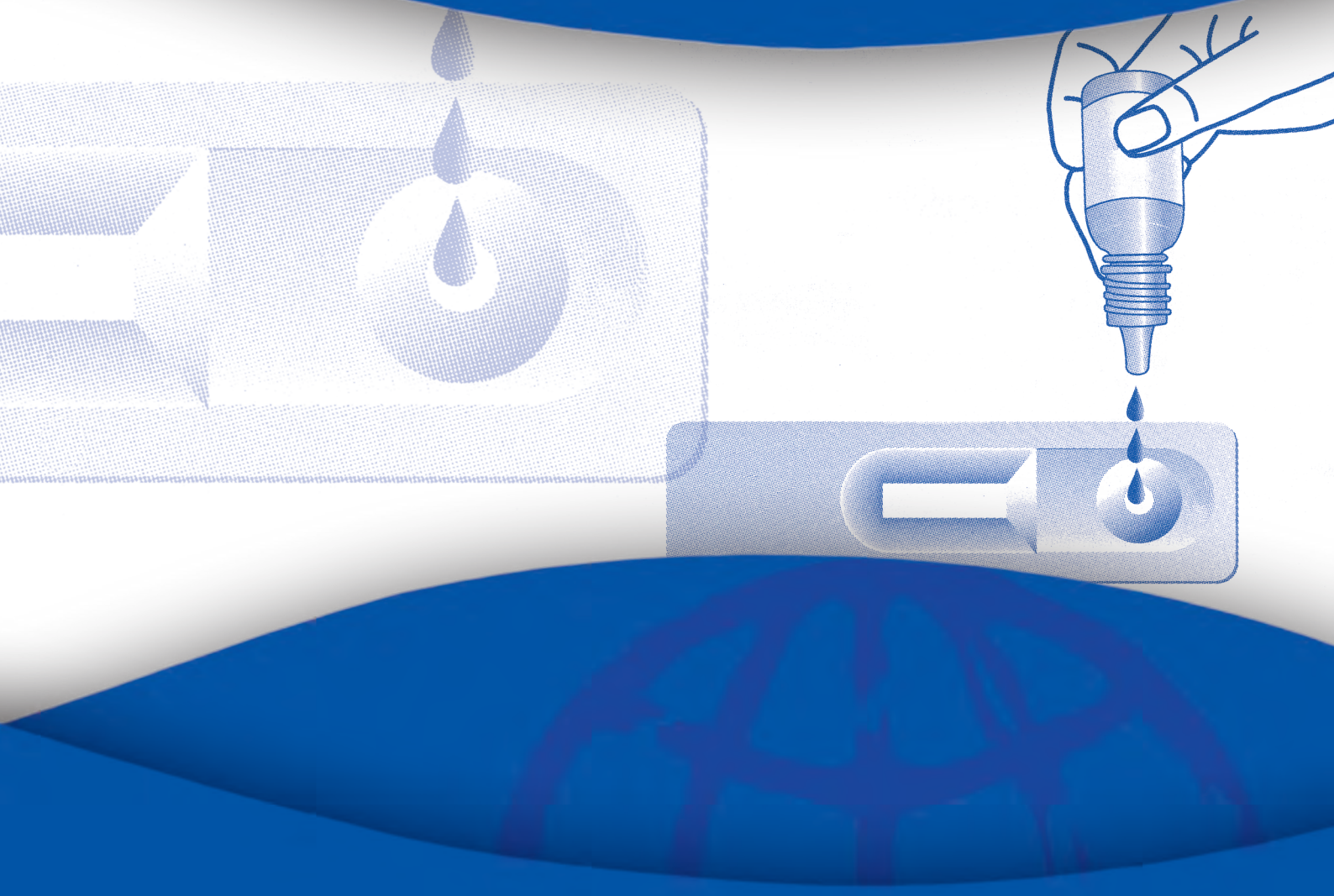
World Health Organization

HIV ASSAYS

OPERATIONAL CHARACTERISTICS

HIV rapid diagnostic tests (detection of HIV-1/2 antibodies)

REPORT 17



Diagnostics and Laboratory Technology

HIV Assays: Operational Characteristics

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CONTENTS

1. SUMMARY

Report 17 summarizes the assessment of the major operational characteristics of commercially available assays to detect HIV-1/2 antibodies. The data presented was obtained in the laboratory evaluation component of the WHO Prequalification of Diagnostics programme of the following five immunochromatographic rapid diagnostic tests (RDTs), and two immunofiltration RDTs carried out between 2011 and 2012:

Immunochromatographic RDTs

- Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd)
- HIV 1/2 STAT-PAK® (Chembio Diagnostic Systems, Inc.)
- HIV 1/2 STAT-PAK® Dipstick (Chembio Diagnostic Systems, Inc.)
- One Step HIV 1/2 Whole Blood/Serum/Plasma Test (Guangzhou Wondfo Biotech Co., Ltd)
- Uni-Gold™ HIV (Trinity Biotech Manufacturing Ltd)
- Anti-human immunodeficiency virus (HIV) antibody diagnostic kit (colloidal gold) (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd)

Immunofiltration RDTs

- INSTI™ HIV-1/HIV-2 Antibody Test (bioLytical™ Laboratories)
- Reveal® Rapid HIV Antibody Test (MedMira Laboratories Inc)

Section 2 of this report provides background information on the WHO Assays: Operational Characteristics series and the WHO Prequalification of Diagnostics programme. Sections 3 and 4 provide an overview of the laboratory diagnosis of HIV and comments on assay selection. Section 5 outlines how the assessments were carried out. Details of the evaluations are contained in the tables in section 6. Cumulative lists of the assays already assessed under the programme and the addresses of their manufacturers are listed in annexes 1, 2, and 3.

2. BACKGROUND INFORMATION

In 1988, World Health Organization (WHO), conscious of the need to advise WHO Member States on the laboratory diagnosis of HIV, initiated the WHO Test Kit Evaluation programme to provide objective assessments of commercially available assays for detecting antibodies to HIV-1 and HIV-2. In 2010, this programme was superseded by the WHO Prequalification of Diagnostics programme. The WHO prequalification assessment is a more robust assessment based on regulatory principles that considers the performance, quality and safety of the assay. It achieves this through dossier assessment (technical file), on-site inspection of manufacturing facilities and the quality management system under which the products are manufactured, and independent laboratory evaluation of performance and operational characteristics. The product testing for this continuing programme is carried out by the WHO Collaborating Centre for HIV/AIDS Diagnostic and Laboratory Support in the Department of Clinical Sciences, Institute of Tropical Medicine, Belgium and coordinated by the Department of Essential Medicines and Health Products, of WHO.

The laboratory evaluations continue to focus on the operational characteristics of these assays, taking into account factors such as ease of use and the performance characteristics, including diagnostic sensitivity and specificity, and analytical aspects including lot to lot variation. The testing utilises a panel of well-characterized sera of diverse geographical origins, commercially sourced seroconversion panels, mixed titre commercial panels and a dilution series (for assessing lot variability).

The evaluations are designed to assess an assay's suitability for use in facilities such as small laboratories and non-facility based testing services including stand-alone HIV testing and counselling sites (HTC) and community settings. This includes all testing conducted in the context of provider-initiated testing and counselling and client-initiated testing and counselling, and in blood transfusion centres in resource-limited settings.

A minimum performance acceptance criteria is set for each format of HIV serology assays. This report contains the results of all evaluations, both those products meeting the acceptance criteria and those products that do not.

Disclaimer: Please note that the status of WHO prequalification is only assigned to products that meet the WHO acceptance criteria for laboratory evaluation, **in addition to** meeting the WHO prequalification requirements for dossier assessment and inspection of site of manufacture(s). All products must satisfactorily meet the WHO requirements for all three components, before they will be WHO prequalified, and therefore eligible for WHO procurement.

INTENDED AUDIENCE

The outcomes of the laboratory evaluations are published in the form of technical reports which are intended for use by health policy makers, directors of blood banks, managers of national HIV/AIDS programmes, end users in testing services and laboratories, and procurement agencies. The comparative data contained in these reports may be used to help select HIV assays that are appropriate for local needs, by

applying country relevant selection criteria, in conjunction with other considerations such as prior experience with a given assay, availability in-country, cost, customer service and technical support from manufacturers.

REPORT DISSEMINATION

The first report was issued in March 1989, and subsequent reports have been issued on a regular basis, details of which are provided in Annexes 1 and 2. Recent reports are also published on the WHO website at the following web address: http://www.who.int/diagnostics_laboratory/en/.

Further copies of this and earlier reports are available by written request to the Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland or by e-mail to diagnostics@who.int. Reports containing information on assays which are currently no longer available are taken out of distribution.

3. LABORATORY DIAGNOSIS OF HIV INFECTION

3.1 A BRIEF OVERVIEW

The diagnosis of HIV infection is usually made on the basis of serology, i.e. the detection of HIV-1/2 antibodies or the simultaneous detection of HIV-1/2 antibodies and HIV-1 p24 antigen. Serological assays for detection HIV-1/2 antibodies or detection of HIV-1/2 antibodies and of HIV-1 p24 antigen are generally classified as either first-line assays (sometimes referred to as screening assays) or second- and third-line assays (sometimes referred to as supplemental assays, or confirmatory assays). First-line assays can provide the presumptive identification of reactive specimens, and thus should have superior sensitivity, and second-line and third-line assays are used to confirm whether specimens found reactive with a first-line assay contains antibodies specific to HIV-1/2 and/or HIV-1 p24 antigen. These should have superior specificity. Simple assays, rapid diagnostic tests and enzyme immunoassays can serve as first line assays. Further discussion on choice of assay format for screening and confirming HIV infection follows.

FORMATS OF DIAGNOSTICS

RAPID DIAGNOSTIC TESTS

Rapid diagnostic tests for HIV include immunochromatographic (lateral-flow tests) and immunofiltration (flow-through tests) formats that detect the presence of HIV-1/2 antibodies and/or HIV-1 p24 antigen. Other analytes may also be added to HIV RDTs such as antibodies to hepatitis C, hepatitis B surface antigen, antibodies to *Treponema pallidum (*syphilis). In brief, specimen (fingerstick/capillary whole blood, venous whole blood, serum, plasma, oral fluid) is added to the test device by a specimen transfer device or pipette. A reactive result is indicated by the appearance of a coloured band, line, spot or dot in the test region and in the control region, the latter which is used to indicate both successful addition of reagent or specimen. Immunochromatographic assays can be performed in less than 30 minutes and immunofiltration assays in less than five minutes and are both referred to broadly as rapid diagnostic tests (RDTs). Results for RDTs are read visually i.e. subjectively read. In general, these assays are most suitable for use in both facility and non-facility based testing services such as stand-alone HIV testing and counselling centres, and laboratories with limited facilities that process low numbers of specimens daily and other community-based testing services.

Due to their simplicity, cost and rapid turn-around time, WHO recommends the use of quality[1] RDTs for resource-limited settings, rather than conventional laboratory-based diagnostics such as enzyme immunoassay (EIA) and Western blotting. RDTs can be performed with capillary blood collected by a simple fingerstick procedure and do not require venipuncture specimen collection. With training, health workers and other non-laboratory staff can perform HIV testing with high accuracy and reliability. The use of RDTs may lower the reliance on laboratory-based techniques; these are assays that use reagents and equipment requiring maintenance and highly skilled staff. However, in instances where there are many tests being carried out per day and patients are retained well, testing by laboratory-based methods, such as EIA, may be more cost-effective and appropriate.

SIMPLE ASSAYS

Simple equipment-free assays for HIV include comb and immunoassays, and particle or latex agglutination assays that detect the presence of HIV-1/2 antibodies and/or HIV-1 p24 antigen. These types of assays are more suited to laboratory or facility-based testing as cool storage of test kits and use of precision pipettes are usually required. Generally, simple assays are not suitable for use with fingerstick/capillary whole blood, venous whole blood, or oral fluid, and thus require phlebotomy to collect an appropriate specimen. Simple assays are less rapid, and require between 30 minutes to two hours to be performed. As for RDTs, simple assays are read visually by the operator.

ENZYME IMMUNOASSAYS

Enzyme immunoassays for HIV are a laboratory-based method to detect the presence of HIV-1/2 antibodies and/or

1 For technical information on quality HIV RDTs, please refer to the list of WHO prequalified diagnostics, accessed 22 August 2012 at http://www.who.int/diagnostics_laboratory/evaluations/en/

HIV-1 p24 antigen. Generally, EIAs are most cost-effective to perform in a laboratory setting with high specimen throughput (greater than 40 per day). EIAs require equipment and an experienced and proficient technician to perform the technique.

EIAs range in format and requirements. For example, EIAs may require the use of EIA plate incubators, EIA plate washers, and EIA plate readers, and varying degrees of hands-on time by the laboratory technician for loading the specimens into the wells, reconstitution and addition of solutions and buffers, plate washing, and plate reading. Simple immunoanalyzers automate a number of the processes and as such require less hands-on time. Random access immunoanalyzers are fully automated and specimens can be placed onto the instrument at any time during the running of the instrument, eliminating the need to wait for the testing cycle to end.

Serology assays for HIV are categorised by generation, being classified on the basis of the antigens and/or conjugate used. First generation EIAs were constructed using viral lysate as the source of antigen, which were relatively sensitive but lacked specificity and thus were highly prone to producing false-reactive results. In order to improve sensitivity and specificity, second generation assays were developed that used synthetic peptides as antigen instead of lysate. The third generation assays utilises recombinant proteins and labelled antigen as conjugate, which further refines sensitivity and specificity. These first three generations of assays only detect antibody to HIV. The latest generation of assays, 4th generation, detects both HIV antigen and antibody to HIV, thus increasing diagnostic sensitivity.

SUPPLEMENTAL ASSAYS

A combination of RDTs, simple assays and EIAs can be used as second-and third-line assays to confirm an initial reactive test result. In addition to these assays, line immunoassays (LIAs) based on recombinant proteins and/or synthetic peptides capable of detecting antibodies to specific HIV-1 and/or HIV-2 recombinant proteins have been widely used to confirm HIV infection. Line immunoassays have replaced Western blotting in many settings and serve a similar purpose, i.e. to give additional information on the pattern of seroreactivity. However, given the cost and relative difficulty to interpret the results, the use of line immunoassays and/or Western blotting to confirm HIV seropositivity is generally not required.

FOURTH GENERATION ASSAYS FOR DISCRIMINATORY OR COMBINED DETECTION OF HIV-1 ANTIGEN AND HIV-1/2 ANTIBODIES

Fourth generation assays that detect both HIV-1 p24 antigen and HIV-1/2 antibodies have the potential to identify infected individuals earlier in the disease course, including individuals in the seroconversion phase (window period). These assays are generally of superior seroconversion sensitivity to assays of earlier generations. Therefore, they should be considered as the first-line (screening) assay where feasible. However recent data show that the HIV-1 antigen detection component of some fourth generation rapid tests may be lacking in sensitivity (Sands et al, 2012).

Certain 4th generation assays can produce a result that indicates whether the assay is reactive to antigen or to antibody, rather than combined detection of these markers. When these discriminatory 4th generation assays are used as the first-line assay and are followed with antibody-detection only assays for second-line and third-line assays, due care should be taken to confirm any initial HIV-1 antigen reactivity by an alternative pathway. This may be done through re-testing of a second specimen taken 14 days later to test for seroconversion (antibody positive) or referral of a specimen for HIV-1 antigen testing at a higher level laboratory.

Circulating p24 antigen appears early in the course of HIV infection, is detectable for 1-2 weeks, and then disappears or falls to very low levels until the onset of clinical illness. Rising titers of HIV p24 antigen late in the illness are correlated with a poor prognosis.

HIV-2 DIAGNOSIS

In order to identify infection with HIV-2, an assay with discriminatory capabilities (separate detection of HIV-1 and HIV-2 antibodies) may be used within the testing algorithm. These may be third or fourth generation assays. However, data generated by laboratory evaluations conducted by the WHO Prequalification of Diagnostics programme shows that the amount of cross reactivity between HIV-1 and HIV-2 may be significant. Dual infection of HIV-1 and HIV-2 within one individual is quite rare. Dual reactivity observed in any given discriminatory assay is more likely to be caused by cross-reactivity given certain homology in the amino acid sequences of HIV-1 and HIV-2. To determine the virus type or diagnose a co-infection, appropriate confirmatory testing must be performed.

USE OF MOLECULAR TECHNOLOGIES

Molecular techniques may be used qualitatively to assist the diagnosis of HIV infection and quantitatively to monitor the progression of HIV infection and the response to antiretroviral therapy (ART). These include molecular techniques that detect the presence of HIV viral nucleic acid i.e. RNA or DNA by means of nucleic acid amplification or signal amplification techniques. Molecular techniques are commonly used for early infant diagnosis in infant under 18 months of age, given the interference of passively transferred maternal antibody on serological methods.

Technologies based on the amplification of viral nucleic acids, such as polymerase chain reaction (PCR) and nucleic

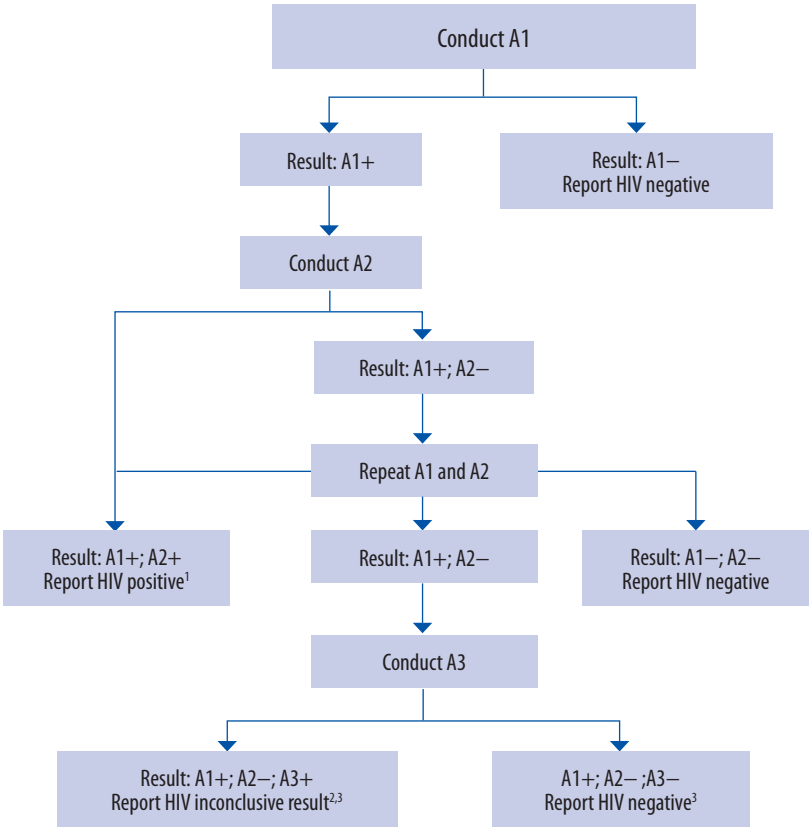
acid sequence-based amplification (NASBA) or amplification of the bound probe signal as in branched-DNA (bDNA) assays have made it possible to detect very low quantities of viral nucleic acid. The detection limit for most assays is about 50 copies/ml. These technologies are suited to early infant diagnosis in the context of mother-to-child transmission and for monitoring the HIV viral load of individuals who are taking ART to determine treatment, or virological failure. Although prices have decreased recently, molecular assays remain expensive, they require sophisticated equipment, rigorous laboratory conditions and highly trained staff. Many of the assays need further refinement as not all HIV-1 subtypes are equally well detected, nor is HIV-2. Therefore, as with serology, it would be unwise to base a diagnosis of HIV infection on a single reactive/positive test result, in the absence of any other detectable marker.

3.2 WHO TESTING STRATEGIES

WHO TESTING STRATEGY FOR DIAGNOSIS IN HIGH PREVALENCE SETTINGS

The following testing strategy applies in high prevalence settings, i.e. above 5% prevalence in the population to be tested. Firstly, all specimens should be tested with the first-line assay. Specimens that are non-reactive (A1-) are considered and reported HIV-negative. Any specimens that are reactive on the first-line assay (A1+) should be tested again using a second-line assay different from the first. For specimens that are reactive on both first and second-line assays (A1+; A2+), the result should be reported as HIV-positive. These individuals should be referred for assessment of eligibility for treatment and entry to care, if these services are not available at the testing site. Specimens that are reactive on the first assay but non-reactive on the second assay (A1+; A2-) **should be repeated** using the same specimen (when serum/plasma) with the same two assays. [When using finger-stick whole blood, a new specimen must be taken.] Repeating the assays is best practice; it may eliminate discrepant results that are due to technical or clerical error or errors inherent to the test device itself. If the new results are concordant (either A1+; A2+ or A1-; A2-), they may be reported as positive or negative, respectively. If the test results remain discrepant (A1+; A2-), the specimen should be further tested using a third-line assay. If the third assay is non-reactive (A1+; A2-; A3-), the test result is considered negative and reported as HIV-negative.

Figure 1. HIV testing strategy for diagnosis in high prevalence settings



Notes:
"Assay A1", "A2", "A3" represent three different assays (of any test format). "Report" = result may be reported.
1 For newly diagnosed individuals, a positive result should be confirmed on a second specimen to rule out laboratory error.
2 Re-testing should be performed on a second specimen taken after 14 days to rule out seroconversion.
3 If A1 is an antigen/antibody detection assay and A2 or A3 is an antibody-detection-only assay, re-testing should be performed with a second specimen taken after 14 days.

If the third assay is reactive (A1+; A2-; A3+), the test result is reported as HIV-inconclusive. The individual should be asked to return in 14 days for further testing. This situation should be rare, if assays with good specificity are used. If the rate of HIV-inconclusive results is high, additional efforts to assure assay and testing quality should be made, and the selection of assays might be reconsidered. If A1 is an antigen/antibody detection assay and A2 and/or A3 are antibody-detection-only assays, re-testing should be performed with a second specimen taken after 14 days.

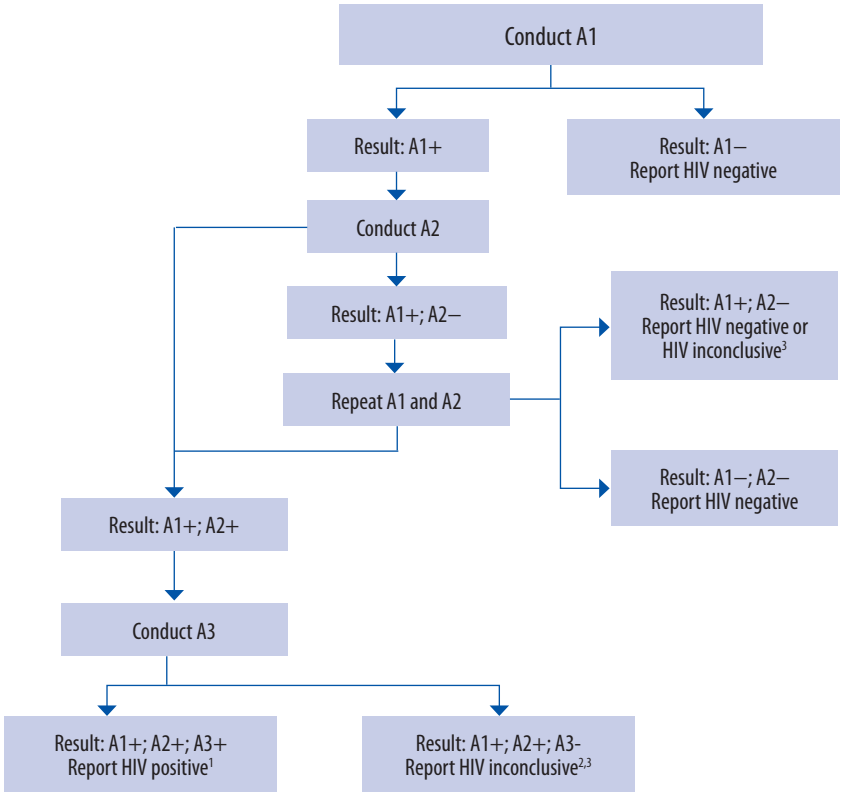
WHO TESTING STRATEGY FOR DIAGNOSIS IN LOW PREVALENCE SETTINGS

The following testing strategy applies in low prevalence settings, i.e. below 5% prevalence in the population to be tested. In these low prevalence populations, the inherent problems of all assays means that there can be less confidence in a positive result.

Firstly, all specimens are tested with one first-line screening assay, and specimens that are non-reactive (A1-) are considered and reported HIV-negative. Any specimens that are reactive on the first-line assay (A+) should be retested using a second-line assay different from the first.

Specimens that are reactive on the first assay but non-reactive on the second assay (A1+; A2-) **should be repeated** using the same specimen (when serum/plasma) with the same two assays. [When using finger-stick whole blood, a new specimen must be taken.] Repeating the assays is best practice; it may eliminate discrepant results that are due to technical or clerical errors or errors inherent to the test device itself. Any specimens that are reactive on the first assay but **non-reactive on the second assay (A1+; A2-) are considered HIV-negative**, and results are reported as such. For these specimens, the positive predictive value will be low for the first-line result, however, the negative predictive value for the second-line result will be high. If A1 is an antigen/antibody detection assay and A2 is an antibody-detection-only assay, the result is inconclusive and re-testing should be performed with a second specimen taken after 14 days.

Figure 2. HIV testing strategy for diagnosis in low prevalence settings



Notes:

"Assay A1", "A2", "A3" represent three different assays (of any test format). "Report" = result may be reported.

[1] For newly diagnosed individuals, a positive result should be confirmed on a second specimen to rule out laboratory error.

[2] Re-testing should be performed with a second specimen taken after 14 days to rule out potential seroconversion.

[3] If A1 is an antigen/antibody detection assay and A2 or A3 is an antibody-detection-only assay, re-testing should be performed with a second specimen taken after 14 days.

In a low prevalence population, even the positive predictive value based on two test results remains too low. Therefore, for specimens that are reactive on both first and second assays (A1+; A2+), a third-line assay should be used to confirm HIV-reactive specimens. If the third test result is also reactive (A1+; A2+; A3+), the result can be reported as HIV-positive. Such individuals should be referred for assessment of their eligibility for treatment and entry to care, if these services are not available at the testing site. If the result of the third assay is non-reactive, (A1+; A2+; A3-), then the result is considered HIV-inconclusive. The individual should be asked to return in 14 days for further testing. This situation should be rare. If the rate of HIV-inconclusive results is high, additional efforts to assure the quality of the testing procedures should be made, and the selection of assays might be re-considered.

3.3 FOLLOW UP AFTER DIAGNOSIS

It is best practice to obtain an additional specimen after a time interval (i.e. not the same day) to retest all newly diagnosed individuals. Retesting is usually performed as part of the clinical and laboratory-based assessment of treatment eligibility and entry to care, for example at the time of CD4 count for ART initiation. This procedure aims to rule out possible technical or clerical errors including specimen mislabelling and transcription errors that may have resulted in an incorrect diagnosis. Further guidance on retesting is available in specific WHO guidance *"Delivering HIV test results and messages for re-testing and counselling in adults,* World Health Organization, Geneva, 2010, ISBN 978 92 4 159911 5" [2]

INCONCLUSIVE RESULTS

Individuals with an inconclusive result should be asked to return for re-testing after 14 days. In particular, if there has been the possibility of HIV exposure within the preceding three months, the discrepancy in the test results may be due to seroconversion, and thus testing a second specimen is advisable. If re-testing results are subsequently concordantly reactive (A1+; A2+), true seroconversion may be highly likely, as the antibody response will have matured and HIV-positive status can be reported. If re-testing results remain either discrepant (A1+; A2-) or resolve to both non-reactive (A1-; A2-), false reactivity is likely to have been the cause, and HIV-negative status can be reported.

Care should be taken with interpreting the results from 4th generation assays, especially in cases when the result was reactive for antigen only and the second-line test only detects antibodies (refer Section 3.1).

Specimens from individuals with clinical signs meeting WHO criteria for stage III or IV may show discrepant testing results due to a decrease of HIV-1/2 antibodies with advanced disease progression and impaired immune function. These instances should not be common. If observed, retesting for HIV antibodies may not be required, but instead additional testing such as CD4 enumeration and HIV virological testing, where available may be carried out to guide clinical decisions.

