



HIV Ab/Ag Combo ELISA

KARDHIV96

Read entire protocol before use.

HIV Ab/Ag Combo ELISA

For research use only. Not for use in diagnostic procedures.

I. INTENDED USE

The kit is a solid phase enzyme immunoassay for the in-vitro diagnostic screening of antibodies to all subtypes of HIV-1 and HIV-2 and HIV-1 antigen (p24) in human serum or plasma.

II. GENERAL INFORMATION

- A. **Proprietary name :** HIV Ab/Ag Combo ELISA
- B. **Catalog number :** KARDHIV96 : 96 tests
- C. **Manufactured by :** DIAsource ImmunoAssays S.A.
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III. INTRODUCTION

Epidemiological evidence indicates that an infectious agent transmitted through intimate contact, intravenous drug use or use of infected blood or blood products leads to Acquired Immunodeficiency Syndrome (AIDS).

This disease affects T-cell mediated immunity, resulting in severe lymphopenia and a reduced subpopulation of helper T-lymphocytes. Destruction of this T-lymphocyte population by the virus causes an immune deficiency, resulting in a reduced or deficient response to subsequent infections.

Consequently, infections become more severe and may cause death. At present, there is no successful treatment for AIDS.

The etiological agent has been identified as a retrovirus, human immunodeficiency virus type 1 (HIV-1).

A closely related, but distinct type of immunodeficiency virus, designated HIV-2, has also been isolated. This virus causes a disease that is indistinguishable from AIDS.

Serological cross-reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample.

This variability requires the inclusion of antigens to both HIV-1 and HIV-2 for the screening of antibodies to HIV-1 and HIV-2.

The presence of anti-HIV-1 and/or anti-HIV-2 and/or HIV p24 antigen in the blood indicates potential infection with HIV-1 and/or HIV-2 and consequently this blood should not be used for transfusion or for manufacture of injectable products.

IV. PRINCIPLES OF THE METHOD

Synthetic peptides representing immunodominant epitopes of HIV 1 and HIV 2 together with a monoclonal antibody to p24 HIV-1 antigen are coated onto wells of a microplate.

The peptides and the antibody have been carefully selected to ensure the screening of antibody and p24 antigen to all HIV-1 subtypes, including subtype O and HIV-2. Serum or plasma samples are added to these wells and, if antibodies specific to HIV-1 and/or HIV-2 (IgG, IgM or IgA) are present in the sample, they will form stable complexes with the HIV peptide antigens in the well. In case HIV-1 p24 is present in the sample, the antigen will be captured by the specific monoclonal antibody.

Antigen-antibody complexes are then identified through the successive addition of: (1) biotinylated peptides, a biotinylated monoclonal antibody to HIV-1 p24, and; (2) horseradish peroxidase HRP Streptavidin conjugate.

The hydrolytic activity of horseradish peroxidase allows for the quantification of these antibody-antigen complexes.

Peroxidase substrate solution is then added.

During incubation, a blue color will develop in proportion to the amount of anti-HIV-1/2 antibodies or HIV-1 p24 antigen bound to the well, thus establishing their presence or absence in the sample.

Wells containing samples negative for anti-HIV antibody and/or p24 antigen remain colorless.

A stop solution is added to each well and the resulting yellow color is read on a microplate reader at 450 nm..

V. REAGENTS PROVIDED

Reagents	96 Tests Kit	Colour Code	Reconstitution
TLP Microtiterplate (12x8 breakable wells) coated with HIV specific gp36, gp41 and gp120 peptides and with a Monoclonal Antibody specific to the HIV-1 p24 Ag. Sealed into a bag with desiccant.	96 wells	Blue	Ready for use
CAL Calibrator : not infectious recombinant p24 antigen in a 10 mM phosphate buffer pH 7.0+/-0.2 with 0.3 mg/ml Gentamicin Sulphate and 0.1% Kathon GC as stabilizers.	1 vial lyophilised	Yellow	The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label
CONTROL - Negative control: animal serum negative for HIV antibodies and for p24 antigen, and 0.1% Kathon GC as preservatives.	1 vial 2 ml	Yellow-brown	Ready for use
CONTROL HIV-1 + Positive control 1: inactivated HIV 1 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservative.	1 vial 2 ml	Light green	Ready for use
CONTROL HIV-2 + Positive control 2: inactivated HIV2 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservatives.	1 vial 2 ml	Dark green	Ready for use
WASH SOLN CONC 20x concentrated solution: 0.1% Kathon GC. Once diluted, the wash solution contains 10 mM phosphate buffer saline pH 7.0+/-0.2 and 0.05% Tween 20.	1 vial 60 ml		To be diluted with EIA water up to grade 1200 ml

