

**CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
de acuerdo con el Anexo IV (excepto punto 4) de la Directiva 98/79/CE**

**EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
in accordance with Annex IV (except Section 4) of Directive 98/79/EC**

PRÓRROGA/EXTENSION — Fecha inicial/ Initial date: 11/12/2003

Fecha de última prórroga/ Last extension date: 27/11/2013

Certificado nº/Certificate no	Fecha de validez/Date of validity	ON nº/NB no
2003 12 0388 CT	Desde/From 27/11/2018 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 los productos/For the products:

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 certificado de examen de diseño: SI

This certificate must be accompanied by design examination certificate: YES

Este certificado es consecuencia de la auditoria del Sistema Completo de Garantía de Calidad y del examen de la documentación técnica contenida en el expediente nº 2003 05 0240, y garantiza que los productos descritos cumplen los requisitos de la Directiva. / *This certificate is issued on the full quality assurance system audit, and the examination of the technical documentation contained in dossier nº 2003 05 02405, and guarantees that the described products fulfil the requirements of the Directive.*

Madrid, 26 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

Localizador: X9GVDEF5C3

Fecha de la firma: 26/11/2018

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CORREO ELECTRÓNICO

on0318@aemps.es

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ORGANISMO NOTIFICADO 0318

C/ CAMPEZO, 1 - EDIFICIO 8

28022 MADRID

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CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
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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*

1. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de Inmunoabsorción enzimática (ELISA)/ *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Enzyme-linked immunosorbent assay (ELISA) [NANDO: IVD 0201; IVD 0202; IVD 0203]*

1.1. HBs Ag one

- SAG1.CE (192 tests) Descrito en el certificado / *Described in the certificate*
- SAG1.CE.96 (96 tests) 2003 12 0389 ED
- SAG1.CE.480 (480 tests)
- SAG1.CE.960 (960 tests)

1.2. HBs Ab

- SAB.CE (96 tests) Descrito en el certificado / *Described in the certificate*
- 2003 12 0390 ED

1.3. HBc Ab

- BCAB.CE (96 tests) Descrito en el certificado / *Described in the certificate*
- 2003 12 0391 ED

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1.4. HBc IgM

- BCM.CE (96 tests) Descrito en el certificado / Described in the certificate 2004 03 0424 ED

1.5. HBe Ag & Ab

- HBE.CE (96 tests) Descrito en el certificado / Described in the certificate 2004 03 0425 ED

1.6. HBs Ag Confirmation

- SCONF.CE (20 tests) Descrito en el certificado / Described in the certificate 2006 11 0511 ED
- SCONF.CE.40 (40 tests)

1.7. HBs Ag one Version ULTRA

- SAG1ULTRA.CE (192 tests) Descrito en el certificado / Described in the certificate 2008 12 0588 ED
- SAG1ULTRA.CE.96 (96 tests)
- SAG1ULTRA.CE.480 (480 tests)
- SAG1ULTRA.CE.960 (960 tests)
- SAG1ULTRA.CE.DB (192 tests)

1.8. HCV Ab

- CVAB.CE (192 tests) Descrito en el certificado / Described in the certificate 2003 12 0392 ED
- CVAB.CE.96 (96 tests)
- CVAB.CE.480 (480 tests)
- CVAB.CE.960 (960 tests)
- CVAB.CE.DB (192 tests)

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1.9. HCV Ab Confirmation

- CCONF.CE (12 tests) Descrito en el certificado / *Described in the certificate*
2005 09 0485 ED

1.10. HCV IgM

- CVM.CE (96 tests) Descrito en el certificado / *Described in the certificate*
2007 09 0532 ED

1.11. HCV Ab (Format 20)

- CVAB.CE.EG (192 tests) Descrito en el certificado / *Described in the certificate*
- CVAB.CE.EG.96 (96 tests) 2015 10 0842 ED
- CVAB.CE.EG.480 (480 tests)
- CVAB.CE.EG.960 (960 tests)

1.12. HDV Ab

- DAB.CE (96 tests) Descrito en el certificado / *Described in the certificate*
2003 12 0393 ED

1.13. HDV Ag

- DAG.CE (96 tests) Descrito en el certificado / *Described in the certificate*
2003 12 0394 ED

1.14. HDV IgM

- DIM.CE (96 tests) Descrito en el certificado / *Described in the certificate*
2003 12 0395 ED

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1.15. HTLV I & II Ab

- HTLVAB.CE (192 tests)
- HTLVAB.CE.96 (96 tests)
- HTLVAB.CE.480 (480 tests)
- HTLVAB.CE.960 (960 tests)

Descrito en el certificado/ *Described in the certificate* 2005 12 0493 ED

1.16. HTLV I & II Ab Version ULTRA

- HTLVABULTRA.CE (192 tests)
- HTLVABULTRA.CE.96 (96 tests)
- HTLVABULTRA.CE.480 (480 tests)
- HTLVABULTRA.CE.960 (960 tests)
- HTLVABULTRA.CE.DB (192 tests)

Descrito en el certificado/ *Described in the certificate* 2011 11 0775 ED

1.17. HIV Ab & Ag

- IVCOMB.CE (192 tests)
- IVCOMB.CE.96 (96 tests)
- IVCOMB.CE.480 (480 tests)
- IVCOMB.CE.960 (960 tests)
- IVCOMB.CE.DB (192 tests)

Descrito en el certificado/ *Described in the certificate* 2008 02 0539 ED

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2. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de PCR en tiempo real/
Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Real-Time PCR [NANDO: IVD 0203]

2.1. HBV DNA Quantitation (QT)

- HBVDNAQT.CE (50 tests)
- HBVDNAQT.CE.25 (25 tests)
- HBVDNAQT.CE.100 (100 tests)
- HBVDNAQT.CE.150 (150 tests)

Descrito en el certificado / *Described in the certificate* 2012 09 0790 ED

2.2. HDV RNA Quantitation (QT)

- DRNA.CE (50 tests)
- DRNA.CE.25 (25 tests)
- DRNA.CE.100 (100 tests)
- DRNA.CE.150 (150 tests)

Descrito en el certificado / *Described in the certificate* 2009 11 0660 ED

3. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante ensayos de quimioluminiscencia (CLIA)/
Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Chemiluminescence Immunoassay (CLIA) [NANDO: IVD 0201; IVD 0202; IVD 0203]

3.1. DIA.CHEMILUX HCV Ab

- RACVAB.CE (100 tests)

Descrito en el certificado / *Described in the certificate* 2015 01 0834 ED

3.2. DIA.CHEMILUX HBs Ag

- RASAG.CE (100 tests)

Descrito en el certificado / *Described in the certificate* 2015 10 0841 ED

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3.3. DIA.CHEMILUX HIV Ab & Ag

- RAIVCOMB.CE (100 tests) Descrito en el certificado / Described in the certificate 2016 02 0844 ED

3.4. DIA.CHEMILUX HBc Ab

- RABCAB.CE (100 tests) Descrito en el certificado / Described in the certificate 2017 07 0863 ED

3.5. DIA.CHEMILUX HTLV I & II Ab

- RAHTLVAB.CE (100 tests) Descrito en el certificado / Described in the certificate 2018 11 0878 ED

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

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HBsAg_{one}

Version ULTRA

**Fourth generation Enzyme
Immunoassay (ELISA)
for the determination of
Hepatitis B surface Antigen or HBsAg
in human serum and plasma**

- for “in vitro” diagnostic use only -



DIA.PRO

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HBsAg One version ULTRA

A. INTENDED USE

Fourth generation Enzyme Immunoassay (ELISA) for the one-step determination of Hepatitis B surface Antigen or HBsAg in human plasma and sera.

The kit is intended for the screening of blood units, is able to detect HBsAg mutants and finds application in the follow-up of HBV-infected patients.

For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood. Chronic hepatitis B in some patients is treated with drugs called *interferon* or *lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programs."

Hepatitis B surface Antigen or HBsAg is the most important protein of the envelope of Hepatitis B Virus, responsible for acute and chronic viral hepatitis.

The surface antigen contains the determinant "a", common to all the known viral subtypes, immunologically distinguished by two distinct subgroups (ay and ad).

The ability to detect HBsAg with high sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

C. PRINCIPLE OF THE TEST

A mix of mouse monoclonal antibodies specific to the determinants "a", "d" and "y" of HBsAg is fixed to the surface of microwells. Patient's serum/plasma is added to the microwell together with a second mix of mouse monoclonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant "a" and against "preS".

The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.

At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.

The chromogen/substrate is then added and, in the presence of captured HBsAg immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.

The color intensity is proportional to the amount of HBsAg present in the sample.

The version ULTRA is particularly suitable for automated screenings and is able to detect "s" mutants.

D. COMPONENTS

The standard configuration contains reagents to perform 192 tests and is made of the following components:

1. Microplate **MICROPLATE**

n° 2. 12 strips of 8 breakable wells coated with anti HBsAg, affinity purified mouse monoclonal antibodies, specific to "a", "y" and "d" determinants, and sealed into a bag with desiccant.

2. Negative Control **CONTROL -**

1x4.0ml/vial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The negative control is pale yellow color coded.

3. Positive Control **CONTROL +**

1x4.0ml/vial. Ready to use control. It contains goat serum, non infectious recombinant HBsAg, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The positive control is color coded green.

4. Calibrator **CAL ...**

n° 2 vials. Lyophilized calibrator. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, non infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO international standard for HBsAg, NIBSC code 00/588), 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives.

Note: 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 .

