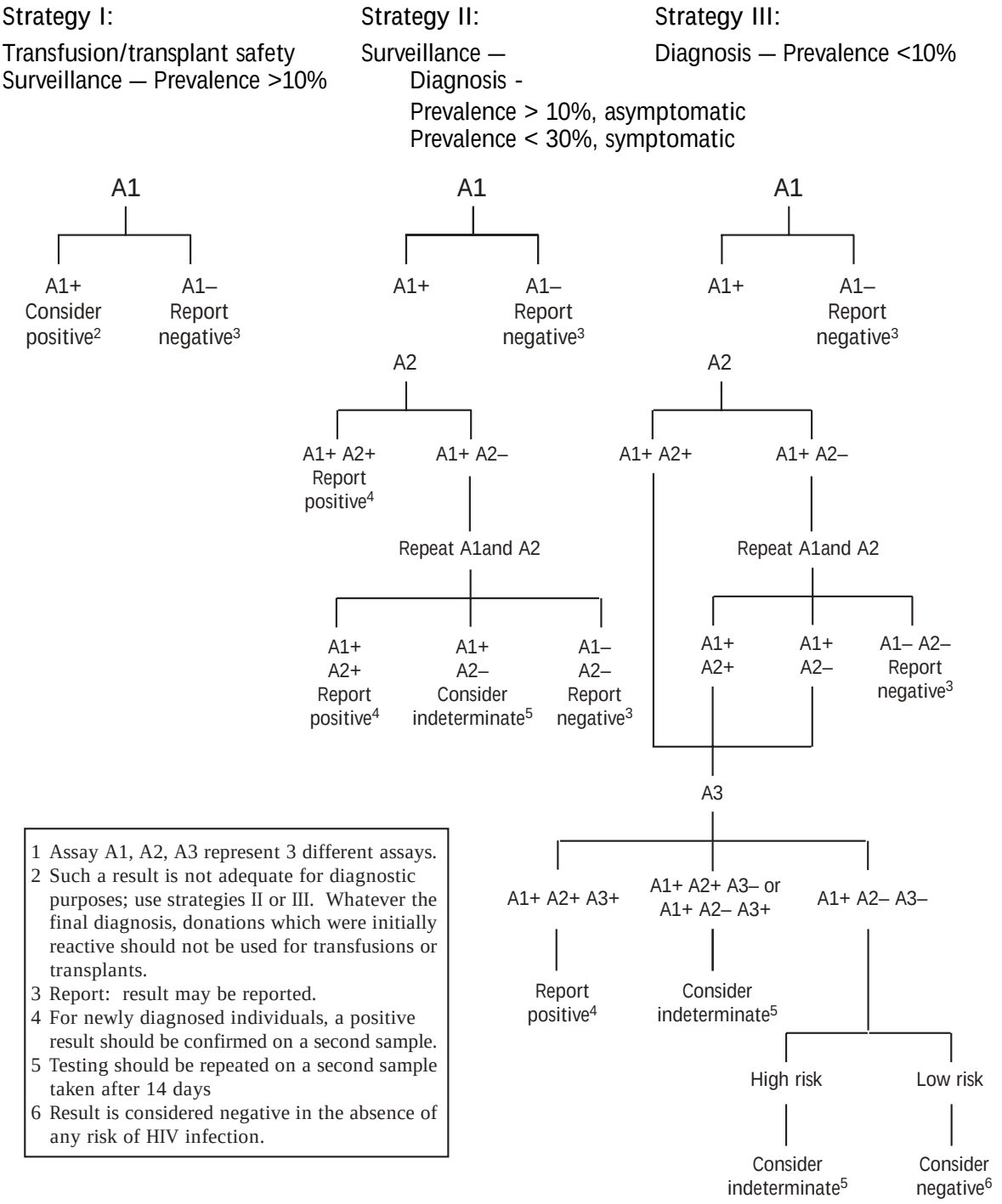
1.5.1 WHO/UNAIDS testing strategies

In considering both serial and parallel testing algorithms, WHO and UNAIDS have recommended three testing strategies (figure 1). Criteria for choosing the appropriate HIV testing strategy (Appendix B) include:

1. Objective of the test (diagnosis, surveillance, blood safety, or research),
2. Sensitivity and specificity of the test(s) being used, and
3. HIV prevalence in the population being tested

Potential testing strategies based on data from several countries can be found in Appendix C. Information on the manufacturers of rapid test kits can be found in Appendix D.

Figure 1. Schematic representation of the WHO/UNAIDS HIV testing strategies



1 Assay A1, A2, A3 represent 3 different assays.
2 Such a result is not adequate for diagnostic purposes; use strategies II or III. Whatever the final diagnosis, donations which were initially reactive should not be used for transfusions or transplants.
3 Report: result may be reported.
4 For newly diagnosed individuals, a positive result should be confirmed on a second sample.
5 Testing should be repeated on a second sample taken after 14 days
6 Result is considered negative in the absence of any risk of HIV infection.

2.0 Rational and Justifications for Conducting Test Evaluations

2.1 Rationale for evaluating assays in Africa

HIV testing algorithms involving the use of supplemental assays such as Western blot (WB) or line immunoassay (LIAs) to confirm infection in samples that are initially reactive on EIA conventional algorithms are still impractical in most African countries due to the high cost of the supplemental assays, long turnaround time, and difficulties related to interpreting WB and EIA strips. To circumvent these limitations, reliable and less expensive HIV serodiagnostic algorithms have been evaluated and shown to be as sensitive and specific as the conventional algorithm [10, 11, 12, 13, 14, 15]. For the testing algorithms to be effective, assays employed in them must be highly sensitive and specific within the context of the HIV situation in each country.

A high degree of genetic diversity exists in several countries in Africa [16]. For instance, HIV-1 circulating recombinant form (CRF_02), and HIV-2 predominate the epidemic in West Africa. In Central Africa, a mixture of subtypes, CRFs, group O and N exists. In East Africa, subtypes A, C, and D predominate; and in Southern Africa, subtype C is most frequent. Although rapid tests continue to improve like EIAs, antigens used for these assays were originally derived from HIV-1 subtype B viruses. Thus, the existence of newly identified aberrant HIV variants in Africa coupled with the high degree of genetic diversity of HIV has historically posed a challenge, especially for persons during early seroconversion. Indeed, some studies have shown a significantly lower sensitivity of some screening assays to detect non-B subtypes antibodies during seroconversion [17]. Moreover, several EIAs were withdrawn from circulation when it was shown that some variants of HIV-1 group O viruses were missed by these assays.

2.2 Justification for evaluating new HIV tests kits

There are many reasons to perform evaluations of HIV tests. Many countries are performing evaluations to determine an algorithm of simple rapid tests that can be used at the point-of-service for VCT, PMTCT, and surveillance. If a country has 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 evaluate 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.

- 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 example, if a test or algorithm has proven efficacy (Se and Sp) within the immediate region, then a country may decide to start the evaluation in the point of service (POS) setting rather than an initial full scale laboratory-based evaluation. Other circumstances requiring a limited evaluation at the POS include revising the order of tests within an approved algorithm or replacing a single test within the algorithm.

Countries should resist pressures to evaluate products solely for in country marketing concerns. 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.

3.0 Laboratory Quality Assurance (QA) and Safety

3.1 Importance of Quality Assurance

Laboratory Quality Assurance (QA) is defined as planned and systematic activities to provide adequate confidence that requirements for quality will be met. It is critical that each facility performing laboratory testing establishes and implements a QA program to monitor and evaluate laboratory functions and services throughout the total testing process. The total testing process is comprised of the pre-analytical, analytical, and post-analytical phases of laboratory testing. Specific activities (although not all inclusive) of the total testing process related to evaluations are outlined below.

Pre-Analytical phase encompasses the following components:

- Test request
- Test selection
- Trained testing personnel
- Patient/client preparation
- Specimen collection, labeling, and transport

Analytical Phase

- Specimen processing and storage
- Reagent preparation
- Preventative 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

Written polices and procedures for each activity will assist in continually assessing the total testing process for areas needing improvement, in identifying problems, and in having defined mechanisms to prevent the reoccurrence of problems. A successful QA program will need the support of the National Reference Lab and requirements should be rigorously complied with to ensure the accuracy of the results from the evaluation and all other assays. Comprehensive QA program guidance is beyond the scope of this document and can be found in an internationally accepted quality management document, e.g., ISO 15189 –Quality Laboratory Management.

3.2 Quality control (QC)

Quality control (QC) refers to those measures that are taken to monitor the quality of the assay itself. QC may 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 (EQA) / Proficiency Testing

Every testing facility must at any time be ready to demonstrate and document its competence in performing HIV serology that is carried out as part of its routine services. External Quality Assessment (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 of a program to evaluate laboratory performance:

- On-site Evaluation
- Proficiency Testing
- Blinded Rechecking

The choices for which type of EQA program to implement will depend on both the available resources and the ability to obtain additional resources as needed to support the EQA program.

Additional information on proficiency testing and the use of Dried Blood Spots (DBS) as a form of EQA are highlighted in section 5.6.2 (Phase III: Implementation and Monitoring of Test Performance - EQA).

3.4 Safety Precautions

Each laboratory or testing site must follow Universal (Standard) Precautions designed to prevent transmission of HIV, hepatitis B virus (HBV), and other bloodborne pathogens. When laboratories adhere to universal precautions, blood and certain body fluids of all patients are considered potentially infectious for HIV, HBV and other bloodborne pathogens. Refer to Appendix E for safety rules [18] that should be followed when working in the laboratory.