3.4 QUALITY ASSURANCE

All laboratories and testing sites carrying out HIV testing should have a well-functioning quality management programme following the twelve quality system essentials (QSEs):

1. Organization
2. Personnel
3. Equipment
4. Purchasing and inventory
5. Process control
6. Information management
7. Documents and records
8. Occurrence management
9. Assessment
10. Process improvement
11. Customer service
12. Facilities and safety

These twelve QSEs can be implemented in varying degrees but the basic principles will still apply to any service providing HIV testing results. Further guidance on quality management systems is available in specific WHO guidance *"Laboratory quality management system: handbook,* World Health Organization, Geneva, 2011, ISBN 978 92 4 154827 4 " .[3] Other guidelines have been developed for application of quality aspects to HIV testing with an emphasis on rapid testing in resource-limited settings. For further details see the *Joint CDC/WHO Guidelines for Assuring the Accuracy and Reliability of HIV Rapid Testing: Applying a Quality System Approach*, World Health Organization, Geneva, 2005 (ISBN 9241593563).[4]

1. Organization – ensure there is an organization chart that describes the roles and responsibilities of all staff in the testing facility, this includes those who may collect specimens, may perform testing, may issue reports, may double-check test results and reports, etc.

2 Delivering HIV test results and messages for re-testing and counselling in adults, World Health Organization, Geneva, 2010. Accessed on 13 November 2012 at http://whqlibdoc.who.int/publications/2010/9789241599115_eng.pdf

3 Laboratory quality management system: handbook, World Health Organization, Geneva, 2011. Accessed on 13 November 2012 at http://whqlibdoc.who.int/publications/2011/9789241548274_eng.pdf

4 Joint CDC/WHO Guidelines for Assuring the Accuracy and Reliability of HIV Rapid Testing: Applying a Quality System Approach, World Health Organization, Geneva, 2005 accessed 21 September 2013 at http://whqlibdoc.who.int/publications/2005/9241593563_eng.pdf

2. Personnel – ensure staff are adequately qualified and trained for the position they are assigned to, including an understanding of quality system essentials. Competency-based pre-service training is preferred, along with on-going in-service training as new tests are introduced, etc.

3. Equipment – maintain an inventory of equipment, and ensure preventive and corrective maintenance is performed, as appropriate. Standard operating procedures (SOPs) should exist for all equipment.

4. Purchasing and inventory – ensure adequate system is in place to track test kits/reagents and consumables (i.e. venous/fingerstick blood collection equipment) that are ordered/received. Then, track consumption and requirements for replenishing stock as this data is important for national quantification of supplies. Take special note of expiry dates and ensure that adequate time is allowed for delivery.

5. Process control – ensure systems are in place to control pre-analytical, analytical and post-analytical steps of the testing procedure, including criteria for specimen rejection, specimen storage/retention/disposal/referral, quality control (QC) for both qualitative and quantitative tests using test kit controls or internal QC with established limits of acceptability, verification of subjectively read tests by a second reader (especially important for RDTs), etc.

6. Information management – ensure a system for recording all test results, lot numbers, expiry dates, operators, as well as overall result given to the client. Standardised laboratory logbooks and/or electronic data capture systems may be useful in this regard. Each specimen should be assigned a unique identifying number, and each individual who enters the service should be assigned a unique patient identifier so that the results of all specimens tested from one individual may be tracked.

7. Documents and recordkeeping – Ensure SOPs exist for all procedures undertaken, including specimen collection and processing requirements, testing algorithms, all test procedures with QC, final reporting (in accordance with validated testing algorithm), etc. Equipment maintenance records, and temperature records for fridges, freezers, and the testing room should be kept. Laboratory notebooks and forms used for recording testing results should be kept.

8. Occurrence management – ensure a system to immediately capture quality issues/problems, then to identify the root cause and implement a corrective action. Indicators such as turnaround times for each test and for an overall testing report, rate of discrepant results, rate of invalid results, rate of specimen rejection may be used.

9. Assessment – ensure some form of internal and external quality assessment is undertaken. An internal audit of certain tasks may also be performed by another staff member not usually performing the task. External assessment may be undertaken in the form of participation in an external quality assessment scheme (EQAS) also known as proficiency testing, or supervisory visits from another facility. WHO, US/CDC, UKNEQAS, RARS Senegal, NICD South Africa, NRL Australia are some of the EQA providers[5] that are able to ship EQAS to resource-limited settings. National reference laboratories should participate in an international EQAS at least twice per year, with the concurrent aim towards implementation of a national EQAS using locally derived and prepared specimens for all testing services. The dried tube specimen approach developed by US/CDC provides a practical means to prepare specimens for distribution to outlying laboratories and testing services.[6]

10. Process improvement – proactively identify opportunities for improvement of services, then relay to higher level management for implementation of better working practices. This may link closely to activities associated with number 8 – occurrence management.

11. Customer service – ensure internal (doctors, nurses, other healthcare workers) and external (clients, accreditation agencies, professional associations, regulatory agencies) customer satisfaction with the testing services provided.

12. Facilities and safety – ensure a person is assigned as safety officer and receives appropriate training on safety. Maintain safe working environment for all staff with necessary procedures in place (universal precautions, how to prevent and/or respond to needle stick injuries or other occupation exposures, chemical and biological safety, spill containment, waste disposal, personal protective equipment, etc.).

5 External Quality Assessment (EQA) Resources. Accessed on 13 November 2012 at http://wwwn.cdc.gov/mlp/eqa.aspx

6 Parekh BS, et al. Dried tube specimens: a simple and cost-effective method for preparation of HIV proficiency testing panels and quality control materials for use in resource-limited settings. J Virol Method, 2010; 163(2):295-300. Accessed on 13 November 2012 at http://www.ncbi.nlm.nih.gov/pubmed/19878697

4. ASSAY SELECTION

There are various operational factors that influence the selection of assays, in addition to their performance characteristics. WHO performance evaluations take these factors into account in assessing suitability for use in both non-facility based testing such as community testing and testing campaigns, and facility-based testing such as in stand-alone HIV testing and counselling sites, and small or lesser-equipped laboratories. The results of the evaluations demonstrate that certain RDTs are more suitable than EIAs in small centres where there are only a limited number of specimens to be tested for HIV (<40 specimens per day) and infrastructure such as electricity, cool storage and clean water are lacking. However for testing large numbers of specimens, EIAs are still the most rapid and most appropriate assay type, where infrastructure and available skilled staff permit.

The choice of the most appropriate HIV assay also depends on the HIV variants (genotypes) present in a particular geographical region (e.g., HIV-1 group O). It is clear, for example, that in areas such as West Africa where HIV-2 is prevalent, an assay capable of detecting antibodies to HIV-2 as well as HIV-1 may be desirable. Furthermore, other factors such as concomitant infections and the underlying prevalence of exogenous and endogenous infections/ substances may affect the performance of certain assays. These factors will likely vary from region to region and country to country. Therefore, testing algorithms should always be validated in the context in which they will be used before large-scale implementation.

HIV assays found to meet minimum quality, safety and performance standards for WHO prequalification are then eligible for WHO, and therefore United Nations (UN), procurement[7]. The UN Bulk Procurement Scheme provides UN agencies and national programmes with access to appropriate diagnostics of good quality at reduced cost. HIV assays other than those purchased by the pooled procurement programme but meeting the minimum WHO performance standards in terms of sensitivity and specificity can also be considered for use with the WHO testing strategies.

In general, for the selection and use of HIV assays to be used within the WHO HIV testing strategies in Figures 1 and 2, the first-line assay should have the highest sensitivity, whereas the second- and third-line assays should have a similar or higher specificity than the first. As assay development has resulted in an increase in terms of performance, it is now frequently the case that many assays have both high sensitivity and specificity.

When validating a potential testing algorithm, six to ten assays should be selected, taking into account the considerations outlined in Table 2. One validated testing algorithm with two additional algorithms validated as back-up options is preferable, in case of stock-outs or product failures. In total three assays will be required, irrespective of the testing strategy, and one back-up assay is preferable to ensure that sufficient alternate testing algorithms can be utilized. In addition to acceptable performance characteristics, the overall positive and negative predictive value of the testing algorithm should be considered. WHO is currently developing more specific guidance on this topic.

7 WHO website, procurement of diagnostics. Accessed 22 August 2013 at http://www.who.int/diagnostics_laboratory/procurement/en/

Table 1. Specific considerations for selection of HIV diagnostics

Parameter	Considerations
Performance characteristics	
Clinical sensitivity	Set the minimum acceptable criteria e.g. ≥99% for RDTs, 100% for EIAs
Clinical specificity	Set the minimum acceptable criteria e.g. ≥98% for RDTs and EIAs
Seroconversion sensitivity	Important for blood screening and suspected highly incident populations
Inter-reader variability, if subjectively read format	Set the minimum acceptable criteria e.g. ≤5%
Invalid rate (devices/test results)	Set the minimum acceptable criteria e.g. ≤5% or ≤1%, depending on the assay format
Operational characteristics	
Test format	RDTs (immunochromatographic, immunofiltration) Simple (comb formats, agglutination assays) EIAs (manual plate-based EIAs, immunoanalysers) Supplemental assays (Western blot, line immunoassays) Nucleic acid testing (molecular) (qualitative)
Specimen type	Serum/plasma, venous or capillary whole blood, oral fluid, or venous/capillary dried blood spots
Detection type	Discriminatory detection of HIV-1 and HIV-2 antibodies or combined detection of HIV-1/2 antibodies Simultaneous or combined detection of HIV-1 antigen and HIV-1/2 antibodies
Subtype detection	M, N, O subtypes
Time to result	Immunochromatographic: Less than 30 minutes with fewer steps Immunofiltration: Less than 5 minutes with more steps
Endpoint stability	How long is the result stable? Is longer reading time or shorter reading time desirable? (depends on service delivery model)
Ease of use	Depends on a combination of: • nature of specimen collection (fingerstick whole blood by lancet or venous whole blood by venipuncture) • number of steps in the test procedure • ease of reading the test band, line, spot • ease of interpretation of testing results • addition of procedural quality control (band appears when human specimen is added versus band appears when running buffer is added)
Degree of laboratory infrastructure required	Refrigeration for storage of test kits and/or reconstituted reagents Temperature controlled work space Electricity/generator
Equipment/consumables required but not provided in the test kit	Lancets, alcohol swabs for fingerstick whole blood Blood collection equipment for venous whole blood Other general laboratory consumables
Specimen through-put and individual testing service delivery models	RDTs if ≤40 specimens per day per operator with limited laboratory infrastructure EIAs if ≥40 specimens per day per operator with laboratory infrastructure
Technical skill of staff conducting testing	Including both laboratory and phlebotomy skills
Availability of test kit controls and compatibility with quality control materials	Some are available but separate from test kit See also note above on procedural in-built quality control
Shelf-life of test kits	Must be negotiated as part of the procurement contract
Access to referral laboratory	Particularly important when fourth generation assays are used

5. MATERIALS AND METHODS OF ASSESSMENT

5.1 ASSAYS

Kits for the commercial assays listed in Section 5.1 were kindly provided free of charge by the manufacturers to WHO for the assessments. The manufacturers and distributors were informed that the assessments were to be carried out and that they were free to visit the assessment site and to demonstrate their assays at their own expense.

ALERE DETERMINE™ HIV-1/2 (ALERE MEDICAL CO., LTD.)

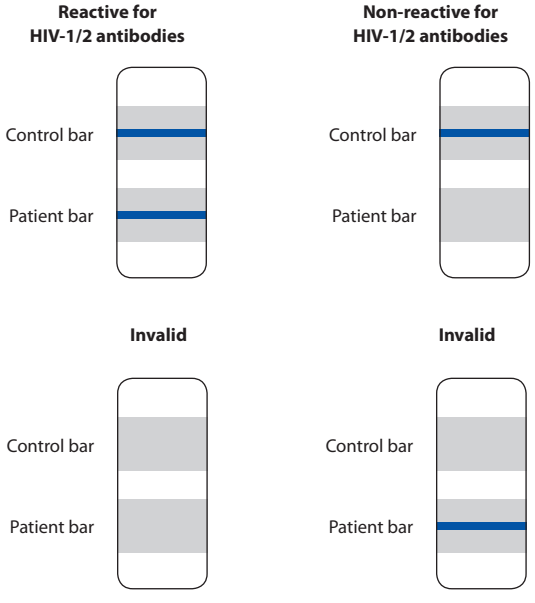
Alere Determine™ HIV-1/2 is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

Shelf life: 12 months
Storage conditions: 2-30 °C
Volume of specimen needed: 50 µL
Time to test one specimen: 16 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for serum/plasma or venous whole blood: precision pipette capable of delivering 50 µl of sample
- for capillary whole blood: EDTA capillary tubes or Microsafe capillary tubes, and Alere Determine™ chase buffer
- for capillary blood collection: lancet, cotton wool, alcohol swab
- for venous blood collection: venipuncture apparatus and appropriate blood collection tubes.



TEST PROCEDURE:

1) Remove the protective foil cover from each test.
2) Label the test with patient or specimen ID number.
3) For serum or plasma specimens:
   a. Using a precision pipette, apply 50µl of specimen to the sample pad (marked by the arrow symbol).
   b. Wait a minimum of 15 minutes (up to 60 minutes) and read result.
4) For venous whole blood (venipuncture) specimens:
   a. Using a precision pipette, apply 50µl of specimen to the sample pad (marked by the arrow symbol).
   b. Wait one minute, then apply one drop of Chase Buffer to the sample pad.
   c. Wait a minimum of 15 minutes (up to 60 minutes) and read result.
5) For capillary whole blood (fingerstick) specimens:
   a. Clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
   b. Using capillary tube, apply 50µl of specimen to the sample pad (marked by the arrow symbol).
   c. Wait until blood is absorbed into the sample pad, then apply one drop of Chase Buffer to the sample pad.
   d. Wait a minimum of 15 minutes (up to 60 minutes) and read result.
6) Interpret results as follows:

Reactive for HIV-1/2 antibodies: a red bar appears in the control reading window *and* a red bar appears in the patient reading window. The intensities of the patient and control bars may differ but any patient bar is reactive, irrespective if it is lighter or darker than the control bar.

Non-reactive for HIV-1/2 antibodies: a red bar appears in the control reading window *but no* red bar appears in the patient reading window.

Invalid: no red bar appears in the control window, even if a red bar appears in the patient reading window.

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

HIV 1/2 STAT-PAK®
(CHEMBIO DIAGNOSTICS SYSTEMS, INC.)

HIV 1/2 STAT-PAK® is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

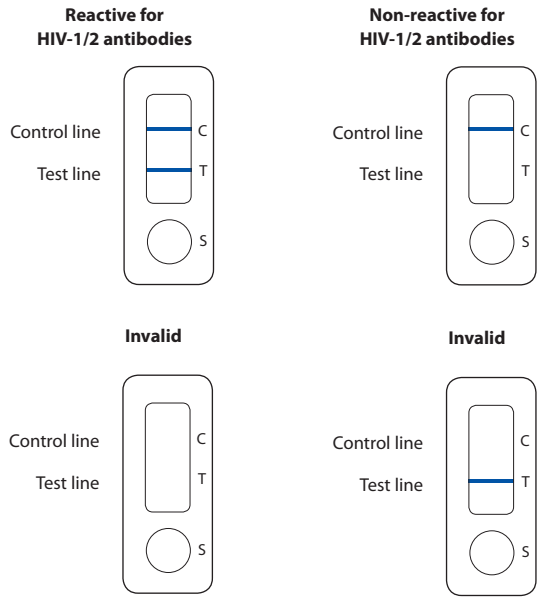
Shelf life: 24 months
Storage conditions: 8-30 °C
Volume of specimen needed: 5 µL
Time to test one specimen: 17 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab
- for venous blood collection: venipuncture apparatus and appropriate blood collection tubes.

TEST PROCEDURE:

1) Remove the test device from its pouch and place it on a flat surface.
2) Label the test device with patient or specimen ID number.
3) For capillary whole blood (fingerstick) specimens: Clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
4) Touch the 5 µl specimen loop provided to the specimen, allowing the opening of the loop to fill with the liquid.
5) Holding the specimen loop vertically, touch it to the sample pad in the center of the (S) well of the device to dispense 5 µl of specimen (serum, plasma, or whole blood) onto the specimen pad.
6) Invert the Running Buffer bottle and hold it vertically (not at an angle) over the specimen well. Add 3 drops (105 µl) of buffer slowly, drop wise, into the (S) well.
7) Read the test result 15 minutes after the addition of the Running Buffer. In some cases, a test line may appear in less than 15 minutes, but 15 minutes are needed to report a nonreactive result. Read results in a well-lit area. Do not read results after 20 minutes.
8) Interpret results as follows:



Reactive for HIV-1/2 antibodies: a pink/purple line appears in the control area *and* a pink/purple line appears in the test area. The intensities of the test and control lines may differ but any test line is reactive, irrespective if it is lighter or darker than the control line.

Non-reactive for HIV-1/2 antibodies: a pink/purple line appears in the control area *but no* pink/purple line appears in the test area.

Invalid: no pink/purple line appears in the control area, even if a pink/purple line appears in the test area.

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

HIV 1/2 STAT-PAK® DIPSTICK
(CHEMBIO DIAGNOSTICS SYSTEMS, INC.)

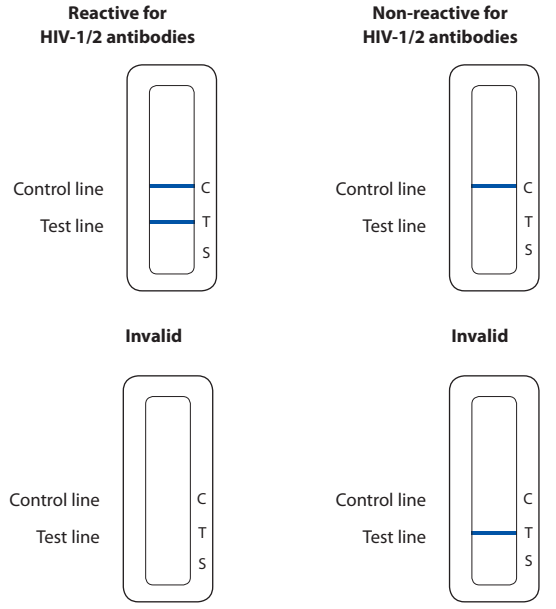
HIV 1/2 STAT-PAK® DIPSTICK is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

Shelf life: 24 months
Storage conditions: 8-30 °C
Volume of specimen needed: 5 µL
Time to test one specimen: 17 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab
- for venous blood collection: venipuncture apparatus and appropriate blood collection tubes.



TEST PROCEDURE:

1) Tear one backing card off for each test to be run.
2) Label the card with the patient or specimen ID.
3) Remove the HIV 1/2 STAT-PAK DIPSTICK test strip from the vial. The vial with the test strips should only be opened to remove dipsticks for testing and should be closed tightly immediately thereafter.
4) Being careful not to touch the membrane, turn the test strip so the green tape is facing away and remove the red liner from the adhesive strip(s) on the back of the test strip. Discard the red liner.
5) Place the test strip onto the backing card in the marked space with the green tape facing up and the arrows on the tape facing in the same direction as the arrows on the backing card. Place the backing card and dipstick assembly on a clean, flat surface.
6) For serum/plasma/venous whole blood specimens:
   a. Touch the 5 µl specimen loop provided to the specimen to fill the circular opening of the loop with specimen. Follow steps 8-11.
7) For capillary whole blood specimens:
   a. Clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
   b. Collect the specimen by touching the 5µl specimen loop provided to the drop of blood. Follow steps 8-11.
8) Holding the specimen loop vertically, touch it to the sample pad in the center of the (S) area of the dipstick to dispense 5 µl of specimen onto the sample pad.
9) Invert the Running Buffer bottle and hold it vertically (not at an angle) over the sample area. Add 3 drops (about 105 µl) of buffer slowly, dropwise, onto the (S) area being sure each drop is absorbed by the sample pad before adding the next. NOTE: In the case that liquid does not start to flow onto the membrane within 2 minutes, add one additional drop of Running Buffer.
10) Read the test results between 15 and 20 minutes after the addition of Running Buffer.
11) Interpret results as follows:

Non-reactive for HIV-1/2 antibodies: a pink/purple line appears in the control area *but no* pink/purple line appears in the test area. *Special note: A red cell line may appear in the test area on rare occasions when using whole blood. This should not be interpreted as a reactive test result.*

Invalid: no pink/purple line appears in the control area, even if a pink/purple line appears in the test area.

Reactive for HIV-1/2 antibodies: a pink/purple line appears in the control area *and* a pink/purple line appears in the test area. The intensities of the test and control lines may differ but any test line is reactive, irrespective if it is lighter or darker than the control line.

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

ONE STEP HIV 1/2 WHOLE BLOOD/SERUM/PLASMA TEST (GUANGZHOU WONDFO BIOTECH CO. LTD.)

One Step HIV1/2 Whole Blood/Serum/Plasma Test is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

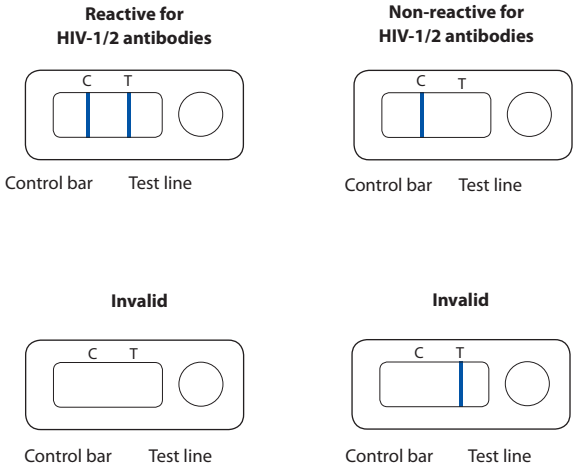
Shelf life: 24 months
Storage conditions: 4-30 °C
Volume of specimen needed: 50 µL
Time to test one specimen: 16 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab
- for venipuncture blood collection: venipuncture apparatus and appropriate blood collection tubes.

TEST PROCEDURE:

1) Remove the test device from the foil pouch by tearing at the notch and place it on a level surface before testing (Note: test kit should be used immediately after removing out of the pouch).
2) Label the test with patient or specimen ID number.
3) For capillary and venous whole blood specimens:
   a. If capillary blood, clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
   b. Holding a sample dropper vertically, add two drops of whole blood (about 50µl) into the sample well (with an arrow marked).
   c. Add two drops (about 50µl) of dilution buffer from the bottle directly into the sample well. Follow steps 5-6.
4) For serum/plasma specimens:
   a. Holding a sample dropper vertically, add four drops (80 µl-100 µl) of the serum or plasma into the sample well. Follow steps 5-6.
5) Wait for 15 minutes and read the results. Do not read the results after 30 minutes.
6) Interpret results as follows:



Reactive for HIV-1/2 antibodies: a rose pink line appears in the control area *and* a rose pink line appears in the test area. The intensities of the test and control lines may differ but any test line is reactive, irrespective if it is lighter or darker than the control line.

Non-reactive for HIV-1/2 antibodies: a rose pink line appears in the control area *but no* rose pink line appears in the test area.

Invalid: no rose pink line appears in the control area, even if a rose pink line appears in the test area.

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

UNI-GOLD™ HIV
(TRINITY BIOTECH PLC)

Uni-Gold™ HIV is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

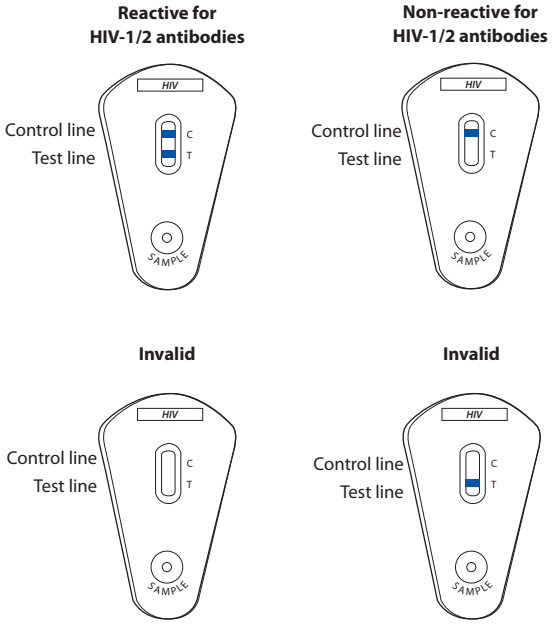
Shelf life: 12 months
Storage conditions: 2-27 °C
Volume of specimen needed: 60 µL
Time to test one specimen: 11 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab
- for venipuncture blood collection: venipuncture apparatus and appropriate blood collection tubes.

TEST PROCEDURE:

1) Remove the test device from the protective wrapper.
2) Label test with patient or specimen ID number.
3) If capillary blood, clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
4) Using one of the disposable pipettes supplied, fill with specimen (serum/plasma/whole blood).
5) Holding the pipette over the sample port, add two drops of specimen (approx. 60µl) carefully.
6) Add 2 drops (approx. 60µl) of wash reagent to sample port.
7) Allow 10 minutes from the time of wash reagent addition for the reaction to occur. The result should be read immediately after the end of the 10 minute incubation time but is stable for a further 10 minutes after the incubation time. Do not read results after 20 minutes following sample addition.
8) Interpret results as follows:



Reactive for HIV-1/2 antibodies: a pink/red band appears in the control area *and* a pink/red band appears in the test area. The intensities of the test and control bands may differ but any test band is reactive, irrespective if it is lighter or darker than the control band.

Non-reactive for HIV-1/2 antibodies: a pink/red band appears in the control area *but no* pink/red band appears in the test area.

Invalid: no pink/red band appears in the control area, even if a pink/red band appears in the test area.

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

ANTI-HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY DIAGNOSTIC KIT (COLLOIDAL GOLD) (BEIJING WANTAI BIOLOGICAL PHARMACY ENTERPRISE CO., LTD)

Anti-human immunodeficiency virus (HIV) antibody diagnostic kit (colloidal gold) is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

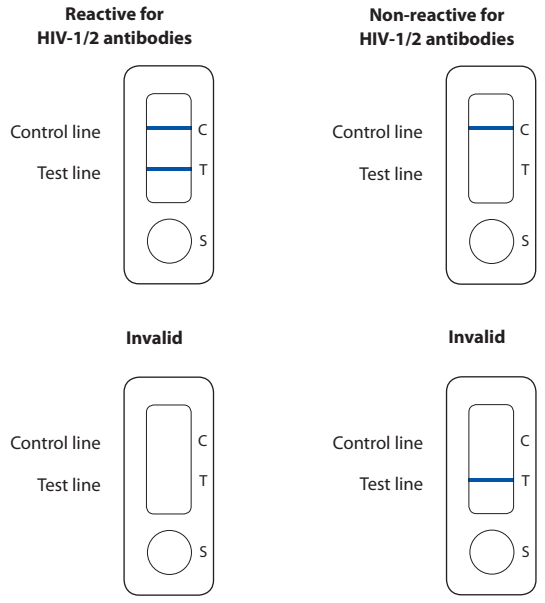
Shelf life: 18 months
Storage conditions: 2-30 °C
Volume of specimen needed: 50-80 µL
Time to test one specimen: 11 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- timer
- appropriate biohazard waste containers
- for capillary blood collection: cotton wool, alcohol swab
- for venipuncture blood collection: venipuncture apparatus and appropriate blood collection tubes.

TEST PROCEDURE:

1) Open pouch and place test device on flat surface, label test with patient or specimen ID number.
2) For serum/plasma specimens:
   a. Add three drops (or pipette 80µl) of serum or plasma into the sample window (S).
3) For venous whole blood specimens:
   a. Add two drops (or pipette 50µl) of specimen into the sample window (S). Add 50µl (about 2 drops) of Diluent Buffer into the sample window (S).
4) For capillary whole blood specimens:
   a. Clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
   b. Add two drops (or pipette 50µl) of specimen into the sample window (S). Add 50µl (about 2 drops) of Diluent Buffer into the sample window (S).
5) Avoid dropping sample or buffer in the observation window. Do not allow the sample to overflow. Place cassette on flat surface.
6) Read the results within 10 to 30 minutes.
7) Interpret results as follows:



Reactive for HIV-1/2 antibodies: a red line appears in the control zone *and* a red line appears in the test zone. The intensities of the test and control lines may differ but any test line is reactive, irrespective if it is lighter or darker than the control line.

Non-reactive for HIV-1/2 antibodies: a red line appears in the control zone *but no* red line appears in the test zone.

Invalid: no red line appears in the control zone, even if a red line appears in the test zone.

