MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

agencia española de medicamentos y productos sanitarios

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CERTIFICADO DE EXAMEN CE DE DISEÑO
de acuerdo con el Anexo IV, punto 4, de la Directiva 98/79/CE

EC DESIGN-EXAMINATION CERTIFICATE
in accordance with Annex IV, Section 4, Directive 98/79/EC

PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: **14/02/2008**
Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2008 02 0539 ED	**Desde/*From* 19/11/2018 Hasta/*To* 18/11/2023**	**0318**

A favor de /*In favour of:*

Fabricante/*Manufacturer*:
Nombre/*Name:* DIA. Pro Diagnostic Bioprobes S.r.l.
Dirección/*Address:* Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/*Authorized EU representative*:
Nombre/*Name:* Idem Dirección/*Address:* Idem

Para el producto/*For the product:*

Categoría/*Category:* Productos Sanitarios para Diagnóstico "In Vitro" / *In Vitro Diagnostic Medical Devices*
Grupo genérico/*Generic group*: Diagnóstico de enfermedades infecciosas / *Diagnostic of infectious diseases*
Tipo/*Type*: Especificados en Anexos de este Certificado/*Specified in Annexes to this Certificate.*

Elaborado en/*In the facilities:*

Dia. Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).

Este certificado debe ir acompañado por el certificado CE de Sistema de Garantía de Calidad Total Nº 2003 12 0388 CT/ *This certificate must be accompanied by the EC Full Quality Assurance System Certificate Nº 2003 12 0388 CT.*

Este certificado es consecuencia de la evaluación de la documentación técnica del diseño contenida en el expediente Nº 2003 05 0240, y garantiza que el diseño de los productos descritos cumple los requisitos de la Directiva/ *This certificate is issued on the assessment of the design documentation contained in dossier Nº 2003 05 0240, and guarantees that the design of the described products fulfil the requirements of the Directive.*

Madrid, 19 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 19/11/2018
Localizador: 6XZPBYWEFC

Puede comprobar la autenticidad del documento en la aplicación Localizador de la Web de la AEMPS

CORREO ELECTRÓNICO
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Nombre/*Name:* Idem Dirección/*Address:* Idem

Tipo de producto / *Device type*: **Reactivos y productos reactivos, calibradores y materiales de control para el diagnóstico de enfermedades infecciosas /** *Reagents, and reagent products, calibrators and control materials for diagnostic of human infectious diseases.*

Clasificación/*Classification:* **Lista A, Anexo II /** *List A, Annex II*

Reactivos y productos reactivos para la determinación, confirmación y cuantificación en muestras humanas de marcadores de infección por HIV 1 y 2, mediante técnicas de Inmunoabsorción enzimática (ELISA)/ *Reagents and reactive products for the determination, confirmation and quantification in human specimens of markers of HIV 1 and 2 infection, by Enzyme-linked immunosorbent assay (ELISA)* **[NANDO: IVD 0201]**

HIV Ab & Ag ELISA cualitativo / *ELISA qualitative*

- IVCOMB.CE (192 tests)
- IVCOMB.CE.96 (96 tests)
- IVCOMB.CE.480 (480 tests)
- IVCOMB.CE.960 (960 tests)
- IVCOMB.CE.DB (192 tests - For Dia.Blood application)

Este certificado ampara todas las marcas de estos productos incluidas por el fabricante en su declaración de conformidad. / *This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity*.