4.0 Planning an Evaluation

4.1 Responsibilities of a National Reference Laboratory

The Ministry of Health (MOH) and the national authority responsible for HIV/AIDS control, e.g., National AIDS Control Program (NACP), should designate a National Reference Laboratory (NRL) or other recognized laboratory in the country that is assigned overall responsibility for coordinating and conducting evaluations of HIV tests. The NRL should work closely with the National AIDS control authorities in each country to ensure coordination of efforts and activities. Each country will need to evaluate its support structure and available resources in order to determine the most effective way to conduct the evaluations.

Responsibilities of the NRL

The NRL should:

- Be mandated by the government to either coordinate or perform test evaluations
- Have sufficient resources to conduct or oversee country test evaluations
- Strive to adhere to internationally recognized quality standards, e.g., ISO 15189; Quality management in the medical laboratory, UK Standards for the Medical Laboratory, etc.
- Advise the government about making recommendations and setting policy
- Maintain existing reference methods, such as EIA, and perform or provide access to additional reference methods, e.g., WB, PCR, etc.
- Support the NACP and other laboratories meet the increased need for simple/rapid tests in an environment of decentralization
- Establish and oversee implementation of a national QA program for HIV testing
- Write standard operating procedures for distribution to all testing sites
- Characterize and maintain evaluation and reference panels

4.2 Program coordination

Evaluation of HIV test kits should always be coordinated with the NACP and any other organizations that will be using the tests and/or results. Program staff should help pre-select test methods, especially if rapid tests are being evaluated for use at POS locations and non-laboratory staff might perform tests. Shared decisions in the planning stages might include the costs of tests, test result reporting, ease of use, storage, data-sharing mechanisms, in addition to test performance characteristics.

4.3 Funding considerations

Evaluation of tests will require funding over and above the normal operating costs of performing diagnostic testing. One component of planning involves developing an itemized budget for each additional cost and ensuring that funds are available before initiating an evaluation. The itemized budget should include estimates for the additional test kits, supplies, any necessary equipment for testing or storage, transport of specimens during field-testing, and any additional staff costs (Appendix - F).

4.4 Test Selection Criteria for Country-level Evaluations

Having appropriate justification for conducting test evaluations, guidelines for selecting assays for evaluation include:

- Assays that have been previously reviewed by WHO, CDC or other independent international organizations with relevant expertise
- Published regional test performance data from:
  Journal Publications
  WHO/UNAIDS
  Manufacturer-provided data
  Websites: WHO/AFRO – www.AFRO.WHO.INT
  CDC – http://www.phppo.cdc.gov/DLS/default.asp
- Documented ability of the test to detect HIV-1 (group M and O) and HIV-2
- Documented ability to detect IgG and IgM antibodies
- Cost per test and possibility for bulk purchase
- Storage requirements
- Equipment and maintenance requirement
- Required technical skill
- Ease of use; Simplicity of test procedure
- Experience with the assay(s)
- Availability
- Shelf-life and robustness
- Service and trouble-shooting provided locally by manufacturers
- Laboratory infrastructure

4.5 Overview of Planning Activities and Timeline

The following list of activities and timeline (figure # 2) represents a typical process for conducting laboratory test evaluations. Details of each phase of the evaluation are explained in detail in section 5.0 (Conducting the Evaluation). Sample contents of and evaluation protocol can be found in Appendix G.

- Determine capacity to conduct evaluations
- List kits available in country and/or kits approached to evaluate
- Conduct literature and data review
- Conduct situation analysis
- Conduct needs analysis
  Select kits worth assessing
  Conduct consensus meetings to gain cooperation of stakeholders
- Develop Evaluation Protocol

- Obtain ethics clearance
- Procure kits, supplies, etc.
- Conduct Training
  Clinic and lab staff
- Pilot test logistics of plan
- Implement phase I
- Evaluate phase I
- Analyze phase I data
- Decide which kits to use in phase II / Determine algorithm
- Publish phase I findings
- Select sites for phase II
- Implement phase II
- Evaluate phase II
- Analyze phase II findings
- Decide which kits/ algorithm to use in the country/setting
- Publish phase II findings
- Implement phase III = ongoing monitoring
  Build capacity for this during phase I and II trials

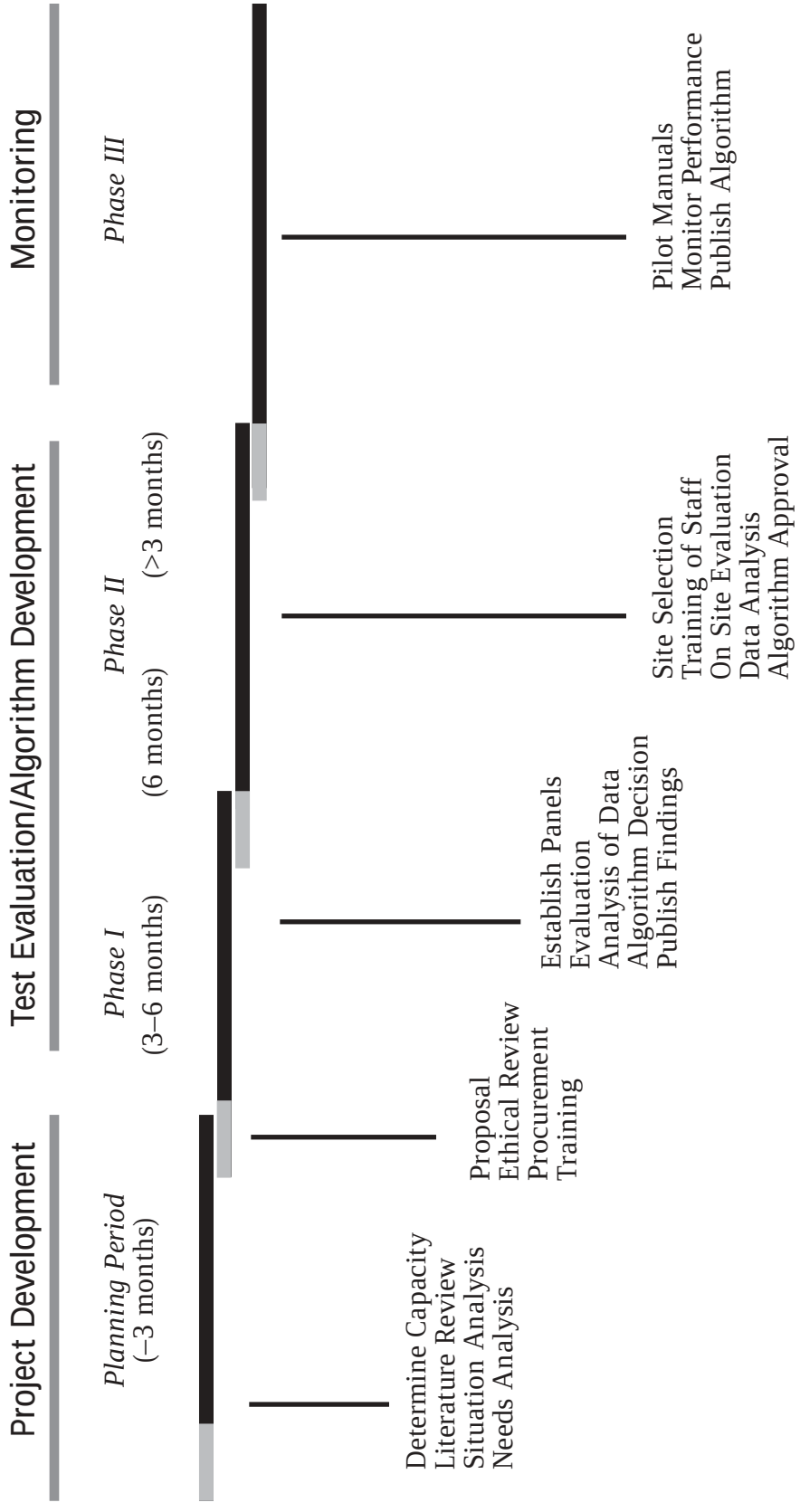
4.6 Technical Training Requirements:

Training should be provided for laboratory and POS testing staff, ideally at the site in which testing will occur, rather than at a centralized venue. Training should also be provided for assessors responsible for monitoring EQA activities of testing sites.

Every effort should be made to ensure continuity of training throughout the evaluation process through the use of documented processes and procedures. In addition to performance of assays, the training should include QA, QC, data management, and laboratory safety. Organizers of the training should ensure availability of training venue, test kits, supplies and samples.

Expansion of training activities is further addressed in section 5.6.1 (Phase III – Training Requirements).

Figure 2


Project Development
Test Evaluation/Algorithm Development
Monitoring
Planning Period
(–3 months)
Phase I
(3–6 months)
Phase II
(6 months)
(>3 months)
Phase III
Determine Capacity
Literature Review
Situation Analysis
Needs Analysis
Proposal
Ethical Review
Procurement
Training
Establish Panels
Evaluation
Analysis of Data
Algorithm Decision
Publish Findings
Site Selection
Training of Staff
On Site Evaluation
Data Analysis
Algorithm Approval
Pilot Manuals
Monitor Performance
Publish Algorithm

5.0 Conducting the Evaluation

5.1 Overview evaluation phases

Evaluation of HIV testing performance is an ongoing process that begins prior to implementation of testing and continues after tests have been implemented in the field. The evaluation process is divided into three phases. Although these phases can apply to evaluation of any HIV tests using serum, plasma, saliva, or whole blood, for the purposes of this document, emphasis is focused on evaluating rapid test methods that can be used in the POS setting with whole blood specimens. Evaluation of rapid tests for use in the POS setting is usually more complex than evaluations of standard EIA formats that can be tested in parallel with the existing EIA in a laboratory setting.

Phase I is a laboratory-based evaluation to provide preliminary results on test performance characteristics (Se, Sp) on the same set of samples. Having evaluated the same sample set that may consist of 4-7 rapid tests, an algorithm of 2-3 tests may then be proposed based on the performance of the combination of test methods.