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

INSTI™ HIV-1/HIV-2 ANTIBODY TEST (BIOLYTICAL™ LABORATORIES INC)

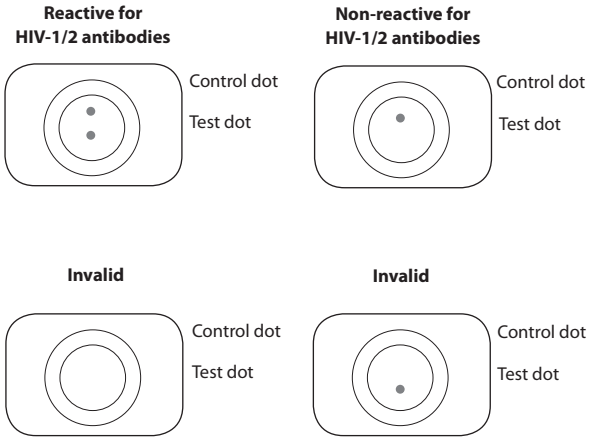
INSTI™ HIV-1/HIV-2 Antibody Test is an immunofiltration rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

Shelf life: 15 months
Storage conditions: 15-30 ˚C
Volume of specimen needed: 50 µL
Time to test one specimen: 2 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- precision pipette capable of delivering 50 µl of specimen (if kit without support materials is ordered)
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab (if kit without support materials is ordered)
- for venipuncture blood collection: venipuncture apparatus and appropriate blood collection tubes.



Reactive for HIV-1/2 antibodies: a blue dot appears in the control zone *and* a blue dot appears in the test zone. The intensities of the test and control dots may differ but any test dot is reactive, irrespective if it is lighter or darker than the control dot.

Non-reactive for HIV-1/2 antibodies: a blue dot appears in the control zone *but no* blue dot appears in the test zone.

Invalid: no blue dot appears in the control zone, even if a blue dot appears in the test zone.

TEST PROCEDURE:

1) To prepare the Colour Developer and Clarifying Solution bottles, remove the caps and seals from the bottles and replace the caps with the droppers provided with the kit.
2) Gather one sealed test pouch containing INSTI Membrane Unit, and one vial of Sample Diluent.
3) For capillary whole blood specimens:
   a. Clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool.
   b. Hold single-use pipette provided with the test kit (if kit with support materials is ordered), horizontally and touch to blood drop and let capillary action draw blood to 50µl fill line.
   c. Add blood to Sample Diluent vial. Recap vial and mix by inversion. Follow steps 5-10.
4) For venous whole blood, serum, plasma specimens:
   a. Using a pipette, add 50 µl of specimen to the Sample Diluent vial. Recap the vial and mix by inversion. Follow steps 5-10.
5) Tear open the pouch and carefully remove the Membrane Unit without touching the center well. Place the unit on a level surface. Label test device with patient or specimen ID number.
6) Remix the Sample Diluent-specimen mixture and pour the entire contents to the center of the Membrane Unit well. Liquid should be absorbed within 30 seconds.
7) Open the Colour Developer and with the blue dropper, slowly draw solution up the 1.5 ml mark. Add the solution to the center of the Membrane Unit well. Liquid should be absorbed within 20 seconds.
8) Open the Clarifying Solution and with the white dropper, slowly draw solution up to the 1.5 ml mark. Add the solution to the center of Membrane Unit well. Immediately read the result while the membrane is still wet. Do not read results if more than 5 minutes has elapsed.
9) Interpret results as follows:

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

REVEAL® RAPID HIV ANTIBODY TEST (MEDMIRA LABORATORIES INC.)

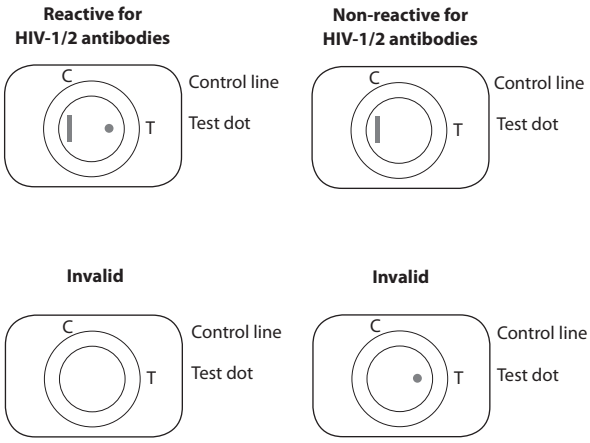
REVEAL® Rapid HIV Antibody Test is an immunofiltration rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens.

KEY INFORMATION:

Shelf life: 18 months
Storage conditions: 2-30 °C
Volume of specimen needed: 35µl
Time to test one specimen: 3 minutes

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- personal protection equipment such as gloves, lab coat or gown
- appropriate biohazard waste containers
- for capillary blood collection: lancet, cotton wool, alcohol swab (if kit without support materials is ordered)
- for venipuncture blood collection: venipuncture apparatus and appropriate blood collection tubes.



Reactive for HIV-1/2 antibodies: a red line appears in the control zone *and* a red dot appears in the test zone. The intensities of the test and control lines/dot may differ but any test dot is reactive, irrespective if it is lighter or darker than the control line.

Non-reactive for HIV-1/2 antibodies: a red line appears in the control zone *but no* red dot appears in the test zone.

Invalid: no red line appears in the control zone, even if a red dot appears in the test zone.

TEST PROCEDURE:

1) Prepare the Colour Developer and Clarifying Solution bottles, remove the caps and seals from the bottles and replace the caps with the droppers provided with the kit.
2) Label one test device with patient or specimen ID number.
3) For capillary whole blood specimens:
   a. Place sample tube on flat surface. Add 4 drops of Universal Buffer to sample tube.
   b. Clean fingertip with alcohol swab, place lancet off-centre and puncture the finger tip. Wipe away first blood drop with cotton wool. Use auto-fill pipette provided to draw one drop of blood to fill line and stop. Add to sample tube containing Universal Buffer. Tap side of the tube. Follow steps 6-9.
5) For venous whole blood specimens:
   a. Place sample tube on flat surface. Add 4 drops of Universal Buffer.
   b. Use transfer pipette provided to dispense 1 drop of whole blood into sample tube. Tap side of the tube. Follow steps 6-9.
6) Using a new transfer pipette, transfer the entire contents in drops to the test cartridge. Wait for specimen to be absorbed.
7) Place the Instant Gold Cap on the test cartridge.
8) Dispense 12 drops of Universal Buffer into cartridge and allow to absorb.
9) Remove the InstantGold Cap and read result immediately. Follow step 15.

For serum, plasma specimens:

10) Add 3 drops of Universal Buffer to cartridge.
11) Add 1 drop of serum or plasma to the cartridge. Wait for specimen to be absorbed.
12) Place the Instant Gold Cap on the test cartridge.
13) Dispense 12 drops of Universal Buffer into cartridge and allow to absorb.
14) Remove the InstantGold Cap and read result immediately.
15) Interpret results as follows:

Disclaimer: these instructions were prepared based on the instructions for use submitted as part of the WHO Prequalification of Diagnostics assessment. These may change over time and so it is recommended to always refer to the most current instructions for use when developing standard operating procedures and/or job aids.

5.2 EVALUATION PANELS

5.2.1 WHO HIV SPECIMEN REFERENCE PANEL

The WHO HIV Specimen Reference Panel consisted of approximately 1079 clinically derived serum/plasma specimens of European, African, Latin America and Asian origin. There were 421 anti-HIV positive specimens, of which 16 were anti-HIV-2 positive, and 658 anti-HIV negative specimens.

5.2.2 COMMERCIALLY ACQUIRED PANELS

5.2.2.1 SEROCONVERSION PANELS

Eight anti-HIV 1 seroconversion panels: PRB914, PRB925, PRB926, PRB930, PRB935, PRB965, PRB968 and PRB969 (sourced from SeraCare Life Science Inc) were tested to determine the sensitivity of the assay during the seroconversion period.

5.2.2.2 HIV MIXED TITER PANEL

One anti-HIV mixed titer performance panel containing 25 members: PRB205 (sourced from SeraCare Life Science Inc) was tested to determine the sensitivity of the assay in specimens with low antibody titer, including from early seroconversion specimens.

5.2.3 LOT-TO-LOT VARIATION PANEL

A panel of ten anti-HIV positive specimens was diluted 2-fold in normal human serum until the antibody end-point titer to make 16 member dilution series (n=160). These were tested to determine lot-to-lot variability in two production lots.

5.2.4 WHO REFERENCE PREPARATIONS

The WHO international biological reference preparation panel with the catalogue number 02/210 (Anti-HIV antibodies [HIV-1 subtypes A, B, C, CRF01_AE, group O and HIV-2] was tested to determine subtype sensitivity.

Table 2. WHO reference panel for antibody detection assays

Panel name	Number of specimens
WHO HIV specimen reference panel	421 HIV positive, 658 HIV negative
Lot-to-lot variation panel	16 member dilution series of 10 specimens, (160 in total)
Commercial HIV seroconversion panels	8 panels comprising 52 specimens in total
Commercial HIV performance panels	1 panel comprising of 25 specimens
WHO international reference preparations	1 panel comprising 7 specimens in total

5.3 TEST PERFORMANCE

The assays were performed according to the instructions for use (IFU) supplied within the test kit. One laboratory technician carried out all the testing for any one particular assay. Due to the subjective, visual nature of the reading of RDTs, the tests were read independently by three technicians. Two out of three reading results determined the final outcome, see section 5.5.2 on inter-reader variability.

5.4 REFERENCE ASSAYS

Initially, each specimen was tested on the following two EIAs Vironostika HIV Ag/Ab (bioMérieux) EIA and Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics) EIA, in parallel:

Specimens that were non-reactive on both EIAs were assigned anti-HIV negative.

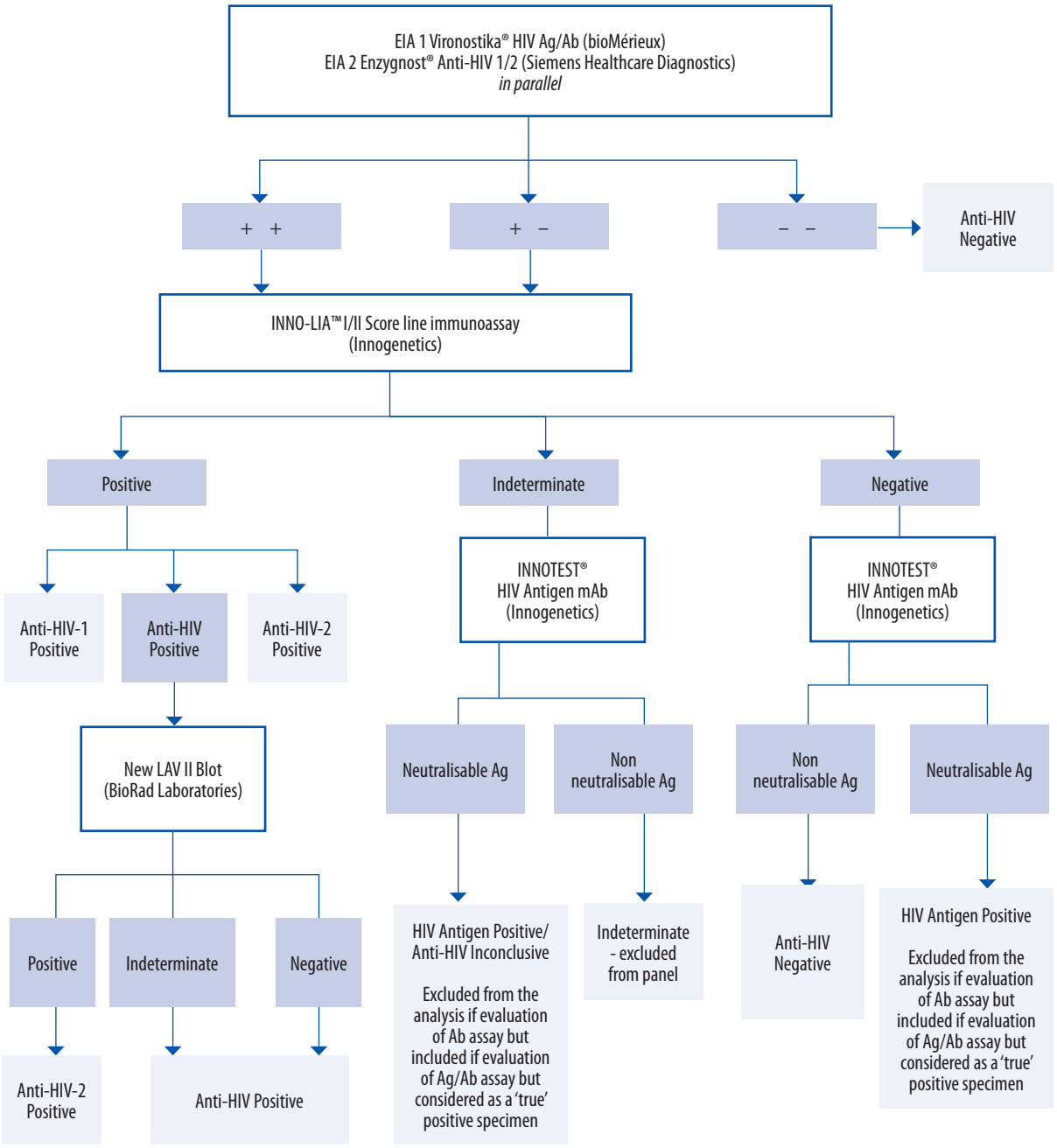
Specimens with discrepant EIA results AND with dually reactive results on both EIAs were tested on the INNO-LIA™ HIV I/II Score (Innogenetics) line immunoassay. Specimens that were negative by line immunoassay were further tested on Innotest® HIV Antigen mAb (Innogenetics) EIA, and if found non-reactive then were assigned anti-HIV negative. If found to be neutralisable for HIV-1 antigen, the specimen was considered HIV-1 antigen positive and anti-HIV negative and was retained for the evaluation of antigen/antibody detection (4th generation) assays but not for antibody detection (2nd and 3rd generation) assays.

Specimens that are indeterminate by line immunoassay were further tested on Innotest® HIV Antigen mAb (Innogenetics) EIA, and if found non-reactive then were excluded from the panel. Specimens that were reactive for antigen (and were neutralisable) were assigned as HIV-1 antigen positive and anti-HIV inconclusive. These specimens were retained for the evaluation of antigen/antibody detection (4th generation) assay but not for antibody detection (2^{nd} and 3^{rd} generation) assays.

Specimens that were only positive by line immunoassay were assigned as anti-HIV-1 positive or anti-HIV-2 positive. Those specimens that could not be discriminated (i.e. anti-HIV positive) were further tested on the NEW LAV II Blot (BioRad Laboratories). Specimens that were indeterminate or negative by the NEW LAV II Blot were assigned as anti-HIV-1 positive. Specimens that were positive by the NEW LAV II Blot were assigned as anti-HIV positive.

All reference assays were interpreted according to IFU as given by the manufacturer. The data obtained with each assay were compared to the reference testing results.

Figure 3. Characterisation testing algorithm for the WHO HIV specimen refernce panel



5.5 ANALYSIS OF THE RESULTS OF THE ASSAYS UNDER EVALUATION

5.5.1 SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUES OF HIV ASSAYS

The following methods were used to calculate the performance characteristics, see Table 3.

Table 3. 2x2 table for calculation of performance characteristics

		Reference testing results		
		+	–	
Results of assay under evaluation	+	a true positives	b false positives	a + b
	–	c false negatives	d true negatives	c + d
		a + c	b + d	

SENSITIVITY

Sensitivity is the ability of the assay under evaluation to detect correctly sera that contain HIV-1/2 antibodies (reference assays positive). Thus sensitivity is the number of true positive sera identified by the assay under evaluation as positive (a), divided by the number of sera identified by the reference assays as positive (a+c), expressed as a percentage.

$$\text{Sensitivity} = \frac{a}{a+c}$$

SPECIFICITY

Specificity is the ability of the assay under evaluation to detect correctly sera that do not contain HIV-1/2 antibodies (reference assays negative). Thus specificity is the number of true negative sera identified by the assay under evaluation as negative (d), divided by the number of sera identified by the reference assays as negative (b+d), expressed as a percentage.

$$\text{Specificity} = \frac{d}{b+d}$$

CONFIDENCE INTERVALS

The 95% confidence intervals were calculated for values in order to assess the level of uncertainty introduced by sample size, etc. Exact 95% confidence intervals for binomial proportions were calculated from the F-distribution (Armitage, 2002; Kirkwood, 2003).

PREDICTIVE VALUES

The positive predictive value (PPV) is the probability that when the test is reactive that the specimen does contain HIV-1/2 antibodies. PPVs were calculated using the following formula.

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

The negative predictive value (NPV) is the probability that when the test is negative that a specimen does not contain HIV-1/2 antibodies. NPVs were calculated using the following formula.

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

The probability that a test result will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. 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 individuals testing false-positive decreases; conversely, the likelihood that a person whose test result is negative is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of individuals testing false-negative. The PPV and NPV are calculated at a prevalence of 0.1%, 1% and 5%.

5.5.2 INTER-READER VARIABILITY

Three individuals independently interpreted each test result. The inter-reader variability was expressed as the percentage of specimens for which initial test results were differently interpreted (i.e. reactive, non-reactive, indeterminate) by the independent readers for the WHO HIV specimen reference panel (clinical specimens) only, excluding the commercially acquired seroconversion and mixed titer panels and lot-to-lot variation panels.

5.5.3 SENSITIVITY IN SEROCONVERSION PANELS

The results obtained with seroconversion panels using the assays under evaluation were compared with those obtained using Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics), the assay arbitrarily designated the reference for determination of relative sensitivity in these panels i.e. the benchmark assay. For each seroconversion series (panel), the first specimen in the sample sequence to become reactive with Enzygnost Anti-HIV 1/2 was assigned the value "0". Results from the assays under evaluation were compared with Enzygnost Anti-HIV 1/2 Plus by determining the difference between the specimen assigned value "0" and the relative position in the sample sequence of the first specimen which showed a reactive result with each of the

assays under evaluation. For example, if an assay became reactive two specimens earlier in a series than Enzygnost Anti-HIV 1/2 Plus, the value assigned for that series in that assay was -2. Similarly, if an assay became reactive one specimen later than Enzygnost Anti-HIV 1/2 Plus, the value assigned was +1. The assigned values over the eight seroconversion series were averaged to determine a mean relative seroconversion sensitivity index for each assay and the 95% confidence limits were determined. These estimates should be interpreted with caution as only eight panels were tested.

5.5.4 INTERPRETATION OF RESULTS FROM HIV MIXED TITER PANEL

The number of specimens detected by the assays on the HIV mixed titer performance panel was determined by comparison with the expected results following interpretation of the combined reference testing results generated by the following assays: Enzgnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics) EIA, Vironostika® HIV Ag/Ab (bioMérieux) EIA, INNO-LIA™ HIV I/II Score (Innogenetics) line immunoassay, and INNOTEST® HIV Antigen mAb (Innogenetics) EIA.

5.5.5 INTERPRETATION OF RESULTS FROM LOT-TO-LOT VARIATION PANEL

The results of the lot-to-lot variation panel on the two lots were compared and a variation of +/– one 2-fold dilution series was considered acceptable.

5.5.6 INTERPRETATION OF WHO REFERENCE PREPARATIONS

The results of the WHO reference preparations were compared for each subtype with the status assigned in the instructions for use that accompanied the preparations.

6. ASSAY EVALUATIONS

6.1 RESULTS OF INDIVIDUAL ASSAYS

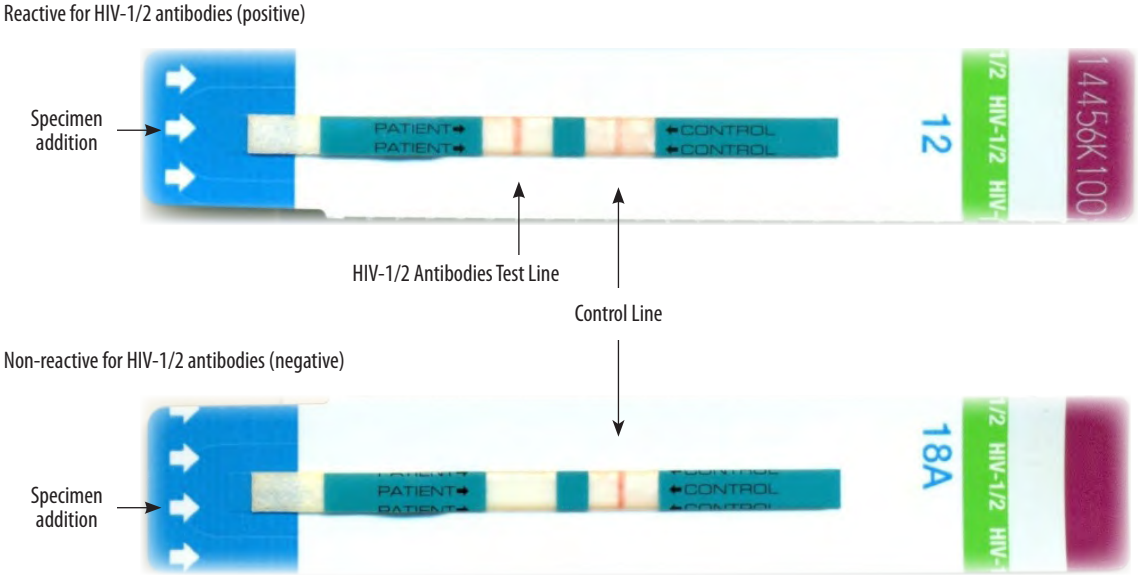
ALERE DETERMINE™ HIV-1/2 (ALERE MEDICAL CO., LTD)

On a panel of 1079 specimens, we observed an initial sensitivity (95% CI) of 100% (99.1–100%) and an initial specificity (95% CI) of 97.9% (96.4–98.8%) compared to the reference assay results. The final sensitivity (95% CI) was 100% (99.1–100.0%) and the final specificity (95% CI) was 98.9% (97.8%–99.6%) compared to the reference assay results. Lot to lot variation was acceptable.

For eight seroconversion panels, Alere Determine™ HIV-1/2 detected on average 0.125 specimens earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]). For the mixed titer panel, Alere Determine HIV-1/2 correctly classified all specimens, one anti-HIV indeterminate/HIV-1 antigen positive specimen was non-reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Alere Determine HIV-1/2 detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 1.4%. The initial invalid rate was 0.28%, and the final invalid rate was 0.1%.

Figure 4. Completed test devices for Alere Determine™ HIV-1/2



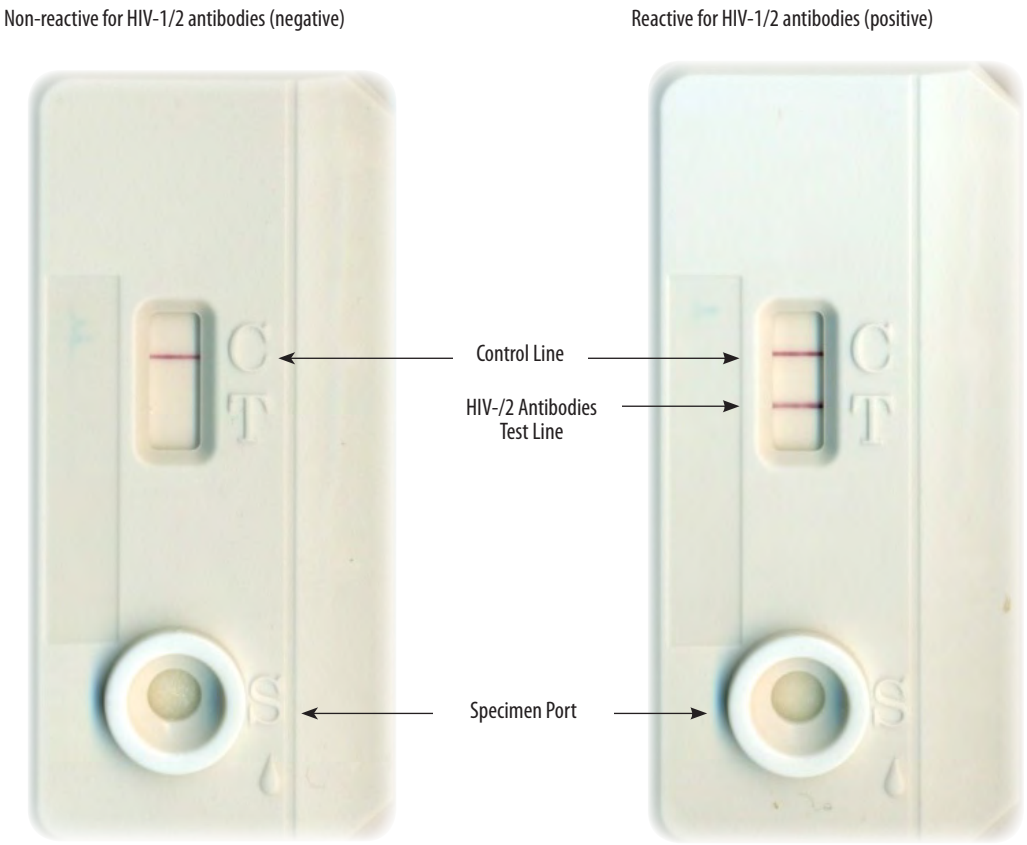
HIV 1/2 STAT-PAK®
(CHEMBIO DIAGNOSTIC SYSTEMS, INC.)

On a panel of 1079 specimens, we observed an initial sensitivity (95% CI) of 99.3% (97.9–100%) and an initial specificity (95% CI) of 100% (99.4–100%) compared to the reference assay results. The final sensitivity (95% CI) was 99.5% (98.3–100%) and the final specificity (95% CI) was 100% (99.4–100%) compared to the reference assay results. Lot to lot variation was acceptable.

For eight seroconversion panels, HIV 1/2 Stat-Pak detected on average 0.625 specimens later than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]). For the mixed titer panel, HIV 1/2 Stat-Pak correctly classified all specimens, three anti-HIV indeterminate/HIV-1 antigen positive specimens were non-reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], HIV 1/2 Stat-Pak detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.2%. The invalid rate was 0%.

Figure 5. Completed test devices for HIV 1/2 STAT-PAK®



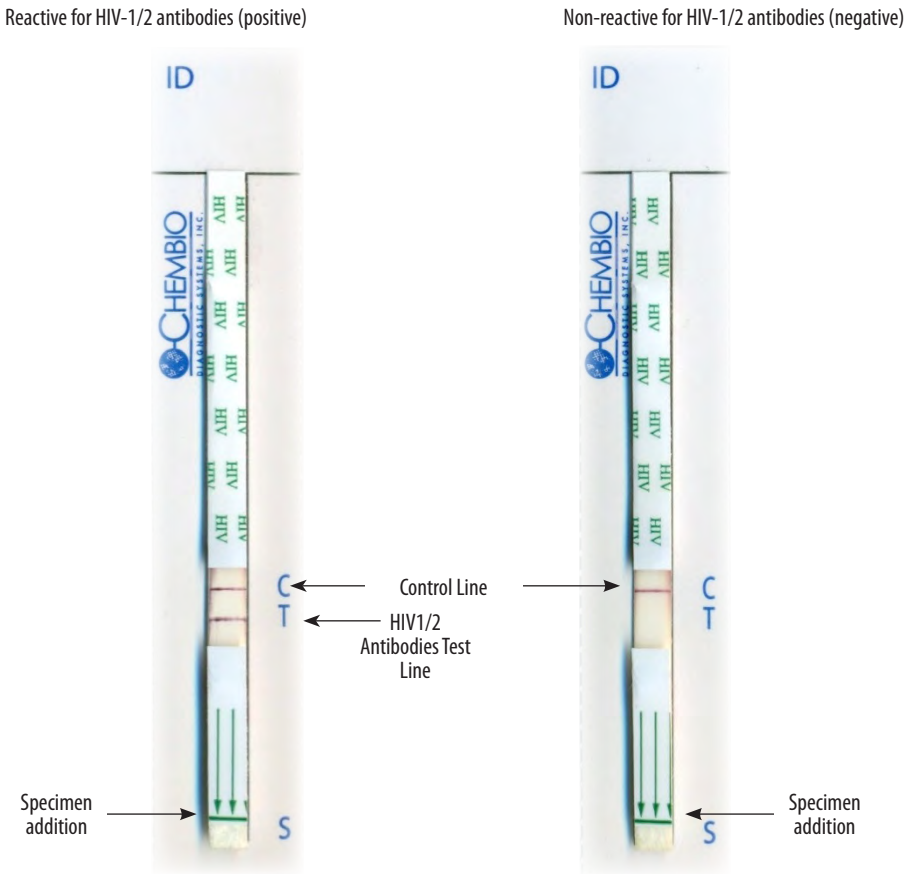
HIV 1/2 STAT-PAK® DIPSTICK (CHEMBIO DIAGNOSTIC SYSTEMS INC)

On a panel of 1079 specimens, we observed an initial sensitivity (95% CI) of 100% (99.1%–100%) and an initial specificity (95% CI) of 99.5% (98.7%–99.9%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1%–100%) and the final specificity (95% CI) was 99.7% (98.9%–99.9%) compared to the reference assays. Lot to lot variation was acceptable.