Madrid, 19 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

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Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HIV Ab&Ag** CODES: **IVCOMB.CE (192 tests)** IVCOMB.CE.96 (96 tests) IVCOMB.CE.480 (480 tests) IVCOMB.CE.960 (960 tests) IVCOMB.CE.DB (192 tests)
CLASSIFICATION	ANNEX II – LIST A
CONFORMITY ASSESSMENT ROUTE	ANNEX IV

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

NOTIFIED BODY	AEMPS – n° 0318
(EC) CERTIFICATE(S)	• FULL QUALITY ASSURANCE SYSTEM N° 2003 12 0388 CT (in accordance with Annex IV – except Section IV) of the Directive 98/79/EC), RELEASED BY EC NOTIFIED BODY N° 0318 • DESIGN CERTIFICATE N° 2008 02 0539 ED RELEASED BY EC NOTIFIED BODY N° 0318 • UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY EC NOTIFIED BODY N° 0318

PLACE & DATE OF FIRST ISSUE	MILANO – FEBRUARY 2008
PLACE & DATE OF CURRENT EMISSION	SESTO SAN GIOVANNI (MI) – MARCH 2018
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	DIA.PRO DIAGNOSTIC BIOPROBES srl

Rev:	0318

HIV Ab&Ag

Fourth generation Enzyme Immunoassay for the determination of antibodies to Human Immunodeficiency Virus or HIV type 1&2&O and P24 HIV-1 Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
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20099 Sesto San Giovanni
(Milano) - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF IVCOMB.CE
96/192/480/960 Tests

HIV Ab&Ag

A. 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.
This kit is intended exclusively for ***In vitro*** diagnostic use in an authorized clinical laboratory and the test has to be carried out by specifically trained health-care professional personnel.
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B. 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.

C. PRINCIPLE OF THE TEST

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.

D. COMPONENTS

The standard format of the product code IVCOMB.CE contains reagents for 192 tests.

1. Microplate MICROPLATE
n° 2 microplates. 12 strips of 8 breakable wells coated with HIV specific gp36, gp41 and gp120 peptides and with a Monoclonal Antibody specific to the HIV-1 p24 Ag. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains animal serum negative for HIV antibodies and for p24 antigen, and 0.1% Kathon GC as preservatives. The negative control is yellow-brown color coded.

3. Positive Control HIV-1 Ab CONTROL 1+
1x4.0ml/vial. Ready to use control. It contains inactivated HIV 1 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservative. The Positive Control is light green color coded.
Important Note: *The positive control has 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.*

4. Positive Control HIV-2 Ab CONTROL 2+
1x4.0ml/vial. Ready to use control. It contains inactivated HIV2 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservatives. The Positive Control is dark green color coded.
Important Note: *The positive control has 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.*

5. HIV-1 P24 Ag Calibrator CAL Ag
2 vials. Lyophilized. It contains not infectious recombinant p24 antigen in a 10 mM phosphate buffer pH 7.0+/-0.2 with 0.3 mg/ml Gentamicine Sulphate and 0.1% Kathon GC as stabilizers. This component 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).
Important Notes:
1) The Calibrator contains p24 recombinant Ag with a concentration of about 100 pg/ml, corresponding to about 4 IU/ml.
2) 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 .

6. Wash buffer concentrate WASHBUF 20X
2x60ml/bottle. 20x concentrated solution. It contains 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.

7. Conjugate # 1 CONJ 1
8 vials. The vial contains lyophilized biotinylated HIV1&2&0 gp36, gp41 and gp120 peptides and a biotinylated monoclonal antibody specific for HIV 1 p24 antigen. Vials are to be resuspended with 6 ml of the Conjugate # 1 diluent.

8. Conjugate 1 Diluent CONJ 1 DIL
1x60ml/bottle. Used to dissolve the lyophilized powder of Conjugate # 1, it contains Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA.

9. Conjugate # 2 CONJ 2
1x25ml/bottle The solution contains HRP conjugated with streptavidin in Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA. This component is color coded in pink/red.

10. Chromogen/Substrate SUBS TMB
1x45ml/bottle Ready-to-use component. It contains 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.
Note: *To be stored protected from light as sensitive to strong illumination.*

11. Sulphuric Acid H_2SO_4 O.3 M
1x25ml/bottle It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

12. Sample Diluent: DILSPE
1x14ml/vial Contains Tris saline buffer supplemented with 0.05% Kathon GC, anti HAMA blocker, and Tween 20; used for specimen dilution. This component is color coded in light blue.