5. Wash buffer concentrate **WASHBUF 20X**

2x60ml/bottle. 20X concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

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6. Enzyme Conjugate Diluent CONJ DIL

2x16ml/vial. Ready to use and pink/red color coded reagent. It contains 10 mM Tris buffer pH 6.8+/-0.1, 1% normal mouse serum, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The solution is normally opalescent.

7. Enzyme Conjugate CONJ 20X

2x1ml/vial. 20X concentrated reagent. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HBsAg, determinant "a" and "preS", 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

8. Chromogen/Substrate SUBS TMB

2x25ml/bottle. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid H₂SO₄ 0.3 M

1x25ml/bottle. It contains 0.3 M H₂SO₄ solution.

Note: Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils n° 4

11. Package insert

Important note:

Only upon specific request, Dia.Pro can supply reagents for 96, 480, 960 tests, as reported below:

Microplates	N°1	N°5	N°10
Negative Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Positive Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Calibrator	N° 1 vial	N° 5 vials	N° 10 vials
Wash buffer concentrate	1x60ml/vial	5x60ml/vial	4x150ml/vial
Enzyme conjugate	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Conjugate Diluent	1x16ml/vial	2x40ml/vial	2x80ml/vial
Chromogen/Substrate	1x25ml/vial	3x42ml/vial	2x125ml/vial
Sulphuric Acid	1x15ml/vial	2x40ml/vial	2x80ml/vial
Plate sealing foils	N° 2	N° 10	N° 20
Package insert	N° 1	N° 1	N° 1
Number of tests	96	480	960
Code SAG1ULTRA.CE	96	480	960

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled 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 (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +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 (TMB) 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. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
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 Stop Solution is an 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.

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G. SPECIMEN: PREPARATION AND WARNINGS

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 lipemic ("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 well as they could give rise to false positive results. Specimens with an altered pathway of coagulation, presenting particles after blood collection and preparation of serum/plasma as those coming from hemodialized patients, could give origin to false positive 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 sample should not be frozen/thawed more than once as this may generate particles that could affect the test result. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8µ filter to clean it up for testing or use the two-steps alternative method.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months.

1. Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation. In this case, call Dia.Pro's customer service. Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Positive Control:

Ready to use. Mix well on vortex before use. The positive control does not contain any infective HBV as it is composed of recombinant synthetic HBsAg.

4. Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex. The solution is not stable. Store the Calibrator frozen in aliquots at -20°C.

5. 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.

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

6. Enzyme conjugate:

The working solution is prepared by diluting the 20X concentrated reagent into the Conjugate Mix well on vortex before use.

Avoid any contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic sterile disposable containers.

Important note: The working solution is not stable. Prepare only the volume necessary for the work of the day. As an example when the kit is used in combination with other instruments or manually, dilute 0.1 ml 20X Conjugate with 1.9 ml Conjugate Diluent into a disposable plastic vial and mix carefully before use.

7. Chromogen/Substrate:

Ready to use. Mix well by end-over-end mixing.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

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

8. Sulphuric Acid:

Ready to use. Mix well by end-over-end mixing.

Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

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.

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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The **ELISA incubator** has to be set at +37°C (tolerance of ±1°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. In case of **shaking** during incubations, the instrument has to ensure 350 rpm ±150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. 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/calibrator 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

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is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

5. **Incubation times** have a tolerance of $\pm 5\%$.
6. The **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 $\geq 1\%$. 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.
7. When using **ELISA automated workstations**, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) 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 by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
8. 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 $2..8^{\circ}\text{C}$, firmly capped.
9. **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 full compliance with the essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination 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. 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. Dilute the 20X concentrated Enzyme Conjugate with its Diluent as reported.
5. Dissolve the Calibrator as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at $+37^{\circ}\text{C}$ 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.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 ul controls & calibrator, then all the samples and finally 100 ul diluted Enzyme Conjugate.

For the pre-washing step (point 1 of the assay procedure) and all 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.

Manual Assay:

1. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.

Important note: *Pre washing (1 cycle: dispensation of 350ul/well of washing solution+ aspiration) is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit it !*

2. Leave the A1 well empty for blanking purposes.
3. Pipette 150ul of the Negative Control in triplicate, 150ul of the Calibrator in duplicate and then 150ul of the Positive Control in single followed by 150ul of each of the samples.
4. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).
5. Dispense 100ul diluted Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

6. Following addition of the conjugate, check that the color of the samples have changed from yellowish to pink/red and then incubate the microplate for **120 min at $+37^{\circ}\text{C}$** .

Important notes:

- a. *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*
- b. *If the procedure is carried out on shaking, be sure to deliver the rpm reported for in Section I.3 as otherwise intra-well contamination could occur.*
7. When the first incubation is over, wash the microwells as previously described (section I.4)

8. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included.

Important note: Do not expose to strong direct light as a high background might be generated.

9. Incubate the microplate protected from light at **18-24°C for 30 min**. Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
10. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow/brown.
11. Measure the color intensity of the solution in each well, as described in section I.6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

Important general notes:

- If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate false positive results on reading.
- Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.
- When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended as long as it is far less sensitive to interferences due to hemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles. The assay is carried out in two-steps at +37°C on shaking at 350 rpm ± 150 as follows:
 - dispense 100 µl of controls, calibrator and samples
 - incubate 60 min at +37°C on shaking
 - wash according to instructions (section I.4)
 - dispense 100 µl diluted enzyme tracer
 - incubate 30 min at +37°C on shaking
 - wash
 - dispense 100 µl TMB&H₂O₂ mix
 - incubate 30 min at r.t. on shaking
 - stop and read

In this procedure the pre-wash can be omitted.

This method shows performances similar to the standard one and therefore can be used in alternative.

4. 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

Operations	Procedure
Pre-Washing step	n° 1 cycle
Controls&Calibrator&samples	150 µl
Diluted Enzyme Conjugate	100 µl
1st incubation	120 min
Temperature	+37°C
Washing steps	n° 4-5
Chromogen/Substrate	200µl
2nd incubation	30 min
Temperature	room
Sulphuric Acid	100 µl
Reading OD	450nm

An example of dispensation scheme is reported in the following section:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.

Ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator 0.5 IU/ml	S/Co $\geq$ 2
Positive Control	> 1.000 OD450nm value

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 perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.050 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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of calibrator) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.050). 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.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

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

$$NC + 0.050 = \text{Cut-Off (Co)}$$

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 performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

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

S/Co	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by HBV and that the blood unit may be transfused. Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused.

A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
- Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 µ filter to remove any microparticles interference. Then, if still

positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.

- When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.
- Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor.

An example of calculation is reported below.

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

Negative Control: 0.012 – 0.008 – 0.010 OD450nm
Mean Value: 0.010 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.489 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.010+0.050 = 0.060
Calibrator: 0.350 - 0.370 OD450nm
Mean value: 0.360 OD450nm S/Co = 6.0
S/Co higher than 2.0 – Accepted
Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS

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). Version ULTRA proved to be at least equivalent to the original design in a study conducted for the validation of the new version.

1. Analytical Sensitivity

The limit of detection of the assay has been calculated on the 2nd WHO international standard, NIBSC code 00/588.

In the following table, results are given for three lots (P1, P2 and P3) of the version ULTRA in comparison with the reference device (Ref.):

WHO IU/ml	Lot # P1 S/Co	Lot # P2 S/Co	Lot # P3 S/Co	Ref. S/Co
0.4	4.6	4.8	4.6	4.6
0.2	2.3	2.4	2.4	2.4
0.1	1.4	1.4	1.5	1.2
0.05	0.8	0.8	1.0	0.7
0.025	0.6	0.6	0.6	0.4
FCS (NC)	0.3	0.2	0.3	0.1

The assay shows an Analytical Sensitivity better than 0.1 WHO IU/ml of HBsAg.

In addition two panels of sensitivity supplied by EFS, France, and by SFTS, France, were tested and gave in the best conditions the following results:

Panel EFS Ag HBs HB1-HB6 lot n° 04

Sample ID	Characteristics	ng/ml	S/Co
HB1	diluent	/	0.2
HB2	adw2+ayw3	0.05	0.6
HB3	adw2+ayw3	0.1	1.0
HB4	adw2+ayw3	0.2	1.8
HB5	adw2+ayw3	0.3	2.4
HB6	adw2+ayw3	0.5	4.2

Sensitivity panel SFTS, France, Ag HBs 2005

Sample ID	Characteristics	ng/ml	S/Co
171	Adw2 + ayw3	2.21 ± 0.15	15,4
172	Adw2 + ayw3	1.18 ± 0.10	8,7
173	Adw2 + ayw3	1.02 ± 0.05	6,1
174	Adw2 + ayw3	0.64 ± 0.04	4,0
175	Adw2 + ayw3	0.49 ± 0.03	3,4
176	Adw2 + ayw3	0.39 ± 0.02	2,6
177	Adw2 + ayw3	0.25 ± 0.02	2,0
178	Adw2 + ayw3	0.11 ± 0.02	1,3
179	Adw2 + ayw3	0.06 ± 0.01	0,9
180	Adw2 + ayw3	0.03 ± 0.01	0,8
181	Adw2	0.5 – 1.0	4,7
182	Adw4	0.5 – 1.0	3,6
183	Adr	0.5 – 1.0	4,5
184	Ayw1	0.5 – 1.0	5,1
185	Ayw2	0.5 – 1.0	6,4
186	Ayw3	0.5 – 1.0	7,3
187	Ayw3	0.5 – 1.0	5,8
188	Ayw4	0.5 – 1.0	6,9
189	Ayr	0.5 – 1.0	6,1
190	diluent	/	0,6

The panel # 808, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.
Results in the best conditions are as follows :

BBi panel PHA 808

Sample ID	Characteristics	ng/ml	S/Co
01	ad	2,49	10,2
02	ad	1,17	4,8
03	ad	1,02	4,3
04	ad	0,96	3,8
05	ad	0,69	2,9
06	ad	0,50	2,2
07	ad	0,41	1,5
08	ad	0,37	1,3
09	ad	0,30	1,2
10	ad	0,23	1,0
11	ay	2,51	11,2
12	ay	1,26	5,9
13	ay	0,97	4,1
14	ay	0,77	3,7
15	ay	0,63	2,0
16	ay	0,48	2,4
17	ay	0,42	2,0
18	ay	0,33	1,8
19	ay	0,23	1,6
20	ay	0,13	1,1
21	negative	/	0,6

2. Diagnostic Sensitivity:

The diagnostic sensitivity was tested according to what required by Common Technical Specifications (CTS) of the directive 98/79/EC on IVD for HBsAg testing.