For eight seroconversion panels, HIV 1/2 Stat-Pak Dipstick detected on average 0.125 specimens later than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]). For the mixed titer panel, HIV 1/2 Stat-Pak Dipstick correctly classified all specimens, two anti-HIV indeterminate/HIV-1 antigen positive specimens were non-reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], HIV 1/2 Stat-Pak Dipstick detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.2%. The invalid rate was 0%

Figure 6. Completed test devices for HIV 1/2 STAT-PAK® dipstick



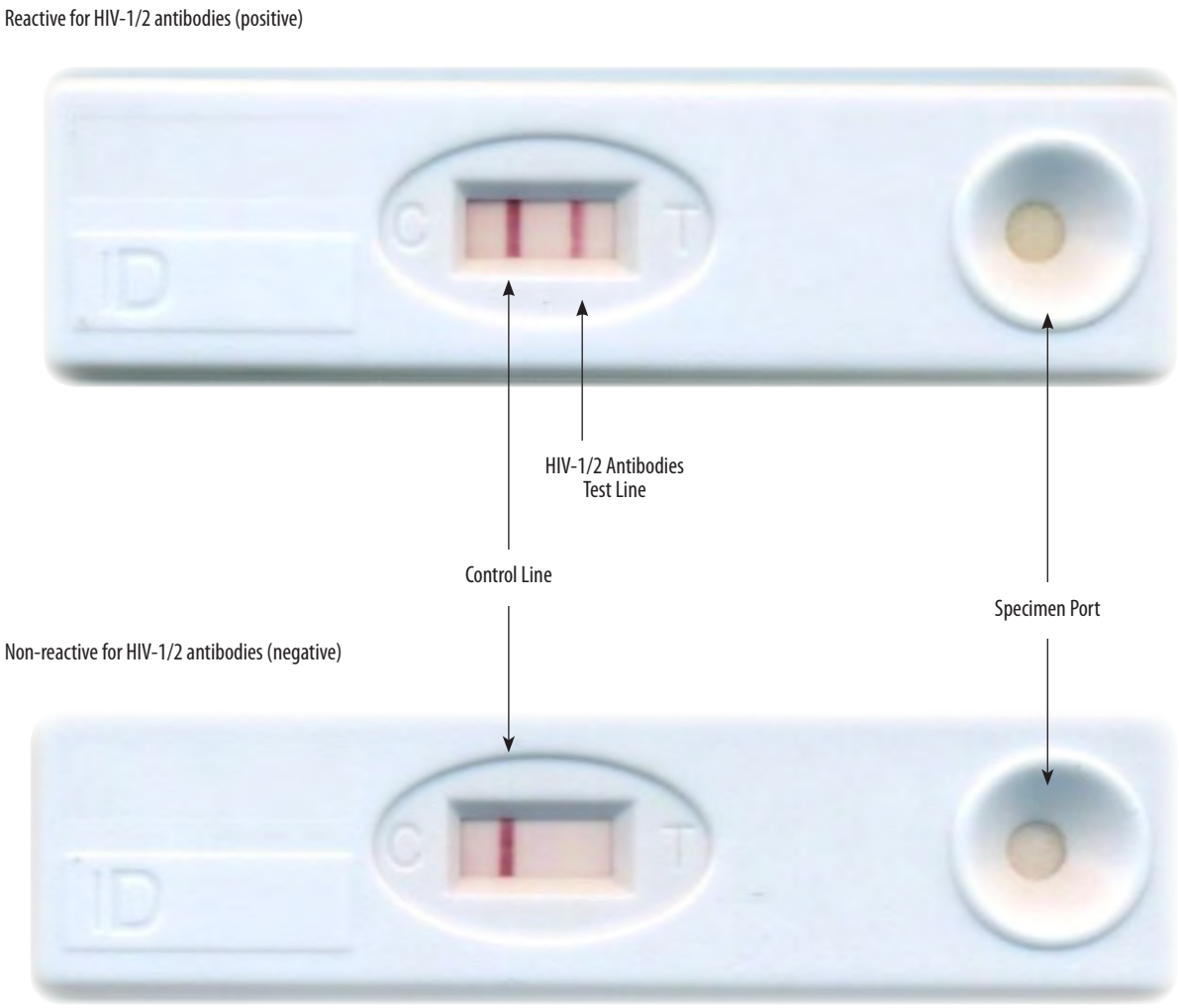
ONE STEP HIV1/2 WHOLE BLOOD/SERUM/PLASMA TEST (GUANGZHOU WONDFO BIOTECH CO., LTD.)

On a panel of 1079 clinically-derived specimens, we observed an initial sensitivity (95% CI) of 100% (99.1%–100%) and an initial specificity (95% CI) of 99.1% (98–99.7%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1–100%) and the final specificity (95% CI) was 99.9% (99.2–100%) compared to the reference assays. Lot to lot variation was acceptable.

For eight seroconversion panels, One Step HIV1/2 Whole Blood/Serum/Plasma Test detected on average 0.375 specimens later than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]). For the mixed titer panel, One Step HIV1/2 Whole Blood/Serum/Plasma Test correctly classified all specimens, one anti-HIV indeterminate/HIV-1 antigen positive specimen was non-reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], One Step HIV1/2 Whole Blood/Serum/Plasma Test detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

In this study, 0.09% of the results were recorded as indeterminate upon initial testing, with repeat testing 0% results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.2%. The invalid rate was 0 %.

Figure 7. Completed test devices for one step HIV1/2 whole blood/serum/plasma test



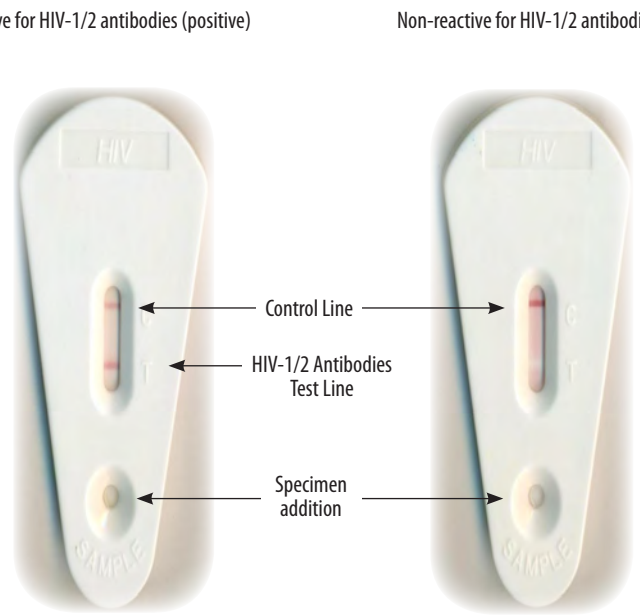
UNI-GOLD™ HIV (TRINITY BIOTECH PLC)

On a panel of 1079 clinically-derived specimens, we observed an initial sensitivity (95% CI) of 99.8% (98.7%–100%) and an initial specificity (95% CI) of 99.9% (99.2%–100%) compared to the reference assay results. The final sensitivity (95% CI) was 99.8% (98.7%–100%) and the final specificity (95% CI) was 99.9% (98.7%–100%) compared to the reference assay results. Lot to lot variation was acceptable.

For eight seroconversion panels, Uni-Gold™ HIV detected on average 0.125 specimens later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, Uni-Gold™ HIV correctly classified all specimens, two anti-HIV indeterminate/HIV-1 antigen positive specimens were non-reactive. Four out of the six anti-HIV indeterminate/HIV-1 antigen positive specimens were identified as anti-HIV-1/2 reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Uni-Gold™ HIV correctly classified all subtypes with the exception of subtype O (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2 were tested).

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.1%. The invalid rate was 0.1%.

Figure 8. Completed test devices for UNI-GOLD™ HIV



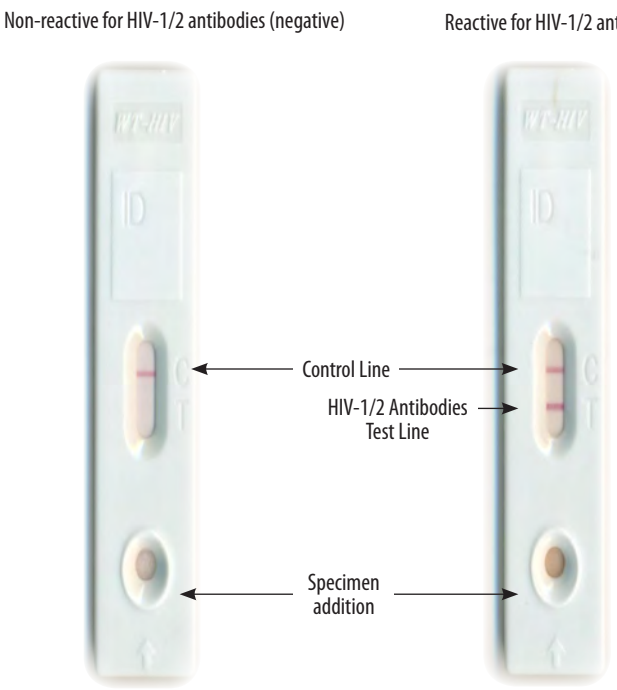
ANTI-HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY DIAGNOSTIC KIT (COLLOIDAL GOLD) (BEIJING WANTAI BIOLOGICAL PHARMACY ENTERPRISE CO., LTD.)

On a panel of 1079 clinically-derived specimens, we observed an initial sensitivity (95% CI) of 99.8% (99.7%–100%) and an initial specificity (95% CI) of 98.3% (97.0%–99.2%) compared to the reference assay results. The final sensitivity (95% CI) was 99.8% (98.7%–100%) and the final specificity (95% CI) was 98.5% (99.1%–100%) compared to the reference assay results. Lot to lot variation was acceptable with the exception of one dilution series for which there was a 2-fold difference between lots.

For eight seroconversion panels, Anti-human Immunodeficiency virus (HIV) antibody diagnostic kit (colloidal gold) detected on average 0.5 specimens later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, Anti-human Immunodeficiency virus (HIV) antibody diagnostic kit (colloidal gold) correctly classified all specimens. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Anti-human Immunodeficiency virus (HIV) antibody diagnostic kit (colloidal gold) correctly classified all subtypes (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0.1%. The invalid rate was 0.1%.

Figure 9. Completed test devices for ANTI-HUMAN immunodeficiency virus (HIV) ANTIBODY diagnostic kit (COLLOIDAL GOLD)



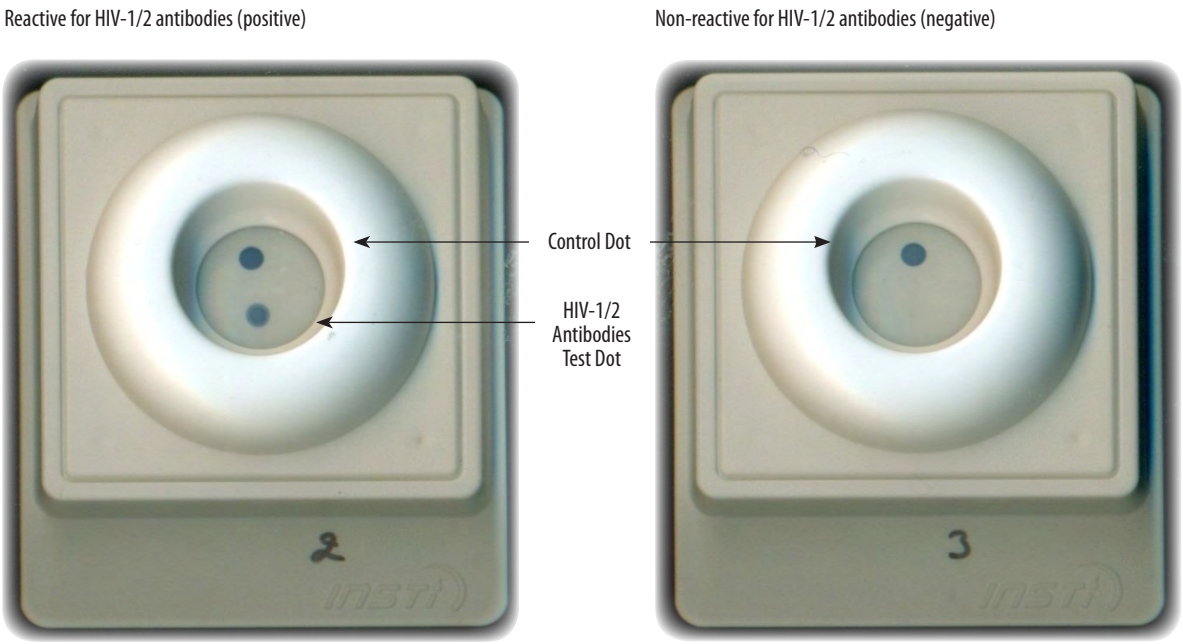
INSTI™ HIV-1/HIV-2 ANTIBODY TEST (BIOLYTICAL™ LABORATORIES INC)

On a panel of 1079 clinically-derived specimens, we observed an initial sensitivity (95% CI) of 100% (99.1%–100%) and an initial specificity (95% CI) of 99.7% (98.9%–100%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1%–100%) and the final specificity (95% CI) was 99.7% (98.9%–100%) compared to the reference assays. Lot to lot variation was acceptable with the exception of one dilution series for which there was a 2-fold difference between lots.

For eight seroconversion panels, INSTI™ HIV-1/HIV-2 Antibody Test detected on average 0.25 specimens later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, INSTI™ HIV-1/HIV-2 Antibody Test correctly classified all specimens, two anti-HIV indetermine/HIV-1 antigen positive specimens were non-reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], INSTI™ HIV-1/HIV-2 Antibody Test correctly classified all but two subtypes, specifically HIV-1 subtype C and HIV-1 type O were not detected (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2 were tested).

In this study, 0% of the results were recorded as indeterminate. Results were interpreted independently by three technicians; the inter-reader variability was 0%. The invalid rate was 0%.

Figure 10. Completed test devices for INSTI™ HIV-1/HIV-2 ANTIBODY test



REVEAL® RAPID HIV ANTIBODY TEST (MEDMIRA INC.)

On a panel of 1079 clinically-derived specimens, we observed an initial sensitivity (95% CI) of 98.5% (96.8%–99.5%) and an initial specificity (95% CI) of 99.5% (98.6%–99.5%) compared to the reference assay results. The final sensitivity (95% CI) was 99.8% (98.6%–100%) and the final specificity (95% CI) was 99.9% (99.2%–100%) compared to the reference assay results. Lot to lot variation was acceptable.

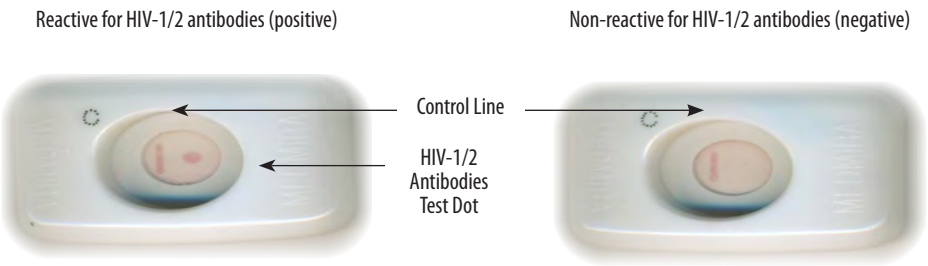
For eight seroconversion panels, Reveal® Rapid HIV Antibody Test detected on average 0.625 specimens later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, Reveal® HIV Rapid HIV Antibody Test correctly classified 19 specimens. A further five anti-HIV indeterminate/HIV-1 antigen positive specimens and one anti-HIV negative/HIV-1 antigen positive specimen were non-reactive. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Reveal® Rapid HIV Antibody Test correctly classified all subtypes (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2).

In this study, 0.3% of the results were initially recorded as indeterminate, upon repeat testing all results could be resolved to either reactive or not-reactive. Results were interpreted independently by three technicians; the inter-reader variability was 1%. The initial invalid rate was 3.1%, and the final invalid rate was 1%.

6.2 TABLES OF COMPARATIVE PERFORMANCE DATA

The assays are grouped into two for ease of reading. Tables 1/Table 10 summarize the general characteristics of each of the assays. The performance characteristics (i.e. the results of the assays evaluated as compared to the reference tests) are given in Table 2/Table 11. Table 3/Table 12 provides further details of operational aspects. Factors taken into account in the calculation of ease of performance and suitability for use in small laboratories are listed in Table 4/Table 13 and Table 5/Table 14, respectively. Performance of the assays evaluated on commercial seroconversion panels and the relative performance of the evaluated assays as compared to the benchmark assay are given in Table 6/Table 15. Performance on commercial anti-HIV 1 mixed titer panels is given in Table 7/Table 16. Performance on lot-to-lot variation panels is given in Table 8/17. Performance on WHO reference preparations is given in Table 9/18. Explanatory notes are provided at the end of each of the assay evaluation tables

Figure 11. Completed test devices for REVEAL® RAPID HIV ANTIBODY test



ASSAY EVALUATIONS

GROUP I

Table 1. General charateristics and operational aspects

PARAMETER	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/Serum/Plasma Test
Manufacturer (Address)	Alere Medical Co. Matsudo-shi, Chiba, Japan	Chembio Diagnostic Systems, Inc. Medford, NY, United States	Chembio Diagnostic Systems, Inc. Medford, NY, United States	Guangzhou Wondfo Biotech Co. Ltd. Luogang District, Guangzhou, China
Assay type	Lateral flow (immunochromatographic) rapid diagnostic test	Lateral flow (immunochromatographic) rapid diagnostic test	Lateral flow (immunochromatographic) rapid diagnostic test	Lateral flow (immunochromatographic) rapid diagnostic test
Antigen type	Recombinant and synthetic peptides for HIV-1 gp41 and HIV-2 gp36	Synthetic peptides for HIV-1 gp41, HIV-1 gp120, and HIV-2 gp36	Synthetic peptides for HIV-1 gp41, HIV-1 gp120, and HIV-2 gp36	Recombinant proteins gp41, gp120 and gp36
Detection type	Non-discriminatory (combined HIV 1/2 antibodies)	Non-discriminatory (combined HIV 1/2 antibodies)	Non-discriminatory (combined HIV 1/2 antibodies)	Non-discriminatory (combined HIV 1/2 antibodies)
Solid phase	Nitrocellulose membrane strip	Nitrocellulose membrane strip	Nitrocellulose membrane strip	Nitrocellulose membrane strip
Specimen type	Serum/plasma, venous/capillary whole blood	Serum/plasma, venous/capillary whole blood	Serum/plasma, venous/capillary whole blood	Serum/plasma, venous/capillary whole blood
Number of tests per kit (product code)	20 (7D2342), 100 (7D2343) Chase Buffer (7D2243) separate order	20 (HIV101), Control Set (HIV104) separate order	30 (HIV 303), Control Set (HIV104) separate order	25 (W6-C), 40 (no product code given)
Lot numbers evaluated (expiry date)	14696K100 (17/06/2012) 14734K100 (17/06/2012)	44032411 (02/02/2013) 44051011 (26/04/2013)	33042211 (24/03/2013) 33011411 (15/11/2012	WO611001W (10/2013) WO611002W (10/2013)
Shelf life upon manufacture	12 months	24 months	24 months	24 months
Storage conditions	2 to 30 ℃	8 to 30 ℃	8 to 30 ℃	4 to 30 ℃
Volume of specimen needed	50µl	5µl	5µl	80-100µl (for S/P), 50µl (for WB)
Time to test 1 specimen Time to test 1 run (h:min)	0:16 0:20	0.17 0.22	0:17 0:25	0:16 0:20
Reading	Visual	Visual	Visual	Visual
Indicative price/test	US$ 0.80–1.25	US$ 1.50	US$ 0.85–0.90	US$ 0.42–0.65

Notes for Table 1 **General characteristics and operational aspects of the assays**

Specimen type — The nature of specimen(s) that may be used in the assay but note that these laboratory evaluations were carried out using serum/plasma specimens, see section 5.2.1.

Non-discriminatory HIV-1/2 or discriminatory HIV-1 & HIV-2 reactivity — Non-discriminatory: No ability to differentiate between HIV-1 and HIV-2 reactivity i.e. one combined test line/band/spot/dot. Discriminatory: Ability to differentiate between HIV-1 and HIV-2 reactivity i.e. two separate test lines/bands/spots/dots.

Total time to perform the assay — Reflects the time needed to carry out 1 specimen and 1 test run, i.e. the most economical use of the technique.

Indicative price/test in US$ — As given at the time of the evaluation by the manufacturer, or converted to USD using the currency conversion rate at the time (2011-2012). The prices stated are meant to be indicative only.

Table 2. Comparison of the assays under evaluation with reference assays

PARAMETER	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/ Serum/Plasma Test
Initial Sensitivity % (95% CI)	100 (99.1–100) (n=420)	99.3 (97.9–100) (n=421)	100 (99.1–100) (n=421)	100 (99.1–100) (n=421)
Final Sensitivity % (95% CI)	100 (99.1–100) (n=421)	99.5 (98.3–99.9) (n=421)	100 (99.1–100) (n=421)	100 (99.1–100) (n=421)
Initial Specificity % (95% CI)	97.9 (96.4–98.8) (n=656)	100 (99.4–100) (n=658)	99.5 (98.7–99.9) (n=658)	99.1 (98–99.7) (n=658)
Final Specificity % (95% CI)	98.9 (97.8–99.6) (n=657)	100 (99.4–100) (n=658)	99.7 (98.9–99.9) (n=658)	99.9 (99.2–100) (n=658)
Initial indeterminate results %	0	0	0	0.1
Final indeterminate results %	0	0	0	0
Initial invalid rate %	0.3	0	0	0
Final invalid rate %	0.1	0	0	0
Initial inter-reader variability %	1.4	0.2	0.1	0.2
PPV 0.1% prevalence 1% prevalence 5% prevalence	8.3 47.9 82.7	100 100 100	25.0 77.1 94.6	40.0 87.1 97.2
NPV 0.1% prevalence 1% prevalence 5% prevalence	100 100 100	99.9 99.9 99.9	100 100 100	100 100 100

Notes for Table 2	Comparison of the results of the assays with reference assays
Sensitivity	Calculated as described on section 5.5.1 of this document.
Specificity	Calculated as described on section 5.5.1 of this document.
95% Confidence intervals (CI)	Calculated as described on section 5.5.1 of this document.
Indeterminate results	Rapid diagnostic tests - test results which could not be interpreted as clearly reactive or non-reactive were considered indeterminate.
Inter-reader variability	Calculated as described on section 5.5.3 of this document.
PPV and NPV	Calculated as described on section 5.5.1 of this document.

Table 3. Detailed operational aspects

PARAMETER	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/Serum/Plasma Test
Dimension (cm) of kit: w-l-h	27–2–16	16.5–13–7	14–5.5–6	16–14–7
Incubation temperature	Not stated	18 to 30 °C	18 to 30 °C	4 to 30 °C
Minimum incubation time (minutes)	15	15	15	15
Reading endpoint stability (minutes)	No more than 60 minutes after specimen or buffer has been added (stable for additional 45 minutes)	No more than 20 minutes after specimen/buffer has been added (stable for additional 5 minutes)	No more than 20 minutes after specimen/buffer has been added (stable for additional 5 minutes)	No more than 30 minutes after specimen/buffer has been added (stable for additional 15 minutes)
Stability after dilution/ opening				
test device	Test kit expiry date (2 to 30°C)	Test kit expiry date (8 to 30°C)	Test kit expiry date (8 to 30°C)	Test kit expiry date (4 to 30°C)
controls	N/A	Vial expiry date (2 to 8°C)	Vial expiry date (2 to 8°C)	N/A
sample diluent/running buffer	Test kit expiry date (2 to 30°C)	Test kit expiry date (8 to 30°C)	Test kit expiry date (8 to 30°C)	Test kit expiry date (4 to 30°C)
conjugate	N/A	N/A	N/A	N/A
substrate	N/A	N/A	N/A	N/A
wash buffer	N/A	N/A	N/A	N/A
No. of sera per run, min–max	1–10	1–10	1–10	1–10

Table 3. Detailed operational aspects (Continued)

PARAMETER	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/Serum/Plasma Test
Number of controls per run	Control samples not available from the manufacturer.	Control samples not supplied within the kit but available on order from manufacturer.	Control samples not supplied within the kit but available on order from manufacturer.	Control samples not available from the manufacturer.
negative	N/A	1	1	N/A
positive	N/A	1x HIV-1, 1x HIV-2	1x HIV-1, 1x HIV-2	N/A
internal control:				
reagent addition control	Yes	Yes	Yes	Yes
specimen addition control	No	Yes	Yes	No
Equipment/items required but not provided in the kit:				
washer	-	-	-	-
incubator (water-bath)	-	-	-	-
spectrophotometric reader	-	-	-	-
refrigerator (storage)	±	±	±	±
agitator, rocker	-	-	-	-
aspiration device	-	-	-	-
precision pipette (µl)	+ (if serum/plasma, 50µl)	-	-	-
multichannel (µl)	-	-	-	-
disposable tips	+ (if serum/plasma, 50µl)	-	-	-
dilution tubes/rack	-	-	+ (if alternate procedure)	-
microtiter plate	-	-	-	-
distilled or deionised water	-	-	-	-
plate covers	-	-	-	-
graduated pipette; cylinder (ml)	-	-	-	-
sulphuric acid/sodium hydroxide	-	-	-	-
absorbent paper	-	-	-	-
disinfectant	-	-	-	-
gloves	+	+	+	+
reagent trough	-	-	-	-
timer	+	+	+	+
centrifuge	+ (if serum/plasma)	+ (if serum/plasma)	+ (if serum/plasma)	+ (if serum/plasma)
alcohol swabs/cotton wool	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)
lancets	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)
specimen transfer devices	+ (if capillary whole blood)	-	-	-
venous blood collection equipment	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)
centrifuge	+ (if serum/plasma)	+ (if serum/plasma)	+ (if serum/plasma)	+ (if serum/plasma)
alcohol swabs/cotton wool	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)
lancets	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)

Table 3. Detailed operational aspects (Continued)

PARAMETER	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/Serum/Plasma Test
specimen transfer devices	+ (if capillary whole blood)	-	-	-
venous blood collection equipment	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)
Definition of reactive result	Red bar appears in the PATIENT window & the CONTROL window	Pink/purple coloured line appears in the TEST and CONTROL areas	Pink/purple coloured line appears in the TEST and CONTROL areas	Rose-pink band appears in the TEST and CONTROL areas
Definition of nonreactive result	One red bar appears in the CONTROL window but no red bar in the PATIENT window	One pink/purple coloured line appears in the CONTROL area but no line in the TEST area	One pink/purple coloured line appears in the CONTROL area but no line in the TEST area	Rose-pink band appears in the CONTROL area but no band in the TEST area
Definition of invalid result	No red bar in the CONTROL window	No pink/purple line in the CONTROL area	No pink/purple line in the CONTROL area	No distinct rose-pink band in the CONTROL area

Notes for Table 3	Detailed operational aspects of the assays
Reading endpoint stability	The time period after the completion of the test procedure, including any stated incubation period, within which the result may be read. Assays which show a time period of 0 must be read immediately upon completion of the test procedure.
Minimum - maximum number of sera	Minimum number = one specimen, in addition to the required controls. Maximum number = the maximum number of specimens, in addition to the required controls, which can be simultaneously tested within the limits of the test procedure.
Number of controls per test run	The number of controls shows the number of replicates of each control required for each test run.
Internal control:	
specimen addition control	The following assays have a control line that shows both that the specimen has been added and the reagents functioned correctly : HIV 1/2 Stat-Pak, HIV 1/2 Stat-Pak Dipstick.
reagent addition control	The following assay has a control line that which shows that the reagents have been added: Alere Determine HIV-1/2, One Step HIV 1/2 Whole Blood/ Serum/Plasma Test.
Definition of reactive, nonreactive, invalid results	A specimen is interpreted as reactive, non-reactive or invalid according to the criteria set by the manufacturer and summarized in the table.

+ : not provided in the kit but necessary to perform the test; - :provided in the kit or not necessary to perform the test; ± : use is optional; * comes with some kit configurations

Table 4A.Technician's appraisal of the test kit

PARAMETER	Score	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/Serum/Plasma Test
Number of steps in the test procedure:					
1–2 steps	6	6	6	6	6
3–5 steps	3				
> 5 steps	1				
Clarity of kit instructions:					
Good	2	2	2	2	2
Needs improvement	1				
Kit and reagent packaging and labelling:					
Good	2	2	2	2	2
Needs improvement	1				
Total (out of 10)	10	10	10	10	10
Comments on the test kit		None stated	The presence of serum/ plasma specimens in the specimen loop is difficult to verify given the small volume and clearish colour.	None stated	For one test device, the control band was not visible as the strip had become displaced. For two other test devices, the strip was displaced but the control band was still visible.