BIOT CONJ CONC Biotinylated HIV1&2&0 gp36, gp41 and gp120 peptides and a biotinylated monoclonal antibody specific for HIV 1 p24 antigen.	4 vials lyophilised		Reconstitute with 6.0 ml of conjugate buffer
CONJ BUF Conjugate buffer: Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA	1 vial 30 ml		Ready for use
SAV HRP HRP conjugated with streptavidin in Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA	1 vial 15 ml	Pink/red	Ready for use
CHROM TMB Chromogenic solution: 50 mM citrate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.	1 vial 25 ml		Ready for use
STOP SOLN Stop solution: 0.3 M H2SO4 solution	1 vial 15 ml		Ready for use
DIL SPE Specimen diluent: Tris saline buffer supplemented with 0.05% Kathon GC, anti-HAMA blocker, and Tween 20	1 vial 7 ml	Light blue	Ready for use

Note : The positive controls HIV-1 and HIV-2 have been inactivated using B-propionolactone BPL/UV. This does not fully ensure the absence of viable pathogens, and therefore, the control should be handled as potentially biohazardous, in accordance with good laboratory practices.

The calibrator is calibrated against the NIBSC 1st International HIV-1 p24 Ag reference sample 90/636 (diluted 1:256) as well as the EFS HIV Ag performance panel (3015-3022). The Calibrator contains p24 recombinant Ag with a concentration of about 100 pg/ml, corresponding to about 4 IU/ml.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Calibrated Micropipettes (200µl and 10µl) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blinking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

VII. SPECIMEN PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°.8°C for up to seven days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present filter using 0.2-0.8 µfilters to clean up the sample for testing.

7. Do not use heat inactivated samples as they could give origin to false reactivity.

VIII. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not shown any relevant loss of activity up to 2 months.

Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the pouch is not broken or that some defect is present indicating a problem of storage. In this case call DIAsource ImmunoAssay's customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°.8°C. When opened the first time, residual strips are stable up to two months.

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Controls:

Positive controls are ready to use. Handle Positive Controls Ab as potentially infective, even if HIV, if present in the control, has been chemically inactivated.

Calibrator

The Lyophilized Calibrator Ag contains a non-infectious recombinant p24 antigen. The volume of EIA grade water to be used for its dissolution and to reach the appropriate p24 concentration (about 100 pg/ml) is written on the vial label. To help dissolve the lyophilized pellet, vortex a few times, at regular intervals. Complete dissolution should be achieved within 2-5 minutes.

Note: *When dissolved the Calibrator is not stable. Store in aliquots at -20°C.*

Wash buffer concentrate:

The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.

In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Important Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Biotinylated conjugate concentrated:

The Biotinylated conjugate concentrated mix solution must be prepared immediately before starting the test. Add 6 ml of Conjugate Buffer 1 diluent directly to one Biotinylated conjugate concentrated vial to dissolve the lyophilized powder. This preparation (a total of 6 ml in one vial) is sufficient for 32 tests, or 4 complete vertical strips of the microplate. To help dissolve the lyophilized powder, vortex a few times, at regular intervals.

Important Note: *Any unused portion of this reconstituted Biotinylated conjugate concentrated Solution may be stored at +2..8°C for no more than 12 hours. After this time it has to be discarded and the empty, used container has to be washed with EIA grade water and kept dry for any following re-use.*

HRP conjugated with streptavidin:

Ready to use reagent. Mix well end-over-end before use.