Positive samples, including HBsAg subtypes and a panel of “s” mutants from most frequent mutations, were collected from different HBV pathologies (acute, a-symptomatic and chronic hepatitis B) or produced synthetically, and were detected positive in the assay.

All the HBsAg known subtypes, “ay” and “ad”, and isoforms “w” and “r”, supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.

An overall value of 100% has been found in a study conducted on a total number of more than 400 samples positive with the original reference IVD code SAG1.CE, CE marked.

A total of 30 sero-conversions were studied, most of them produced by Boston Biomedica Inc., USA.

Results obtained by examining eight panels supplied by Boston Biomedica Inc., USA, are reported below for the version ULTRA in comparison with the reference device code SAG1.CE.

Panel ID	1 st sample positive	HBsAg subtype	HBsAg ng/ml	Version ULTRA S/Co	Ref. device S/Co
PHM 906	02	ad	0.5	3.7	1.4
PHM 907 (M)	06	ay	1.0	4.4	2.9
PHM 909	04	ad	0.3	1.2	0.8
PHM 914	04	ad	0.5	1.1	1.1
PHM 918	02	ad	0.1	1.8	0.5
PHM 923	03	ay	< 0.2	2.2	1.2
PHM 925	03	Ind.	n.d.	1.4	0.9
PHM 934	01	ad	n.d.	1.0	0.8

3. Diagnostic Specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where more than 5000 negative samples from blood donors (two blood centers), classified negative with a CE marked device in use at the laboratory of collection were examined, the diagnostic specificity was recently assessed by testing a total of 2288 negative blood donors on seven different lots. A value of specificity of 100% was found.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

Samples derived from patients with different viral (HCV, HAV) and non viral pathologies of the liver that may interfere with the test were examined. No cross reaction were observed.

4. Precision:

It has been calculated for the version ULTRA on two samples examined in 16 replicates in 3 different runs for three lots.

Results are reported in the following tables:

Average values	Negative Sample	Calibrator 0.5 IU/ml
Total n = 144		
OD450nm	0.026	0.332
Std.Deviation	0.004	0.027
CV %	16%	8%

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

Repeatable false positive results were assessed on freshly collected specimens in less than 0.1% of the normal population, mostly due to high titers Heterophilic Anti Mouse Antibodies (HAMA).

Interferences in fresh samples were also observed when they were not particles-free or were badly collected (see chapter G).

Old or frozen samples, presenting fibrin clots, cryoglobulins, lipid-containing micelles or microparticles after storage or thawing, can generate false positive results.

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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.

Manufacturer:
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CE
0318

HBsAb

**Enzyme Immunoassay for
qualitative/quantitative determination of
antibodies to Hepatitis B surface Antigen
in human serum and plasma**

- for “in vitro” diagnostic use only -



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HBs Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for both the quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or

cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon* or *lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV). This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.

Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.

Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.

The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.

After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.

The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.

The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.

Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin and sealed into a bag with desiccant.

Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: **CAL N° ...**

5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml. Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. Standards are blue coloured.

3. Wash buffer concentrate: **WASHBUF 20X**

1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : **CONJ**

1x16.0 ml/vial. Ready-to-use solution and red color coded. It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

5. Chromogen/Substrate: **SUBS TMB**

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂). **Note: To be stored protected from light as sensitive to strong illumination.**

6. Sulphuric Acid: **H₂SO₄ 0.3 M**

1x15ml/vial. Contains 0.3 M H₂SO₄ solution. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: **DILSPE**

1x8ml. 10 mM Tris Buffered solution pH 7.4 +/-0.1, suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide as preservatives.

8. Control Serum: **CONTROL ...ml**

1 vial. Lyophilized. Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled 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 (dry or wet), set at +37°C (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) 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. 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.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at 2.8°C into a temperature controlled refrigerator or cold room.

6. 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.

7. 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.

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.

9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.

10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.

11. 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.

12. 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.

13. 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..

14. 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.

15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water

16. 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 WARNINGS

1. Blood is drawn aseptically by venipuncture 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. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.

3. 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.

4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be freeze/thawed more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 µl of each specimen with 450 µl of Cal 0 (1:10). Then 50 µl of the 1:10 dilution are diluted with 450 µl of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

1. Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.

In this case, call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Calibration Curve

Ready to use. Mix well on vortex before use.

3. Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.

Note: The control after dissolution is not stable. Store frozen in aliquots at -20°C.

4. Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

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

5. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

6. Specimen Diluent:

Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.

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

8. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

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.

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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.

2. The ELISA incubator has to be set at +37°C (tolerance of ±1°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/calibrator 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/calibrator 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 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; 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 workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the

values reported in the sections "Validation of Test" and "Assay Performances". 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 samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

7. 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 full 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 Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. 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.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

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.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed. Then Dispense in all the wells to be used for the test, except for A1 and B1, 50µl of the Specimen Diluent.

Important note: This additive is added before distributing samples and controls into specific wells and is particularly

intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.

2. Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100ul of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

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

3. Wash the microplate as reported in section I.3.
4. In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes**.

Important note:

- 1) Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.
- 2) Mix thoroughly the Enzyme Conjugate on vortex before use.

5. Wash the microplate as described.
6. Pipette 100µl TMB/H₂O₂ mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dispense 50 ul Specimen Diluent in all the wells, except for the blank A1. Then pipette 100µl of the Calibrator 0 mIU/ml in duplicate, 100µl of the Calibrator 10 mIU/ml in duplicate, 100µl of the Calibrator 250 mIU/ml in single, and then 100ul of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes**.

Important note:

- 3) Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.
- 4) Mix thoroughly the Enzyme Conjugate on vortex before use.

5. Wash the microplate as described.
6. Pipette 100µl TMB/H₂O₂ mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells.

Important general 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 should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.
3. The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.

N. ASSAY SCHEME (standard procedure)

Specimen Diluent	50 ul
Calibrators	100 ul
Control Serum	100 ul
Samples	100 ul
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme Conjugate	100 ul
2nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂ mix	100 ul
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm & 620nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS	S7									
F	CAL2	CS	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3	S11									
B	CAL1	S4	S12									
C	CAL1	S5	S13									
D	CAL2	S6	S14									
E	CAL2	S7	S15									
F	CAL5	S8	S16									
G	S1	S9	S17									
H	S2	S10	S18									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 WHO mIU/ml	< 0.200 OD450nm after blanking
Calibrator 10 WHO mIU/ml	OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0.100
Calibrator 250 WHO mIU/ml	> 1.500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mIU/ml ± 10%
Coefficient of variation	< 30% for the Calibrator 0 mIU/ml

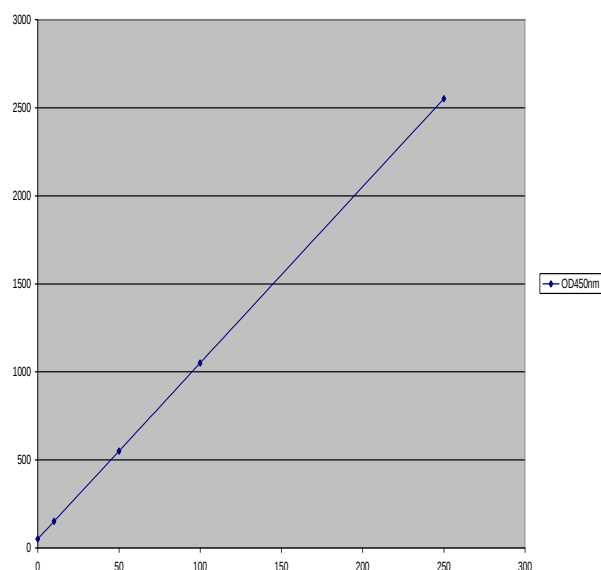
If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 mIU/ml > 0.200	1. that the washing procedure and the washer settings are as validated in the pre qualification study;
coefficient of variation > 30%	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 when the dispensation of standards is carried out;
	4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate;
	5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate
	6. that the washer needles are not blocked or partially obstructed.

Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Calibrator 250 mIU/ml < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: a) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. b) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

Example of Calibration Curve :



Important Note:

Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation:

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

Calibrator 0 mIU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted

Calibrator 10 mIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 250 mIU/ml: 2.845 OD450nm
Higher than 1.500 – Accepted

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti HBsAg antibody in samples.

An example of Calibration curve is reported in the next page.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 WHO mIU/ml are considered negative for anti HBsAg antibody by most of the international medical literature.

Samples with a concentration higher than 10 WHO mIU/ml are considered positive for anti HBsAg antibody.

In the follow up of vaccination recipients, however, the value of 20 WHO mIU/ml is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

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).