Phase II involves evaluation of the selected algorithm under field conditions that may include test performance and interpretation by non-laboratory clinic staff. Phase II is often referred to as the field trials, and typically is conducted in at least 2-3 POS sites. Tests under evaluation in this phase should be performed in the same manner in which it is to be used, e.g., finger stick specimens.

Phase III represents ongoing evaluation of performance through EQA programs that not only monitor the performance of individual clinic and/or staff, but also provide aggregate data for ongoing assessment of test performance.

5.2 Objectives of Evaluation Phases

Objectives of Phase I:

- Provide preliminary performance characteristics on tests under evaluation
- Develop a panel of well-characterized serum for future use
- Review performance of each test combination to develop 2-3 test algorithm

Objectives of Phase II:

- Evaluate the performance of the 2-3 test algorithm in the POS setting
- Perform a demonstration study in selected sites and conditions that will provide a reasonable/reliable indication of how the testing methods and algorithm will perform when implementation is expanded to multiple sites through the country

Objectives of Phase III:

- Ensure each new testing site has appropriate training and preliminary observation of performance prior to reporting results
- Assess clinic/ staff performance through EQA
- Monitor aggregate test performance

5.3 Evaluation Scenarios

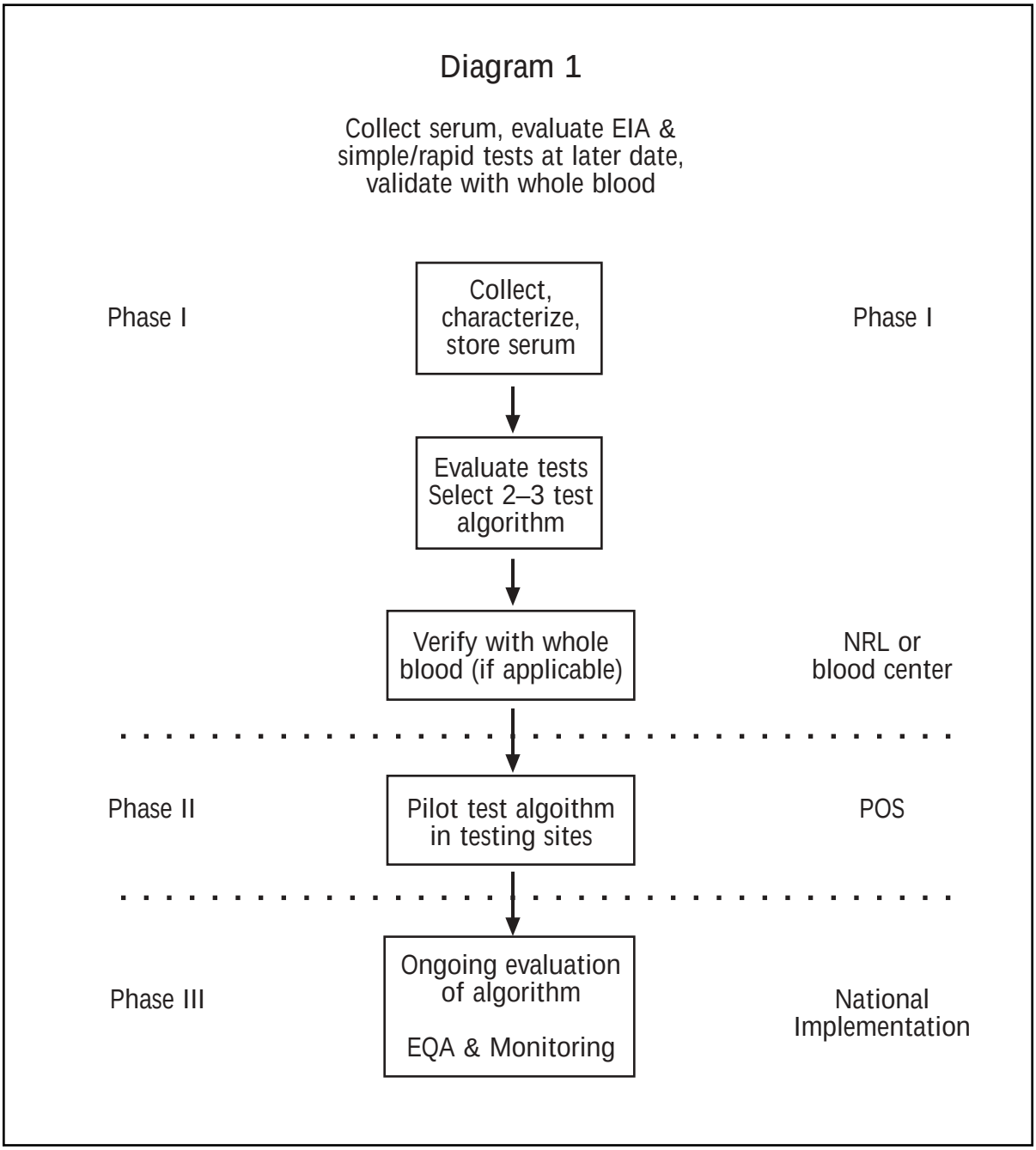


Diagram 1 is a scenario in which the NRL is preparing for the evaluation by collecting, characterizing and storing serum specimens for later evaluation. This allows the NRL to collect and store approximately 500 specimens over a period of weeks to months and then separately evaluate several new tests in a few days.

Advantages

- The NRL can pick and choose the appropriate number of positive and negative specimens for evaluating tests from all the specimens received over time.
- This scenario avoids unnecessary testing of excess negatives or specimens that cannot be characterized with the tests that are under evaluation.
- The evaluation panel can be collected without making major changes to the laboratory workload.
- Multiple tests can be evaluated with stored sera in a short time (e.g., <1 week).

Disadvantages

- Evaluation with stored serum may be sub-optimal, as additional requirements must be met for sample preparation and storage, and different performance characteristics may result in testing fresh sera compared with testing stored sera.
- Different performance characteristics may be observed with whole blood after initial evaluation with serum.
- For whole blood-based rapid tests, an additional step using whole blood to provide preliminary validation data of performance characteristics is required before implementing phase II.
- The laboratory must have sufficient resources to meet quality standards for storing specimens. At a minimum, a subset of stored specimens should be retested to ensure validity of earlier results. If any deviation is found in the subset of re-tested specimens, then all stored samples must be retested.

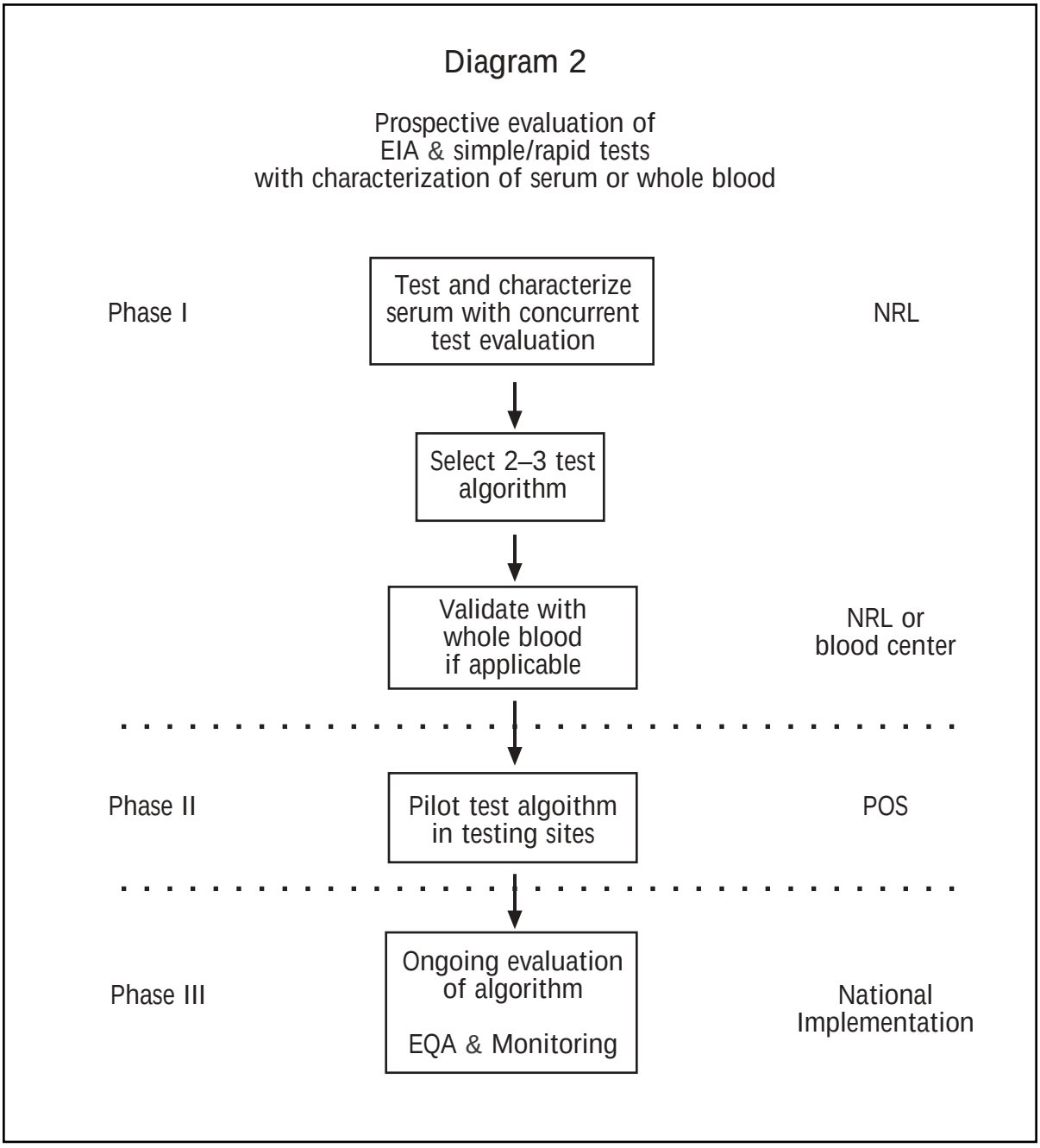


Diagram 2 is a scenario in which the tests being evaluated are performed concurrently with standard test methods. This scenario still represents the use of serum due to limited availability and logistical difficulty in transporting whole blood to the NRL. Since the tests are performed concurrently, less is required for managing the storage and retrieval of specimens.