Chromogenic solution:

Ready to use. Mix well end-over-end before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container.

Stop solution:

Ready to use. Mix well end-over-end before use.

Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P364).

Legend:

Warning H statements:

H315 – Causes skin irritation.

H319 – Causes serious eye irritation.

Precautionary P statements:

P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.

P332 + P313 – If skin irritation occurs: Get medical advice/attention.

P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337 + P313 – If eye irritation persists: Get medical advice/attention.

P362 + P363 – Take off contaminated clothing and wash it before reuse.

Specimen Diluent:

Ready to use. Mix well end-over-end before use.

IX. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.

2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

4. Incubation times have a tolerance of +5%.

5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to > 2.0; (c) linearity to > 2.0; (d) repeatability > 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.

7. DIAsource ImmunoAssay's S.A. offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

X. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.

2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colourless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminium pouch, containing the microplate, is not punctured or damaged.

3. Dilute all the content of the 20x concentrated Wash Solution as described above.

4. Dissolve the Calibrator.

5. Dissolve the Biotinylated Conjugate Concentrate vial containing lyophilized powder with the Conjugate Buffer (1 lyophilized Biotinylated Conjugate Concentrate + 6ml Conjugate Buffer) to obtain the Conjugate Mix as described in the proper section.

6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.

7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer's instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.

8. Check that the ELISA reader has been turned on at least 20 minutes before reading.

9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.

10. Check that the micropipettes are set to the required volume.

11. Check that all the other equipment is available and ready to use.

12. In case of problems, do not proceed further with the test and advise the supervisor.

XI. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense 50 µl Specimen Diluent first and then 150 µl controls and samples.

Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples or tips have to be changed.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

The correct number of lyophilized Biotinylated Conjugate Concentrate must be dissolved each with 6 ml Conjugate Buffer. Once the lyophilized powders are dissolved and mixed well, they are to be mixed together into a plastic container and the assay may begin.

2. Manual assay:

1. Dissolve the right number of lyophilized Biotinylated Conjugate Concentrate with Conjugate Buffer before starting to dispense the samples and controls of the test.
2. Place the required number of strips in the microwell holder. Leave the 1st well empty for the operation of blanking.
3. Dispense 50 µl Specimen Diluent in all the wells, except A1 used for blanking.
4. Dispense 150 µl of Negative Control in triplicate, 150 µl HIV1 Positive Control, 150 µl HIV2 Positive Control and 150 µl of Calibrator in duplicate in proper wells.
5. Dispense 150 µl of Sample in each properly identified well. Mix gently the plate on the work surface, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into the diluent.
6. Incubate the microplate for 60 min at +37°C.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

7. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section IX.3).
8. Pipette 150 µl Conjugate Mix, prepared as described before, into each well, except the 1st blanking well, and cover with the sealer.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Conjugate. Contamination might occur.*

9. Incubate the microplate for 30 min at +37°C.
10. Pipette 100 µl of SAV-HRP in all the wells, except A1, and gently agitate the microplate to mix the two conjugates.

Important Note: *This solution must be added to the bottom of each well to ensure proper performance. Inadequate mixing of the two solutions (Biotinylated Conjugate Concentrate and SAV-HRP) may reduce the binding of streptavidin HRP (SAV-HRP) to the biotinylated reagents and consequently affect the performance of the assay. Be sure to provide an adequate mixing when the SAV-HRP is added, both in the manual and in the automated procedures.*

11. Incubate the microplate sealed for 30 min at +37°C.
12. Wash as in section 7.
13. Dispense 200 µl of Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-25°C) for 30 minutes. Start the timing immediately after addition of this component to the first well.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

14. Pipette 100 µl Stop Solution into all the wells using the same pipetting sequence as in step 13 to stop the enzymatic reaction. Addition of acid will turn the positive controls and positive samples from blue to yellow/brown.
15. Measure the colour intensity of the solution in each well, as described in section IX.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 30 minutes after its addition. Some self-oxidation of the chromogen can occur leading to high background.*