Table 4B. Calculation of ease of performance

PARAMETER	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK®		HIV 1/2 STAT-PAK® DIPSTICK		One Step HIV 1/2 Whole Blood/Serum/Plasma Test	
	Serum/ plasma	Capillary WB	Serum/ plasma	Capillary WB	Serum/ plasma	Capillary WB	Serum/ plasma	Capillary WB
Need to prepare:								
1 = reagent needs no preparation								
0 = reagent needs preparation								
antigen	1	1	1	1	1	1	1	1
substrate	1	1	1	1	1	1	1	1
wash solution	1	1	1	1	1	1	1	1
conjugate	1	1	1	1	1	1	1	1
pre-dilution of specimen	1	1	1	1	1	1	1	0
Stability after dilution/opening:								
1 = expiry date or N/A								
0 = less than kit expiry date								
test device	1	1	1	1	1	1	1	1
controls	1	1	1	1	1	1	1	1
sample diluent/running buffer	1	1	1	1	1	1	1	1
conjugate	1	1	1	1	1	1	1	1
substrate	1	1	1	1	1	1	1	1
wash buffer	1	1	1	1	1	1	1	1
sufficient reagents	1	1	1	1	1	1	1	1
wash (yes=1; no =0)	0	0	0	0	0	0	0	0
Items required but not provided in the kit:								
1 = item provided in kit or N/A								
0 = item not provided in kit								
reagent trough	1	1	1	1	1	1	1	1
precision pipette	0	1	1	1	1	1	1	1
dilution tubes, rack/microtiter plate	1	1	1	1	1/0**	1	1	1
distilled or deionised water	1	1	1	1	1	1	1	1
plate covers	1	1	1	1	1	1	1	1
graduated pipette, cylinder	1	1	1	1	1	1	1	1
sulphuric acid/sodium hydroxide	1	1	1	1	1	1	1	1
lancets, alcohol swabs, cotton wool	1	0	0	1	0	1	0	1
specimen transfer devices, running buffer	0	0***	1	1	1	1	1	1
Technician's appraisal of the test kit – see Table 4A (rating out of 10)	10	10	10	10	10	10	10	10
Total (out of possible 30)	29	28	29	30	29/28	30	30	30
Ease of performance:								
less easy < 20								
easy 20 ≤ x < 25								
very easy > 25	very easy		very easy		very easy		very easy	

Notes for Table 4 B **Technician's appraisal and calculation of ease of performance of the assays**

** For HIV-1/2 Stat-Pak Dipstick, if using alternate test procedure, sample tubes and tube rack are required.

Table 5. Technical suitability for use in small laboratories or non-laboratory testing services

PARAMETER	Score	Alere Determine™ HIV-1/2	HIV 1/2 STAT-PAK®	HIV 1/2 STAT-PAK® DIPSTICK	One Step HIV 1/2 Whole Blood/Serum/ Plasma Test
Sensitivity (final)					
100%	5	5		5	5
98–100%	3		3		
< 98%	0				
Specificity (final)					
> 98%	5	5	5	5	5
95–98%	3				
< 95%	0				
Incubation temperature					
room temp °C	3	3	3	3	3
other than room temp °C	1				
Shelf-life					
> 1 year	3		3	3	3
> 6 months < 1 year	2	2			
< 6 months	1				
Storage at					
room temp °C possible (opened kit)	5	5	5	5	5
room temp °C possible (unopened kit)	2				
2–8 °C required	1				
Price per test (US$)					
< 1.0	3			3	3
> 1.0 < 2.0	2	2	2		
> 2.0	1				
Ease of performance					
very easy	5	5	5	5	5
easy	3				
less easy	1				
Rapidity of performance: 1 specimen					
< 10 min	3				
10–30 min	2	2	2	2	2
> 30 min	1				
Washer/agitator					
not needed	3	3	3	3	3
needed	1				
Reading					
visual:					
inter-reader variability ≤ 3%	5	5	5	5	5
inter-reader variability > 3%	3				
reading equipment	1				
Total (out of possible 40)		37	36	39	39
Suitability for use in small labs and non-lab testing sevices					
less suitable < 23					
suitable 23 < x < 30					
very suitable > 30		very suitable	very suitable	very suitable	very suitable

Table 6. Results on commercial HIV seroconversion panels

Panel	Days since 1st bleed	Assays under evaluation				Reference Results									
		Alere Determine HIV-1/2	HIV-1/2 STAT-PAK	HIV-1/2 STAT-PAK DIPSTICK	One Step HIV 1/2 WB/S/P Test	INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV Confirmation[1]						
						OD/CO	OD/CO	OD/CO	Sgp120	gp41	p31	p24	p17	sgp105	gp36	Result
PRB914-01	0	R	NR	R	NR	0.43	4.49	4.68	-	2+	-	1+	-	-	-	HIV-1
PRB914-02	4	R	NR	R	NR	0.41	4.16	7.63	-	2+	-	2+	-	-	-	HIV-1
PRB914-03	7	R	R	R	R	0.39	4.71	8.23	-	2+	-	2+	-	-	-	HIV-1
PRB914-04	25	R	R	R	R	0.46	6.47	18.29	1+	2+	-	2+	1+	-	-	HIV-1
PRB914-05	31	R	R	R	R	0.44	6.61	18.29	2+	2+	-	3+	1+	-	-	HIV-1
PRB925-01	0	NR	NR	NR	NR	0.43	0.10	0.37	-	-	-	-	-	-	-	Neg
PRB925-02	10	NR	NR	NR	NR	0.41	0.08	0.38	-	-	-	-	-	-	-	Neg
PRB925-03	18	NR	NR	NR	NR	0.37	0.08	0.35	-	-	-	-	-	-	-	Neg
PRB925-04	22	NR	NR	NR	NR	0.41	0.09	0.35	-	-	-	-	-	-	-	Neg
PRB925-05	44	R	R	R	R	10.57	6.61	3.88	-	2+	-	-	-	-	-	IND
PRB925-06	49	R	R	R	R	5.62	6.61	6.13	-	3+	-	2+	-	-	-	HIV-1
PRB926-01	0	NR	NR	NR	NR	0.45	0.07	0.37	-	-	-	-	-	-	-	Neg
PRB926-02	2	NR	NR	NR	NR	0.43	0.08	0.37	-	-	-	-	-	-	-	Neg
PRB926-03	7	NR	NR	NR	NR	9.14	0.08	0.77	-	-	-	-	-	-	-	Neg
PRB926-04	9	NR	NR	NR	NR	21.74	0.07	5.42	-	-	-	-	-	-	-	Neg
PRB926-05	27	R	R	R	R	2.26	6.6	10.93	-	3+		2		-	-	HIV-1
PRB926-06	32	R	R	R	R	2.66	661	16.84	-	3+	-	2+	1+	-	-	HIV-1
PRB930-01	0	NR	NR	NR	NR	2.29	0.09	0.59	-	-	-	-	-	-	-	Neg
PRB930-02	3	NR	NR	NR	NR	9.01	0.12	1.05	-	-	-	-	-	-	-	Neg
PRB930-03	7	R	NR	NR	R	15.48	4.57	3.46	-	1+	-	-	-	-	-	IND
PRB930-04	10	R	NR	R	R	19.40	6.61	7.32	-	2+	-	2+	-	-	-	HIV-1
PRB955-01	0	NR	NR	NR	NR	0.49	0.08	0.38	-	-	-	-	-	-	-	Neg
PRB955-02	3	NR	NR	NR	NR	1.95	0.07	0.51	-	-	-	-	-	-	-	Neg
PRB955-03	7	NR	NR	NR	NR	14.95	0.11	1.66	-	-	-	-	-	-	-	Neg
PRB955-04	12	R	NR	R	NR	18.59	1.19	2.41	-	-	-	-	-	-	-	Neg
PRB955-05	14	R	R	R	R	17.88	6.61	6.70	-	1+	-	1+	-	-	-	HIV-1
PRB965-01	0	NR	NR	NR	NR	0.46	0.09	0.37	-	-	-	-	-	-	-	Neg
PRB965-02	5	NR	NR	NR	NR	0.49	0.08	0.52	-	-	-	-	-	-	-	Neg

Table 6. Results on commercial HIV seroconversion panels (Continued)

Panel	Days since 1st bleed	Assays under evaluation				Reference Results									
									INNO-LIA HIV Confirmation[1]						
		Alere Determine HIV-1/2	HIV-1/2 STAT-PAK	HIV-1/2 STAT-PAK DIPSTICK	One Step HIV 1/2 WB/S/P Test	INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]							
						OD/CO	OD/CO	OD/CO	Sgp120	gp41	p31	p24	p17	sgp105	gp36	Result
PRB965-03	7	NR	NR	NR	NR	0.51	0.16	0.63	-	-	-	-	-	-	-	Neg
PRB965-04	12	R	NR	R	R	0.49	6.61	3.45	-	+	-	-	-	-	-	Neg
PRB965-05	14	R	R	R	R	0.50	6.61	6.32	-	2+	-	-	-	-	-	IND
PRB965-06	21	R	R	R	R	0.49	6.61	6.93	-	2+	-	1+	-	-	-	HIV-1
PRB968-01	0	NR	NR	NR	NR	0.41	0.08	0.32	-	-	-	-	-	-	-	Neg
PRB968-02	3	NR	NR	NR	NR	0.38	0.08	0.30	-	-	-	-	-	-	-	Neg
PRB968-03	8	NR	NR	NR	NR	0.42	0.10	0.35	-	-	-	-	-	-	-	Neg
PRB968-04	10	NR	NR	NR	NR	0.42	0.08	0.36	-	-	-	-	-	-	-	Neg
PRB968-05	15	NR	NR	NR	NR	0.45	0.08	0.34	-	-	-	-	-	-	-	Neg
PRB968-06	17	NR	NR	NR	NR	0.52	0.08	0.37	-	-	-	-	-	-	-	Neg
PRB968-07	26	NR	NR	NR	NR	21.74	0.26	3.46	-	-	-	-	-	-	-	Neg
PRB968-08	28	R	NR	NR	NR	21.74	0.78	6.75	-	-	-	-	-	-	-	Neg
PRB968-09	33	R	R	R	R	11.56	6.61	5.07	-	2+	-	2+	-	-	-	HIV-1
PRB968-10	35	R	R	R	R	2.12	6.55	4.50	-	2+	-	2+	1+	-	-	HIV-1
PRB969-01	0	NR	NR	NR	NR	0.41	0.09	0.38	-	-	-	-	-	-	-	Neg
PRB969-02	29	NR	NR	NR	NR	0.41	0.08	0.33	-	-	-	-	-	-	-	Neg
PRB969-03	48	NR	NR	NR	NR	0.44	0.08	0.35	-	-	-	-	-	-	-	Neg
PRB969-04	53	NR	NR	NR	NR	0.45	0.08	0.39	-	-	-	-	-	-	-	Neg
PRB969-05	55	NR	NR	NR	NR	0.44	0.07	0.54	-	-	-	-	-	-	-	Neg
PRB969-06	61	NR	NR	NR	NR	0.64	0.08	0.35	-	-	-	-	-	-	-	Neg
PRB969-07	63	NR	NR	NR	NR	1.02	0.07	0.38	-	-	-	-	-	-	-	Neg
PRB969-08	70	R	R	R	R	2.49	5.96	4.49	-	2+	-	2+	2+	-	-	HIV-1
PRB969-09	72	R	R	R	R	1.86	5.75	8.94	-	2+	-	2+	2+	-	-	HIV-1
PRB969-10	77	R	R	R	R	0.57	2.58	6.15	-	2+	-	2+	2+	-	-	HIV-1

Notes for Table 6 **Performance of the assay on seroconversion panels**

An assay's performance on the seroconversion panels should be viewed against both the sensitivity and specificity of the assay. Caution should be taken when reviewing seroconversion performance of assays tested only in eight seroconversion panels.

Reference results [1]Reference results obtained from I™, Belgium. INNOTEST HIV Antigen mAb (HIV-1 p24 antigen EIA), Enzygnost Anti-HIV1/2 Plus (HIV-1/2 antibody EIA), Vironostika HIV Ag/Ab combo (HIV-1/2 antibody and HIV-1 p24 antigen EIA), and INNO-LIA HIV Confirmation (HIV line immunoassay)

Assays under evaluation RDT1: Alere Determine™ HIV-1/2, RDT2: HIV 1/2 STAT-PAK®, RDT3: HIV 1/2 STAT-PAK® DIPSTICK, RDT4: One Step HIV1/2 Whole Blood/Serum/Plasma Test

See figures 11 and 12 for graphical representation of the relative seroconversion sensitivity of each RDT in comparison to either antibody-detection or antibody and/or antigen-detection EIAs

Table 7. Results on commercial Anti-HIV 1 mixed titer performance panel

	Assays under evaluation				Reference Results										
					INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV I/II Score Immunoblot[1]							
Panel	RDT1	RDT2	RDT3	RDT4	OD/CO	OD/CO	OD/CO	sgp 120	gp41	p31	p24	p17	sgp 105	gp36	Result	Reference Result
PRB205-01	R	R	R	R	0.43	3.90	5.94	-	2+	-	2+	-	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-02	R	R	R	R	15.49	6.68	20.13	3+	3+	2+	3+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-03	R	R	R	R	26.32	6.68	9.74	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-04	R	R	R	R	0.65	6.68	20.13	2+	3+	2+	2+	-	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-05	R	R	R	R	0.39	6.68	20.13	3+	3+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-06	R	NR	NR	R	1.81	4.82	3.42	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-07	R	NR	R	R	9.18	5.80	2.49	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-08	R	R	R	R	26.32	3.90	20.13	-	2+	-	-	-	-	-	IND	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-09	R	R	R	R	0.45	6.68	20.13	2+	2+	2+	2+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-10	R	R	R	R	0.41	6.68	20.13	3+	3+	-	3+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-11	R	R	R	R	4.42	6.68	20.13	3+	2+	2+	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-12	NR	NR	NR	NR	12.85	2.87	1.93	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-13	R	R	R	R	0.39	6.68	20.13	2+	4+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-14	NR	NR	NR	NR	0.39	0.08	0.38	-	-	-	-	-	-	-	Neg	Anti-HIV negative HIV-1 antigen negative
PRB205-15	R	R	R	R	3.53	3.21	13.78	1+	2+	-	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-16	R	R	R	R	0.42	6.68	20.13	3+	3+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-17	R	R	R	R	1.07	4.78	17.20	1+	2+	-	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-18	R	R	R	R	4.68	6.68	4.01	-	2+	-	-	-	-	-	IND	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-19	R	R	R	R	0.46	5.26	20.13	2+	3+	-	2+	1+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-20	R	R	R	R	0.42	6.68	20.13	3+	3+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-21	NR	NR	NR	NR	0.41	0.09	0.43	-	-	-	-	-	-	-	Neg	Anti-HIV negative HIV-1 antigen negative
PRB205-22	R	R	R	R	2.48	6.68	20.13	3+	3+	2+	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-23	R	R	R	R	0.40	6.68	18.22	2+	3+	-	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-24	NR	NR	NR	NR	9.10	0.89	0.82	-	-	-	-	-	-	-	Neg	Anti-HIV negative HIV-1 antigen positive
PRB205-25	R	R	R	R	0.38	6.68	20.13	3+	4+	-	1+	1+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative

Notes for Table 7 **Performance on mixed HIV-1 antibody titer specimens**

Reference results [1] Reference results obtained from ITM, Belgium. INNOTEST HIV Antigen mAb (HIV-1 p24 antigen EIA), Enzygnost Anti-HIV1/2 Plus (HIV-1/2 antibody EIA), Vironostika HIV Ag/Ab combo (HIV-1/2 antibody and HIV-1 p24 antigen EIA), and INNO-LIA HIV Confirmation (HIV line immunoassay).

Assays under evaluation RDT1: Alere Determine™ HIV-1/2, RDT2: HIV 1/2 STAT-PAK®, RDT3: HIV 1/2 STAT-PAK® DIPSTICK, RDT4: One Step HIV1/2 Whole Blood/Serum/Plasma Test

Table 8. Results on lot-to-lot variation panel

Dilution	Specimen ID	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK ®		HIV 1/2 STAT-PAK ® DIPSTICK		One Step HIV 1/2 Whole Blood/ Serum/Plasma Test		Reference Results
		Lot 14696K100	Lot 14734K100	Lot 44032144	Lot 44051011	Lot 33042211	Lot 33011411	Lot WO611001W	Lot WO611002W	Vironstika HIV Ag/Ab
Neat	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/32	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/64	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/128	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/256	WHO3-0690	R	R	NR	NR	R	R	R	R	15.63
1/512	WHO3-0690	R	R	NR	NR	R	R	R	R	9.75
1/1024	WHO3-0690	R	R	NR	NR	NR	R	NR	R	7.20
1/2048	WHO3-0690	NR	R	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0690	R	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		0		1		1		
Neat	WHO3-0736	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0736	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0736	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0736	R	R	NR	R	R	R	R	R	> 17.8
1/16	WHO3-0736	R	R	NR	NR	R	R	R	R	> 17.8
1/32	WHO3-0736	R	NR	NR	NR	NR	NR	R	NR	> 17.8
1/64	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	1.89

Dilution	Specimen ID	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK ®		HIV 1/2 STAT-PAK ® DIPSTICK		One Step HIV 1/2 Whole Blood/ Serum/Plasma Test		Reference Results
		Lot 14696K100	Lot 14734K100	Lot 44032144	Lot 44051011	Lot 33042211	Lot 33011411	Lot WO611001W	Lot WO611002W	Vironstika HIV Ag/Ab
1/8192	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		1		1		1		
Neat	WHO3-0789	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0789	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0789	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0789	R	R	NR	NR	R	R	R	R	> 17.8
1/16	WHO3-0789	R	R	NR	NR	R	R	R	R	> 17.8
1/32	WHO3-0789	R	R	NR	NR	NR	NR	R	R	> 17.8
1/64	WHO3-0789	NR	R	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		0		0		0		
Neat	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/32	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/64	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/128	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/256	WHO3-0634	R	R	R	R	R	R	R	R	15.63
1/512	WHO3-0634	R	R	NR	NR	R	R	R	R	9.75
1/1024	WHO3-0634	R	R	NR	NR	R	NR	R	R	7.20

Dilution	Specimen ID	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK ®		HIV 1/2 STAT-PAK ® DIPSTICK		One Step HIV 1/2 Whole Blood/ Serum/Plasma Test		Reference Results
		Lot 14696K100	Lot 14734K100	Lot 44032144	Lot 44051011	Lot 33042211	Lot 33011411	Lot WO611001W	Lot WO611002W	Vironstika HIV Ag/Ab
1/2048	WHO3-0634	R	R	NR	NR	NR	NR	R	R	4.41
1/4096	WHO3-0634	R	R	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0634	R	R	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0634	R	R	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0634	NR	NR	NR	NR	NR	NR	NR	NR	0.66
1/65536	WHO3-0634	NR	NR	NT	NT	NR	NR	NT	NT	1.15
1/131072	WHO3-0634	NR	NR	NT	NT	NR	NR	NT	NT	0.86
1/262144	WHO3-0634	NR	NR	NT	NT	NR	NR	NT	NT	0.62
2-fold Difference		0		0		1		0		
Neat	WHO3-0577	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0577	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0577	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0577	R	R	R	NR	R	R	R	R	> 17.8
1/16	WHO3-0577	R	R	NR	NR	R	R	NR	NR	> 17.8
1/32	WHO3-0577	R	R	NR	NR	NR	R	NR	NR	> 17.8
1/64	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		1		1		0		
Neat	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/32	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8

Table 8. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK ®		HIV 1/2 STAT-PAK ® DIPSTICK		One Step HIV 1/2 Whole Blood/ Serum/Plasma Test		Reference Results
		Lot 14696K100	Lot 14734K100	Lot 44032144	Lot 44051011	Lot 33042211	Lot 33011411	Lot WO611001W	Lot WO611002W	Vironstika HIV Ag/Ab
1/64	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/128	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/256	WHO3-0584	R	R	NR	NR	R	R	R	R	15.63
1/512	WHO3-0584	R	R	NR	NR	R	NR	R	R	9.75
1/1024	WHO3-0584	R	R	NR	NR	NR	NR	R	R	7.20
1/2048	WHO3-0584	R	R	NR	NR	NR	NR	R	NR	4.41
1/4096	WHO3-0584	R	R	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0584	NR	R	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	0.66
1/65536	WHO3-0584	NR	NR	NT	NT	NR	NR	NT	NT	0.74
1/131072	WHO3-0584	NR	NR	NT	NT	NR	NR	NT	NT	0.57
1/262144	WHO3-0584	NR	NR	NT	NT	NR	NR	NT	NT	0.45
2-fold Difference		1		0		1		1		
Neat	990885	R	R	R	R	R	NT	R	R	> 17.8
1/2	990885	R	R	NR	NR	R	R	R	R	> 17.8
1/4	990885	R	R	NR	NR	NR	R	R	R	> 17.8
1/8	990885	R	R	NR	NR	NR	NR	R	R	> 17.8
1/16	990885	R	R	NR	NR	NR	NR	R	R	> 17.8
1/32	990885	R	R	NR	NR	NR	NR	R	NR	> 17.8
1/64	990885	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	990885	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	990885	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	990885	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	990885	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	990885	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	990885	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	990885	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	990885	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	990885	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		0		1		1		

Table 8. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK ®		HIV 1/2 STAT-PAK ® DIPSTICK		One Step HIV 1/2 Whole Blood/ Serum/Plasma Test		Reference Results
		Lot 14696K100	Lot 14734K100	Lot 44032144	Lot 44051011	Lot 33042211	Lot 33011411	Lot WO611001W	Lot WO611002W	Vironstika HIV Ag/Ab
Neat	990814	R	R	NR	NR	NT	R	R	R	> 17.8
1/2	990814	R	R	NR	NR	NR	NR	R	R	> 17.8
1/4	990814	R	R	NR	NR	NR	NR	R	R	> 17.8
1/8	990814	R	R	NR	NR	NR	NR	R	R	> 17.8
1/16	990814	R	R	NR	NR	NR	NR	R	R	> 17.8
1/32	990814	R	NR	NR	NR	NR	NR	R	R	> 17.8
1/64	990814	NR	NR	NR	NR	NR	NR	R	R	> 17.8
1/128	990814	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	990814	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	990814	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	990814	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	990814	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	990814	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	990814	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	990814	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	990814	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		0		1		0		
Neat	990831	R	R	R	R	NT	R	R	R	> 17.8
1/2	990831	R	R	R	R	R	R	R	R	> 17.8
1/4	990831	R	R	R	R	R	R	R	R	> 17.8
1/8	990831	R	R	R	R	R	R	R	R	> 17.8
1/16	990831	R	R	R	R	R	R	R	R	> 17.8
1/32	990831	R	R	R	R	R	R	R	R	> 17.8
1/64	990831	R	R	R	R	R	R	R	R	> 17.8
1/128	990831	R	R	NR	NR	R	R	R	NR	> 17.8
1/256	990831	R	R	NR	NR	NR	NR	NR	NR	15.63
1/512	990831	R	R	NR	NR	NR	NR	NR	NR	9.75
1/1024	990831	R	R	NR	NR	NR	NR	NR	NR	7.20
1/2048	990831	R	R	NR	NR	NR	NR	NR	NR	4.41
1/4096	990831	R	R	NR	NR	NR	NR	NR	NR	1.89
1/8192	990831	R	R	NR	NR	NR	NR	NR	NR	1.59

Table 8. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	Alere Determine™ HIV-1/2		HIV 1/2 STAT-PAK ®		HIV 1/2 STAT-PAK ® DIPSTICK		One Step HIV 1/2 Whole Blood/ Serum/Plasma Test		Reference Results
		Lot 14696K100	Lot 14734K100	Lot 44032144	Lot 44051011	Lot 33042211	Lot 33011411	Lot WO611001W	Lot WO611002W	Vironstika HIV Ag/Ab
1/16384	990831	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	990831	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		0		0		1		
Neat	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0788	R	R	NR	NR	R	R	R	R	> 17.8
1/32	WHO3-0788	NR	NR	NR	NR	NR	NR	R	R	> 17.8
1/64	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		0		0		0		

Table 9. Results on WHO reference preparations

	Assays under evaluation				Reference Results									
					INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV Confirmation[1]						
Subtyp	Alere Determine HIV-1/2	HIV-1/2 STAT-PAK	HIV-1/2 STAT-PAK DIPSTICK	One Step HIV 1/2 WB/S/P Test	OD/CO	OD/CO	OD/CO	Sgp120	gp41	p31	p24	p17	sgp105	gp36	Result
HIV-1 A	R	R	R	R	> 17.1	> 6.7	0.4	4+	4+	2+	3+	4+	-	-	HIV-1
HIV-1 B	R	R	R	R	> 17.1	> 6.7	0.4	3+	3+	2+	2+	-	-	-	HIV-1
HIV-1 C	R	R	R	R	> 17.1	> 6.7	0.4	2+	2+	1+	2+	2+	-	-	HIV-1
HIV-1 CRF01_AE	R	R	R	R	> 17.1	> 6.7	0.4	2+	2+	2+	2+	2+	-	-	HIV-1
HIV-1 O	R	R	R	R	> 17.1	> 6.7	0.6	2+	2+	1+	-	-	-	-	HIV-1
HIV-2	R	R	R	R	> 17.1	> 6.7	0.4	-	-	1+	3+	1+	2+	2+	HIV-2

Notes for Table 9 **Performance of assays on WHO reference preparations**

Reference results [1] Reference results obtained from ITM, Belgium. INNOTEST HIV Antigen mAb (HIV-1 p24 antigen EIA), Enzygnost Anti-HIV1/2 Plus (HIV-1/2 antibody EIA), Vironostika HIV Ag/Ab combo (HIV-1/2 antibody and HIV-1 p24 antigen EIA), and INNO-LIA HIV Confirmation (HIV line immunoassay).

Assays under evaluation RDT1: Alere DetermineTM HIV-1/2, RDT2: HIV 1/2 STAT-PAK®, RDT3: HIV 1/2 STAT-PAK® DIPSTICK, RDT4: One Step HIV1/2 Whole Blood/Serum/Plasma Test

ASSAY EVALUATIONS

GROUP II

Table 10. General charateristics and operational aspects

PARAMETER	INSTI™ HIV-1/HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Manufacturer (Address)	bioLytical™ Laboratories Inc. Richmond, BC, Canada	MedMira Laboratories Inc. Halifax, Canada	Trinity Biotech PLC, Wicklow, Ireland	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. Beijing, China
Assay type	Flow through (immunofiltration) rapid diagnostic test	Flow through (immunofiltration) rapid diagnostic test	Lateral flow (immunochromatographic) rapid diagnostic test	Lateral flow (immunochromatographic) rapid diagnostic test
Antigen type	Recombinant proteins for HIV-1 gp41 and HIV-2 gp36	Synthetic peptides for HIV-1 gp41, gp 120 and (group O) and HIV-2 gp36	Recombinant proteins for HIV-1 gp41, HIV-1gp120 and HIV-2 gp36	Recombinant HIV-1 gp120 and gp41 and HIV-2 gp36 antigens
Detection type	Non-discriminatory (combined HIV 1/2 antibodies)	Non-discriminatory (combined HIV 1/2 antibodies)	Non-discriminatory (combined HIV 1/2 antibodies)	Non-discriminatory (combined HIV 1/2 antibodies)
Solid phase	Synthetic filtration membrane atop of absorbent material	Nitrocellulose membrane unit	Nitrocellulose membrane strip	Nitrocellulose membrane strip
Specimen type	Serum/plasma, venous/capillary whole blood	Serum/plasma, venous/capillary whole blood	Serum/plasma, venous/capillary whole blood	Serum/plasma, venous/capillary whole blood
Number of tests per kit (product code)	1 test (90-1012) with consumables, 24 tests (90-1013) with consumables, 24 tests (90-1010) without consumables, 48 tests (90-1022) with consumables, 48 tests (90-1021) without consumables, controls (80-1037) for 8 tests	30 tests (815311000768) with consumables 30 tests (815311000775) with consumables 30 tests (815311000782) without consumables controls (815311005510) for 5 tests	20 tests	10 tests (WJ-1810) with support accessories, 50 tests (WJ-1850)
Lot numbers evaluated (expiry date)	B1M134 (02/02/2013) B1M135 (02/02/2013)	RUIA0013 (09/2013) RUIA0014 (09/2013)	HIV 1100043 (07/06/2013) HIV 1100022 (25/05/2013)	V 20111201 (06/2013) V 20120101 (07/2013)
Shelf life upon manufacture	15 months	18 months	12 months	18 months
Storage conditions	15 to 30 °C (no refrigeration)	2 to 30 °C	2 to 27 °C	2 to 30 °C
Volume of specimen needed	50µl	35µl	60µl	80µl for serum/plasma 50µl for whole blood
Time to test 1 specimen Time to test 1 run (h:min)	0:02 0:08	0:03 0:07	0:11 0:15	0:11 0:13
Reading	Visual	Visual	Visual	Visual
Indicative price/test	US$ 1.50–2.50 US$ 10.00 (Controls)	US$ 2.40–4.00	US$ 1.60–2.80	US$ 0.35–0.60

Notes for Table 10

General characteristics and operational aspects of the assays

Specimen type	The nature of specimen(s) that may be used in the assay but note that these evaluations were carried out using serum/plasma specimens, see section 5.2.1.
Non-discriminatory or discriminatory HIV-1 & HIV-2 reactivity	Non-discriminatory: No ability to differentiate between HIV-1 and HIV-2 reactivity, i.e. one combined test line/band/spot/dot. Discriminatory: Ability to differentiate between HIV-1 and HIV-2 reactivity, i.e. two separate test lines/bands/spots/dots.
Shelf life (at °C)	The maximum shelf life of the product if stored within the given temperature range.
Total time to perform the assay	Reflects the time needed to carry out 1 specimen and 1 test run, i.e. the most economical use of the technique.
Indicative price/test in US$	As given at the time of the evaluation by the manufacturer, or converted to USD using the currency conversion rate at the time (2011-2012). The prices stated are meant to be indicative only.