Advantages

- By testing fresh sera, the NRL avoids the necessity of previous rigid requirements for aliquoting and storing specimens prior to beginning the evaluation.
- Since evaluation tests are performed concurrently, there will be earlier indications of unacceptable performance. Given these early indicators, one may stop evaluation of tests as soon as a statistically significant sample size is reached.

Disadvantages

- In a lower prevalence setting, the laboratory may have to perform preliminary tests on excess negatives; leading to an increase in the length of time before phase I is completed.
- Evaluation with stored sera may be sub-optimal for whole blood-based rapid tests. There is the possibility of observing different performance characteristics when used with whole blood in phase II.
- For whole blood-based rapid tests, an additional step is required to provide preliminary validation of performance characteristics data before implementing phase II.

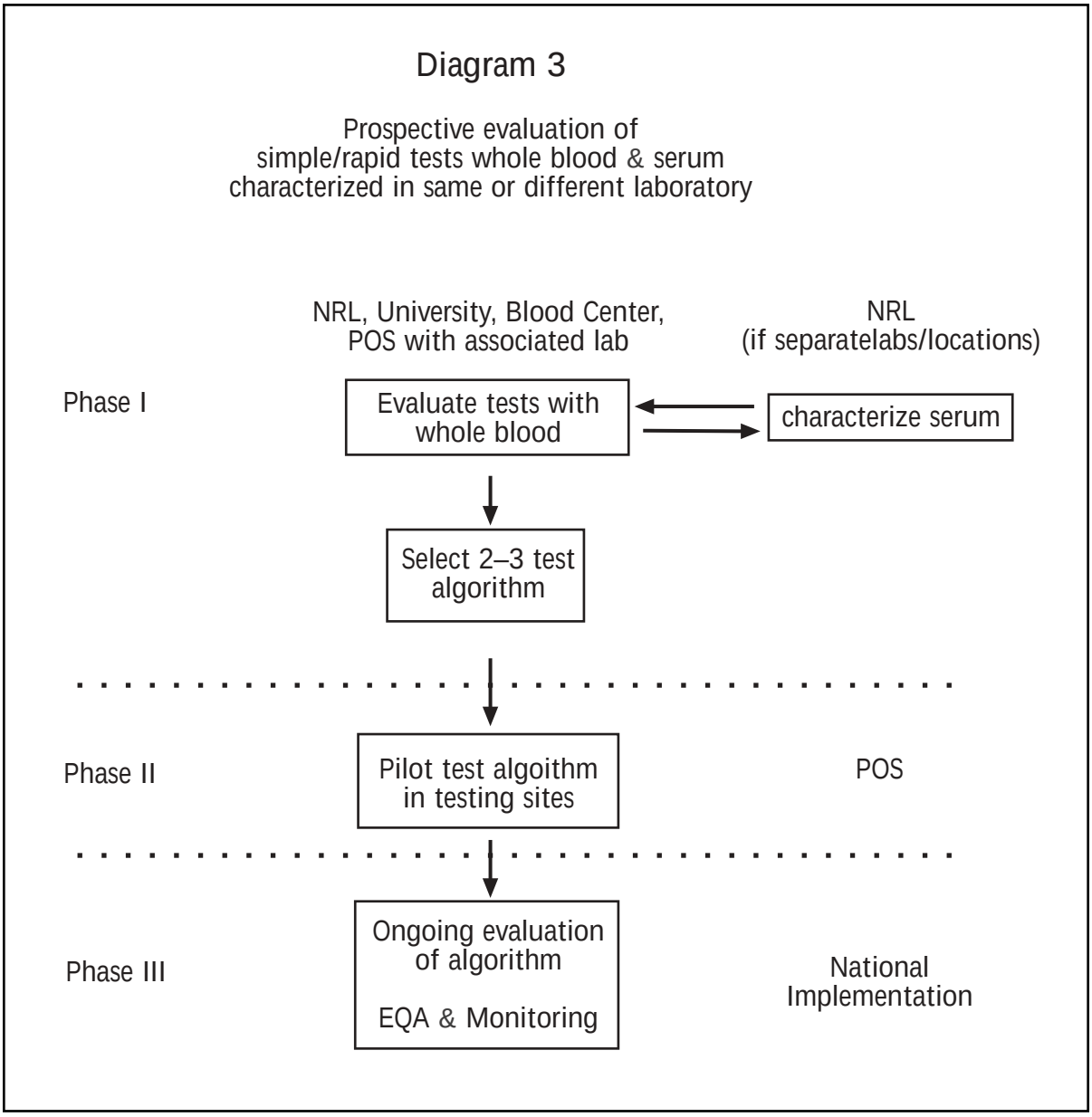


Diagram 3 is a scenario in which the laboratory can perform a concurrent prospective evaluation using whole blood prior to characterizing the serum with the gold standard methods. This type of evaluation is possible when there are laboratory resources to perform 3-5 tests in a clinic setting, such as blood center, where whole blood is immediately available.

Advantages

- Using whole blood to evaluate rapid tests that will be used in POS settings with whole blood is the best method in directly determining the performance characteristics and selecting an algorithm
- This scenario negates the need for an additional step of validating using whole blood specimens

Disadvantages

- The whole blood is often retrieved from a venous sample and may not mimic all the aspects of test performance when used with a fingerstick specimen
- Performing a concurrent evaluation of several tests with whole blood may be logistically difficult due to requiring a laboratory in the POS setting
- Performing 3-4 tests concurrently directly from fingersticks may be logistically difficult, particularly if non-lab staff are performing tests

5.4 Phase I: Laboratory Evaluation

5.4.1 Use of Stored Serum

Fresh sera are the preferred specimens for evaluation of serum-based tests and preliminary evaluation of whole blood tests when whole blood is not immediately available. If sera are frozen before the evaluation, there should be some standards and practices to ensure that the quality of the thawed serum has not been impaired by freeze/thawing, contamination, excess particulate matter, etc. The sera should be aliquoted in separate vials to avoid multiple freezing/thawing. For monitoring the quality of frozen storage, a percentage of specimens should be retested with standard tests prior to performing the evaluation to ensure that test results do not change.

5.4.2 Sample Size

A test evaluation should include a minimum of approximately 200 HIV-positive and 200 HIV-negative specimens to provide 95% confidence intervals of less than ± 2% for both the estimated sensitivity and specificity. Lower numbers of HIV-positive and HIV negatives specimens may be used, but this will increase the confidence interval for sensitivity and specificity. The total number of specimens included in the evaluation will depend on whether the HIV reactivity of the specimens is known prior to evaluating the test. In a prospective evaluation, such as using whole blood in the clinic setting where the HIV reactivity is unknown, the evaluation would be performed until a minimum of 200 positives are obtained. For instance, in a setting with 20% prevalence this might require testing upwards of 1000 specimens until 200 positives are obtained (Appendix H). In a laboratory-based evaluation where the HIV reactivity of specimens is known, such as with previously tested and stored serum or plasma specimens, it is preferable from a cost perspective to select 200 HIV-positives and 200 HIV- negatives.

When a whole blood or serum-based rapid/simple test is initially evaluated in phase 1 with serum, then an additional validation step is required to provide some reassurance that the

performance in whole blood is similar to that obtained in serum before initiating a more extensive evaluation in Phase 2. This assessment does not need to be as extensive as the serum-based evaluation, The test methods representing a 2-3 test algorithm should be validated with 50-100 whole blood specimens (containing a minimum of 20 positives).

5.4.3 Sample Population

Selecting the sample population for a test evaluation will include several considerations. Although there are considerations of having a sample that is representative of the various areas of the country, this may not be feasible in phase I when the NRL is limited to available specimens. If specific concerns exist about how HIV-1 and HIV-2 or specific subtypes are distributed, these might be addressed by selected specimens in a panel. In most instances the primary goal should be selecting a population with a high prevalence of infection to obtain a sufficient number of positives.

5.5 Phase II – Field Evaluation / Pilot testing

5.5.1 Number of Sites

In Phase II, the selecting testing sites from different areas of the country should be balanced with the need and logistics of monitoring on-site testing and transport of specimens to the NRL for characterization by the gold standard method. At a minimum, 2-3 sites should be considered for inclusion in Phase II of the evaluation. Some larger countries may need to consider up to 4-5 sites that are implemented sequentially to allow for training at each site. Managing the logistics of transporting specimens and reporting may be difficult with more than 3 sites.

5.5.2 Sample Size

The same sample size for Phase II evaluation should be used as in phase I. This will require finding a sufficient number of field test sites with high prevalence to obtain the minimum of 200 positives distributed across all sites.

5.5.3 Sample Population

If a country has specific concerns about having a representative population for test evaluation, these should be addressed through the selection of testing sites in Phase II. The primary concern should be about representative testing conditions.

5.6 Phase III – Implementation and Monitoring

5.6.1 Training Requirements

When tests and algorithms have been evaluated in phase II and considered acceptable, there is continued need to provide training and support for systematic implementation in additional sites. The NRL and NAP must develop a plan than involves training and evaluation of staff at new sites prior to reporting results to patients. In many cases, implementation will involve merging testing practices, evaluation, and quality assessment into counseling programs and settings.