3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

XII. ASSAY SCHEME

Method	Operations
Specimen Diluent	50 µl
Controls and calibrator	150 µl
Samples	150 µl
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Biotinylated conjugate Mix	150 µl
2nd incubation	30 min
Temperature	+37°C
HRP conjugated with streptavidin	100 µl
3rd incubation	30 min
Temperature	+37°C
Wash step	4-5 cycles
Chromogenic solution	200 µl
4th incubation	30 min
Temperature	r.t.
Stop solution	100 µl
Reading OD	450nm

XIII. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	≤ 0.100 OD450nm value
Negative Control (NC)	≤ 0.200 mean OD450nm value after blanking Absorbance of individual negative control values must be less than or equal to 0.200. If one value is outside this range, discard this value and recalculate mean. If two values are outside this range the run should be repeated.
HIV-1 Ab Positive Control	Mean OD450nm ≥ 0.700.
HIV-2 Ab Positive Control	Mean OD450nm ≥ 0.700.
HIV Ag Calibrator	S/Co > 1

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Controls < 0.700 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.200, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
HIV Ag Calibrator S/Co < 1	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of Calibrator Ag. In this case, the negative control will have an OD450nm value > 0.200, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred. 5. that the lyophilize powder was dissolved correctly with the correct volume of water written on the vial label.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

XIV. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

$$NC + 0.125 = \text{Cut-Off (Co)}$$

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: *When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

XV. INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1	Negative
> 1	Positive

A negative result indicates that the patient has not been infected by HIV.

If the initial absorbance value is equal to or greater than the cut-off value, retest the sample in duplicate. If both retest values are less than the cut-off, the interpretation is not reactive for HIV antibody and/or antigen (negative).

If one or both retest values are equal to or greater than the cut-off the interpretation of the test results is repeatedly reactive. The sample should be considered reactive or positive for HIV antibody and/or antigen according to the criteria of this HIV ELISA test.

A positive result is indicative of HIV infection and therefore the patient should be treated accordingly.

Important notes:

1. Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.
2. Repeatedly reactive specimens should be submitted to a Confirmation Assay before diagnosis of HIV infection is released.
3. When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.
4. Diagnosis of HIV infection has to be done and released to the patient only by a qualified medical doctor.

An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.110 – 0.120 - 0.115

OD450nm Mean Value: 0.115 OD450nm

Lower than 0.200 – Accepted

HIV 1 Ab Positive Control: 2.000 OD450nm mean value

Higher than 0.700 – Accepted

HIV 2 Ab Positive Control: 2.100 OD450nm mean value

Higher than 0.700 – Accepted

Calibrator : 0.322 OD450nm mean value

S/Co > 1 - Accepted

Cut-Off = 0.115 + 0.125 = 0.240

Sample 1: 0.070 OD450nm

Sample 2: 1.690 OD450nm

Sample 1 S/Co < 1 = negative

Sample 2 S/Co > 1 = positive

XVI. PERFORMANCES

The performance evaluation was carried out both in an external center of excellence for HIV diagnosis, that examined the device on a population of antibody positive and negative samples against a CE-marked kit used in the laboratory as reference, and in DIAsource ImmunoAssay's laboratories as well to complete the study.

A. Analytical sensitivity

The limit of detection (or analytical sensitivity) of the assay has been calculated by means of preparations specific for HIV-1 and HIV-2 antibody and HIV-1 p24 Ag detection, supplied by NIBSC Blanche Lane South Mimms Potters Bar Hertfordshire EN6 3QG, UK.

Samples were diluted in HIV Ab&Ag negative plasma to generate limiting dilution curves and examined in duplicate.

The tables below reports the mean OD450nm values and the S/Co index:

NIBSC anti-HIV 2 monitor sample
code 99/674 – 005

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	over	>14.5	over	>15.0	over	>15.1
2x	3.838	14.1	3.765	14.5	3.774	14.4
4x	2.371	8.7	2.268	8.7	2.319	8.9
8x	1.253	4.6	1.097	4.2	1.140	4.4
16x	0.700	2.6	0.712	2.7	0.735	2.8
32x	0.462	1.7	0.439	1.7	0.483	1.8
64x	0.281	1.0	0.260	1.0	0.294	1.1
128x	0.189	0.7	0.171	0.7	0.174	0.7
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The device shows a limiting dilution value at 64x.