Table 11. Comparison of the assays under evalution with reference assays

PARAMETER		INSTI™ HIV-1/HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Initial Sensitivity % (95% CI)		100 (99.1–100) n=421	98.5 (96.8–99.5) n=402	99.8 (98.7–100) n=421	99.8 (98.7–100) n=421
Final Sensitivity % (95% CI)		100 (99.1–100) n=421	99.8 (98.6–100) n=408	99.8 (98.7–100) n=421	99.8 (98.7–100) n=421
Initial Specificity % (95% CI)		99.7 (98.9–100) n=658	99.5 (98.6–99.9) n=643	99.9 (99.2–100) n=658	98.3 (97.0–99.2) n=658
Final Specificity % (95% CI)		99.7 (98.9–100) n=658	99.9 (99.2–100) n=655	99.9 (99.2–100) n=658	98.5 (97.2–99.3) n=658
Initial indeterminate results %		0	0.3	0	0
Final indeterminate results %		0	0	0	0
Initial invalid rate %		0	3.7	0.1	0.1
Final invalid rate %		0	1.5	0	0
Initial inter-reader variability %		0	1.6	0.1	0.1
PPV	0.1%	25.0	39.9	39.9	6.2
	1%	77.1	87.0	87.0	39.9
	5%	94.6	97.2	97.2	77.6
NPV	0.1%	100	100	100	99.9
	1%	100	100	100	99.9
	5%	100	99.9	97.2	99.9

Notes for Table 11	Comparison of the results of the assays with reference assays
Sensitivity	Calculated as described on section 5.5.1 of this document.
Specificity	Calculated as described on section 5.5.1 of this document.
95% Confidence intervals (CI)	Calculated as described on section 5.5.1 of this document.
Indeterminate results	Rapid diagnostic test - test results which could not be interpreted as clearly reactive or non-reactive were considered indeterminate.
Inter-reader variability	Calculated as described on section 5.5.3 of this document.
PPV and NPV	Calculated as described on section 5.5.1 of this document.

Table 12. Detailed operational aspects

PARAMETER	INSTI™ HIV-1/HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Dimension (cm) of kit: w-l-h	22–29–16.5	20–17–14.5	23–14–8	13.5–9.5–7.5
Incubation temperature	15 to 42 °C	Not stated	15 to 27 °C	Not stated
Incubation time (minutes)	0	0	10	10-30
Reading endpoint stability (minutes)	No more than 5 minutes after clarifying solution has been added (stable for additional 5 minutes)	0 (read immediately)	No more than 20 minutes after specimen/buffer has been added (stable for additional 10 minutes)	No more than 30 minutes after specimen/buffer has been added (stable for additional 20 minutes)
Stability after dilution/ opening				
test device	Test kit expiry date (15 to 30°C)	Test kit expiry date (2 to 30°C)	Test kit expiry date (2 to 27°C)	Test kit expiry date (2 to 30°C)
controls	28 days (2 to 8°C)	21 days (2 to 8°C)	N/A	N/A
sample diluent/running buffer	Test kit expiry date (15 to 30°C)	Not stated	Test kit expiry date (2 to 27°C)	1 month (room temperature)
conjugate	N/A	N/A	N/A	N/A
substrate	1 month (15 to 30°C)****	N/A	N/A	N/A
wash buffer	N/A	N/A	N/A	N/A
No. of sera per run, min –max	1–5	1–5	1–10	1–10

Table 12. Detailed operational aspects (Continued)

PARAMETER	INSTI™ HIV-1/HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Number of controls per test run	Controls not supplied within the kit but available on order from manufacturer.	Controls not supplied within the kit but available on order from manufacturer.	Controls not available from manufacturer.	Controls not available from manufacturer.
negative	1	1	N/A	N/A
positive	1 x HIV-1 and 1x HIV-2	1x HIV-1/2	N/A	N/A
internal controls :				
reagent addition control	Yes	Yes	Yes	Yes
specimen addition control	Yes	Yes	No	No
Equipment required but not provided in the kit:				
washer	-	-	-	-
incubator (water-bath)	-	-	-	-
spectrophotometric reader	-	-	-	-
refrigerator (storage)	-	±	±	±
agitator, rocker	-	-	-	-
aspiration device	-	-	-	-
precision pipette (µl)	+ (if serum/plasma/venous WB) (50µl)	-	-	+ (if serum/plasma)
multichannel (µl)	-	-	-	-
disposable tips	+ (if serum/plasma/venous WB) (50µl)	-	-	+ (if serum/plasma)
dilution tubes/rack	-	-	-	-
microtiterplate	-	-	-	-
distilled or deionised water	-	-	-	-
plate covers	-	-	-	-
graduated pipette; cylinder (ml)	-	-	-	-
sulphuric acid/sodium hydroxide	-	-		
absorbent paper	-	-	-	-
disinfectant	-	-	-	-
gloves	+	+	+	+
reagent trough	-	-	-	-
timer	+	+	+	+
centrifuge	+ (if serum/plasma)	+ (if serum/plasma)	+ (if serum/plasma)	+ (if serum/plasma)
alcohol swabs	+* (if capillary whole blood)	+* (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)
lancets	+* (if capillary whole blood)	+* (if capillary whole blood)	+ (if capillary whole blood)	+ (if capillary whole blood)
specimen transfer devices	+*(if capillary whole blood)	+*(if capillary whole blood)	-	-
blood collection equipment	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)	+ (if serum/plasma, venous WB)
Definition of reactive result	Blue dots appear that are discernible above any background tint in the TEST and CONTROL areas.	One vertical red line appears under C and a red dot appears next to the T on the test membrane.	A line of any intensity forming in the test region, plus a line forming in the control region indicates a positive result.	One red line next to the Test Zone (T) indicates that antibodies to HIV 1+2 have been detected using this test.

Table 12. Detailed operational aspects (Continued)

PARAMETER	INSTI™ HIV-1/HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Definition of nonreactive result	Appearance of one blue dot that is discernible above any background tint in the CONTROL area and no dot in the TEST area	One vertical red line appears under C.	A line in the control region only indicates a negative test result.	No red line appears within 30 minutes next to the Test Zone (T) indicating that no antibodies to HIV 1+2 have been detected with this HIV 1+2 Rapid Test.
Definition of invalid result	No distinct dot in the CONTROL area OR uniform tint across the membrane OR only appearance of blue specks on the membrane.	If no red line appears under C even if a dot appears in the test region.	No line appears in the control region. The test should be repeated with a fresh device, irrespective of a line developing in the test region.	One red line will always appear next to the Control Zone (C) indicating the validity of the test. If no red line appears, the test is invalid – discard the test and repeat with new sample and new cassette.

Notes for Table 12	**Detailed operational aspects of the assays**
Reading endpoint stability	The time period after the completion of the test procedure, including any stated incubation period, within which the result may be read. Assays which show a time period of 0 must be read immediately upon completion of the test procedure.
Stability after dilution/opening	**** For Insti HIV-1/HIV-2 Antibody Test, the colour developer and clarifying solution have one month stability after opening.
Minimum - maximum number of sera	Minimum number = one specimen, in addition to the required controls. Maximum number = the maximum number of specimens, in addition to the required controls, which can be simultaneously tested within the limits of the assay procedure.
Number of controls per test run	The number of controls shows the number of replicates of each control required for each assay run.
Internal control:	
- specimen addition control	The following assays have a control line that shows both that the specimen has been added and the reagents functioned correctly: Reveal® Rapid HIV Antibody Test, INSTI™ HIV-1/HIV-2 Antibody Test.
- reagent addition control	The following assay has a control line that which shows that the reagents have been added but do not confirm if the specimen has been added: Uni-Gold™ HIV, Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold).
Definition of reactive, non-reactive, invalid results	A specimen is interpreted as reactive according to the criteria set by the manufacturer and summarized in the table.

+ : not provided in the kit but necessary to perform the test; - :provided in the kit or not necessary to perform the test; ± : use is optional ; * comes with some kit configurations

Table 13A. Technician's appraisal of the test kit

PARAMETER	Score	INSTI™ HIV-1/HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Number of steps in the test procedure:					
1–2 steps	6			6	6
3–5 steps	3	3	3		
>5 steps	1				
Clarity of kit instructions:					
Good	2	2	2	2	2
Needs improvement	1				
Kit and reagent packaging and labelling:					
Good	2	2	2	2	2
Needs improvement	1				
Total (out of 10)	10	7	7	10	10
Comments on the test kit		For less than 1% of the test devices, the control dot had a larger diameter than for the others.	12 drops of buffer must be dispensed into the InstantGold cap; a graduated dropper would make this easier. As mentioned in the IFU, the use of cloudy or viscous specimens can cause incorrect test results as observed during this evaluation.	None stated.	None stated.

Table 13B. Calculation of ease of performance

PARAMETER	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
	With accessories	Without accessories	With accessories	Without accessories	Serum/ plasma	Capillary WB	
Need to prepare:							
1 = reagent needs no preparation							
0 = reagent needs preparation							
antigen	1	1	1	1	1	1	1
substrate	1	1	0	0	1	1	1
wash solution	1	1	1	1	1	1	1
conjugate	1	1	1	1	1	1	1
pre-dilution of specimen	0	0	0	0	1	1	1
Stability after dilution/opening:							
1 = expiry date							
0 = less than kit expiry date							
test device	1	1	1	1	1	1	1
controls	0	0	0	0	1	1	1
sample diluent/running buffer	1	1	1	1	1	1	0
conjugate	1	1	1	1	1	1	1
substrate	0	0	0	0	1	1	1
wash buffer	1	1	1	1	1	1	0
sufficient reagents	1	1	1	1	1	1	1
wash (yes=1; no =0)	1	1	1	1	1	1	1
Item needed but not provided in the kit:							
1 = item provided in kit or N/A							
0 = item not provided in kit							
reagent trough	1	1	1	1	1	1	1
precision pipette	1	0	1	0	1	1	1
dilution tubes, rack/microtiter plate	1	1	1	1	1	1	1
distilled or deionised water	1	1	1	1	1	1	1
plate covers	1	1	1	1	1	1	1
graduated pipette, cylinder ml	1	1	1	1	1	1	1
sulphuric acid/sodium hydroxide	1	1	1	1	1	1	1
lancets, alcohol swabs, cotton wool	1	0	1	0	1	0	1
specimen transfer devices, running buffer	1	1	1	1	1	1	1
Technician's appraisal of the test kit – see Table 13 A (rating out of 10)	7	7	7	7	10	10	10
Total (out of 30)	24	22	25	23	32	31	30
Ease of performance:							
less easy < 20							
easy 20 ≤ x < 25	easy	easy	easy	easy			
very easy > 25					very easy	very easy	very easy

Notes for Table 13A and 13B Technician's appraisal and calculation of ease of performance of the assays
The criteria for this calculation are given in the respective tables.

Table 14. Technicial suitability for use in small laboratories or non-laboratory testing services

PARAMETER	Score	INSTI™ HIV-1/ HIV-2 Antibody Test	Reveal® Rapid HIV Antibody Test	Uni-Gold™ HIV	Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)
Sensitivity (final)					
100%	5	5			
98–100%	3		3	3	3
<98%	0				
Specificity (final)					
>98%	5	5	5	5	5
95–98%	3				
<95%	0				
Incubation temperature					
room temp °C	3	3	3	3	3
other than room temp °C	1				
Shelf-life					
>1 year	3	3	3	3	3
> 6 months < 1 year	2				
< 6 months	1				
Storage at					
room temp °C possible (opened kit)	5	5	5	5	5
room temp °C possible (unopened kit)	2				
2–8 °C required	1				
Price per test (US$)					
< 1.0	3				3
>1.0 < 2.0	2			2	
> 2.0	1	1	1		
Ease of performance					
very easy	5			5	5
easy	3	3	3		
less easy	1				
Rapidity of performance: 1 specimen					
< 10 min	3	3	3	3	
10–30 min	2				2
> 30 min	1				
Washer/agitator					
not needed	3	3	3	3	3
needed	1				
Reading					
visual:					
inter-reader variability ≤ 3%	5	5	5	5	5
inter-reader variability > 3%	3				
reading equipment	1				
Total (out of possible 40)		36	35	37	37
Suitability for use in small labs or non-lab testing services					
less suitable < 23					
suitable 23 < x < 30					
very suitable > 30		very suitable	very suitable	very suitable	very suitable

Table 15. Results on commercial HIV seroconversion panels

Panel	Days since 1st bleed	Assays under evaluation				Reference Results									
		Insti HIV-1/ HIV-2 Ab Test	Reveal Rapid HIV Ab Test	Uni-Gold HIV	Anti-HIV Ab Diagnostic Test (colloidal gold)	INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV Confirmation[1]						
						OD/CO	OD/CO	OD/CO	Sgp120	gp41	p31	p24	p17	sgp105	gp36	Result
PRB914-01	0	R	R	R	NR	0.43	4.49	4.68	-	2+	-	1+	-	-	-	HIV-1
PRB914-02	4	R	R	R	R	0.41	4.16	7.63	-	2+	-	2+	-	-	-	HIV-1
PRB914-03	7	R	R	R	R	0.39	4.71	8.23	-	2+	-	2+	-	-	-	HIV-1
PRB914-04	25	R	R	R	R	0.46	6.47	18.29	1+	2+	-	2+	1+	-	-	HIV-1
PRB914-05	31	R	R	R	R	0.44	6.61	18.29	2+	2+	-	3+	1+	-	-	HIV-1
PRB925-01	0	NR	NR	NR	NR	0.43	0.10	0.37	-	-	-	-	-	-	-	Neg
PRB925-02	10	NR	NR	NR	NR	0.41	0.08	0.38	-	-	-	-	-	-	-	Neg
PRB925-03	18	NR	NR	NR	NR	0.37	0.08	0.35	-	-	-	-	-	-	-	Neg
PRB925-04	22	NR	NR	NR	NR	0.41	0.09	0.35	-	-	-	-	-	-	-	Neg
PRB925-05	44	R	R	R	R	10.57	6.61	3.88	-	2+	-	-	-	-	-	IND
PRB925-06	49	R	R	R	R	5.62	6.61	6.13	-	3+	-	2+	-	-	-	HIV-1
PRB926-01	0	NR	NR	NR	NR	0.45	0.07	0.37	-	-	-	-	-	-	-	Neg
PRB926-02	2	NR	NR	NR	NR	0.43	0.08	0.37	-	-	-	-	-	-	-	Neg
PRB926-03	7	NR	NR	NR	NR	9.14	0.08	0.77	-	-	-	-	-	-	-	Neg
PRB926-04	9	NR	NR	NR	NR	21.74	0.07	5.42	-	-	-	-	-	-	-	Neg
PRB926-05	27	R	R	R	R	2.26	6.61	10.93	-	3+		2		-	-	HIV-1
PRB926-06	32	R	R	R	R	2.66	6.61	16.84	-	3+	-	2+	1+	-	-	HIV-1
PRB930-01	0	NR	NR	NR	NR	2.29	0.09	0.59	-	-	-	-	-	-	-	Neg
PRB930-02	3	NR	NR	NR	NR	9.01	0.12	1.05	-	-	-	-	-	-	-	Neg
PRB930-03	7	NR	NR	R	R	15.48	4.57	3.46	-	1+	-	-	-	-	-	IND
PRB930-04	10	R	R	R	R	19.40	6.61	7.32	-	2+	-	2+	-	-	-	HIV-1
PRB955-01	0	NR	NR	NR	NR	0.49	0.08	0.38	-	-	-	-	-	-	-	Neg
PRB955-02	3	NR	NR	NR	NR	1.95	0.07	0.51	-	-	-	-	-	-	-	Neg
PRB955-03	7	NR	NR	NR	NR	14.95	0.11	1.66	-	-	-	-	-	-	-	Neg
PRB955-04	12	NR	NR	NR	NR	18.59	1.19	2.41	-	-	-	-	-	-	-	Neg
PRB955-05	14	R	NR	R	R	17.88	6.61	6.70	-	1+	-	1+	-	-	-	HIV-1
PRB965-01	0	NR	NR	NR	NR	0.46	0.09	0.37	-	-	-	-	-	-	-	Neg

Table 15. Results on commercial HIV seroconversion panels (Continued)

Panel	Days since 1st bleed	Assays under evaluation				Reference Results									
		Insti HIV-1/ HIV-2 Ab Test	Reveal Rapid HIV Ab Test	Uni-Gold HIV	Anti-HIV Ab Diagnostic Test (colloidal gold)	INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV Confirmation[1]						
						OD/CO	OD/CO	OD/CO	Sgp120	gp41	p31	p24	p17	sgp105	gp36	Result
PRB965-02	5	NR	NR	NR	NR	0.49	0.08	0.52	-	-	-	-	-	-	-	Neg
PRB965-03	7	NR	NR	NR	NR	0.51	0.16	0.63	-	-	-	-	-	-	-	Neg
PRB965-04	12	R	NR	R	R	0.49	6.61	3.45	-	+	-	-	-	-	-	Neg
PRB965-05	14	R	R	R	R	0.50	6.61	6.32	-	2+	-	-	-	-	-	IND
PRB965-06	21	R	R	R	R	0.49	6.61	6.93	-	2+	-	1+	-	-	-	HIV-1
PRB968-01	0	NR	NR	NR	NR	0.41	0.08	0.32	-	-	-	-	-	-	-	Neg
PRB968-02	3	NR	NR	NR	NR	0.38	0.08	0.30	-	-	-	-	-	-	-	Neg
PRB968-03	8	NR	NR	NR	NR	0.42	0.10	0.35	-	-	-	-	-	-	-	Neg
PRB968-04	10	NR	NR	NR	NR	0.42	0.08	0.36	-	-	-	-	-	-	-	Neg
PRB968-05	15	NR	NR	NR	NR	0.45	0.08	0.34	-	-	-	-	-	-	-	Neg
PRB968-06	17	NR	NR	NR	NR	0.52	0.08	0.37	-	-	-	-	-	-	-	Neg
PRB968-07	26	NR	NR	NR	NR	21.74	0.26	3.46	-	-	-	-	-	-	-	Neg
PRB968-08	28	NR	NR	NR	NR	21.74	0.78	6.75	-	-	-	-	-	-	-	Neg
PRB968-09	33	R	R	R	R	11.56	6.61	5.07	-	2+	-	2+	-	-	-	HIV-1
PRB968-10	35	R	R	R	R	2.12	6.55	4.50	-	2+	-	2+	1+	-	-	HIV-1
PRB969-01	0	NR	NR	NR	NR	0.41	0.09	0.38	-	-	-	-	-	-	-	Neg
PRB969-02	29	NR	NR	NR	NR	0.41	0.08	0.33	-	-	-	-	-	-	-	Neg
PRB969-03	48	NR	NR	NR	NR	0.44	0.08	0.35	-	-	-	-	-	-	-	Neg
PRB969-04	53	NR	NR	NR	NR	0.45	0.08	0.39	-	-	-	-	-	-	-	Neg
PRB969-05	55	NR	NR	NR	NR	0.44	0.07	0.54	-	-	-	-	-	-	-	Neg
PRB969-06	61	NR	NR	NR	NR	0.64	0.08	0.35	-	-	-	-	-	-	-	Neg
PRB969-07	63	NR	NR	NR	NR	1.02	0.07	0.38	-	-	-	-	-	-	-	Neg
PRB969-08	70	R	NR	R	R	2.49	5.96	4.49	-	2+	-	2+	2+	-	-	HIV-1
PRB969-09	72	R	R	R	R	1.86	5.75	8.94	-	2+	-	2+	2+	-	-	HIV-1
PRB969-10	77	R	R	R	R	0.57	2.58	6.15	-	2+	-	2+	2+	-	-	HIV-1

Notes for Table 15 **Performance of the assay on seroconversion panels**

An assay's performance on the seroconversion panels should be viewed against both the sensitivity and specificity of the assay. Caution should be taken when reviewing seroconversion performance of assays tested only in eight seconversion panels

Reference results [1] Results obtained from ITM, Belgium. INNOTEST HIV Antigen mAb (HIV-1 p24 antigen EIA), Enzygnost Anti-HIV1/2 Plus (HIV-1/2 antibody EIA), Vironostika HIV Ag/Ab combo (HIV-1/2 antibody and HIV-1 p24 antigen EIA), and INNO-LIA HIV Confirmation (HIV line immunoassay).

Assays under evaluation RDT1: INSTI™ HIV-1/HIV-2 Antibody Test, RDT2: Reveal® Rapid HIV Antibody Test, RDT3:Uni-Gold™ HIV ® RDT4: Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)

Figure 11. Relative seroconversion sensitivity compared to anti body-detection enzyme immunoassay (Enzygnost Anti-HIV-1/2 plus [Siemens Healthcare Diagnostics])

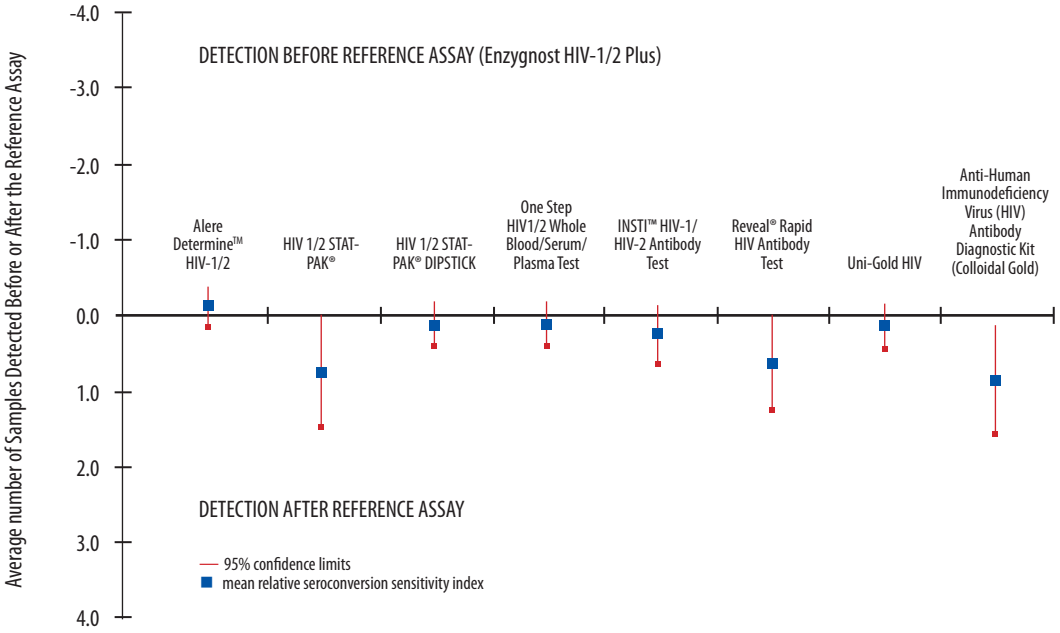


Figure 12. Relative seroconversion sensitivity compared to an antigen/antibody combined detection enzyme immunoassay (Vironostika Ag/Ab Combo [bioMérieux])

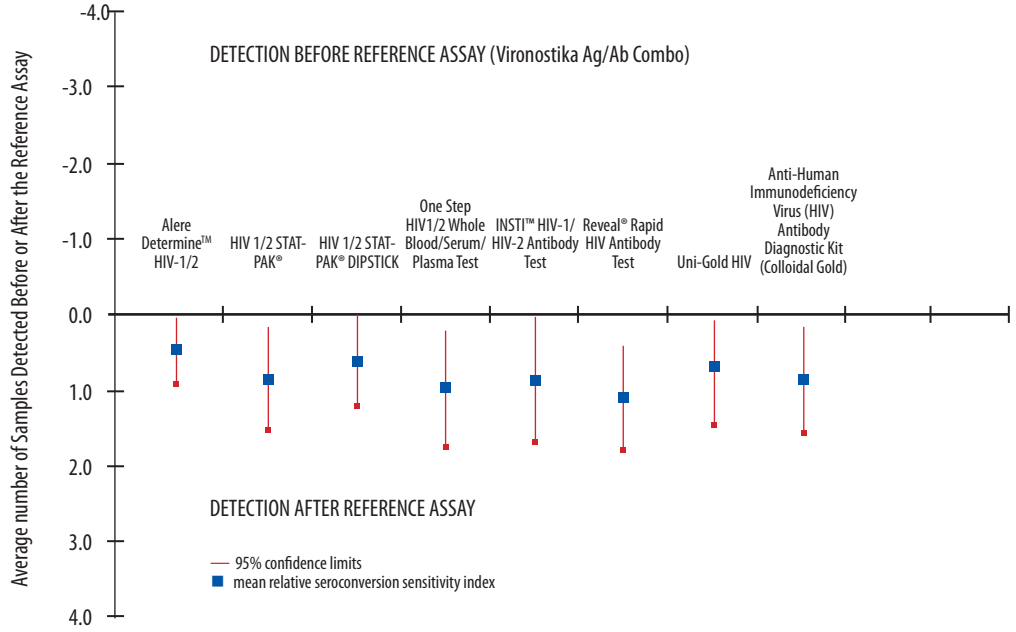


Table 16. Results on commercial Anti-HIV 1 mixed titer performance panel

	Assays under evaluation				Reference Results											
					INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV Confirmation[1]								
Panel	RDT1	RDT2	RDT3	RDT4	OD/CO	OD/CO	OD/CO	sgp 120	gp41	p31	p24	p17	sgp 105	gp 36	Result	Reference Result
PRB205-01	R	R	R	R	0.43	3.90	5.94	-	2+	-	2+	-	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-02	R	R	R	R	15.49	6.68	20.13	3+	3+	2+	3+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-03	R	R	R	NR	26.32	6.68	9.74	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-04	R	R	R	R	0.65	6.68	20.13	2+	3+	2+	2+	-	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-05	R	R	R	R	0.39	6.68	20.13	3+	3+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-06	R	R	R	NR	1.81	4.82	3.42	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-07	R	R	R	NR	9.18	5.80	2.49	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-08	NR	NR	R	NR	26.32	3.90	20.13	-	2+	-	-	-	-	-	IND	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-09	R	R	R	R	0.45	6.68	20.13	2+	2+	2+	2+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-10	R	R	R	R	0.41	6.68	20.13	3+	3+	-	3+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-11	R	R	R	R	4.42	6.68	20.13	3+	2+	2+	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-12	NR	NR	R	NR	12.85	2.87	1.93	-	-	-	-	-	-	-	Neg	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-13	R	R	R	R	0.39	6.68	20.13	2+	4+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-14	NR	NR	NR	NR	0.39	0.08	0.38	-	-	-	-	-	-	-	Neg	Anti-HIV negative HIV-1 antigen negative
PRB205-15	R	R	R	R	3.53	3.21	13.78	1+	2+	-	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-16	R	R	R	R	0.42	6.68	20.13	3+	3+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-17	R	R	R	R	1.07	4.78	17.20	1+	2+	-	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-18	R	R	R	R	4.68	6.68	4.01	-	2+	-	-	-	-	-	IND	Anti-HIV indeterminate HIV-1 antigen positive
PRB205-19	R	R	R	R	0.46	5.26	20.13	2+	3+	-	2+	1+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-20	R	R	R	R	0.42	6.68	20.13	3+	3+	2+	3+	3+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-21	NR	NR	NR	NR	0.41	0.09	0.43	-	-	-	-	-	-	-	Neg	Anti-HIV negative HIV-1 antigen negative
PRB205-22	R	R	R	R	2.48	6.68	20.13	3+	3+	2+	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen positive
PRB205-23	R	R	R	R	0.40	6.68	18.22	2+	3+	-	2+	2+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative
PRB205-24	NR	NR	NR	NR	9.10	0.89	0.82	-	-	-	-	-	-	-	Neg	Anti-HIV negative HIV-1 antigen positive
PRB205-25	R	R	R	R	0.38	6.68	20.13	3+	4+	-	1+	1+	-	-	HIV-1	Anti-HIV positive HIV-1 antigen negative

Notes for Table 16 **Results for mixed HIV-1 antibody titer specimens**

Reference results [1] Results obtained from ITM, Antwerp. INNOTEST HIV Antigen mAb (HIV-1 p24 antigen EIA), Enzygnost Anti-HIV1/2 Plus (HIV-1/2 antibody EIA) Vironostika HIV Ag/Ab combo (HIV-1/2 antibody and HIV-1 p24 antigen EIA), and INNO-LIA HIV Confirmation (HIV line immunoassay).