1. LIMIT OF DETECTION:

The limit of detection of the assay has been calculated by means of the HBsAb international preparation supplied by CLB on behalf of WHO (1st reference preparation 1977, lot 17-2-77), on which Calibration Curve has been calibrated. HBV negative serum was used as diluent, as recommended by the supplier. Results of Quality Control are given in the following table:

WHO mIU/ml	SAB.CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

A Performance Evaluation has been conducted on a total number of more than 700 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.

More than 500 negative specimens were tested, internally and externally, against a European company.

A diagnostic specificity of 98.8% was assessed. .

Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.

Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.

More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

The mean values obtained from a study conducted on three samples of different anti-HBsAg reactivity, examined in 16 replicates in three separate runs is reported below:

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.038	0.038	0.039	0.039
Std.Deviation	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.250	0.243	0.244	0.246
Std.Deviation	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.998	3.000	3.259	3.085
Std.Deviation	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.048	0.048	0.050	0.049
Std.Deviation	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.249	0.252	0.242	0.248
Std.Deviation	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	3.544	3.653	3.612	3.603
Std.Deviation	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.050	0.051	0.050	0.050
Std.Deviation	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.226	0.238	0.239	0.234
Std.Deviation	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	3.526	3.457	3.499	3.494
Std.Deviation	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

The variability shown in the tables did not result in sample misclassification.

4. ACCURACY

The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 10.000 mIU/ml.

5. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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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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HDV Ab

**Competitive Enzyme Immunoassay
for the qualitative determination of
antibodies to Hepatitis Delta Virus
in human serum and plasma**

- for “in vitro” diagnostic use only -



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HDV Ab

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with a "two-steps" methodology.

The kit is used for the follow-up of patients infected by HDV.

For "in vitro" diagnostic use only.

B. INTRODUCTION

The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication.

Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation.

The determination of HDV specific serological markers (HDV Ag, HDV Ab, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment. The detection of HDV total antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST

Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with peroxidase (HRP), for a fixed amount of rec-HDV coated on the microplate. The test is carried out with a two steps incubation competitive system. First the sample is added to the plate and specific anti HDV antibodies bind to the adsorbed antigen. After washing, an enzyme conjugated antibody to HDV is added and binds to the free portion of the antigen coated. After washing a chromogen/substrate mixture is dispensed. The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by the added chromogen/substrate. The concentration of HDV-specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti-HDV antibodies.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

8x12 microwell strips coated with recombinant HDV-specific antigen and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use. Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The negative control is colour coded pale yellow.

3. Positive Control: CONTROL +

1x2.0ml/vial. Ready to use. Contains goat serum proteins, high titer anti HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The positive control is colour coded green.

4. Calibrator: CAL

n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low titer human antibodies to HDV, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Note: 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.

5. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme conjugate: CONJ

1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The component is colour coded red.

7. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H₂O₂.

Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. Contains 0.3 M H₂SO₄ solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Plate sealers n° 2

Instructions for Use n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionized, 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 (dry or wet) set at +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. 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.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H₂O₂) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at +2..8°C into a temperature controlled refrigerator or cold room.
6. 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.
7. 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.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. 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.
12. The use of disposable plastic labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. 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. 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..
14. Accidental spills 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.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
16. 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.
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 in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at -20°C for at least 12 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, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Antigen coated microwells:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing. In this case, call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Positive Control:

Ready to use. Mix well on vortex before use.

4. Calibrator:

Low positive control. Add precisely the volume of EIA grade water, reported on its label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C. When thawed do not freeze again; discard it.

5. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

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

6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

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

8. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

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.

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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of +2%.
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 in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution. The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
5 washing cycles (aspiration + dispensation of 350µl/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
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 of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to 4; (c) linearity to 4; 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, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". 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 samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

7. 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 full 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 (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). 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 as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. 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 reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. 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. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at +2..8°C, sealed.
2. Pipette 100 µl of Negative Control in triplicate, 100 µl Positive Control in single and then 100 µl of samples. Check that controls and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min.**
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Then incubate the microplate at **+37°C for 60 min.**

Important note: Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H₂O₂ mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 min**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step n° 6 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.

8. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
2. Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.
3. The use of the Calibrator, a low positive control, is not mandatory for the assay as the CAL does not enter into the cut-off calculation. The CAL may be used as a low titer positive control when a laboratory internal quality verification is required by the management. When used for such purpose, dispense 100 µl of it, possibly in duplicate.

N. ASSAY SCHEME

Controls/Calibrator	100 µl
Samples	100 µl
1st incubation	60 min
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂ mix	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm / 620-630nm

An example of dispensation scheme (including CAL) is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the negative and positive controls any time, and on the Calibrator in addition when the kit is used for the first time, in order to verify whether the expected OD450nm / 620-630nm or Co/S values have been matched in the analysis. Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking If lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation < 30%
Positive Control (PC)	OD450 nm < NC/10
Calibrator (CAL)	PC ≤ OD450nm < (NC+PC)/5

If the results of the test match the requirements stated above, proceed to the next section.

If they don't, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 30%	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 the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm Outside the range	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of Calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 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.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:

The analysis must be done proceeding as the reading step described in the section M, point 8.

P. RESULTS

The results are calculated by means of a cut-off value determined with the following formula:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 5$$

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

Results are interpreted as ratio between the cut-off value and the sample OD450nm / 620-630nm or Co/S. Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

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

Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.

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

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 8).

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

Negative Control: 2.100 – 2.200 – 2.000 OD450nm

Mean Value: 2.100 OD450nm

Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm

Lower than NC/10 – Accepted

$$\text{Cut-Off} = (2.100 + 0.100) / 5 = 0.440$$

Calibrator: 0.300-0.260 OD450nm

Mean value: 0.280 OD450nm

Within the range $\text{PC} \leq \text{OD450nm} < (\text{NC} + \text{PC})/5$ – Accepted

Sample 1: 0.020 OD450nm

Sample 2: 1.900 OD450nm

Sample 1 Co/S > 1.1 positive

Sample 2 Co/S < 0.9 negative

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)

1. LIMIT OF DETECTION:

In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA . The table below reports the OD450nm shown by this preparation when diluted in Fetal Calf Serum to prepare a limiting dilution curve, in three different lots.

Co/S values

	DAB.CE	Lot #	DAB.CE	Lot #	DAB.CE	Lot #
	OD450 nm	Co/S value	OD450 nm	Co/S value	OD450 nm	Co/S value
Accurun # 127						
1x	0.171	3.0	0.163	2.9	0.156	2.8
2x	0.187	2.7	0.176	2.6	0.179	2.5
4x	0.230	2.2	0.220	2.1	0.202	2.2
8x	0.298	1.7	0.285	1.6	0.271	1.6
16x	0.417	1.2	0.405	1.1	0.402	1.1
32x	0.514	1.0	0.490	0.9	0.482	0.9
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	////////	2.261	////////	2.114	////////

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The diagnostic performances were evaluated in a clinical trial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference kit.

Negative, positive and potentially interfering samples were examined in the trial.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Results are briefly reported in the tables below:

Sensitivity	> 98 %
Specificity	> 98 %

3. PRECISION

The mean values obtained from a study conducted on two samples of different anti-HDV antibody reactivity, examined in 16 replicates in three separate runs for three lots of product, is reported below:

DAB.CE: lot #1102

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.342	2.428	2.433	2.401
Std.Deviation	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.298	0.289	0.286	0.291
Std.Deviation	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/S	1.6	1.7	1.7	1.7

DAB.CE: lot #0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.208	2.237	2.246	2.230
Std.Deviation	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.269	0.277	0.266	0.271
Std.Deviation	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/ S	1.7	1.7	1.7	1.7

DAB.CE: lot # 0403

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.246	2.221	2.182	2.216
Std.Deviation	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.286	0.273	0.280	0.280
Std.Deviation	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/S	1.6	1.7	1.6	1.6

The variability shown in the tables did not result in sample misclassification.

Important note:

The performance data have been obtained proceeding as the reading step described in the section M, point 8.

S. LIMITATIONS

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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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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HDV Ab

**Ensayo inmunoenzimático competitivo
para la determinación cualitativa de
anticuerpos frente al Virus de la
Hepatitis Delta
en plasma y suero humanos**

Uso exclusivo para diagnóstico "in vitro"



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REF DAB.CE
96 pruebas

HDV Ab

A. OBJETIVO DEL EQUIPO.

Ensayo inmunoenzimático competitivo (ELISA) para la determinación cualitativa de anticuerpos frente al Virus de la Hepatitis Delta (HDV) en plasma y suero humanos con una metodología de "dos pasos".

El equipo ha sido desarrollado para el seguimiento de pacientes infectados con HDV.

Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

El Virus de la Hepatitis Delta es un virus ARN defectivo. Se compone de un núcleo con los antígenos delta específicos, y está encapsulado por el HBsAg. Para su replicación necesita ayuda funcional de HBV.

La infección por HDV ocurre en presencia de una infección aguda o crónica por HBV. Cuando se presenta simultáneamente la infección aguda por los dos virus, la enfermedad es grave y el cuadro clínico, así como las características bioquímicas son prácticamente indistinguibles de una infección por HBV. Sin embargo, una persona infectada por HBV de forma crónica puede soportar indefinidamente la replicación por HDV, normalmente la enfermedad es menos severa y aparece como exacerbadión clínica.

La determinación de los marcadores serológicos específicos de HDV (HDV Ag, HDV Ab, HDV IgM y HDV IgG) representa una herramienta importante para los clínicos en la clasificación del agente etiológico, en el seguimiento de los pacientes así como en el tratamiento.