Training all staff, laboratory or non-laboratory, who will perform the test(s) is a necessary and important prerequisite to expanding the testing sites. Training topics should include at a minimum, test performance, quality control, safety, and also include some measure of test performance with standard competency proficiency panels established by the NRL. Successful participants should receive a certificate acknowledging their competency. The certificate, however, should recognize that the training and competency are limited to specific tests performed during the training.

Every new testing site should receive a laboratory visit that combines training and evaluation by observation. This visit should be a standard component of implementation and occur before any patient test results are reported. Each site should be provided with SOPs for testing either during training or as part of the initial visit. When appropriate, the NRL should provide control materials for the specific tests.

Initial evaluation of the pInitial evaluation of the performance of testing personnel

The performance of individuals at each site should be evaluated before results are reported. For rapid tests, this should involve taking an additional venous sample on the first 50-100 patients and comparing the rapid test results obtained in the POS with the standard EIA results. The results reported to the patient/client should be based on results from the standard EIA.

5.6.2 EQA

There should be at least one or more methods available to assess the quality of testing within a country. This should include every NRL establishing a program for monitoring different manufactured lots of tests kits that are received/purchased by the country. This will require using a standard reference panel to assess lot-to-lot performance for each individual test. Special consideration should be given to including weak positives for adequately assessing any lot-to-lot variations in test sensitivity.

5.6.2.1 Onsite Evaluation

External quality assessment programs should provide onsite evaluation of each testing site in addition to methods that will assess testing performance. Having onsite evaluation is necessary to review QC, record keeping, and observation of test performance. Additionally, this evaluation is an opportunity to directly administer a proficiency test to each individual performing testing during the visit. A program of onsite evaluation should include a standard checklist of laboratory indicators and evaluators should be trained to perform consistent reviews of laboratories and other POS sites. Standard checklists and evaluation methods allow for collecting and comparing consistent information from multiple sites.

5.6.2.2 Proficiency Testing

Proficiency testing (PT) is the most common form of EQA and involves development of specimen panels by the NRL for distributing to POS sites. Laboratories administering PT panels should strive to adhere to international guidelines, e.g., ISO Guide 43. There are standard methods available to develop PT samples and might be the easiest type of program for implementation at sites where serum-based tests are performed. The limitations of PT are that it usually involves only a few specimens and the test results may not represent the routine test performance. This may be due in part to the greater care in handling PT specimens.

5.6.2.3 Blinded Rechecking

Retesting selected specimens in a reference/referral laboratory may also assess the quality of testing. This can be accomplished by forwarding all positive and 10% of negative specimens for standard EIAs when a venous specimen is available. Another systematic sampling method may be considered to reduce the potential bias of selecting test specimens for referral.

5.6.2.4 Dried Blood Spots (DBS)

The use of dried blood spots (DBS) is one method that is being developed as EQA for whole blood tests where it may be impractical to refer specimens for additional testing or where there is limited or no access to serum PT specimens for monitoring test performance. The DBS are collected at the time of patient testing (e.g. fingerstick) on filter paper and easily transported to a reference laboratory. The use of DBS will require a reference laboratory that has demonstrated proficiency with eluting the DBS specimens and performing standard EIA methods. Additional concerns include the logistics and methods of collecting DBS in the testing protocol. Although a statistical sample of specimens re-tested by DBS based on testing volume may be desirable, this may be difficult to implement in the flow of testing and counseling of patients. Additionally, testing a percentage of specimens, such as 10% may be problematic. Countries may consider random sampling of DBS such as bimonthly, or at a given time or day. Further development of DBS protocols, proficiency testing and EQA guidelines are necessary to assist with the expansion and monitoring of rapid testing.

5.6.3 Remediation / Corrective Measures

When deficiencies are noted during on-site visits, corrective measures should be taken to ensure the quality of results. This may include additional training and discontinuation of services.

6.0 Evaluation Materials

6.1 Types of evaluation materials

These guidelines describe several types of evaluation panels that may differ by the composition of negatives and positives, and by degree of characterization. Specimen library is a term given to the source or collection of all specimens that may be selected and retrieved for evaluation purposes. In some instances, this might represent a large collection of stored sera from which a set of positives and negatives is selected and retested for inclusion in the evaluation. The specimen library could also represent all fresh specimens tested in the laboratory where only a subset of specimens is selected for evaluation.

The evaluation panel consists of those specimens that are tested by the gold standard method and evaluation test methods and included in calculating the sensitivity and specificity for individual tests and algorithms. The evaluation panel should usually consist at a minimum of 400-500 total specimens including at least 200 positives.

A laboratory may also have available several special reference panels. These panels may represent a collection of difficult or unusual specimens that provide a unique challenge to the tests being evaluated. Samples from uninfected and infected persons, which represent unusual screening results and have been further tested to resolve serostatus, may be used in the panel as challenges to the sensitivity and specificity of an assay under evaluation. Because the sensitivity of some antibody tests is less for sera collected early in HIV infection for persons infected with non-B subtypes, it is important to evaluate the assays on panels containing specimens from persons recently infected with the HIV-1 or HIV-2 subtypes circulating in the country.

Each specimen in the reference panel should be tested with multiple EIAs and positives confirmed with Western Blot and when possible, additional tests including p24, PCR, genotype, etc.

Because of repetitive use of reference panels during Phase II and Phase III, stability and storage of samples are critical. Samples should be aliquoted into storage vials and preferably frozen at -70^{o} (minimum standard is -20^{o} when molecular procedures are not used).

6.2 Specimen Collection and Handling

6.2.1 Specimen Collection

Plasma

Collect up to 10 ml of blood from the patient's vein into a sterile anticoagulated tube. Choice of anticoagulant should be appropriate to the test being evaluated according to the manufacturer's insert. Using an evacuated blood collection system is recommended for safety. The blood drawn is immediately mixed by gently inverting the tube 10 times. Shaking should be avoided to prevent hemolysis.

The specimen should be centrifuged at 300-400g for 10 minutes to separate the plasma. After centrifugation, the separated plasma should be withdrawn using a clean pipette and transferred to a storage tube. Ideally, specimens are prepared for storage in 0.5ml aliquots.

Serum

Collect up to 10 ml of blood from the patient's vein into a sterile serum separation tube, preferably an evacuated blood collection tube without anticoagulants. Again, shaking should be

avoided to prevent hemolysis. Let the blood stand for 20-30 minutes at room temperature to allow for clot formation. Serum can be separated from the clot by centrifugation at 300-400 g for 10 minutes. Alternatively, gently draw the serum off the clot using a sterile pipette. The serum can be subsequently clarified further by centrifugation at a remote site. Specimens should be prepared for storage in 0.5 ml aliquots.

Whole Blood

Collect up to 10 ml of blood from the patient's vein into a sterile tube containing an anticoagulant. Again, choice of anticoagulant should follow test manufacturer's recommendations. Immediately draw off sufficient quantities of whole blood to run the tests under evaluation. The remaining blood should be used for preparing of plasma as described above.

6.2.2 Transfer and Storage of Specimens

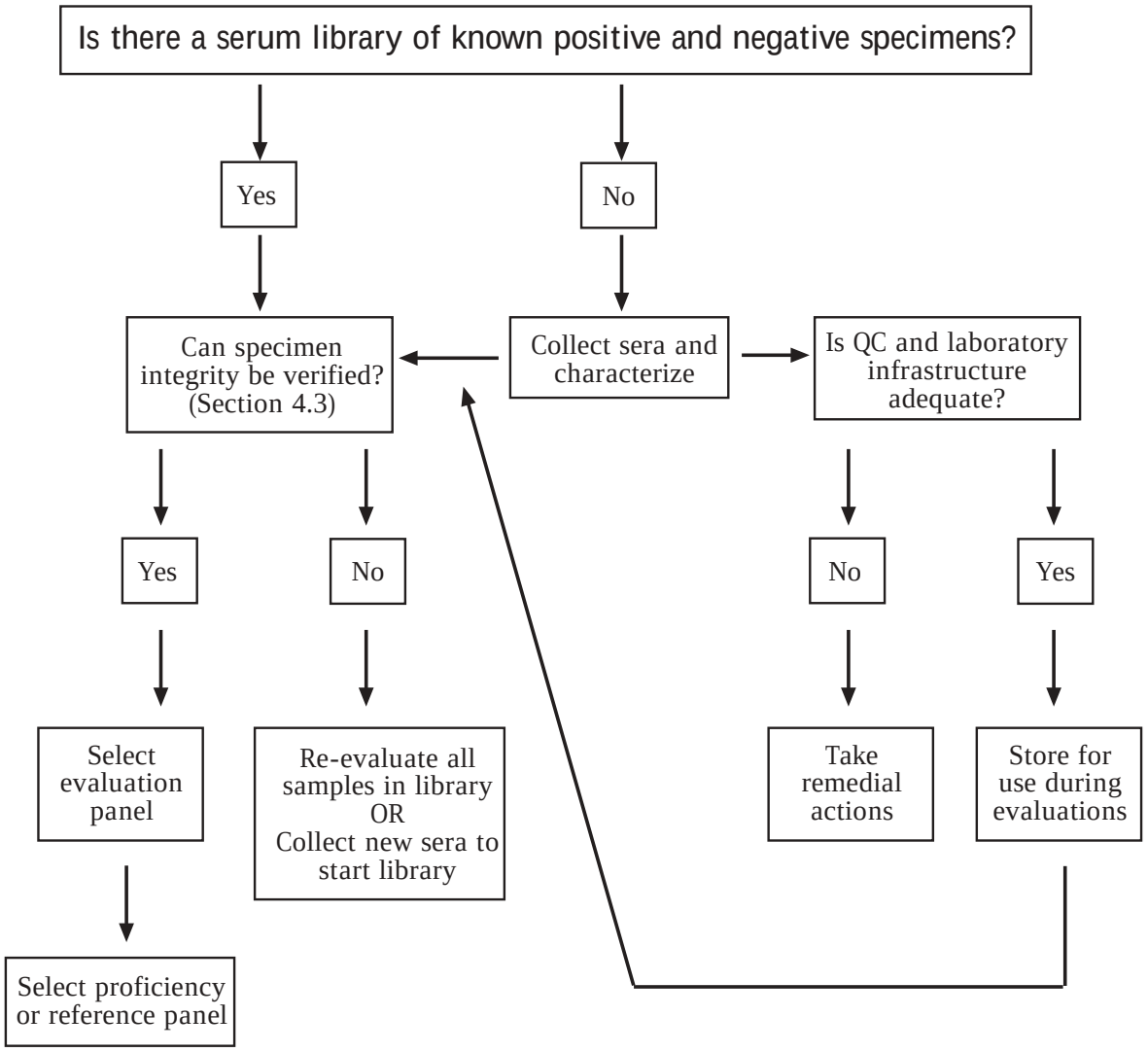
Ideally, aliquoted serum or plasma specimens should be stored immediately at –20^{o}C. If specimens are to be transferred to a central facility they should be maintained at 4^{o}C and shipped on cold packs to the storage site. If cold packs are not available, serum specimens can remain at room temperature for up to 3 days, whereas whole blood hemolyzes over time. Signed specimen transfer sheets should accompany specimens during shipment. Upon receipt at the central facility, specimens should be immediately transferred to a non-self-defrosting freezer for storage. Specimens should be stored uniformly aliquoted and stored in a polypropylene tube. Specimen identifiers should be labeled directly on the tube, and not on the screw-cap top. Specimen inventories should be maintained for storage freezers that are specifically reserved for reposited specimens. Every effort should be made to limit the number of freeze-thaw cycles, since repetitive thaws may result in loss of antibody titer and formation of serum flocculates. For long-term storage, specimens should be frozen at –70^{o}C.