Assays under evaluation RDT1: INSTI™ HIV-1/HIV-2 Antibody Test, RDT2: Reveal® Rapid HIV Antibody Test, RDT3:Uni-Gold™ HIV ®, RDT4: Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold).

Table 17. Results on lot-to-lot variation panel

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
Neat	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/32	WHO3-0690	R	R	R	R	R	R	R	R	> 17.8
1/64	WHO3-0690	R	R	NR	R	NR	R	R	R	> 17.8
1/128	WHO3-0690	NR	R	NR	NR	NR	NR	R	R	> 17.8
1/256	WHO3-0690	NR	NR	NR	NR	NR	NR	R	R	15.63
1/512	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0690	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		1		1		0		
Neat	WHO3-0736	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0736	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0736	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0736	R	R	R	R	R	NR	R	R	> 17.8
1/16	WHO3-0736	R	R	NR	R	NR	NR	R	R	> 17.8
1/32	WHO3-0736	R	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/64	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	15.63

Table 17. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
1/512	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0736	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		1		1		0		
Neat	WHO3-0789	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0789	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0789	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0789	R	R	NR	R	R	R	R	R	> 17.8
1/16	WHO3-0789	R	R	NR	NR	NR	NR	R	R	> 17.8
1/32	WHO3-0789	NR	R	NR	NR	NR	NR	NR	NR	> 17.8
1/64	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0789	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		1		0		0		

Table 17. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
Neat	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/32	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/64	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/128	WHO3-0634	R	R	R	R	R	R	R	R	> 17.8
1/256	WHO3-0634	R	R	NR	R	R	R	R	R	15.63
1/512	WHO3-0634	NR	R	NR	NR	NR	NR	R	R	9.75
1/1024	WHO3-0634	NR	NR	NR	NR	NR	NR	R	R	7.20
1/2048	WHO3-0634	NR	NR	NR	NR	NR	NR	R	R	4.41
1/4096	WHO3-0634	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0634	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0634	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0634	NR	NR	NR	NR	NR	NR	NR	NR	0.66
1/65536	WHO3-0634	NT	NT	NT	NT	NT	NT	NT	NT	1.15
1/131072	WHO3-0634	NT	NT	NT	NT	NT	NT	NT	NT	0.86
1/262144	WHO3-0634	NT	NT	NT	NT	NT	NT	NT	NT	0.62
2-fold Difference		1		1		0		0		
Neat	WHO3-0577	R	R	R	R	NR	NR	R	R	> 17.8
1/2	WHO3-0577	R	R	R	R	NR	NR	R	R	> 17.8
1/4	WHO3-0577	R	R	R	R	NR	NR	R	R	> 17.8
1/8	WHO3-0577	R	R	R	R	NR	NR	NR	NR	> 17.8
1/16	WHO3-0577	R	R	NR	R	NR	NR	NR	NR	> 17.8
1/32	WHO3-0577	R	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/64	WHO3-0577	R	NR	NR	NR	NR	NR	NR	NR	> 17.8

Table 17. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
1/128	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0577	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		2		1		0		0		
Neat	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/32	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/64	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/128	WHO3-0584	R	R	R	R	R	R	R	R	> 17.8
1/256	WHO3-0584	R	R	NR	NR	R	R	R	R	15.63
1/512	WHO3-0584	NR	NR	NR	NR	NR	NR	R	R	9.75
1/1024	WHO3-0584	NR	NR	NR	NR	NR	NR	R	NR	7.20
1/2048	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0584	NR	NR	NR	NR	NR	NR	NR	NR	0.66
1/65536	WHO3-0584	NT	NT	NT	NT	NT	NT	NT	NT	0.74

Table 17. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
1/131072	WHO3-0584	NT	NT	NT	NT	NT	NT	NT	NT	0.57
1/262144	WHO3-0584	NT	NT	NT	NT	NT	NT	NT	NT	0.45
2-fold Difference		0		0		0		1		
Neat	990885	R	R	R	R	R	R	R	R	> 17.8
1/2	990885	R	R	R	R	R	R	R	R	> 17.8
1/4	990885	R	R	R	NR	R	R	R	NR	> 17.8
1/8	990885	R	NR	NR	NR	R	NR	R	NR	> 17.8
1/16	990885	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/32	990885	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/64	990885	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	990885	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	990885	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	990885	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	990885	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	990885	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	990885	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	990885	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	990885	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	990885	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		1		1		2		
Neat	990814	R	R	R	R	R	R	R	R	> 17.8
1/2	990814	R	R	NR	R	R	R	R	R	> 17.8
1/4	990814	R	R	NR	R	R	R	R	R	> 17.8
1/8	990814	NR	NR	NR	NR	R	R	R	R	> 17.8
1/16	990814	NR	NR	NR	NR	NR	NR	R	R	> 17.8
1/32	990814	NR	NR	NR	NR	NR	NR	R	R	> 17.8

Table 17. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
1/64	990814	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	990814	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	990814	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	990814	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	990814	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	990814	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	990814	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	990814	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	990814	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	990814	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		2		0		0		
Neat	990831	R	R	R	R	R	R	R	R	> 17.8
1/2	990831	R	R	R	R	R	R	R	R	> 17.8
1/4	990831	R	R	R	R	R	R	R	R	> 17.8
1/8	990831	R	R	R	R	R	R	R	R	> 17.8
1/16	990831	R	NR	R	R	R	R	R	R	> 17.8
1/32	990831	NR	NR	R	NR	R	NR	R	R	> 17.8
1/64	990831	NR	NR	NR	NR	NR	NR	R	R	> 17.8
1/128	990831	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	990831	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	990831	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	990831	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	990831	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	990831	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	990831	NR	NR	NR	NR	NR	NR	NR	NR	1.59
1/16384	990831	NR	NR	NR	NR	NR	NR	NR	NR	0.91

Table 17. Results on lot-to-lot variation panel (Continued)

Dilution	Specimen ID	INSTI™ HIV-1/HIV-2 Antibody Test		Reveal® Rapid HIV Antibody Test		Uni-Gold™ HIV		Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)		Reference Results
		Lot B1M134	Lot B1M135	Lot RUIA0013	Lot RUIA0014	Lot HIV 1100043	Lot HIV 1100022	Lot V 20111201	Lot V 20120101	Vironstika HIV Ag/Ab
1/32768	990831	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		1		1		1		0		
Neat	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/2	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/4	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/8	WHO3-0788	R	R	R	R	R	R	R	R	> 17.8
1/16	WHO3-0788	R	R	NR	R	R	NR	R	R	> 17.8
1/32	WHO3-0788	R	R	NR	NR	NR	NR	NR	NR	> 17.8
1/64	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/128	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	> 17.8
1/256	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	15.63
1/512	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	9.75
1/1024	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	7.20
1/2048	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	4.41
1/4096	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	1.89
1/8192	WHO3-0788	NR	NR	NR	.NR	NR	NR	NR	NR	1.59
1/16384	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	0.91
1/32768	WHO3-0788	NR	NR	NR	NR	NR	NR	NR	NR	0.66
2-fold Difference		0		1		1		0		

Table 18. Results on WHO reference preparations

	Assays under evaluation				Reference Results									
					INNOTEST HIV Antigen mAb[1]	Enzygnost Anti-HIV1/2 Plus[1]	Vironostika HIV Ag/Ab[1]	INNO-LIA HIV Confirmation[1]						
Subtype	RDT1	RDT2	RDT3	RDT4	OD/CO	OD/CO	OD/CO	Result	Sgp120	gp41	p31	p24	p17	sgp105	gp36
HIV-1 A	R	R	R	R	> 17.1	> 6.7	0.4	HIV-1	4+	4+	2+	3+	4+	-	-
HIV-1 B	R	R	R	R	> 17.1	> 6.7	0.4	HIV-1	3+	3+	2+	2+	-	-	-
HIV-1 C	NR	R	R	R	> 17.1	> 6.7	0.4	HIV-1	2+	2+	1+	2+	2+	-	-
HIV-1 CRF01_AE	R	R	R	R	> 17.1	> 6.7	0.4	HIV-1	2+	2+	2+	2+	2+	-	-
HIV-1 O	NR	R	NR	R	> 17.1	> 6.7	0.6	HIV-1	2+	2+	1+	-	-	-	-
HIV-2	R	R	R	R	> 17.1	> 6.7	0.4	HIV-2	-	-	1+	3+	1+	2+	2+

Notes for Table 18 **Performance of the assay on WHO reference preparations**

Reference results [1] Reference results obtained from ITM, Belgium. INNOTEST HIV Antigen mAb (HIV-1 p24 antigen EIA), Enzygnost Anti-HIV1/2 Plus (HIV-1/2 antibody EIA) ,Vironostika HIV Ag/Ab combo (HIV-1/2 antibody and HIV-1 p24 antigen EIA), and INNO-LIA HIV Confirmation (HIV line immunoassay).

Assays under evaluation RDT1: INSTI™ HIV-1/HIV-2 Antibody Test, RDT2: Reveal® Rapid HIV Antibody Test, RDT3: Uni-GoldTM HIV ®, RDT4: Anti-Human Immunodeficiency Virus (HIV) Antibody Diagnostic Kit (Colloidal Gold)

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8. ANNEXES

ANNEX 1 - CUMULATIVE LIST OF ASSAYS EVALUATED WHOSE PRODUCTION HAS BEEN DISCONTINUED

The names (and manufacturers) of the assays evaluated to date under the WHO Test Kit Evaluation programme and WHO Prequalification of Diagnostics programme are listed in the table below. The number of the report in which each assay is covered is given, as well as sensitivity and specificity with 95% confidence intervals, δ values for HIV antibody-positive and antibody-negative specimen populations, cost per test, ease of performance and suitability for use in small blood collection centers.

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	δ Values		Cost per test (US$) /year	nm[h]	Ease of performance	Suitability	Indeterminate results (%)
				Pos	Neg					
Enzyme immunoassays										
For the detection of antibody to HIV-1										
Dupont HIV-1 Recombinant ELISA (Dupont de Nemours)	1	100.0 (98.7–100.0)	97.0 (92.7–98.8)	N/A	N/A	0.9/'88	450/410	LE	LS	N/A
Enzygnost Anti-HIV Micro (Behringwerke)	1	100.0 (97.8–100.0)	100.0 (98.1–100.0)	N/A	N/A	1.8/'88	450 630	LE	LS	0.0
HIV-TEK G (Sorin Biomedica)	1	100.0 (96.0–100.0)	86.5 (79.5–91.8)	N/A	N/A	1.0/'88	450	LE	LS	N/A
Vironostika Anti-HIV Uni-Form (Organon Teknika)	1	100.0 (97.6–100.0)	99.5 (97.3–100.0)	N/A	N/A	2.2/'88	492 630	LE	LS	N/A
Ortho HIV ELISA System (Ortho Diagn. Systems)	1	100.0 (97.8–100.0)	98.0 (95.0–99.4)	N/A	N/A	1.8/'88	490	LE	LS	N/A
HIV-1 env Peptide EIA (Labsystems)	2	96.0 (90.8–98.7)	97.0 (93.5–98.9)	N/A	N/A	3.9/'89	405 630	LE	LS	N/A
Wellcozyme HIV Recombinant (Wellcome Diagnostics)	2	100.0 (98.2–100.0)	99.1 (96.8–99.9)	N/A	N/A	1.5/'89	450	LE	LS	N/A
REC VIH-KCOI (Heber Biotec)	3	97.0 (93.5–98.9)	100.0 (98.3–100.0)	2.1	-4.14	N/A	492	LE	LS	N/A
UBI HIV-1 EIA (United Biomedical)	6	100.0 (99.9–100.0)	88.2 (87.1–89.3)	7.5	-1.12	1.0/'92	492 620–690	LE	S	N/A

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	δ Values		Cost per test (US$) /year	nm[h]	Ease of performance	Suitability	Indeterminate results (%)
				Pos	Neg					
Peptide HIV-1 ELISA Test System (Sero-Immuno Diagnostics)	6	82.1	94.1	N/A	N/A	0.6/'92	visual	E	VS	0.0
		(76.5–87.6)	(91.0–97.2)							
Peptide HIV-2 ELISA Test (Sero-Immuno Dianostics)	6	97.1	98.1	N/A	N/A	0.6/'92	visual	E	VS	N/A
		(93.0–100.0)	(96.3–99.9)							
UBI HIV-2 EIA (United Biomedical)	7	100.0	96.1	10.5	-1.7	1.2/'93	492	LE	S	N/A
		(97.4–100.0)	(93.4–98.8)				620–630			
Enzygnost Anti-HIV-1 (Behringwerke)	7	100.0	100.0	7.4	-3.3	N/A	450	LE	LS	0.0
		(98.1–100.0)	(98.8–100.0)				615–690			
Enzygnost Anti-HIV-2 (Behringwerke)	8	100.0	99.5	23.8	-3.5	6.2/'93	450	LE	LS	N/A
		(96.7–100.0)	(98.5–100.0)				630			
For the detection of antibody to HIV-1 and HIV-2										
Enzygnost Anti-HIV -1+2 (Behringwerke)	2	100.0	97.4	11.3	-2.15	2.3/'89	450	LE	LS	0.0
		(98.4–100.0)	(94.0–99.2)				615–690			
Biochrom HIV-1/HIV-2 ELISA Modul-test (Biochrom)	3	100.0	96.3	6.20	-1.69	0.9/'89	405	LE	LS	1.0
		(98.6–100.0)	(92.5–98.5)							
DuPont HIV-1/HIV-2 ELISA (DuPont de Nemours)	3	100.0	85.6	9.34	-0.96	1.3/'90	405 or 410	LE	LS	N/A
		(98.7–100.0)	(79.8–90.2)	N/A	N/A	N/A	620 or 630			
Vironostika HIV MIXT (Organon Teknika)	3	100.0	100.0	10.10	-2.94	1.8/'90	492	LE	LS	N/A
		(98.7–100.0)	(98.1–100.0)							
Elavia Mixt (Diagnostics Pasteur)	4	100.0	95.1	54.33	-2.31	2.1/'90	492	LE	LS	0.0
		(98.7–100.0)	(91.3–97.8)				620			
Anti-HIV-1/HIV-2 EIA (F. Hoffman-LaRoche)	4	100.0	96.9	11.30	-2.37	1.7/'90	492	LE	LS	N/A
		(98.7–100.0)	(93.4–98.9)							
Clonatec HIV (1+2) Ab EIA (Clonatec)	6	99.6	95.9	7.47	-1.68	2.7'91	492	LE	S	0.0
		(98.8–100.0)	(93.1–98.7)							
Enzymun-Test Anti-HIV-1+2 (Boehringer Mannheim)	6	100.0	100.0	5.50	-2.48	3.0'92	405	LE	S	0.0
		(98.7–100.0)	(98.6–100.0)							
UBI HIV-1/2 EIA (United Biomedical)	6	100.0	88.7	7.18	-1.24	1.2/'92	492	LE	S	N/A
		(99.9–100.0)	(84.2–93.1)				620–690			

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	δ Values		Cost per test (US$) /year	nm[h]	Ease of performance	Suitability	Indeterminate results (%)
				Pos	Neg					
Enzygnost Anti-HIV-1/HIV-2 (Behringwerke)	6	100.0 (99.9–100.0)	99.5 (98.5–100.0)	26.53	-3.50	2.6'92	450 615–690	LE	LS	0.0
Cobas Core Anti-HIV-1/HIV-2 EIA (Hoffmann-La Roche)	7	100.0 (98.6–100.0)	89.2 (84.6–93.8)	10.8	-1.0	2.2'93	450	LE	LS	0.0
Biochrom HIV-1/HIV-2 ELISA Version 2 (Biochrom)	7	99.5 (99.0–100.0)	100.0 (98.6–100.0)	7.5	-7.3	1.0/'93	450	LE	LS	0.0
Wellcozyme HIV-1 + 2 (Wellcome Diagnostics)	4	100.0 (98.7–100.0)	96.9 (93.3–98.9)	38.51	-1.99	1.5/'90	492	LE	LS	N/A
Peptide HIV ELISA (Cal-Tech Diagnostics)	5	72.6 (69.4–77.6)	95.4 (91.3–97.9)	N/A	N/A	0.9/'91	visual	E	S	0.2
Genelavia Mixt (Sanofi Diagnostics Pasteur)	5	100.0 (98.6–100.0)	98.5 (95.6–99.8)	16.77	-2.10	1.5/'91	492 620	LE	LS	0.0
Biotest Anti-HIV-1/-2 Recombinant (Biotest)	5	100.0 (98.6–100.0)	97.9 (94.9–99.4)	50.47	-3.08	1.2/'91	492 570–650	LE	LS	0.0
Innotest HIV-1/HIV-2 Ab (Innogenetics)	6	100.0 (98.8–100.0)	97.9 (95.9–99.9)	7.22	-2.30	1.9'91	450 620–690	LE	LS	N/A
Peptide HIV-1 & HIV-2 ELISA Test (Sero-Immuno Dianostics)	6	97.6 (95.7–99.5)	98.5 (96.7–100.0)	N/A	N/A	0.6'92	visual	E	VS	N/A
UBI HIV-1/2 EIA 2nd (United Biomedical)	7	99.5 (98.6–100.0)	92.4 (88.6–96.2)	4.8	-1.5	1.2'93	492 620,630	LE	S	N/A
VIDAS HIV-1+2 (Bio Merieux)	8	100.0 (98.5–100.0)	97.8 (95.6–100.0)	N/A	N/A	3.6/'93	450	VE	S	0.3
HIV 1+2 env Peptide EIA (Labsystems OY)	8	100.0 (98.6–100.0)	76.2 (70.0–82.4)	N/A	N/A	08/2.8/'93	450	LE	LS	0.0
Enzygnost Anti-HIV 1/-HIV 2 (Behringwerke)	9	100.0 (99.6–100.0)	99.5 (98.7–100.0)	24.8	-2.55	2.6'92	450 615–690	LE	LS	0.0
VIRONOSTIKA HIV Uni-Form II (Organon Teknika)	9	100.0 (99.6–100.0)	98.8 (97.6–100.0)	7.4	-3.0	1.7/'94	450 660 + 40	LE	LS	N/A
BIOTEST Anti-HIV-1/-2 recombinant (Biotest AG)	9	100.0 (99.6–100.0)	99.1 (98.1–100.0)	74.9	-3.3	1.2/'94	492 570–650	LE	LS	0.0
INNOTEST HIV-1/HIV-2 Ab s.p. (Innogenetics n.v.)	9	100.0 (99.6–100.0)	98.8 (97.6–100.0)	14.0	-3.8	1.5/'94	450 620–690	LE	LS	N/A

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	δ Values		Cost per test (US$) /year	nm[h]	Ease of performance	Suitability	Indeterminate results (%)
				Pos	Neg					
Genelabs Diagnostics HIV-1/HIV-2 ELISA (Genelabs Diagnostics)	10	100 (99.6–100.0)	97.3 (95.6–99.0)	72.2	-2.7	0.9'94	492	LE	LS	N/A
HIV SCREEN (Labsystems OY)	10	100.0 (99.6–100.0)	99.7 (99.1–100.0)	21.51	-4.11	0.6'95	450	LE	LS	N/A
HIVvisual 1 & 2 (Immuno Diagnostics Inc.)	10	90.9 (87.4–94.4)	94.5 (92.5–97.3)	1.88	-1.15	N/A	450	LE	LS	N/A
ETI-AB-HIV-1/2 K (Sorin Biomedica)	10	100.0 (99.6–100.0)	98.8 (97.6–100.0)	10.4	-2.5	1.5/'94	450 630	LE	LS	N/A
ICE * HIV-1.0.2 (Murex Biotech Ltd.)	11	100.0 (99.6–100.0)	99.4 (98.6–100.0)	16.8	-4.3	0.6'95	450 620–690	LE	LS	N/A
GENSCREEN HIV 1/2 (Sanofi Diagnostics Pasteur)	11	100.0 (99.6–100.0)	98.5 (97.2–99.8)	22.8	-2.7	1.5'95	450 620	LE	LS	0.0
HIVA TEST (Lupin Laboratories Ltd)	11	100.0 (99.5–100.0)	93.7 (91.0–96.4)	12.2	-1.1	0.6'98	450	LE	LS	1.5
HIV-1 and/or HIV-2 Recombigen EIA (Trinity Biotech plc)	7	100.0 (98.6–100.0)	100.0 (98.6–100.0)	10.4	-5.0	1.7/'93	490 630	LE	LS	N/A
For the detection of antibody to HIV-1 and HIV-2 and HIV-1 p24 antigen										
Genscreen® Plus HIV Ag/Ab (Bio-Rad Laboratories)	15	100 (97.7–100)	98.3 (96.1–99.4)	N/A	N/A	0.62-0.68/'04	450 620–700	LE	LS	0.0
EIA and Western blot - Evaluations on urine specimens										
For the detection of HIV-1										
Calypte™HIV-1 Urine EIA, Fc (Calypte™ Biomedical Corporation)	13	97.8 (92.4–99.7)	100.0 (98.2-100)	2.4	-4.4	3.00-4.25/'02	405 630	E	LS	N/A
Calypte™ HIV-1 Urine EIA (Recombinant) (Calypte™ Biomedical Corporation)	13	98.9 (94.2–100)	98.6 (95.8-99.7)	2.28	-2.42	3.00-4.25/'02	405 630	E	LS	N/A
Cambridge Biotech HIV-1 Urine Western Blot Kit (Calypte™ Biomedical Corporation)	13	98.9 (94.2–100)	N/A	N/A	26.00/'02	Visual	E	LS/S		N/A

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
Simple/Rapid assays								
For the detection of antibody to HIV-1								
HIV CHEK/HIVSPOT (Genelabs Diagnostics)	1	94.5 (89.7–97.4)	99.0 (96.4–99.9)	12.3	2.5/'88	VE	VS	
Recombigen HIV-LA (Cambridge BioScience)	1	95.2 (88.3–98.7)	96.1 (92.6–98.2)	6.0	3.0/'88	VE	S	N/A
Immunocomb (PBS Orgenics)	1	98.8 (95.7–99.9)	98.9 (96.0–99.9)	2.8	2.5/'89	VE	VS	N/A
Serion Immuno Tab HIV-1 (Serion Immunodiagnostica)	2	98.9 (96.9–99.9)	100.0 (98.3–100.0)	7.1	2.5/'90	LE	LS	1.2
Genie HIV-1 (Genetic Systems)	4	99.5 (97.4–100.0)	99.1 (96.7–99.9)	1.1	3.5/'90	VE	VS	0.2
SimpliRed HIV-1 Ab (Agen Biomedical)	5	97.5 (94.2–99.2)	91.2 (86.6–94.7)	10.5	7.8/1.5/'91	VE	S	0.7
Healthtest HIV-1 Assay (Akers Research Corp.)	6	58.7 (49.2–68.2)	89.4 (84.9–93.9)	7.0	1.4/2.3/'92	VE	S	0.2
Entebe HIV Dipstick (Hepatika Laboratories)	6	97.0 (94.4–99.6)	99.1 (97.8–100.0)	N/A	N/A	E	VS	N/A
Abbott Retrocell HIV 1 (Abbott GmbH)	9	100.0 (99.6–100.0)	100.0 (99.7–100.0)	2.2	1.45/'94	VE	S	0.6
PATH HIV Dipstick (PATH)	4	99.5 (97.3–100.0)	98.2 (97.1–99.1)	1.3	<1.5'91	E	VS	0.0
SUDS Murex HIV-1 Ab test (Murex Corporation)	5	100.0 (98.5–100.0)	75.1 (69.3-80.9)	22.9	4.5/'91	VE	S	11.7
Serodia-HIV (Fujirebio)	1	100.0 (97.6–100.0)	96.9 (93.4–99.0)	0.8	1.1/'88	E	S	0.0
For the detection of antibody to HIV-1/HIV-2								
Test Pack HIV-1/HIV-2 Ab (Abbott)	2	100.0 (98.5–100.0)	95.9 (92.0–98.2)	1.4	4.8/'89	VE	VS	0.0
Immunocomb Bi-Spot (PBS Orgenics)	3	98.5 (96.3–99.6)	100.0 (98.1–100.0)	7.6	4.0/'90	VE	VS	0.9
HIV CHEK 1+2/HIVSPOT 1+2 (DuPont de Nemours)/ (Genelabs Diagnostics)	3	99.3 (97.4–99.9)	100.0 (98.1–100.0)	7.2	4.0/'90	E	VS	1.0

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
Recodot (Waldheim Pharmazeutika)	4	98.9 (97.0–99.8)	88.6 (82.2–93.3)	31.7	2.0/'90	LE	LS	12.3
Genie HIV-1 and HIV-2 (Genetic Systems)	4	99.3 (97.5–99.9)	99.5 (97.2–100.0)	11.8	3.5/'90	VE	VS	0.0
Clonatec rapid HIV 1-HIV 2 Ab (Clonatec)	5	98.9 (96.8–99.8)	99.5 (97.2–99.8)	15.9	4.3/'91	E	VS	0.4
Recobead LA Assay (Waldheim Pharmazeutika)	6	59.8 (53.9–65.7)	94.8 (91.7–97.9)	22.3	1.7/2.2/'91	VE	S	0.4
Recombigen HIV-1/HIV-2 Rapid Test Device (Trinity Biotech plc)	7	100.0 (98.7–100.0)	94.5 (91.2–97.8)	11.4	4.0/'93	E	VS	2.8
MicroRed HIV-1/HIV-2 Ab Test (Agen Biomedical)	9	98.5 (97.0–100.0)	95.5 (93.2–97.7)	1.5	1.5/1.0/'94	VE	S	0.5
SimpliRed HIV-1 /HIV-2Ab Test (Agen Biomedical)	9	99.2 (98.2–100.0)	87.3 (83.7–90.9)	9.5	4.0/3.0/'94	VE	S	0.3
HIV (Sav) 1&2 Rapid Sero Test (Diatech (Savyon) Diagnostica Ltd.)	10	97.7 (95.9–99.5)	96.7 (94.8–98.6)	5.1	1.9'94	VE	S	0.2
ENTEBE HIV Dipstick (Hepatika Laboratories)	10	100.0 (99.6–100.0)	96.4 (94.4–98.4)	5.0	0.8'94	VE	VS	1.3
Dipstick-HIV 1 + 2 (Pacific Biotech Co., Ltd.)	10	100.0 (99.6–100.0)	98.2 (96.8–99.6)	1.0	0.5'94	E	VS	0.3
DIA (Dot Immuno Assay) HIV 1 + 2 (Weiner Lab.)	10	99.6 (98.8–100.0)	99.4 (98.6–100.0)	0.8	<1.0'94	VE	VS	0.2
SERO•STRIP HIV-1/2 (Saliva Diagnostic Systems)	11	98.9 (97.6–100.0)	100.0 (99.7–100.0)	1.5	1.5'95	VE	VS	0.0
RED-DOT HIV 1&2 (Cal-Test Diagnostics Inc.)	11	100.0 (99.6–100.0)	94.9 (92.5–97.3)	9.5	2.9'94	VE	S	1.9
HIVCHEK System 3 Test Kit (Ortho Diagnostic Systems)	11	99.6 (98.9–100.0)	99.7 (99.1–100.0)	1.0	4.35'95	E	VS	0.2
AccuSpot HIV-1 and 2 (Specialty BioSystems Inc.)	11	100.0 (99.6–100.0)	86.3 (82.5–90.1)	10.8	2.5'95	VE	S	5.0
SEROCARD HIV (Trinity Biotech plc)	11	100.0 (99.6–100.0)	97.9 (96.4–99.1)	1.5	4.0/'94	VE	VS	0.2