La detección de anticuerpos totales permite la clasificación de la enfermedad y el seguimiento de la seroconversión.

C. PRINCIPIOS DEL ENSAYO.

El ensayo es de tipo competitivo, donde los anticuerpos anti-HDV de la muestra compiten con un anticuerpo policlonal (IgG) específico para el virus y conjugado con peroxidasa (HRP), por el antígeno recombinante-HDV de la fase sólida.

El ensayo se realiza mediante un sistema de dos pasos con incubación competitiva. La muestra se añade a la placa y los anticuerpos específicos anti-HDV se combinan con el antígeno de la fase sólida. Después del lavado, se añade un anticuerpo conjugado con peroxidasa (HRP) que se une al antígeno libre en la placa. Previo lavado, se añade el substrato cromogénico.

La concentración de la enzima conjugada, unida a la fase sólida es inversamente proporcional a la cantidad de anticuerpos al HDV presentes en la muestra y su actividad se detecta por la adición del substrato cromogénico.

La concentración de anticuerpos específicos al HDV en la muestra se determina de manera semicuantitativa a través del cálculo de un valor de corte.

D. COMPONENTES.

Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE

12 tiras de 8 pocillos recubiertos con antígeno recombinante específico de HDV, en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 4°C.

2. Control Negativo: CONTROL -

1x2.0ml/vial. Listo para el uso. Contiene proteínas del suero de cabra, tampón Tris-HCl 100 mM pH 7.4 +/-0.1, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control negativo está codificado con el color amarillo pálido.

3. Control Positivo: CONTROL +

1x2.0ml/vial. Listo para el uso. Contiene proteínas del suero de cabra, alto título de anticuerpos anti-HDV, tampón Tris-HCl 100 mM pH 7.4 +/-0.1, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control positivo está codificado con el color verde.

4. Calibrador: CAL ...

n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero bovino fetal, bajo título de anticuerpos humanos al HDV, además de sulfato de gentamicina 0.02 mg/ml y ProClin 300 0.045% como conservantes.

Nota: El volumen necesario para disolver el contenido del frasco, varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Tampón de Lavado Concentrado: WASHBUF 20X

1x60ml/botella. Solución concentrada 20x.

Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%

6. Conjugado CONJ

1x16ml/vial. Solución lista para el uso. Contiene 5% de albúmina de suero bovino, tampón Tris 10mM a pH 6.8 +/- 0.1, anticuerpo anti-HDV conjugado con peroxidasa (HRP) en presencia de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservante. El conjugado está codificado con el color rojo.

7. Cromógeno/Substrato SUBS TMB

1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H₂O₂) 0.02% así como dimetilsulfóxido 4%.

Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

8. Ácido Sulfúrico: H₂SO₄ 0.3M

1x15ml/vial. Contiene solución de H₂SO₄ 0.3M

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Sellador adhesivo, n° 2

Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (10-1000 µl) y puntas plásticas desechables.
2. Agua de calidad EIA (Bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. Timer con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37°C.
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal

debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.

3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del sustrato (TMB/H₂O₂) a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 °C, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben de ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar el uso de conservantes, en particular azida sódica, ya que pudiera afectar la actividad enzimática del conjugado.

3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para el pesquaje en unidades de sangre, se recomienda el uso del código de barras.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a -20°C durante al menos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.

1. Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8°C. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Control Negativo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Control Positivo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

4. Calibrador:

Control positivo bajo. Añadir de manera precisa al polvo liofilizado el volumen de agua de calidad EIA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.

Note: Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a -20°C. Cuando se descongele, descartar el agua en lugar de congelarla nuevamente.

5. Solución de Lavado Concentrada:

Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada hasta 1200ml y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.

Nota: Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8°C.

6. Conjugado:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

7. Cromógeno/ Substrato:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Ácido Sulfúrico:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**

H315 – Provoca irritación cutánea.

H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**

P280 – Llevar guantes/prendas/gafas/máscara de protección.

P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.

P332 + P313 – En caso de irritación cutánea: Consultar a un médico.

P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.

P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.

P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

- Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
- La incubadora de ELISA debe ser ajustada a 37°C (+/- 0.5°C) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
- El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible

remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.

- Los tiempos de incubación deben tener un margen de $\pm 5\%$.
- El lector de microplaca ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630 nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a 4, c) Linealidad a 4, reproducibilidad $\geq 1\%$. El blanco se prueba en el pocillo indicado en la sección "Procedimiento del ensayo". El sistema óptico del lector debe ser calibrado periódicamente para garantizar que se mide la densidad óptica correcta. Periódicamente debe procederse al mantenimiento según las instrucciones del fabricante.
- En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones "Control interno de calidad" y "Procedimiento del ensayo". El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
- El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

- Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
- Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
- Diluir totalmente la solución de lavado 20x concentrada, como se ha descrito anteriormente.
- Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente usando un vórtex.
- Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
- Ajustar la incubadora de ELISA a 37°C y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
- Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
- En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
- Comprobar que las micropipetas estén fijadas en el volumen requerido.
- Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.

11. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

1. Poner el número necesario de tiras en el soporte plástico. Dejar el pocillo A1 vacío para el blanco. Almacenar las tiras restantes en la bolsa con el desecante a temperaturas entre 2 y 8°C.

2. Dispensar 100µl del Control Negativo, por triplicado, 100µl del Control Positivo una vez y, posteriormente, añadir 100µl de muestras. Comprobar que los controles y muestras se han añadido correctamente.

Después incubar la microplaca durante **60 minutos a +37°C**.

3. Lavar la microplaca según lo descrito previamente (sección I.3).

4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1; comprobar que los reactivos se han añadido correctamente. Incubar la microplaca durante **60 minutos a +37°C**.

Nota importante: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.

5. Lavar la microplaca según lo descrito previamente (sección I.3).

6. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1.

Incubar la microplaca protegida de la luz a **temperatura ambiente (18-24°C) durante 20 minutos**.

Nota importante: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.

7. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 6. La adición de la solución de parada cambia el color del Control Negativo y las muestras negativas de azul a amarillo.

8. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

- Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.
- La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.
- El uso del calibrador (CAL), un control negativo bajo, no es obligatorio para el ensayo ya que el calibrador (CAL) no afecta al cálculo del valor de corte. El calibrador (CAL) puede usarse como un control negativo bajo si la gestión requiere un control interno de calidad del laboratorio. Dispensar 100µl del calibrador (CAL), posiblemente por duplicado, cuando se utilice para este propósito.

N. ESQUEMA DEL ENSAYO.

Controles/Calibrador	100 µl
Muestras	100 µl
1ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ª incubación	20 min
Temperatura	t.a.*
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

t.a.* temperatura ambiente

A continuación se describe un ejemplo del esquema de dispensado (incluido el calibrador (CAL):

		Microplaca											
		1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M2											
B	CN	M3											
C	CN	M4											
D	CN	M5											
E	CAL	M6											
F	CAL	M7											
G	CP	M8											
H	M1	M9											

Leyenda: BL = Blanco CN = Control Negativo
CAL = Calibrador CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles negativo y positivo cada vez que se usa el equipo, y con el calibrador la primera vez que se usa el equipo, para verificar si los valores DO450nm o Co/M son los esperados.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	valor < 0.100 DO450nm
Control Negativo (CN)	> 1.000 DO450nm después de leer el blanco Si es menor, controle cuidadosamente el proceso de lavado y disminuya los ciclos o el tiempo entre los mismos. Coeficiente de variación < 30%
Control Positivo (CP)	DO450 nm < CN/10
Calibrador (CAL)	CP ≤ DO450nm < (CN+CP)/5

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, no siga adelante y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control	1. el proceso de lavado y los parámetros

Negativo (CN) < 1.000 DO450nm después de leer el blanco Coeficiente de variación > 20%	del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador DO450nm Fuera de rango	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Control Positivo DO450nm > CN/10	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, informe al responsable para tomar las medidas pertinentes.

Nota importante:

El análisis debe seguir el paso de lectura descrito en la sección M, punto 8.

P. RESULTADOS.

Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

$$\text{Valor de corte} = (\text{CN} + \text{CP}) / 5$$

Nota Importante: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.

Q. INTERPRETACIÓN DE LOS RESULTADOS.

La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm / 620-630nm de las muestras y el Valor de corte Co/M.

Los resultados se interpretan según la siguiente tabla:

Co/M	Interpretación
< 0.9	Negativo
0.9 - 1.1	Equívoco
> 1.1	Positivo

Un resultado negativo indica que el paciente no está infectado por HDV.

Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.

Un resultado positivo es indicativo de infección por HDV y por consiguiente el paciente debe ser tratado adecuadamente.

Notas importantes:

1. La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.
2. Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
3. El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.

A continuación se incluye un ejemplo de los cálculos (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 8).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 2.100 – 2.200 – 2.000 DO450nm

Valor medio: 2.100 DO450nm

Mayor de 1.000 – Válido

Control Positivo: 0.100 DO450nm

Menor de CN/10 – Válido

$$\text{Valor de corte} = (2.100 + 0.100) / 5 = 0.440$$

Calibrador: 0.300-0.260 DO450nm

Valor medio: 0.280 DO450nm

Dentro del rango $CP \leq DO450nm < (CN+CP)/5$ – Válido

Muestra 1: 0.020 DO450nm

Muestra 2: 1.900 DO450nm

Muestra 1 Co/M > 1.1 positiva

Muestra 2 Co/M < 0.9 negativa

R. FUNCIONAMIENTO.

La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. LÍMITE DE DETECCIÓN.