It is important to store these specimens with all pertinent detailed information concerning specimens in a computer database or bound logbook, which is periodically updated to reflect specimen use or transfer. Maintaining this database and deposited specimens will facilitate additional evaluations at a later date.

6.2.3 To improve quality of sera for storage, the following steps may be followed:

- Centrifuge
- Pipette serum from clot rather than pouring the serum
- Filter the serum
- Make aliquots of serum to avoid multiple cycles of freezing and thawing
- Store at –70 degrees centigrade in non-self defrosting freezer
- Keep good daily freezer logs
- Exclude specimens that are:
  - Particulate
  - Lipemic
  - Hemolyzed
  - Contaminated with bacteria

6.3 Serum Library: Collection and use of stored serum



6.3.1 Characterization of Evaluation Panel

Characterization of the library of specimens used in the evaluation should be based on a multi-test algorithm that allows for establishing a gold standard to determine serostatus. Consideration should be given for confirmation of only the positive samples by the Western blot for the following reasons:

- Use of WB allows for characterization of sera to develop a panel for repeated use;
- Use of the WB is recommended to allow countries to share evaluation data that represent standard confirmation methods and a more complete and accurate characterization of specimens for evaluation.

Although some countries may currently evaluate tests using only an EIA algorithm, countries should strive to adopt the WB for standardization and to increase data sharing.

6.4 Trouble-shooting of problematic specimens

Occasionally, assays produce results that are difficult to interpret and are erroneous, which may be due to factors inherent with the specimens or clerical errors. If such results occur, consider the following:

- Check specimen integrity for evidence of bacteria contamination, hemolysis, and lipidic substances
- Verify labeling, paper-work, and procedures
- Re-check equipment and reagents
- Have the same technologist re-test the specimen
- Repeat testing blindly by another technologist
- Repeat on reference test blindly
- Repeat at different laboratory or reference laboratory
- Determine true status by other assays (PCR testing, p24)

7.0 Data Analysis

7.1 Data Management

Before collecting blood specimens, it is important to design a simple questionnaire and tracking records for specimen management, which should include a unique specimen number, date, and site of draw. They may also include limited demographic information such as age, sex, profession, and home district. Tracking documents should include an inventory of specimens being shipped, their origin, destination, and time and date of transfer. Also create a database that will allow the variables to be entered and linked with the associated specimen. Such variables will include the 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 such as WB pattern, and final determination of sero-status (positive or negative).

7.2 Resolving Discordants

There are two types of discordant results in an evaluation: One such discordant is a specimen that does not meet the criteria of positive or negative using the gold standard method/definition. Before the evaluation, the laboratory should determine the gold standard for positives and negatives. In the case of an evaluation this may differ from normal testing practices, such as the use of WB to confirm positive obtained in an evaluation setting. An example of a discordant result may be a specimen that is positive by EIA(s), but indeterminate on WB. In the case of prospective evaluation, the laboratory must ensure that the reason for the discordant is not sample mix-up or transcription error before deciding to perform additional testing to resolve these types of discordants, such as p24 antigen testing or PCR. Only the 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.

The second type of discordant result occurs when the result of the test(s) being evaluated differs from the result of the gold standard. An example might be a specimen that is negative with the gold standard algorithm of EIA(s), but positive on one or more of the tests being evaluated. Once again the laboratory may decide 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.

7.3 Sensitivity, Specificity, PPV, NPV, Confidence Interval, Delta value, Reproducibility, Inter-reader variability

Several key parameters need to be evaluated for each assay: sensitivity, specificity, positive and negative predictive values, and delta values. The sensitivity and specificity of each assay are calculated using the gold standard.

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).

Example:

Evaluation of a rapid test on a panel of specimens that have been tested by the gold standard is shown to contain HIV antibodies to 300 serum samples and no HIV antibodies to 200 samples (Figure 3). Of the 300 serum samples that were antibody positive, the rapid test classified 275 of the samples as positive. Of the 200 samples that were HIV antibody negative by the gold standard, 125 were classified by the rapid test as not containing HIV antibodies

Figure 3: Results of Evaluation Panel Using Gold Standard

		Gold Standard Results +	Gold Standard Results –	
Results of assay	+	A True-positives 275	B False positives 75	A + B 350
Under evaluation	-	C False-negatives 25	D True-negatives 125	C + D 150
		A + C = 300	B+D = 200	500

Sensitivity=A/(A+C), 275/(275 + 25) = 91.67%
Specificity=D/(B+D), (125/(75 + 125) = 62.5%
Positive Predictive value=A/(A+B), 275/(275 + 75) = 78.57%
Negative Predictive value=D/(C+D), 125/(25 + 125) = 83.33%

Positive Predictive Value (PPV): 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:

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

Negative Predictive Value (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:

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

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 positive predictive value (PPV). Thus, with increasing prevalence, the

proportion of positive results that are false-positive 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.

Confidence Interval (CI): The 95% confidence interval is an estimate of a population parameter computed so that the statement "the population parameter lies in this interval" will be true at a stated confidence, e.g., 95%.

95% CI of the calculated sensitivity and specificity are calculated using the formula:

$$P \pm 1.96 \sqrt{\frac{P(1-P)}{N}}$$

where P is the sensitivity or specificity

where N is the number of sera analyzed.

Delta value (∂)

Delta values are used to determine the ability of EIAs to separate the negative and positive anti-HIV serum populations from the cut-off. Delta (∂) values of the anti-HIV positive and negative sample populations are calculated by dividing the mean Optical Density (OD) ratio (log10) by the standard deviation of each population. OD ratios are calculated by dividing by the relevant cut-off:

$$\text{OD ratio} = \frac{\text{OD sample}}{\text{OD cutoff}}$$

In case of overflow, usually denoted as "****" in the print out, an OD of 3.000 is attributed to the specimen. The higher the positive (∂+) and negative (∂ -) values, the higher the probability that the test will clearly distinguish antibody positive and negative specimens.

Reproducibility

To determine reproducibility, retest approximately 10% of the initially reactive and non-reactive samples. Reproducibility, expressed as a percentage, is calculated by dividing the number of concordant results by the total number of samples retested.

Inter-reader variability of rapid test

It is important to determine the inter-reader variability of rapid tests. Three persons independently interpret each test result, and the reader variability is expressed as percentage of sera for which different readers interpret test results differently.

8.0 Reporting Results, Conclusions, Recommendations

8.1 Developing an Algorithm

Evaluation data should be analyzed to determine the performance of individual tests and the combination of tests used in a proposed algorithm. In phase I, this will involve determining the performance of various test combinations in addition to the individual test performance. An important point to consider in the analyzing potential algorithms is whether the tests will be performed in a parallel or serial testing algorithm. Most standard EIAs will be used in a serial algorithm in which the use of the second test is dependent on a reactive result in the first test. Many rapid tests that are used in POS, however, may be tested in parallel logistical reasons. A typical example might involve determining the concordance of 2 tests performed in combination and then evaluating the results when both tests agree (concordance) and when a 3rd test is required as a tiebreaker because the first 2 tests have discordant results (Figure 4).

Figure 4: Evaluation Methods

Panel	EIA1	EIA2	Status	Screening	Conf	Tiebreaker	Algorithm
296	N	N	N	N	N	N	N
297*	P	P	P	N	N	N	N
667	P	P	P	P	P	P	P
16	P	P	P	P	P	P	P
660	P	P	P	P	N	N	N
506	N	N	N	N	P	P	N
668	P	P	P	P	P	N	P
1,005	N	N	N	N	P	N	N

Raw dataset = 1,022 records
Final Panel = 972 specimens (360 positives / 612 negatives)

Samples in panel # 660 and #506 would have completely different interpretations in the algorithm based on whether the tests were performed sequentially (Figure 4) or in parallel. This is also true if the algorithm is a two test only or three tests with tiebreaker. The probable cause of the difference in results in the EIA status vs. the rapid tests results is sample mix-up.