NIBSC British working standard for anti-HIV 1
code 99/750 – 007

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	2.206	8.1	2.086	8.0	2.142	8.2
2x	0.999	3.7	0.925	3.6	1.027	3.9
4x	0.475	1.7	0.486	1.9	0.486	1.9
8x	0.295	1.1	0.301	1.2	0.289	1.1
16x	0.212	0.8	0.206	0.8	0.220	0.8
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The device shows a limiting dilution value at 8x.

NIBSC British working standard for anti-HIV 1
code 99/710 – 007

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	0.588	2.2	0.611	2.4	0.607	2.3
2x	0.301	1.1	0.309	1.2	0.312	1.2
4x	0.198	0.7	0.210	0.8	0.192	0.7
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The device shows a limiting dilution value at about 2x.

NIBSC HIV-1 p24 Antigen Monitor Sample
code 02/146-002

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	3.664	13.4	3.650	14.0	3.552	13.5
2x	2.151	7.9	2.133	8.2	2.086	8.0
4x	1.209	4.4	1.178	4.5	1.214	4.6
8x	0.734	2.7	0.729	2.8	0.780	3.0
16x	0.388	1.4	0.342	1.3	0.351	1.3
32x	0.259	0.9	0.236	0.9	0.229	0.9
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The device shows a limiting dilution value at about 16x.

NIBSC 1st International reference Reagent for HIV 1 Ag code 90/636 – (Version 4, 12 May 2009)

Sample	Lot #	1	Lot #	2	Lot #	3
IU/ml	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
16	2.053	14.8	2.048	14.8	2.053	14.5
8	1.118	8.0	1.121	8.1	1.124	8.0
4	0.571	4.1	0.574	4.1	0.576	4.1
2	0.290	2.1	0.291	2.1	0.290	2.0
1	0.160	1.2	0.162	1.2	0.160	1.1
0.5	0.104	0.7	0.105	0.8	0.103	0.7
diluent	0.014	//	0.014	//	0.014	//

Note: Lot 1 = C2E2T2/6 – Lot 2 = C2E2T2/5 – Lot 3 = C2E2T2/4

The device shows a sensitivity < 2 IU/ml as required by CTS:2009.

B. Diagnostic specificity and sensitivity

Diagnostic Specificity: In addition to the first study, where a total of more than 5000 unselected blood donors, more than 200 hospitalized patients, (under examination for non-HIV pathologies) and more than 100 potentially interfering specimens (other infectious diseases, E.coli antibody positive, patients affected by non-viral hepatic diseases, dialysis patients, pregnant women, hemolysed, lipemic, etc.) were tested, the diagnostic specificity was recently assessed by testing a total of 3268 negative samples on four different lots. A value of diagnostic specificity of 100% was observed.

No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity. Frozen specimens have been tested, as well, to check for interferences due to collection and storage. No interference was observed.

Diagnostic Sensitivity: The diagnostic sensitivity of the test was determined on a population of HIV positive specimens.

Etablissement Francais du Sang
Mixed titer Performance
Panel HIV Ab (1-6) Lot # 11

ID	Composition	Lot # 0506	Lot # 0706	Lot # 0906	S/Co
		S/Co	S/Co	S/Co	mean
1	HIV2(1/200)	>14.5	>15.0	>15.1	>14.9
2	HIV2(1/800)	10.0	10.2	10.2	10.1
3	negative	0.4	0.4	0.4	0.4
4	HIV1(1/700)	8.9	9.0	9.1	9.0
5	HIV1(1/160)	>14.5	>15.0	>15.1	>14.9
6	HIV1(1/200)	1.9	1.9	1.8	1.9