En ausencia de un estándar internacional, la sensibilidad del ensayo ha sido calculada por medio de un producto denominado Accurun n° 127 suministrado por Boston Biomedical Inc., Estados Unidos.

La siguiente tabla muestra los valores de DO450nm para esta preparación, diluido en suero bovino fetal (SFB), para construir la curva de dilución límite en tres lotes diferentes:

Valores Co/M						
	DAB.CE	Lote #	DAB.CE	Lote #	DAB.CE	Lote #
	DO450	Co/M	DO450	Co/M	DO450	Co/M
Accurun	nm	valor	nm	valor	nm	valor
# 127						
1x	0.171	3.0	0.163	2.9	0.156	2.8
2x	0.187	2.7	0.176	2.6	0.179	2.5
4x	0.230	2.2	0.220	2.1	0.202	2.2
8x	0.298	1.7	0.285	1.6	0.271	1.6
16x	0.417	1.2	0.405	1.1	0.402	1.1
32x	0.514	1.0	0.490	0.9	0.482	0.9
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	////////	2.261	////////	2.114	////////

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICA.

La evaluación del procedimiento diagnóstica se realizó mediante un ensayo con más de 400 muestras frente a un equipo de referencia. Este ensayo clínico fue conducido por el Prof. M. Rizzetto, Departamento de Gastro-Hepatología del hospital S. Giovanni Battista de Turín, Italia.

Se examinaron muestras negativas, positivas y otras que pudieran provocar interferencia.

Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

A continuación se muestran brevemente los resultados obtenidos:

Sensibilidad	> 98 %
Especificidad	> 98 %

3. PRECISIÓN.

Se realizó un estudio con 3 lotes y dos muestras de diferente reactividad anti-HDV, examinadas en 16 réplicas, en tres tandas separadas. Los valores medios obtenidos se reportan a continuación:

DAB.CE: lote #1102

Control Negativo (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	2.342	2.428	2.433	2.401
Desviación estándar	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrador (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	0.298	0.289	0.286	0.291
Desviación estándar	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/M	1.6	1.7	1.7	1.7

DAB.CE: lote #0103

Control Negativo (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	2.208	2.237	2.246	2.230
Desviación estándar	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrador (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	0.269	0.277	0.266	0.271
Desviación estándar	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/M	1.7	1.7	1.7	1.7

DAB.CE: lote # 0403

Control Negativo (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	2.246	2.221	2.182	2.216
Desviación estándar	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrador (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	0.286	0.273	0.280	0.280
Desviación estándar	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/M	1.6	1.7	1.6	1.6

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

Nota importante:

Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 8.

S. LIMITACIONES.

La contaminación bacteriana de las muestras o la inactivación por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito. Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.

El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideración la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:

Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Mi) – Italia



0318

HBcAb

**Competitive Enzyme Immunoassay for
the determination of antibodies
to Hepatitis B core Antigen
in human serum and plasma**

- for “in vitro” diagnostic use only -



DIA.PRO

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HBcAb

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis B core Antigen in human plasma and sera.

The kit is intended for the screening of blood units and the follow-up of HBV-infected patients.

For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child-to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic

infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon* or *lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of HBV.

HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregating the core particles; the antigen contains at least one immunological determinant.

Upon primary infection, anti HBcAg antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, slightly later than HBsAg, the viral surface antigen.

Anti HBcAg antibodies are produced usually at high titers and their presence is detectable even years after infection. Isolated HBcAb, in absence of other HBV markers, have been observed in infected blood units, suggesting the use of this test for screening HBV, in addition of HBsAg.

The determination of HBcAb has become important for the classification of the viral agent, together with the detection of the other markers of HBV infection, in sera and plasma.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with a monoclonal antibody for a fixed amount of antigen on the solid phase.

A purified recombinant HBcAg is coated to the microwells.

The patient's serum/plasma is added to the microwell together with an additive able to block interferences present in the sample.

In the second incubation after washing, a monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and specific for HBcAg is added and binds to the free rec-HBcAg coated on the plastic.

After incubation, microwells are washed to remove any unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product.

The color intensity is inversely proportional to the amount of antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate **MICROPLATE**

8x12 microwell strips coated with recombinant HBcAg and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control **CONTROL -**

1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The negative control is pale yellow color coded.

3. Positive Control **CONTROL +**

1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, anti HBcAg antibodies at a concentration of about 10 PEI U/ml, (calibrated on PEI HBc Reference Material 82), 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The positive control is green color coded.

4. Calibrator **CAL ...**

n°1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, human antibodies to HBcAg at a concentration of 2 PEI U/ml +/-10% (calibrated on PEI HBc Reference Material 82) and 0.1% Kathon GC as preservative.

Note: 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 .

5. Wash buffer concentrate **WASHBUF 20X**

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

6. Enzyme Conjugate **CONJ**

1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated mouse monoclonal antibody to HBcAg in presence of 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives. The component is red colour coded .

7. Chromogen/Substrate **SUBS TMB**

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.6 +/-0.1, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H₂O₂) and 4% dimethylsulphoxide

Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent **DILSPE**

4x3ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.1% Kathon GC for the pre-treatment of samples and controls in the plate, blocking interference.

Note: Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid **H₂SO₄ 0.3 M**

1x15ml/vial. Contains 0.3 M H₂SO₄ solution.

Attention: Irritant (Xi R36/38; S2/26/30)

10. Plate sealing foil n°2

11. Instruction manual n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled 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 (dry or wet) set at +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 (TMB) 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.
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 external (primary 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 washing solution or in transferring components into other containers of automated workstations, in order to avoid 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. 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 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 an 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.
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 five 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, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months.

1. Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storage. In this case, call Dia.Pro's customer service. Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°.8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Positive Control:

Ready to use. Mix well on vortex before use.

4. Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C.

5. Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

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

6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

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

8. Specimen Diluent

Ready to use solution. Mix gently on vortex before use. Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (Xi R36/38; S2/26/30)

Legenda: R 36/38 = Irritating to eyes and skin.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
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/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µl/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/calibrator 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 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; 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.

- When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". 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 samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
- 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 full 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

- Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
- Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
- Dilute all the content of the 20x concentrated Wash Solution as described above.
- Dissolve the Calibrator as described above and gently mix.
- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- 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.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- Check that all the other equipment is available and ready to use.
- In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

- Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
- Leave the A1 well empty for blanking purposes.
- Dispense 50 µl Specimen Diluent into all the control and sample wells.
- Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Calibrator in duplicate and then 50 µl of the Positive Control in single. Then dispense 50 µl of each of the samples.
- Incubate the microplate for **60 min at +37°C**.

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

- When the first incubation is finished, wash the microwells as previously described (section I.3)
- Pipette 100 µl Enzyme Conjugate in all the wells, except A1; incubate the microplate for **60 min at +37°C**.

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

- When the second incubation is finished, wash the microwells as previously described (section I.3)
- Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: Do not expose to strong direct light. as a high background might be generated.

- Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with negative control and negative samples will turn from clear to blue (competitive method).
- Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.
- Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

- 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.
- Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.

N. ASSAY SCHEME

Specimen Diluent	50 µl
Controls&calibrator and samples	50 µl
1st incubation	60 min
Temperature	+37°C
Wash	n° 4-5
Enzyme Conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash	n° 4-5
TMB/H2O2 mix	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or Co/S values have been matched in the analysis.

Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 20%
Calibrator (about 2 PEI U/ml)	Co/S > 1
Positive Control	< 0.200 OD450nm

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 perform the following checks:

Problem	Check
Blank well > 0.050 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 20%	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 the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator Co/S < 1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control > 0.200 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 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.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. RESULTS

The results are calculated by means of a cut-off value determined with the following formula:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 5$$

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

Results are interpreted as ratio between the cut-off value and the sample OD450nm or Co/S.

Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

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

Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample. The blood unit should not be transfused.

A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.

An example of calculation is reported below.

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

Negative Control: 2.000 – 2.200 – 2.000 OD450nm

Mean Value: 2.100 OD450nm

Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm

Lower than 0.200 – Accepted

$$\text{Cut-Off} = (2.100 + 0.100) / 5 = 0.440$$

Calibrator: 0.400-0.360 OD450nm

Mean value: 0.380 OD450nm

Co/S>1 – Accepted

Sample 1: 0.028 OD450nm

Sample 2: 1.890 OD450nm

Sample 1 Co/S > 1.1 positive

Sample 2 Co/S < 0.9 negative

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).

1. LIMIT OF DETECTION:

The sensitivity of the assay has been calculated by means of the reference preparation for HBcAb supplied by Paul Erlich Institute (PEI HBc Reference Material 82). The assay shows a sensitivity of about 1.25 PEI U/ml.

The table below reports the Co/S values shown by the PEI standard diluted as suggested by the manufacturer to prepare a limiting dilution curve in Fetal Calf Serum (FCS).

PEI U/ml	Lot 1001	Lot 0702	Lot 0702/2	Lot 1202
5	22.6	18.0	19.0	17.7
2.5	8.0	5.5	5.4	5.0
1.25	1.1	1.3	1.0	1.0
0.625	0.4	0.4	0.4	0.4

In addition Accurun 1 – series 3000 – supplied by Boston Biomedica Inc., USA, was tested to determine its Co/S value. Results are reported in the table below:

Accurun 1 – series 3000

Value	Lot 1001	Lot 0702	Lot 1202
Co/S	2.9	2.3	2.2

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 6000 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. A total of more 5000 unselected donors, including 1st time donors, were examined.

In a first study 2023 samples were tested against a US company as reference. A specificity of 99.5% was found. In a second study 1588 samples were examined against a European company. A specificity of 99.7% was found. In the last study 1565 samples were assayed against the same US company; a value of 99.8% was found.