13. Plate sealing foils n° 4

14. Package insert n° 1

Important note: Upon request, Dia.Pro can supply reagents for 96, 480, 960 tests, as reported below **:**

1.Microplate	n°1	n°5	n°10
2.Negative Control	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
3.Positive Control 1	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
4.Positive Control 2	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
5.HIV p24 Calibrator	n°1 vial	n° 5 vials	n° 10 vials
6.Wash buff conc	1x60ml/bottle	5x60ml/bottles	4x150ml/bottles
7.Conjugate # 1	n°4 vials	n°20 vials	n°40 vials
8.Conjugate 1 Diluent	1x30ml/vial	3x50ml/bottles	2x150ml/bottles
9.Conjugate # 2	1x15ml/vial	2x38ml/bottle	2x75ml/bottle
10.Chrom/Substrate	1x25ml/vial	3x42ml/bottle	2x125ml/bottles
11.Sulphuric Acid	1x15ml/vial	2x40ml/bottles	2x80ml/bottles
12.SampleDiluent	1x7ml/vial	1x35ml/bottle	1x70ml/bottle
Plate seal foils	n° 2	n° 10	n° 20
Pack. insert	n° 1	n°1	n° 1
Number of tests	**96**	**480**	**960**
Code	**IVCOMB.CE.96**	**IVCOMB.CE.480**	**IVCOMB.CE.960**

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (200ul and 10ul) 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 (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMENDATIONS
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 0.2-0.8u filters to clean up the sample for testing.
7. Do not use heat inactivated samples as they could give origin to false reactivity.

H. 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 Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum 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 Ab:
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 Ag
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.

Conjugate # 1:
The Conjugate # 1 mix solution must be prepared immediately before starting the test. Add 6 ml of Conjugate 1 diluent directly to one Conjugate # 1 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 Conjugate # 1 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.

Conjugate # 2:
Ready to use reagent. Mix well end-over-end before use.

Chromogen/Substrate:
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.

Sulphuric Acid:
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).

Legenda:

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.

Sample Diluent:
Ready to use. Mix well end-over-end before use.

I. 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 350ul/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 "Validation of Test" and "Assay 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 $\pm$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 $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; (d) repeatability $\geq$ 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. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. Dia.Pro's customer service 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.

L. 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 colorless 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 aluminum 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 Ag.
5. Dissolve the Conjugate # 1 vial containing lyophilzed powder with the Conjugate 1 Diluent (1 lyophilized Conjugate # 1 + 6ml Conjugate # 1 Diluent) to obtain the Conjugate # 1 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 manufacturers 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.

M. 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 ul Sample Diluent first and then 150 ul 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 Conjugate # 1 must be dissolved each with 6 ml Conjugate # 1 Diluent. 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 Conjugate # 1 with Conjugate # 1 Diluent 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 ul Sample Diluent in all the wells, except A1 used for blanking.
4. Dispense 150 ul of Negative Control in triplicate, 150 ul HIV1 Positive Control, 150 ul HIV2 Positive Control and 150 ul of Calibrator Ag in duplicate in proper wells.
5. Dispense 150 ul 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 350ul/well of diluted washing solution as reported previously (section I.3).
8. Pipette 150 ul Conjugate # 1 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 ul of Conjugate # 2 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 (Conjugate 1 and Conjugate 2) may reduce the binding of streptavidin HRP (Conjugate 2) to the biotinylated reagents and consequently affect the performance of the assay. Be sure to provide an adequate mixing when the Conjugate # 2 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 ul 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 ul Sulphuric Acid 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 color intensity of the solution in each well, as described in section I.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.*