8.2 Reporting Results

Analysis of evaluation data should be completed and reported to the NAP, MOH, and other partners immediately following the phase of evaluation in which it was performed, and before beginning the next phase.

The report for Phase I evaluation typically includes the data presented in a table that itemizes the test methods, and the Se, Sp, PPV, NPV for each method and combination of methods evaluated (Figure 5). Phase II reports should include the on-site performance data in addi-

tion to the subjective input of the client/patient flow. Having completed Phase III of the evaluation, countries should consider including the following recommendations in the final report.

- The names and manufacturers of all EIA or rapid tests evaluated with documented test performance
- The name and required specimen type for each test approved for use in POS settings
- The name of the test to be used as the tiebreaker for resolving discordant specimens and justification for use
- The names and manufacturers of each test with demonstrable testing performance, but excluded for use in POS setting. Justifications for excluding tests should be noted.
- Summary of individual test data

Figure 5.

Test Method	Sensitivity	Specificity	PPV	NPV
A	95% (190/200)	98% (294/300)		
B	97% (194/200)	98.5% (295/300)		
C	96% (192/200)	99% (297/300)		

Algorithm	Concordance	Sensitivity (concordant results)	Specificity (concordant results)	PPV	NPV
A and B	93% (475/500)				
B and C	92% (460/500)				
A and C					

Example of evaluation of algorithm of tests performed in parallel

Test combination	Tiebreaker	Discordants	Combined sensitivity of concordant (2 tests) and discordant (3 tests)	Combined specificity of concordant (2 tests) and discordant (3 tests)
A and B	93% (475/500)			
B and C	92% (460/500)			
A and C				

Test method combination with tiebreaker test for discordant results

8.3 Aggregation and dissemination of evaluation data

Conclusions and recommendations from evaluation of tests should be submitted to WHO for access and dissemination to other countries within the region. This compilation of test performance will allow countries to review data from neighboring countries which should limit the need for full-scale evaluations.

The following should be included in the report to WHO:

- Protocol for evaluating tests, including designation of gold standard
- Discordant results as tested by WB, if part of country's gold standard

	Phase I (n = # of samples)		Phase II (n)		Phase III (n)	
Tests	Se	Sp	Se	Sp	Se	Sp
A						
B						
C						
D						

Summary reports should be submitted to:

Dr. Guy-Michel Gershy-Damet or Designate
Regional Advisor For Laboratory
Regional Program on AIDS
WHO Regional Office For Africa
PO BOX BE 773
Harare -Zimbabwe

Tel: 263-4- 746342/827/323/359
Fax: 263-4-746867
Email: gershyg@whoafr.org

Appendix A

Testing Algorithms

Parallel testing algorithm

In a parallel testing algorithm, sera are simultaneously tested by two assays. True-positive sera are concordantly reactive by two different initial assays. A true-negative specimen in the algorithm is defined as being concordantly negative in the two initial assays. Sera yielding discordant results between the two assays are tested in a third assay, and the outcome of the latter assay is considered definitive.

Serial testing algorithm

The serial testing algorithm is most consistent with the proposed testing strategies of WHO/UNAIDS [19]. In the serial algorithm, all specimens are tested by a first test that is highly sensitive. Specimens are considered as true negative if they react negatively in the first test. Specimens reactive in this assay are retested by a second assay that has a high specificity (this second assay must be one which possesses dissimilar antigen presentations than that of the first assay. If specimens are concordantly positive by the two assays, they are considered as true-positives. Discordantly reactive sera are further tested by a third assay, whose outcome is considered as definitive. This algorithm is recommended for identification of asymptomatic seropositive persons in areas with an HIV seroprevalence of more than 10% [20].

Appendix B

Summary of WHO Testing Strategies

WHO Strategy I:

- Requires one test.
- For use in diagnostic testing in populations with an HIV prevalence >30% among persons with clinical signs or symptoms of HIV infection.
- For use in blood screening, for all prevalence rates.
- For use in surveillance testing in populations with an HIV prevalence >10% (e.g., unlinked anonymous testing for surveillance among pregnant women at antenatal clinics). No results are provided.

WHO Strategy II:

- Requires up to two tests.
- For use in diagnostic testing in populations with an HIV prevalence <30% among persons with clinical signs or symptoms of HIV infection or >10% among asymptomatic persons.
- For use in surveillance testing in populations with an HIV prevalence <10% (e.g., unlinked anonymous testing for surveillance among patients at antenatal clinics or sexually transmitted infection clinics). No results are provided.

WHO Strategy III:

- Requires up to three tests.
- For use in diagnostic testing in populations with an HIV prevalence = 10% among asymptomatic persons.
- Alternative approaches that address limitations to these strategies are addressed in WHO/UNAIDS and surveillance documents.

Appendix C

Potential Testing Strategies

	Screening	Confirmation
Whole Blood	Determine HIV 1/2	HemaStrip HIV 1/2
		UniGold HIV Recombinant
		OraQuick HIV –1/2
	HemaStrip HIV 1/2	UniGold HIV Recombinant
		OraQuick HIV – 1/2
	OraQuick HIV1/2	HemaStrip HIV1/2
		UniGold HIV Recombinant
Serum / Plasma	Capillus HIV 1/2	SeroCard HIV
		MultiSpot HIV 1/2
		HIVChek System 3
		SeroStrip HIV 1/2
		HIVSav 1&2
		DoubleCheck HIV 1&2
		Genie II HIV1/2
		HIVSpot HIV
	HIVSpot HIV	SeroCard HIV
		SeroStrip HIV 1/2
		DoubleCheck HIV 1/2
		Genie II HIV 1/2
		HIVSav 1&2
	Determine HIV 1/2	SeroCard HIV
		SeroStrip HIV 1/2
		DoubleCheck HIV 1/2
		Genie II HIV 1/2
		HIVSpot HIV
		MultiSpot HIV 1/2
Oral Fluids	OraQuick HIV 1/2	Saliva-Strip HIV1/2
		SalivaCard HIV

Note:

1. This is a very limited review based on experiences of CDC investigators and collaborators.
2. Choice of screening/confirmation order should be based on review of sensitivity and specificity in country. Examples above are starting points based on work in several countries. (Tests like Determine and Capillus have high sensitivity and are designed as screening tests but consistently give some false positives, therefore, are not recommended as confirmatory tests).

Appendix D

Manufacturer/Distributor	Product Description	Sensitivity	Specificity	Field Use	Comments[5]	Status	Cost/Test[6]
Abbott Laboratories Abbott Park, IL, USA www.abbottdiagnostics.com Manufacturer: Dainabot Co., Ltd. Tokyo, Japan	Determine HIV-1/2 Immunochromatographic Recombinant antigens and synthetic peptides for HIV-1/2 Single reagent Uses whole blood, plasma, or serum	97.9-100%[1]	100%[1]	Limited	Complexity: 1 Store at 2-30°C	For sale	$3.80 Negotiable
BIONOR A/S Skien, Norway6g www.bionor.no	Bionor HIV-1&2 Magnetic particle-bound EIA Synthetic peptides for HIV-1/2 Multiple reagents Uses whole blood, plasma, or serum	99.8-100%[1,2]	95.6-100%[1,2]	Moderate	Complexity: 3 Store at 2-8℃ Requires Bionor equipment	For sale WHO-evaluated	Negotiable
Sayvon Diagnostics Ltd. Ashdod, Isreal www.hctech.com/savyon	HIVSav 1&2 Microfiltration-bound EIA Recombinant antigens and synthetic peptides for HIV-1/2 Multiple reagents Uses plasma or serum	95-99%[1,3]	96-99.9%[1,3]	Extensive	Complexity: 2 Store at 2-25℃[4] Optional: centrifuge	For sale WHO-evaluated	Negotiable
Orasure Technologies, Inc. Bethlehem, Pennsylvania, USA www.orasure.com	OraQuick HIV-1/2 Immunochromatographic Synthetic peptides for HIV-1/2 Single reagent Uses oral fluid, whole blood, plasma, or serum	100%[1,3]	100%[1,3]	Limited	Complexity: 1 Store at 18-30°C	Evaluations ongoing	Negotiable
Genelabs Diagnostics, Pte, Ltd. Singapore Parent Company: Genelabs Technologies, Inc. Redwood City, CA, USA www.genelabs.com.sg	HIV SPOT Microfiltration-bound EIA Recombinant antigens and synthetic peptides for HIV-1/2 Multiple reagents Uses plasma or serum	97-99%[1,3]	96-99%[1,3]	Extensive	Complexity: 2 Store at 2-25℃[4] Optional: centrifuge	For sale WHO-evaluated	$1.20-1.80
Orgenics, Ltd. Yavne, Isreal www.orgenics.com	DoubleCheck HIV 1&2 Immunochromatographic Recombinant antigens for HIV-1/2 Single reagent Use with plasma, or serum	100%[1,3]	99.5-100[1,3]%	Moderate	Complexity: 2 Store at 2-30℃[4] Optional: centrifuge	For sale WHO-evaluated	negotiable

Appendix D (continued)