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
EasiDot HIV/EasiSpot HIV (Nubenco Diagnostics)	11	95.3 (92.7–97.9)	71.3 (66.4–76.2)	23.7	N/A	VE	S	12.5
CAPILLUS HIV-1/HIV-2 (Trinity Biotech plc)	9	100.0 (99.6–100.0)	98.8 (97.6–100.0)	0.0	2.2/'94	VE	VS	0.0
HIV 1 & 2 DoubleCheck (Orgenics)	11	100 (99.6–100.0)	99.4 (98.6–100.0)	0.8	2.0/'96	VE	VS	0.2
GENIE II HIV-1/HIV-2 (Bio-Rad)	14	100 (97.7–100)	99.7 (98.1–100)	0.7	2.55/'03	E	VS	0.2
Efoora HIV Rapid (Efoora Inc)	14	96.2 (91.9–98.6)	98.9 (95.6–99.3)	3.8	0.75–2.60/'03	VE	S	0.4
Hema • Strip(r) HIV 1/2 (Chembio Diagnostics Inc)	14	98.1 (94.5–99.6)	100.0 (98.8–100.0)	3.3	1.85–2.5/'03	VE	VS	0.0
DoubleCheckGold™ HIV 1&2 (Orgenics Ltd)	14	Lot A 99.4 (96.5–100.0) Lot B 100.0 (97.7–100.0)	Lot A 95.6 (92.6–97.6) Lot B 94.6 (91.4–96.9)	2.4	0.65–0.70/'03	VE	VS	Lot A 0.9 Lot B 2.0
DoubleCheckGold™ HIV 1&2 Whole Blood (Orgenics Ltd)	16	100 (98.8–100)	99.3 (98.1–99.9)	0.13	1.20–1.32/''05	VE	VS	0.0
InstantScreen™ HIV-1/2 (Gen 2) (Gaifar GmbH)	12	100.0 (90.0–100.0)	100.0 (97.0–100.0)	0.7	8.00–12.00/'01	VE	VS	0.0
CAPILLUS™ HIV-1/HIV-2 (Trinity Biotech PLC)	12	100.0 (95.5–100.0)	100.0 (97.9–100.0)	0.0	2.20/'01	VE	VS	0.0
SMLX Technologies Diagnostic test (SMLX Technologies)	13	62.7 (51.0–74.0)	74.8 (67.0–82.0)	22.5	NA	E	S	7.7
OraScreen HIV Rapid Test (Beacon Diagnostics Inc)	13	56.0 (44.0–68.0)	98.6 (95.0–100.0)	11.3	NA	E	S	4.1
Salivax™-HIV (ImmunoScience Inc)	13	79.4 (67.0–89.0)	96.0 (91.0–99.0)	8.5	NA	E	S	2.7
Wellcozyme HIV 1+2 GACELISA (Murex Biotech Ltd)	13	100 (95.2–100.0)	99.0 (95.0–100.0)	NA	NA	LE	LS	0.0
Supplemental assays								
For the detection of antibody to HIV-1 or HIV-2								
RIBA HIV-1 (Chiron)	1	99.4 (96.6–100.0)	100.0 (97.9–100.0)	N/A	27.6/'88	E	S	N/A

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
HIV Western Blot Kit (Organon Teknika)	3	100.0	100.0	N/A	21.0/'90	LE	S	10.5
		(98.2–100.0)	(98.0–100.0					
Wespage HIV-1 Western blot Kit (Bio Genex)	6	100.0	100.0	N/A	21.6/'92	LE	VS	12.8
		(99.9–100.0)	(99.9–100.0)					
Wespage HIV-1 Western blot Kit II (Bio Genex)	7	100.0	100.0	N/A	17.7/'93	LE	S	12.4
		(98.5–100.0)	(98.7–100.0)					
CBC HIV-2 Western blot kit (Cambridge Biotech)	7	100.0	100.0	N/A	16/'93	LE	S	13.9
		(97.0–100.0)	(98.5–100.0)					
INNO-LIA HIV-1/HIV-2 Ab (Innogenetics)	2	100.0	100.0	NA	18.4/'89	LE	S	4.3
		(98.6–100.0)	(98.0–100.0)					
Ancoscreen (Ancos)	2	100.0	90.4	NA	10.8/21.5/'89	LE	LS	31.4
		(97.8–100.0)	(82.6–95.5)					
IFA anti-HIV-1 (Waldheim Pharmazeutika)	5	98.9	100.0	13.8	5.6/'91	LE	LS	0.7
		(96.9–99.8)	(98.3–100.0)					
IFA anti-HIV-2 (Waldheim Pharmazeutika)	5	98.7	100.0	11.0	6.0/'91	LE	LS	1.8
		(93.1–99.7)	(98.2–100.0)					
HIV-1 Western Blot Kit (Open Tray Procedure) (Bio Genex)	7	100.0	100.0	NA	17.7/'93	LE	S	6.7
		(98.5–100.0)	98.7–100.0)					
Speedscreen HIV (British Bio-Technology)	4	100.0	66.4	NA	17.0/'90	LE	S	16.9
		(99.4–100.0)	(57.9–74.1)					

ANNEX 2 - CUMULATIVE LIST OF ASSAYS EVALUATED; CURRENTLY COMMERCIALLY AVAILABLE

The names (and manufacturers) of the assays evaluated to date under the WHO Test Kit Evaluation programme and WHO Prequalification of Diagnostics programme are listed in the table below. The number of the report in which each assay is covered is given, as well as sensitivity and specificity with 95% confidence intervals, δ values for HIV antibody-positive and antibody-negative specimen populations, cost per test, ease of performance and suitability for use in small blood collection centers.

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	δ Values[e]		Cost per test (US$) /year	nm[h]	Ease of performance	Suitability	Indeterminate results (%)
				Pos	Neg					
Enzyme immunoassays - Evaluations on serum/plasma										
For the detection of antibodies to HIV-1 and HIV-2										
Detect-HIV™ (Biochem Immunosystemes now Adaltis)	3	100.0	97.4	12.65	-2.21	2.5/'90	450	LE	LS	N/A
		(98.6–100.0)	(94.0–99.2)				600–650			
Abbott Recombinant HIV-1/HIV-2 3rd Generation (Abbott)	7	100.0	100.0	11.5	-4.3	1.7/1.8'93	492	LE	LS	N/A
		(98.5–100.0)	(98.5–100.0)							
UBI HIV 1/2 EIA (United Biomedical Inc.)	9	100.0	100.0	10.8	-3.2	1.0/'94	492	LE	LS	N/A
		(99.6–100.0)	(99.7–100.0)				620–690			
HIV EIA (Labsystems OY now Anilabsystems)	10	100	99.4	14.20	-3.85	0.6'95	450	LE	LS	N/A
		(99.6–100.0)	(98.6–100.0)							
IMx HIV-1/HIV-2 3rd generation Plus (Abbott GmbH Diagnostika)	11	99.6	97.9	9.1	-2.1	3-4'95	Imx system	VE	S	0.3
		(98.9–100.0)	(96.4–99.4)							
Enzygnost Anti-HIV 1/2 Plus (Behringwerke AG now Dade Behring)	11	100.0	99.7	19.1	-6.6	1.0'95	450	LE	LS	0.0
		(99.6–100.0)	(99.1–100.0)				615–690			
Vironostika Uni-Form II plus O (Organon Teknika nv (now bioMérieux)	11	100.0	100.0	17.2	-4.1	1.5'97	450	LE	LS	N/A
		(99.6–100.0)	(99.7–100.0)				620–700			
For the detection of antibody to HIV-1 and HIV-2 and HIV-1 p24 antigen										
Enzygnost HIV Integral II (Dade Behring)	15	100	100	N/A	N/A	N/A/'04	450	LE	LS	0.0
		(97.7–100)	(98.7–100)				650			
Genedia® HIV Ag-Ab ELISA (Green Cross)	15	100	99.7	N/A	N/A	0.40–0.45/'04	450	LE	LS	0.0
		(97.7–100)	(98.1–100)				620			
Murex HIV Ag/Ab Combination (Abbott Diagnostics)	15	100	99.3	N/A	N/A	0.80–1.20/'04	450	LE	LS	0.0
		(97.7–100)	(97.6–99.9)				620–690			
Vironostika® HIV UniForm II Ag/Ab (bioMérieux)	15	100	99.0	N/A	N/A	1.48–1.95/'04	450	LE	LS	0.0
		(97.7–100)	(97.1–99.8)				620–700			

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
Rapid diagnostic tests - Evaluations on serum/plasma								
For the detection of antibody to HIV-1 and HIV-2								
Serodia-HIV-1/2 (Fujirebio)	8	100 (98.5–100.0)	100 (98.5–100.0)	6.3	2.8/'93	LE	S	0.0
SPAN COMBAIDS VISUAL (Span Diagnostics.)	8	96.5 (93.5–99.5)	100.0 (98.3–100.0)	0.8	0.4/'93	E	VS	0.0
Immunocomb II BiSpot HIV 1&2 (PBS Orgenics)	9	100.0 (99.6–100.0)	99.7 (99.1–100.0)	4.5	1.7/'94	VE	VS	0.2
SPAN COMBAIDS VISUAL (Span Diagnostics Ltd.)	10	100.0 (99.6–100.0)	88.0 (84.5–91.5)	6.3	0.5'94	E	S	3.2
HIV TRI-DOT (J. Mitra & Co. Ltd.)	11	99.6 (98.9–100.0)	99.7 (99.1–100.0)	3.2	2.0'96	VE	VS	0.2
BIONOR HIV-1&2 (Bionor A/S)	11	100.0 (99.6–100.0)	98.8 (97.6–100.0)	1.0	2.5/'95	VE	S	0.2
InstantCHEK™-HIV 1+2 (EY Laboratories Inc)	14	99.4 (96.5–110.0)	97.6 (95.2–99.0)	4.6	1.0'03	E	S	0.0
OraQuick HIV-1/2 Rapid HIV-1/2 Antibody Test (OraSure Technologies Inc)	14	98.1 (94.5–99.6)	100.0 (98.8–100)	2.4	NA	VE	VS	0.4
SD Bioline HIV 1/2 3.0 (Standard Diagnostics)	14	100.0 (97.7–100.0)	99.3 (97.6–99.9)	3.5	1.10/'03	VE	VS	0.0
HIV 1/2 STAT-PAK (Chembio Diagnostics Inc)	14	97.6 (93.6–99.3)	100.0 (98.8–100.0)	0.7	0.75–1.25/'03	VE	VS	0.0
HIV (1+2) Antibody (Colloidal Gold) (Shanghai Kehua Bioengineering Co. Ltd)	14	100 (97.7–100.0	100.0 (98.8–100.0)	0.2	1.50/'03	VE	VS	0.0
GENEDIA® HIV 1/2 Rapid 3.0 (Green Cross Life Science Corp)	14	100 (97.7–100.0)	99.7 (98.1–100.0)	1.8	0.93–1.15/'03	VE	VS	0.0
HIV 1/2 Stat-Pak (Chembio Diagnostics Systems)	16	100 (98.8–100)	99.3 (98.1–99.9)	1.7	1.10–1.35/'05	VE	VS	0.0
HIV 1/2 Stat-Pak Dipstick (Chembio Diagnostic Systems)	16	99.4 (97.7–99.9)	100 (99.2–100)	0.3	0.80–0.95/'05	VE	VS	0.0
ADVANCED QUALITY™ HIV Rapid Test (InTec Products)	16	99.7 (98.2–99.8)	99.8 (98.8–100)	0.8	0.80–0.90/'05	VE	VS	0.0

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
Retrocheck HIV WB / Core HIV 1&2 (Qualpro Diagnostics / Core Diagnostics)	16	100 (98.8–100)	99.1 (97.8–99.8)	0.4	0.70–0.85/''05	VE	VS	0.1
Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd)	17	100 (99.1–100)	98.9 (97.8–99.6)	1.4	0.80–1.25/'11	VE	VS	0.0
HIV 1/2 STAT-PAK® (Chembio Diagnostic Systems, Inc.)	17	99.5 (98.3–99.9)	100 (99.4–100)	0.2	1.50/'12	VE	VS	0.0
HIV 1/2 STAT-PAK® Dipstick (Chembio Diagnostic Systems, Inc.)	17	100 (99.1–100)	99.7 (98.9–99.9)	0.1	0.85–0.90/'11	VE	VS	0.0
One Step HIV 1/2 Whole Blood/Serum/ Plasma Test (Guangzhou Wondfo Biotech Co., Ltd)	17	100 (99.1–100)	99.9 (99.2–100)	0.2	0.42–0.65/'12	VE	VS	0.1
Uni-Gold™ HIV (Trinity Biotech Manufacturing Ltd)	17	99.8 (98.7–100)	99.9 (99.2–100)	0.1	1.60–2.80/'12	VE	VS	0.0
Anti-human immunodeficiency virus (HIV) antibody diagnostic kit (colloidal gold) (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd)	17	100 (99.1–100)	98.5 (97.2–99.3)	0.1	0.35–0.60/'12	VE	VS	0
INSTI HIV-1/HIV-2 Antibody Test (bioLytical Laboratories)	17	100 (99.1–100)	99.7 (98.9–100)	0.0	1.50–2.50/'13	E	VS	0
Reveal Rapid HIV Antibody Test (MedMira Laboratories Inc)	17	99.8 (98.6–100)	99.9 (99.2–100)	1.6	2.40–4.00/'12	E	VS	0.3
Supplemental assays- Evaluations on serum/plasma								
For the detection of antibody to HIV-1								
New Lav-Blot-I (Sanofi Diagnostics Pasteur)	5	100.0 (98.1–100.0)	100.0 (96.8–100.0)	N/A	11.6/'91	E	S	30.6
For the detection of antibody to HIV-1 and HIV-2								
Pepti-Lav 1-2 (Sanofi Diagnostics Pasteur)	4	99.3 (96.4–99.9)	100.0 (98.1/100.0)	N/A	21.5/'90	LE	S	0.7
Rapid diagnostic tests - Evaluations on whole blood specimens								
For the detection of antibody to HIV-1 and HIV-2								
Determine™ HIV-1/2 (Abbott Laboratories Dainabot Co. Ltd)	12	100.0 (95.5–100.0)	99.4 (96.7–100.0)	1.6	1.20/'01	VE	VS	0.0
ADVANCED QUALITY™ Rapid HIV Test (InTec Products Inc.)	12	98.8 (93.2–100.0)	100.0 (97.9–100.0)	2.0	0.80-1.20/'01	VE	VS	0.8

Assay (manufacturer)	Report No [a]	Sensitivity (%)[b,c]	Specificity (%)[c,d]	Inter-reader variability %	Cost per test (US$) /year	Ease of performance	Suitability	Indeterminate results (%)
MedMira Rapid HIV Test (MedMira Laboratories Inc.)	12	100.0	97.6	14.4	3.00/'00	E	VS	0.8
		(95.5–100.0)	(94.1–99.6)					
First Response™ HIV-1/HIV-2 WB (PMC Premier Medical Corporation)	12	100.0	98.8	0.4	1.15/'01	VE	VS	0.0
		(95.5–100)	(95.8–99.9)					
Uni-Gold™ HIV (Trinity Biotech PLC)	12	100.0	100.0	0.4	2.34/'01	VE	VS	0.0
		(95.5–100.0)	(97.9–100.0)					

Legend for Annexes 1 and 2

a: Operational characteristics of commercially available assays to detect antibodies to HIV-1 and/or HIV-2 in human sera:
Report 1- GPA/RES/BMR/89.4
Report 2- GPA/RES/BMR/90.1
Report 3- GPA/RES/BMR/91.1
Report 4- GPA/RES/DIA/91.6
Report 5- GPA/RES/DIA/92.8
Report 6 - GPA/RES/DIA/93.4
Report 7 - GPA/RES/DIA/93.6
Report 8- GPA/RES/DIA/94.4
Report 9,10 -WHO/BLS/98.1
Report 11 -WHO/BTS/99.1
Report 14 - ISBN 92 4 159216 8
Report 15 - ISBN 92 4 159237 0
Report 16 - ISBN 978 92 4 159769 2

b,c,d: Sensitivity, specificity and 95% confidence intervals were calculated as described section 5.5.1 of this document.
e: δ-values were calculated as described in previous documents , see above.
f: Inter-reader variability was calculated as described section 5.5.3 of this document.
g: Prices quoted are those in effect at the time of the evaluation.
h: The wavelength(s) of the spectrophotometer (single and/or double) is specified by the manufacturer.
i: Ease of performance is defined on table 4b.
j: Suitability for use in small laboratories is defined on table 5.
k: Indeterminate results.

ANNEX 3 - CUMULATIVE LIST OF ASSAY MANUFACTURERS' ADDRESSES

Name	Address
Abbott GmbH, Diagnostika	Max-Planck-Ring 2, 65205 Wiesbaden, Germany. Tel: +49 6122 58 16 23; Telex: 4182555; Fax: +49 6122 58 16 12
Adaltis	10900 rue Hamon, Montréal (Québec), Canada H3M 3A2. Tel: +1 514 335 9922; Telex: 058-27642 IAF BCM MTL; Fax: +1 514 335 9919
Agen Biomedical Ltd	11 Durbell Street, P.O. Box 391, Acacia Ridge, Queensland 4110, Australia. Tel: +61 7 173 6266; Fax: +61 7 273 6224
Akers Laboratories Inc.	201 Grove Road, Thorofare, New Jersey 08086, USA. Tel: +1 609 848 8698; Fax: +1 609 848 0269
Ancos Denmark ApS	Tengslemarkvej 4, 4573, Hfjby, Denmark. Tel: +45 59 30 65 55; Telex: 42580 ancos dk; Fax: +45 59 30 60 45
Anilabsystems	Pulttitie 8, P. O. Box 8, 00881 Helsinki, Finland. Tel: +358 0 75821; Telex: 123569 Labsy sf; Fax: +358 0 7557610
Biochem Immunosystèmes	See Adaltis
Biochrom KG	Leonorenstrasse 2-6, 12247 Berlin, Germany. Tel: +49 30 77 99 06 00; Telex: 185 821 bio d; Fax: +49 30 771 0012
Bio Genex	4600 Norris Canyon Road, San Ramon, CA 94583, USA. Tel: +1 510 275 0550, Fax: +1 510 276 0580
BioMérieux S.A.,	69280 Marcy-l'Etoile, France. Tel: +33 78 87 20 00; Fax: +33 78 87 20 90
BIONOR A/S	P.O. Box 1868, N-3705 Skien, Norway Tel: +47 35 53 84 88; Fax: +47 35 53 71 30
Bio-Rad Laboratories	3, boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, France Tel: +33 1 47 95 60 00; Fax: +33 1 47 41 91 33
Biotest AG	Landsteiner Str. 5, 63303 Dreieich, Germany. Tel: +49 6103 80 10; Telex: 4185429; Fax: +49 6103 80 11 30
Boehringer Mannheim GmbH	Sandhofer Strasse 116, 68298 Mannheim, Germany. Tel: +49 621 759 8838; Telex: 463193 bmd/462420 bmd; Fax: +49 621 759 8842
British Bio-Technology Ltd	Watlington Road, Cowley, Oxford OX4 5LY, England. Tel: +44 865 748747; Telex: 838083 BIOTEC G; Fax: +44 865 717598
Cal-Tech Diagnostics	1580 A. West San Bernardino Road, Covina, CA 91722, USA. Tel: +1 818 331 9763, (1 818) 571 6826, (1 818) 369 3755; Fax: +1 818 331 1882, (1 818) 280 4846; Telex: 9102409630 Cal-Tech UQ.
CAL-TEST DIAGNOSTICS	13760 Mountain Avenue, Chino, CA 91710, USA Tel: +1 909 902 0550, Fax: +1 909 902 0044
Cambridge Diagnostics Ireland Ltd.	See Trinity Biotech plc
Catalina Bio-Diagnostic Consulting, Inc.	5595 E. 7th Street, Long Beach, CA 90804, USA. Tel: +1 310 983 8111; Fax: +1 310 987 0670
Chembio Diagnostic Systems Inc.	3661 Horseblock Road, Medford, BY 11763, USA Tel: +1 631 924 1135; Fax: +1 631 924 6033
Chiron Corporation	4560 Horton Street, Emeryville, CA 94608-2916, USA. Tel: +1 510 655 8730; Fax +1 510 655 9910
Clonatec Diagnostics S.A.	60 rue de Wattignies, 75580 Paris Cedex 12, France. Tel: +33 1 43 42 43 88; Telex: 214044F; Fax: +33 1 43 40 48 86
Core Diagnostics	Aspect Court, 4 Temple Row, Birmingham B2 5HG, United Kingdom. Tel: + 44 121 609 4720; Fax: + 44 121 609 4721
Dade Behring Marburg GmbH	Postfach 1149, 35001 Marburg, Germany. Tel: +49 6421 39 4478; Fax: +49 6421 66064
Efoora Inc.	900 Asbury Drive, Buffalo Grove, Illinois, USA 60089 Tel: +1 847 634 6400; Fax: +1 847 634 0476
EY Laboratories, Inc.	P.O. Box 1787, 107 N. Amphlett Blvd., San Mateo, CA 94401, USA Tel: +1 650 342 3296; Fax: +1 650 342
Fujirebio Inc.	19th floor, Shinjuku Daiichi Seimei Building, 7-1 Nishi-Shinjuku 2-Chome, Shinjuku-Ku, Tokyo 163-07, Japan. Tel: +81 3 3348 0947; Telex: J 28612; Fax: +81 3 3342 6220
Genelabs Diagnostics	See MP Biomedicals
Genetic Systems Corporation	3005 First Avenue, Seattle, WA 98121, USA. Tel: +1 206 728 4900; Telex: 532050 Genetic Systems; Fax: +1 206 728 495
Green Cross Life Science Corporation	227-3, Gugal-li, Giheung-eup, Yongin-shi, Kyonggi-do, Korea Tel: +82 31 260 9300; Fax: +82 31 260 9491

Name	Address
Heber Biotec S.A.	Calle 8, No. 306, Miramar, Havana, Cuba. Tel: +537 291187; Telex: 511269 cimex cu; Fax: +537 222261
Hepatika Laboratories	Yayasan Hati Sehat, Jalan Bung Hatta 3A, Mataram, Lombok, Indonesia, under license from the Concept Foundation Program for Appropriate Technology in Health (PATH), Seattle, WA, USA. Tel: +62 3 64 31 662; Fax: +62 3 64 35642
Hoffmann-La Roche F. AG	Grenzacherstr 124, 4058 Basel, Switzerland. Tel: +41 61 688 55 55; Fax: +41 61 681 98 67
Human GmbH	Max-Planck-Ring 21, D 65205, Wiesbaden, Germany. Tel: +49 6122 99880; Fax: +49 6122 9988 100/99
Immuno-Chemical Laboratories.	See Pacific Biotech Co.Ltd.
Immuno Diagnostics, Inc.	85 Great Arrow Avenue., Buffalo, New York 14216, USA. Tel: +1 716 873 9400; Fax: +1 716 876 7919
InTec Products Inc.	332 Xinguang Road, Xinyang Industry Area, Haicang, Xiamen, 361022 P R China. Tel: +86 5926 807 188; Fax: +86 592 651 9161
Innogenetics S.A.	Technologiepark 6, 9052 Ghent, Belgium Tel: +32 9 329 1329; Fax: +32 9 329 1911
J. Mitra & Co. Ltd	A-180, Okhla Industrial Area, Phase-1, New Delhi-110 020, India Tel: +91 11 681 8971, +91 11 681 8973, +91 11 681 3995, +91 11 681 3989; Fax: +91 11 681 0945, +91 11 681 8970
Johnson & Johnson International	Roissy Pole B.P. 10784, 1, Place de Londres, F-95727 Roissy CDG Cedex, France. Tel: +33 1 48 62 08 75; Fax: +33 1 48 62 00 54
KHB Shanghai Kehua Bio-engineering Co. Ltd.	1189 N Qinzhou Road, Shanghai, 200233, People's Republic of China Tel: +86 21 64851188 +86 21 64853370 +86 21 8203370; Fax: +86 21 64854051
Labsystems OY	See Anilabsystems
Lupin Laboratories Ltd.	159, CST Road, Kalina, Santacruz (E), Mumbai 400098, India. Tel: +91 22 611 3391; Fax: +91 22 611 4008
Murex Biotech Limited	Central Road, Temple Hill, Dartford, Kent DA1 5LR, England. Tel: +44 1322 27 77 11; Telex MUREX G 896113; Fax: +44 1322 27 32 88
MP Biomedicals	Halle de Frêt, P. O. Box 1015, 1215 Geneva 15 Airport, Switzerland. Tel: +41 22 788 1908; Fax +41 22 788 1986
Nubenco Enterprises, Inc.	One Kalisa Way, Suite 207 Paramus, New Jersey 07652-3508, USA. Tel: +1 201 967 9000; Fax +1 201 967 9444
OraSure Technologies, Inc.	150 Webster Street, Bethlehem, PA 18015, USA Tel: +1 610 882 1820
Organon Teknika N.V.	See bioMérieux
Orgenics Ltd.	P.O. Box 360, Yavne 70650, Israel Tel: +972 8 9429212; Fax: +972 8 9438758
Ortho Diagnostic Systems Inc.,	US Route 202, Raritan, N.J. 08869, USA. Tel: +1 201 218 1300; Telex: 833 425; Fax: +1 201 218 8582
Pacific Biotech Co., Ltd.	6 Ladprao 110 (Sonthiwattana 3), Ladprao Road, Bangkapi, Bangkok 10310, Thailand. Tel: +66 2 530 4608 or 530 2754; Fax: +66 2 530 4619
PBS Orgenics	Parc de l'Innovation, B.P. 209, 67405 Illkvich Cedex, Strasbourg, France. Tel: +33 88 67 08 30; Telex: 890665; Fax: +33 88 67 38 61 North Industrial Zone, P. O. Box 360, Yavne, 70650 Israel. Tel: +972 8 43 87 52-2; Fax: +972 8 43 87 58
Program for Appropriate Technology in Health (PATH)	4 Nickerson Street, Seattle, WA 98109, USA. Tel: +1 206 285 3500; Telex: 47 100 49 PATH UI; Fax: +1 206 285 6619
Qualpro Diagnostics	Plot Nos. 88/89, Phase II C, Verna Industrial Estate, Verna, Goa, 403 722, India. Tel: + 91 832 2783140; Fax: + 91 832 2783139
Saliva Diagnostic Systems (SDS), SDS International Ltd.	11 Sovereign Close, Sovereign Court, London E1)HW, UK Tel: +44 171 415 0550; (Fax: +44 171 415 0553 Saliva Diagnostic Systems, (SDS), 11719 NE 95th Street, Vancouver, WA 98682, USA Tel: +1 360 696 4800; Fax: +1 360 254 7942
Sanofi Diagnostics Pasteur	See Bio-Rad Laboratories
Savyon Diagnostics, LTD	Kiryat Minrav, 3 Habosem, Ashdod 77101, Israel. 5870 Pacific Center Boulevard, Suite A, SanDiego,California 92121 USA. Tel: +1 619 457 9927; Fax: +1 619 457 2425
Serion Immunodiagnostica	Bronnbachergasse 18a, 8700 Würzburg, Germany. Tel: +49 931 14079; Telex: 68480 virion d; Fax: +49 931 52650

Name	Address
Sero-Immuno Diagnostics	P.O. Box 616, 2177-J Flintstone Drive, Tucker, GA 30084, USA. Tel: +1 404 496 1370; Telex: 750747 SERO UD; Fax: +1 404 938 7189
Sorin Biomedica SpA	Divisione Diagnostici, 13040 Saluggia (Vercelli), Italy. Tel: +39 161 487243; Telex: 200064 I SORIN; Fax +39 161 487672
Span Diagnostics PVT-Ltd	173-B New Industrial Estate UDHNA-394210 (SURAT), India. Tel: +91 261 67 71 43; Telex: 0188284 span in; Fax: +91 261 66 57 57
Specialty BioSystems, Inc.	
Standard Diagnostics, Inc.	575-34 Pajang-dong, Jangan-ku, Suwon-si, Kyonggi-do, Korea 440-290 Tel: +82 31 258 2994; Fax: +82 31 258 2995
Trinity Biotech plc,	IDA Business Park, Bray, Co. Wicklow, Ireland. Tel: +353 1276 9800; Fax: +353 1276 9888
United Biomedical Inc.	25, Davids Drive, Hauppauge, NY 11788, USA. Tel: +1 516 273 2828; Fax: +1 516 273 1717
Waldheim Pharmazeutika GmbH	Boltzmanngasse 11, 1091 Vienna, Austria. Tel: +43 1 319 1456; Telex: 116487 wamed a; Fax: +43 1 319 1456-44;
Wiener Laboratories	Riobama 2944, 2000 Rosario, Argentina. Tel: +54 41 39 01 73/8; Fax: +54 41 37 13 77
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