N. ASSAY SCHEME

Method	Operations
Sample Diluent	50 ul
Controls and calibrator	150 ul
Samples	150 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Conjugate # 1	150 ul
2nd incubation	**30 min**
Temperature	+37°C
Conjugate # 2	100 ul
3rd incubation	**30 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	200 ul
4th incubation	**30 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL Ag										
B	NC	CAL Ag										
C	NC	S1										
D	NC	S2										
E	POS 1 Ab	S3										
F	POS 1 Ab	S4										
G	POS 2 Ab	S5										
H	POS 2 Ab	S6										

Legenda: BLK = Blank NC = Negative Control POS 1 Ab = HIV -1 Ab Positive Control, POS 2 Ab = HIV -2 Ab Positive, CAL Ag = HIV p24 Ag Calibrator, S = Sample

O. 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.

P. 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 = 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.*

Q. 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. *Rrepeatedly 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 centre, 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 Ag: 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

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).
The performance evaluation was carried out both in an external centre 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 DiaPro's laboratories as well to complete the study.

R.1 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 devise 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 devise 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 devise 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 devise shows a sensitivity ≤ 2 IU/ml as required by CTS:2009.

R.2 DIAGNOSTIC SPECIFICITY AND SENSITIVITY

R.2.1 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, hemolized, 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.

R.2.2 Diagnostic Sensitivity

The diagnostic sensitivity of the test was determined on a population of HIV positive specimens.
Results are reported in the tables below.

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 #	IVCOMB.CE S/Co	IVAB.CE S/Co
1	**3.5**	**3.1**
2	0.5	0.2
3	**2.3**	**2.5**
4	**11.4**	**10.6**
5	**3.8**	**2.9**
6	**6.9**	**5.3**
7	**4.0**	**3.7**
8	**7.5**	**6.3**
9	**6.3**	**3.2**
10	**2.4**	**1.5**
11	**8.8**	**7.2**
12	**4.1**	**2.9**
13	**3.7**	**2.3**
14	**7.2**	**6.1**
15	**10.9**	**6.3**

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

Member ID #	IVCOMB.CE 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 #	IVCOMB.CE 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 #	IVCOMB.CE 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 #	IVCOMB.CE 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 #	IVCOMB.CE 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 #	IVCOMB.CE 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	IVCOMB.CE 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

BBI HIV p24 Antigen Mixed Titer Performance Panel - PRA 203

Member ID #	IVCOMB.CE 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 IVCOMB.CE lot # 0506. In the table below results are reported.

Seroconversion Panel	**IVCOMB.CE** 4th Generation HIV Ab&Ag	**IVAB.CE** 3rd Generation HIV Ab
ID	**First specimen detected positive in the panel**	
PRB 910 (J)	2	3
PRB 916 (P)	4	5
PRB 917 (Q)	2	5
PRB 919 (S)	1	2
PRB 922 (V)	1	1
PRB 924 (X)	5	6
PRB 926 (Z)	3	5
PRB 927 (AB)	2	3
PRB 928 (AC)	2	3
PRB 929 (AD)	4	6
PRB 930 (AE)	2	3
PRB 931 (AF)	6	6
PRB 933 (AH)	2	2
PRB 935 (AJ)	6	7
PRB 937 (AL)	4	not detected
PRB 938 (AM)	1	3
PRB 939 (AN)	7	9
PRB 940 (AP)	2	4
PRB 941 (AQ)	4	5
PRB 942 (AR)	4	not detected
PRB 944 (AT)	3	5
PRB 946 (AV)	3	not detected
PRB 947 (AW)	2	4
PRB 948 (AX)	4	not detected
PRB 949 (AY)	4	5
PRB 950 (AZ)	3	4
PRB 952 (BB)	3	5
PRB 953 (BC)	3	4
PRB 955 (BE)	3	4
PRB 956 (BF)	5	not detected

The device shows a better sensitivity than the previous generation as it is able to detect the p24 antigen.

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

R.3 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.

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

S. 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), Radioimmunoprecipitation Assay (RIPA) 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 repeatably 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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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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