Manufacturer/Distributor	Product Description	Sensitivity	Specificity	Field Use	Comments[5]	Status	Cost/Test[6]
Ortho – Clinical Diagnostic Systems Parent Company: Johnson & Johnson New Brunswick, New Jersey, USA www.orthoclinical.com	HIVCHEK System 3 Microfiltration-bound EIA Recombinant antigens and synthetic peptides for HIV-1/2 Multiple reagents Uses plasma or serum	98.2-100%[1,2]	98.8-100%[1,2]	Extensive	Complexity: 3 Store at 2-25°C[4] Optional: centrifuge	For sale WHO-evaluated	Negotiable
Saliva Diagnostic Systems, Ltd Medford, NY, USA www.salv.com	Sero-Strip HIV-1/2 Immunochromatographic Synthetic peptides for HIV-1/2 Single reagent and buffer Uses plasma or serum	98.4-99.9%[1,2,3]	99.6-100%[1,2,3]	Moderate	Complexity: 2 Store at 2-25°C[4] Optional: centrifuge	For sale WHO-evaluated	$1.50
	Hema-Strip HIV-1/2 Immunochromatographic Synthetic peptides for HIV-1/2 Single reagent Uses whole blood, plasma, or serum	99.6%[1]	99.9%[1]	Limited	Complexity: 1 Store at 20-33°C	For sale	$3.00 Negotiable
	Saliva-Strip HIV-1/2 Immunochromatographic Synthetic peptides for HIV-1/2 Single reagent and buffer Uses oral fluid	99.4%[1]	99.4%[1]	Moderate	Complexity: 2 Store at 2-25°C[4] Requires vortex	For sale	Negotiable
Sanofi Diagnostics Pasteur, S.A. 92430 MARNES la COQUETTE, FRANCE Parent Company: BioRad Hercules, California, USA www.bio-rad.com	MultiSpot HIV-1/HIV-2 Microfiltration-bound EIA Recombinant antigens and synthetic peptides for HIV-1/2 Multiple reagents Uses plasma or serum	99.3-100%[1,3]	98.5-100%[1,3]	Extensive	Complexity : 3 Store at 2-8°C Optional: centrifuge	For sale WHO-evaluated	$4.00-5.00
	Genie II HIV1/HIV2 Microfiltration- bound EIA Recombinant antigens and synthetic peptides for HIV-1/2 Multiple reagents Uses plasma or serum	97.8-100%[1,2]	99.7-100%[1,2]	Limited	Complexity: 2 Optional: centrifuge	For sale	Negotiable

Appendix D (continued)

Trinity Biotech USA Jamestown, NY, USA Parent Company: Trinity Biotech, Plc Dublin, Ireland www.trinitybiotech.com	Capillus HIV-1/HIV-2 Latex bead agglutination Recombinant antigens and synthetic peptides for HIV-1/2 Multiple reagents Uses plasma or serum	98.6-99.9%[1,2]	98.2-99.6%[1,2]	Extensive	Complexity: 2 Store at 2-8°C Optional: reader & centrifuge	For sale WHO-evaluated	US$1.50
	SalivaCard HIV Microfiltration-bound EIA Synthetic peptides for HIV-1/2 Multiple reagents Uses oral fluid	98.9%[1]	98.8%[1]	Limited	Complexity: 2 Store at 2-8°C Requires: Orapette device	For Sale	Negotiable
	SeroCard HIV Microfiltration-bound EIA Synthetic peptides for HIV-1/2 Multiple reagents Uses whole blood, plasma, or serum	99.8-100%[1,2]	99.5%[1,2]	Extensive	Complexity: 2 Store at 2-8°C	For sale	$1.80
	UniGold HIV Recombinant Immunochromatographic Recombinant antigens for HIV-1/2 Single reagent Uses whole blood, plasma, or serum	99.8%[1]	100%[1]	Limited	Complexity: 1 Store at 2-27°C	For Sale	$2.25 Negotiable

Notes:

1. Sensitivity and specificity data supplied by manufacturer as determined for blood/serum/plasma against panel containing multiple HIV-1/2 subtypes.
2. Published sensitivity and specificity data against multiple HIV-1/2 subtypes.
3. Sensitivity and specificity data from CDC evaluation against multiple HIV-1/2 subtypes.
4. Stable at room temperature (20-33°C), but shelf life improved with refrigeration.
5. Although all tests are designed to be simple, some require multiple steps, including pipetting, and performance may improve with centrifugation. In these instances limited training and laboratory experience are useful. Complexity rating: (1) Specimen may be whole blood, venipuncture not required, sample manipulation limited to application followed by addition of buffer reagent or wash, easily read; (2) Specimen limited to plasma or serum, centrifugation or optional equipment beneficial; (3) Reagent or sample preparation may be required, multi-step assay.
6. Prices vary dependent on market and availability; they are provided solely as a point of reference.

Appendix E

Laboratory Safety Rules

Important rules, not necessarily in order of importance, should be adhered to when working in a laboratory:

1. Pipetting by mouth should be prohibited.
2. Eating, drinking, smoking, storing food and applying cosmetics must not be permitted in the laboratory/testing work areas.
3. Labels must not be licked, materials must not be placed in the mouth
4. The laboratory/testing site should be kept neat, clean, and free of materials that are not pertinent to the work.
5. Work surfaces must be decontaminated imediately after any spill of potentially dangerous material and at the end of the working day.
6. Members of the staff must wash their hands after handling infectious materials, and before they leave the laboratory.
7. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
8. All contaminated materials and specimens must be decontaminated before disposal or cleaning for reuse. They should be placed in a leak-proof, color- coded plastic bag for autoclaving or incineration on the premises. These bags should be supported in rigid containers. If it is necessary to move the bags to another site for decontamination, they should be placed in leak-proof containers e.g., solid-bottomed, that can be closed before they are removed from the laboratory.
9. Laboratory coveralls, gowns or uniforms must be worn for work in the laboratory. This clothing should not be worn in non-laboratory areas such as offices, libraries, staff rooms and canteens. Contaminated clothing must be decontaminated by appropriate methods.
10. Open-toed footwear should not be worn.
11. Protective laboratory clothing should not be stored in the same lockers or cupboards as street clothing.
12. Safety glasses face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes and impacting objects.
13. Only persons who have been advised of the potential hazards and who meet specific entry requirements (e.g. immunization) should be allowed to enter the laboratory working areas. Laboratory doors should be kept closed when work is in progress; children should be excluded from laboratory working areas.
14. There should be an insect and rodent control program.
15. Gloves appropriate for the work must be worn for all procedures that may involve accidental direct contact with blood, and infectious materials. After use, gloves should be removed aseptically and autoclaved with other laboratory wastes before disposal. Hands must then be washed. Do not wash or disinfect surgical or examination gloves for reuse.

16. All spills, accidents and overt or potential exposures to infectious materials must be reported immediately to the laboratory supervisor. A written record of such accidents and incidents should be maintained.
17. Appropriate medical evaluation, surveillance and treatment should be provided.
18. Baseline serum samples may be collected from laboratory staff and other persons at risk. These should be stored as appropriate.
19. The laboratory supervisor should ensure that training in laboratory safety is provided. A safety or operations manual that identify known and potential hazards and that specifies practices and procedures to minimize or eliminate such hazards should be adopted. Personnel should be advised of special hazards and required to read and follow standard practices and procedures. The supervisor should make sure that personnel understand these.

Appendix F

Sample Evaluation Expenditures

Test Kits	Cost
Kit –1	$4,000
Kit –2	$3,000
Kit –3	$6,000
HIV Western Blot	$2,500
Reagents	$2,000
Supplies	
Fine tips	$2,500
Vaccutainer tubes with needles	$2,250
General (expendable) supplies	$2,000
Monitoring and Evaluation	
Laboratory personnel	$1,000
Onsite Checks	$248
Proficiency testing	$2000
Travel expenses	
HIV Western Blot	$2500
HIV Reagents	$2,000
Total Budget for 6 Months	$27,498 US

Appendix G

Sample Contents of an Evaluation Protocol

- Introduction
- Purpose
  - Literature review
  - Limitations
- Methods
  - Specimens required
  - Study sites
  - Study populations
  - Sampling
  - Sample size
- Budget
  - Kits (ELISA, rapids, +/- WB, P24 Ag, PCR)
  - Bench expenses (non-kit reagents, pipette tips, time, technicians, equipment costs.
  - Transport of specimens and personnel
  - Use of panels and libraries
  - Venipuncture and collection equipment
  - Storage cryotubes
  - Training of lab and field staff
  - IQA, IQC, EQA
  - Data management and storage
- Implementation
  - Time frames
  - Staff duties
  - Analysis
  - Reporting and Publishing results
- Results
  - Statistical calculations
  - Ethical issues
  - References
  - Appendices

Appendix H

95% Confidence Ranges for .98 Sensitivity and .98 Specificity

	Prevalence of HIV									
	. 01		.05		.10		.20		.30	
	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec
100	1.7785	0.02767	0.161	0.0295	0.099	0.03054	0.0672	0.0310	0.052	0.0334
200	0.4256	0.01950	0.0987	0.0199	0.0666	0.02045	0.0451	0.0216	0.0361	0.02319
300	0.2570	0.01592	0.0769	0.0162	0.0521	0.01669	0.0361	0.0177	0.0295	0.01893
400	0.1946	0.01378	0.0666	0.0140	0.0451	0.01446	0.0313	0.0153	0.0250	0.01639
500	0.1609	0.01233	0.0596	0.0125	0.0403	0.01293	0.0274	0.0137	0.0224	0.01466
600	0.1400	0.01125	0.0521	0.0114	0.0361	0.01180	0.0250	0.0125	0.0204	0.01338
700	0.1248	0.01042	0.0482	0.0106	0.0334	0.01093	0.0231	0.0115	0.0189	0.01239
800	0.1143	0.00975	0.0451	0.0099	0.0313	0.01022	0.0216	0.0108	0.0177	0.01159
900	0.1054	0.00919	0.0425	0.0093	0.0295	0.00964	0.0204	0.0102	0.0167	0.01093
1000	0.0987	0.00872	0.0403	0.0089	0.0274	0.00914	0.0194	0.0097	0.0158	0.01037

Hence with 500 samples .98 sensitivity and .98 specificity and a prevalence of .05
Sensitivity= 1.00 to .92 /Specificity= 1.00 to .97

Note: These numbers are derived making distributional and data collection assumptions that may not be suited to a particular data set. They are intended to be used as an approximation on the number of samples one needs to use to reach a desired accuracy.

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