BBI Anti-HIV 1 Low Titer
Performance Panel - PRB 108

Member ID #	KARDHIV96 S/Co
1	3.5
2	0.5
3	2.3
4	11.4
5	3.8
6	6.9
7	4.0
8	7.5
9	6.3
10	2.4
11	8.8
12	4.1
13	3.7
14	7.2
15	10.9

BBI Anti-HIV 1 Low Titer
Performance Panel - PRB 107
(modified version)

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
2	5.6	3.7
3	2.1	4.5
5	0.5	0.1
6	4.4	7.3
7	1.4	1.0
8	6.3	7.1
10	1.8	3.5
12	5.9	5.0
13	7.1	3.3
15	3.2	2.7

BBI anti-HIV-1 Low Titer
Performance Panel - PRB 106

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
1	4.5	7.5
2	2.6	1.8
3	5.2	2.0
4	6.1	8.9
5	11.4	17.6
6	0.5	0.1
7	3.9	7.9
8	13.2	15.7
9	>14.5	16.6
10	6.1	12.7
11	4.8	6.8
12	3.0	2.4
13	5.9	10.5
14	7.7	6.0
15	>14.5	5.5

BBI Anti-HIV 1 Mixed Titer
Performance Panel - PRB 204

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
1	1.2	1.0
2	>14.5	65.2
3	0.3	0.2
4	>14.5	65.1

5	>14.5	66.1
6	>14.5	67.5
7	>14.5	66.0
8	>14.5	64.6
9	1.3	2.4
10	>14.5	65.1
11	>14.5	65.4
12	>14.5	63.8
13	4.7	67.0
14	>14.5	63.8
15	>14.5	63.6
16	>14.5	66.6
17	>14.5	68.8
18	>14.5	71.2
19	>14.5	71.5
20	>14.5	68.5
21	>14.5	71.7
22	>14.5	68.5
23	0.3	0.2
24	2.3	2.3
25	0.8	0.9

BBI anti-HIV 1/2 Combo
Performance Panel - PRZ 206

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
1	10.2	>15.7
2	0.4	0.2
3	>14.5	>15.7
4	>14.5	>15.7
5	6.6	9.2
6	>14.5	9.1
7	0.4	0.2
8	>14.5	>15.7
9	11.4	15.5
10	>14.5	>15.7
11	>14.5	>15.7
12	>14.5	12.8
13	>14.5	15.7

BBI HIV-1 Incidence/Prevalence
Performance Panel - PRB 601

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
1	>14.5	18.5
2	>14.5	17.4
3	>14.5	17.4
4	>14.5	17.4
5	>14.5	15.8
6	>14.5	17.4
7	>14.5	18.5
8	>14.5	17.4
9	>14.5	17.4
10	>14.5	17.4
11	>14.5	18.5
12	>14.5	17.4
13	>14.5	17.4
14	>14.5	17.4
15	>14.5	17.4

BBI Worldwide HIV
Performance Panel - WWRB 303

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
1	>14.5	>15.9
2	>14.5	>15.9
3	>14.5	>15.9
4	>14.5	>15.9
5	4.1	>15.9
6	>14.5	>15.9
7	>14.5	>15.9
8	>14.5	>15.9
9	1.1	2.4
10	>14.5	>15.9
11	11.3	0.3
12	0.3	0.4
13	>14.5	>15.9
14	>14.5	>15.9
15	>14.5	>15.9

Etablissement Francais du Sang
Performance Panel
HIV Ag (3015-3022) lot 2004

Sample	KARDHIV96 Lot # 0506 S/Co	Concentration HIV 1 p24 Ag [pg/ml]
3015	8.7	500

3016	4.2	250
3017	1.8	100
3018	1.2	50
3019	0.9	25
3020	0.6	10
3021	0.6	5
3022(diluent)	0.5	diluent

Average values N = 48	Negative Sample	Low Positive
OD450nm	0.136	0.916
Std.Deviation	0.011	0.022
CV %	7.6	4.0