In addition to the above population, 206 samples from hospitalized patients were tested against the European company. A value of 99.3% specificity was found.

Moreover, diagnostic specificity was assessed by testing 164 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolyzed, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.

Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

373 positive specimens were tested against the European company; a diagnostic sensitivity of 99.7 was found.

3. PRECISION

The mean values obtained from a study conducted on three lots and on two samples of different anti-HBcAg reactivity, examined in 16 replicates in three separate runs is reported below:

BCAB.CE: lot # 1202

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	1.943	1.939	1.924	1.935
Std.Deviation	0.081	0.078	0.103	0.087
CV %	4.2	4.0	5.3	4.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.143	0.147	0.148	0.146
Std.Deviation	0.014	0.017	0.018	0.016
CV %	9.8	11.4	12.1	11.1
Co/S	2.8	2.7	2.6	2.7

BCAB.CE: lot # 0702

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.163	2.110	2.106	2.126
Std.Deviation	0.105	0.088	0.139	0.111
CV %	4.9	4.2	6.6	5.2

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.182	0.193	0.195	0.190
Std.Deviation	0.018	0.023	0.019	0.020
CV %	10.0	12.0	9.9	10.6
Co/S	2.5	2.2	2.3	2.3

BCAB.CE: lot # 0702/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.278	2.098	2.130	2.169
Std.Deviation	0.135	0.126	0.159	0.140
CV %	5.9	6.0	7.5	6.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.193	0.190	0.199	0.194
Std.Deviation	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/S	2.4	2.2	2.2	2.3

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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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.

Produced by
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0318

HBe Ag&Ab

**Enzyme Immunoassay (ELISA) for the
determination of Hepatitis B Virus
"e" Antigen and Antibody
in human plasma and sera.**

- for "in vitro" diagnostic use only -



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HBe Ag&Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Hepatitis B "e" Antigen or HBeAg is known to be intimately associated with Hepatitis B Virus or HBV replication and the presence of infectious Dane particles in the blood.

Recently, it has been found that HBeAg is a product of proteolytic degradation of Hepatitis B core Antigen or HBcAg, occurring in hepatocytes, whose expression is under the control of the precore region of HBV genome.

If HBeAg is considered a specific marker of infectivity, the presence of anti HBeAg antibodies in blood is recognised to be a clinical sign of recovery from infection to convalescence.

The determination of these two analytes in samples from HBV patients has become important for the classification of the phase of illness and as a prognostic value in the follow up of infected patients.

C. PRINCIPLE OF THE TEST

HBeAg:

HBeAg, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mix of two specific anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HBeAg.

The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.

The presence of HBeAg in the sample is determined by means of a cut-off value that allows for the semiquantitative detection of the antigen.

HBeAb

Anti HBeAg antibodies, if present in the sample, compete with a recombinant HBeAg preparation for a fixed amount of an anti HBeAg antibody, coated on the microplate wells.

The competitive assay is carried out in two incubations, the first with the sample and rechBeAg, and the second with a tracer, composed of two anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP).

The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti HBeAg antibodies in the sample and its activity is detected by adding the chromogen/substrate in the third incubation.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti HBeAg antibodies.

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

12 strips of 8 breakable wells coated with anti HBeAg specific monoclonal antibody, postcoated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL

1x2.0ml/vial. Ready to use control. It contains bovine serum, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is colorless.

3. Antigen Positive Control: CONTROL + Ag

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, non infectious recombinant HBeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green color coded.

4. Antibody Positive Control: CONTROL + Ab

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, human anti HbeAg positive plasma at about 10 PEI U/ml, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The label is red colored.

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG ...ml

n° 1 vial. Lyophilized calibrator for HBeAg. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, non infectious recombinant HBeAg at 1 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important Note: 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. Antibody Calibrator: CALAB ...ml

n° 1 vial. Lyophilized calibrator for anti HBeAg antibody. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, positive plasma at 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The label is red colored.

Important Note: 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.

7. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

8. Enzyme conjugate: CONJ

1x16ml/vial. Ready to use conjugate. It contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

1x10ml/vial. Ready to use reagent. It contains recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0+/-0.1, 0.045% ProClin 300 and 0.09% sodium azide as preservatives.

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Ready-to-use component. It contains a 50 mM citrate-phosphate buffered solution at 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: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n°2

13. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled 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 (dry or wet) set at +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. 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.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. 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.
7. 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.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. 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.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. 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. 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..

14. Accidental spills 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.

15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water

16. 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; 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.
4. Haemolysed 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 in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at -20°C for at least 12 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, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing. In this case, call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Antigen Positive Control:

Ready to use. Mix well on vortex before use.

4. Antibody Positive Control:

Ready to use. Mix well on vortex before use.

5. Antigen Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C.

6. Antibody Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C.

7. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.

During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

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

8. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

9. HBe Antigen:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

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

11. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

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.

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. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm 2\%$.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm 0.5^\circ\text{C}$) 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 in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution. The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing. An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm 5\%$.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. 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 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 section "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 when the number of samples to be tested exceed 20-30 units per run.
7. 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 (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H₂O₂) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). 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 as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. 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 reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

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

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

A) HBe Antigen:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive Control in single.
4. Then dispense 100 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Incubate the microplate for **60 min at +37°C**.

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

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.

Important note: Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.

9. Check that the reagent has been dispensed properly and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: Do not expose to strong direct light as a high background might be generated.

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.

13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.

14. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

B) HBe Antibody:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody Positive Control in single.
4. Then dispense 50 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Dispense then 50 µl of HBe Antigen in all the wells, except for A1.
7. Incubate the microplate for **60 min at +37°C**.

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

8. When the first incubation is finished, wash the microwells as previously described (section I.3)
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

Important notes:

1. Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
2. Reading should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher 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

HBe antigen test

Controls and calibrator	100 ul
Samples	100 ul
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂ mix	100 ul
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator	50 ul
Samples	50 ul
Neutralising antigen	50 ul
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank // NC = Negative Control
PC = Positive Control // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

HBe Antigen

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2.0
Positive Control (PC)	> 1.500 OD450nm

HBe Antibody

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 10%
Antibody Calibrator	OD450nm < NC/1.5
Positive Control (PC)	OD450nm < NC/10

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, don't proceed any further and perform the following checks:

HBeAg

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	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 the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 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.

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	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 (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm > NC/1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control; 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.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:

The analysis must be done proceeding as the reading step described in the section M, point 14.

P. CALCULATION OF THE CUT-OFF

The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

$$NC + 0.100 = \text{Cut-Off (Co)}$$

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

HBeAb:

$$(NC + PC) / 3 = \text{Cut-Off (Co)}$$

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

Results are interpreted as follows:

HBeAg:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

HBeAb:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Note:

$S = \text{OD450nm}/620\text{-}630\text{nm}$ of the sample

Co = cut-off value

An example of calculation for HBeAg assay is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

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

Negative Control: 0.020 – 0.030 – 0.025 OD450nm

Mean Value: 0.025 OD450nm

Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm

Higher than 1.500 – Accepted

Cut-Off = $0.025 + 0.100 = 0.125$

Calibrator: 0.520 - 0.540 OD450nm

Mean value: 0.530 OD450nm

S/Co higher than 2.0 – Accepted S/Co = 4.2

Sample 1: 0.030 OD450nm

Sample 2: 1.800 OD450nm

Sample 1 S/Co < 0.9 = negative

Sample 2 S/Co > 1.1 = positive

An example of calculation for HBeAb is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

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

Negative Control: 2.100 – 2.200 – 2.000 OD450nm

Mean Value: 2.100 OD450nm

Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm

Lower than NC/10 – Accepted

Cut-Off = $(2.100 + 0.100) / 3 = 0.733$

Calibrator: 0.720-0.760 OD450nm

Mean value: 0.740 OD450nm

OD450nm < NC/1.5 – Accepted

Sample 1: 0.020 OD450nm

Sample 2: 1.900 OD450nm

Sample 1 Co/S > 1.1 positive

Sample 2 Co/S < 0.9 negative

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory director to reduce the risk of judgment errors and misinterpretations.
2. The Identification of the clinical status of a HBV patient (acute, chronic, asymptomatic hepatitis) has to be done on the basis also of the other markers of HBV infection (HBsAg, HBsAb, HBcAb, HBcIgM);
3. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
4. Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAg
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 51, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

BBI's Accurun 51 (S/Co)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	4.1	1.6	0.9	0.6	0.4
0103/2	4.1	1.7	0.9	0.6	0.4
0303	4.0	1.6	0.9	0.5	0.4

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.

Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE S/Co	Abbott EIA S/Co	Sorin EIA S/Co
21	5.4	4.5	6.3
22	3.7	4.3	5.4
23	1.9	3.2	3.1
24	1.1	2.4	1.5
25	1.0	2.1	1.2
26	0.6	1.7	0.7
27	0.2	0.8	0.3
28	0.2	0.6	0.2
29	0.2	0.4	0.2
30	0.2	0.3	0.2
31	0.1	0.3	0.2
32	0.1	0.3	0.2

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for an other commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2

11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved kit.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

Samples derived from patients with different viral (HCV and HAV) and non viral pathologies of the liver that may interfere with the test were examined.

No cross reaction were observed.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98%.