BBI HIV p24 Antigen Mixed Titer
Performance Panel - PRA 203

Member ID #	KARDHIV96 S/Co	Ref. Kit S/Co
1	2.3	4.3
2	4.1	12.2
3	1.4	6.0
4	1.9	3.0
5	Nd	Nd
6	1.0	1.3
7	2.9	5.7
8	3.0	1.5
9	0.4	0.5
10	3.6	12.2
11	2.4	3.1
12	1.6	3.5
13	1.2	2.1
14	0.4	0.9
15	3.3	2.5
16	1.1	1.8
17	2.5	6.3
18	1.7	2.4
19	0.4	0.5
20	4.6	17.0

Moreover, in the external Performance Evaluation a total of 651 positive samples, including HIV type 1, HIV type 2, HIV type 1 mixed subtypes (including 0), HIV 1 Antigen, more than 40 early seroconversion HIV samples and cell culture supernatants were evaluated and a value of 100% was found.

Finally, more than 30 panels of seroconversion containing samples of HIV 1/2/0 Antibodies and/or HIV-1 p24 Antigen positive, obtained from BBI, USA, were evaluated using KARDHIV96 lot # 0506. In the table below results are reported.

Seroconversion Panel	KARDHIV96 HIV Ab&Ag
ID	First specimen detected positive in the panel
PRB 910 (J)	2
PRB 916 (P)	4
PRB 917 (Q)	2
PRB 919 (S)	1
PRB 922 (V)	1
PRB 924 (X)	5
PRB 926 (Z)	3
PRB 927 (AB)	2
PRB 928 (AC)	2
PRB 929 (AD)	4
PRB 930 (AE)	2
PRB 931 (AF)	6
PRB 933 (AH)	2
PRB 935 (AJ)	6
PRB 937 (AL)	4
PRB 938 (AM)	1
PRB 939 (AN)	7
PRB 940 (AP)	2
PRB 941 (AQ)	4
PRB 942 (AR)	4
PRB 944 (AT)	3
PRB 946 (AV)	3
PRB 947 (AW)	2
PRB 948 (AX)	4
PRB 949 (AY)	4
PRB 950 (AZ)	3
PRB 952 (BB)	3
PRB 953 (BC)	3
PRB 955 (BE)	3
PRB 956 (BF)	5

The results of the Performance Evaluation, correlate perfectly with what stated by EU CTS and show an overall value of diagnostic sensitivity of 100%.

C. Precision

The precision of the device was assessed by determining its values in a within and between runs. In the table below results are reported for a negative sample and a low positive sample.

XVII. LIMITATIONS

The user of this kit is advised to carefully read and understand this package insert. Strict adherence to the protocol is necessary in order to obtain reliable test results. In particular, accurate sample and reagent pipetting, along with careful washing and timing of incubation steps is essential for accurate and reproducible detection of HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen.

After the EIA test is performed, repeatedly reactive specimens should be submitted for additional testing using Western Blot (WB), Immunofluorescence Assay (IFA), Radioimmunoassay (RIA) tests and PCR for HIV nucleic acid.

The determination that a person's serum contains antibodies or p24 antigen to HIV has extensive medical, social, psychological and economic implications.

It is recommended that confidentiality, appropriate counselling and medical evaluation be considered an essential aspect of the testing sequence. AIDS and AIDS-related conditions are clinical diseases and their diagnosis can only be established clinically.

EIA testing alone cannot be used to diagnose AIDS.

A non-reactive test result at any point in the testing sequence does not preclude the possibility of exposure to or infection with HIV. The risk of an asymptomatic person, who is repeatedly reactive, developing AIDS and/or AIDS-related conditions is not known.

Falsely reactive test results can be observed with a test kit of this nature. The proportion of reactive samples will depend on the sensitivity and specificity of the test kit and on the prevalence of HIV-1 and HIV-2 antibodies in the population to be screened.

Antibodies to HIV may occur due to voluntary participation in an HIV vaccine study.

Interpretation of this diagnostic test will depend on the type of vaccine given. Correlation with the medical history and additional testing may be necessary to accurately diagnose HIV in vaccine volunteers.

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