4. Precision

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
CV %	7.4	8.2	7.9	7.8

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.569	0.559	0.575	0.568
Std.Deviation	0.027	0.029	0.028	0.028
CV %	4.7	5.3	4.9	4.9
S/Co	4.4	4.4	4.4	4.4

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.033	0.031	0.030	0.032
Std.Deviation	0.003	0.003	0.002	0.003
CV %	7.9	8.5	7.4	8.0

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.565	0.573	0.568	0.569
Std.Deviation	0.026	0.025	0.024	0.025
CV %	4.7	4.3	4.2	4.4
S/Co	4.2	4.4	4.4	4.3

HBE.CE: lot # 0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0.003
CV %	9.7	9.8	9.2	9.6

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.579	0.573	0.564	0.572
Std.Deviation	0.023	0.028	0.025	0.025
CV %	4.1	4.8	4.5	4.5
S/Co	4.5	4.3	4.1	4.3

B) HBe Antibody

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAb
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 52, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

Accurun 52 (Co/S)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	1.0	0.8	0.6	0.4	0.4
0103/2	1.0	0.8	0.6	0.5	0.4
0303	1.0	0.8	0.6	0.4	0.4

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit. Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE Co/S	Abbott EIA Co/S	Sorin EIA Co/S
21	0.4	0.4	0.5
22	0.4	0.5	0.6
23	0.4	0.6	0.5
24	0.4	0.5	0.6
25	0.4	0.6	0.5
26	0.5	0.6	0.6
27	0.6	0.8	0.7
28	0.7	0.9	0.7
29	0.6	0.9	0.7
30	0.8	1.0	0.9
31	1.0	1.3	1.1
32	1.0	1.2	1.0

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for another commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	-	0.3	0.5
2	-	0.2	0.5
3	-	0.2	0.5
4	-	0.2	0.5
5	-	0.3	0.6
6	-	0.3	0.6
7	-	0.2	0.4
8	-	0.2	0.4
9	-	0.2	0.5
10	-	1.9	0.6
11	-	0.3	0.5
12	-	0.4	0.9
13	2	4.4	9.1
14	1	3.8	2.9
15	< 1	1.0	1.5
16	> 50	4.3	120.9
17	< 1	1.0	1.0
18	5	5.6	21.8
19	1	2.7	6.4
20	11	5.0	47.3
21	2	1.9	10.0
22	26	28.1	90.7
23	-	0.3	0.5
24	< 1	0.8	1.3
25	50	28.1	167.4

3. Diagnostic specificity:

The clinical specificity has been determined as described before for HBeAg.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98%.

4. Precision:

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.484	2.420	2.471	2.458
Std.Deviation	0.129	0.160	0.142	0.144
CV %	5.2	6.6	5.7	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.867	0.800	0.878	0.848
Std.Deviation	0.043	0.060	0.050	0.051
CV %	5.0	7.5	5.7	6.1
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.316	2.361	2.413	2.363
Std.Deviation	0.127	0.144	0.146	0.139
CV %	5.5	6.1	6.0	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046
CV %	5.4	6.3	5.8	5.8
Co/S	1.0	1.0	1.0	1.0

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.

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.334	2.415	2.437	2.395
Std.Deviation	0.146	0.155	0.158	0.153
CV %	6.3	6.4	6.5	6.4

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE
0318

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.048	0.050
CV %	6.1	5.9	5.5	5.8
Co/S	0.9	1.0	1.0	1.0

Important note:

The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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HCV Ab

**Version 4.0 Enzyme Immunoassay
for the determination of
anti Hepatitis C Virus antibody
in human serum and plasma**

- for “in vitro” diagnostic use only -



DIA.PRO

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e-mail: info@diapro.it

REF CVAB.CE
96,192,480,960 Tests

HCV Ab

A. INTENDED USE

Version 4.0 Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis C Virus in human plasma and sera. The kit is intended for the screening of blood units and the follow-up of HCV-infected patients. For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) define Hepatitis C infection as follows:

"Hepatitis C is a viral infection of the liver which had been referred to as parenterally transmitted "non A, non B hepatitis" until identification of the causative agent in 1989. The discovery and characterization of the hepatitis C virus (HCV) led to the understanding of its primary role in post-transfusion hepatitis and its tendency to induce persistent infection.

HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily by direct contact with human blood. The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most persons in developing countries to afford. Thus, from a global perspective, the greatest impact on hepatitis C disease burden will likely be achieved by focusing efforts on reducing the risk of HCV transmission from nosocomial exposures (e.g. blood transfusions, unsafe injection practices) and high-risk behaviours (e.g. injection drug use).

Hepatitis C virus (HCV) is one of the viruses (A, B, C, D, and E), which together account for the vast majority of cases of viral hepatitis. It is an enveloped RNA virus in the *flaviviridae* family which appears to have a narrow host range. Humans and chimpanzees are the only known species susceptible to infection, with both species developing similar disease.

An important feature of the virus is the relative mutability of its genome, which in turn is probably related to the high propensity (80%) of inducing chronic infection. HCV is clustered into several distinct genotypes which may be important in determining the severity of the disease and the response to treatment.

The incubation period of HCV infection before the onset of clinical symptoms ranges from 15 to 150 days. In acute infections, the most common symptoms are fatigue and jaundice; however, the majority of cases (between 60% and 70%), even those that develop chronic infection, are a symptomatic. About 80% of newly infected patients progress to develop chronic infection. Cirrhosis develops in about 10% to 20% of persons with chronic infection, and liver cancer develops in 1% to 5% of persons with chronic infection over a period of 20 to 30 years. Most patients suffering from liver cancer who do not have hepatitis B virus infection have evidence of HCV infection. The mechanisms by which HCV infection leads to liver cancer are still unclear. Hepatitis C also exacerbates the severity of underlying liver disease when it coexists with other hepatic conditions. In particular, liver disease progresses more rapidly among persons with

alcoholic liver disease and HCV infection. HCV is spread primarily by direct contact with human blood. Transmission through blood transfusions that are not screened for HCV infection, through the reuse of inadequately sterilized needles, syringes or other medical equipment, or through needle-sharing among drug-users, is well documented. Sexual and perinatal transmission may also occur, although less frequently. Other modes of transmission such as social, cultural, and behavioural practices using percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing) can occur if inadequately sterilized equipment is used. HCV is not spread by sneezing, hugging, coughing, food or water, sharing eating utensils, or casual contact.

In both developed and developing countries, high risk groups include injecting drug users, recipients of unscreened blood, haemophiliacs, dialysis patients and persons with multiple sex partners who engage in unprotected sex. In developed countries, it is estimated that 90% of persons with chronic HCV infection are current and former injecting drug users and those with a history of transfusion of unscreened blood or blood products. In many developing countries, where unscreened blood and blood products are still being used, the major means of transmission are unsterilized injection equipment and unscreened blood transfusions. In addition, people who use traditional scarification and circumcision practices are at risk if they use or re-use unsterilized tools.

WHO estimates that about 170 million people, 3% of the world's population, are infected with HCV and are at risk of developing liver cirrhosis and/or liver cancer. The prevalence of HCV infection in some countries in Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific (when prevalence data are available) is high compared to some countries in North America and Europe.

Diagnostic tests for HCV are used to prevent infection through screening of donor blood and plasma, to establish the clinical diagnosis and to make better decisions regarding medical management of a patient. Diagnostic tests commercially available today are based on Enzyme immunoassay (EIA) for the detection of HCV specific antibodies. EIAs can detect more than 95% of chronically infected patients but can detect only 50% to 70% of acute infections. A recombinant immunoblot assay (RIBA) that identifies antibodies which react with individual HCV antigens is often used as a supplemental test for confirmation of a positive EIA result. Testing for HCV circulating by amplification tests RNA (e.g. polymerase chain reaction or PCR, branched DNA assay) is also being utilized for confirmation of serological results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the presence of active infection and a potential for spread of the infection and or/the development of chronic liver disease.

Antiviral drugs such as interferon taken alone or in combination with ribavirin, can be used for the treatment of persons with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with ribavirin is effective in about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone.

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development. Lack of knowledge of any protective immune response following HCV infection also impedes vaccine research. It is not known whether the immune system is able to eliminate the virus.

Some studies, however, have shown the presence of virus neutralizing antibodies in patients with HCV infection. In the absence of a vaccine, all precautions to prevent infection must be taken including (a) screening and testing of blood and organ donors; (b) Virus inactivation of plasma derived products; (c) implementation and maintenance of infection control practices in health care settings, including appropriate sterilization of medical and dental equipment; (d) promotion of behaviour change among the general public and health care workers to reduce overuse of injections and to use safe injection practices; and (e) Risk reduction counselling for persons with high-risk drug and sexual practices. “

The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing. The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world.

C. PRINCIPLE OF THE TEST

Microplates are coated with HCV-specific antigens derived from “core” and “ns” regions encoding for conservative and immunodominant antigenic determinants (Core peptide, recombinant NS3, NS4 and NS5 peptides).

The solid phase is first treated with the diluted sample and HCV Ab are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound HCV antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti hlgG&M antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HCV antibodies present in the sample. A cut-off value let optical densities be interpreted into HCV antibody negative and positive results.

D. COMPONENTS

Code CVAB.CE contains reagents for 192 tests.

1. Microplate **MICROPLATE**

n° 2 microplates

12 strips of 8 microwells coated with Core peptide, recombinant NS3, NS4 and NS5 peptides. Plates are sealed into a bag with desiccant.

2. Negative Control **CONTROL -**

1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is olive green colour coded.

3. Positive Control **CONTROL +**

1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, human antibodies positive to HCV, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The Positive Control is blue colour coded.

4. Calibrator **CAL ...**

n° 2 vials. Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label. It contains foetal bovine serum proteins, human antibodies to HCV whose content is calibrated on the NIBSC Working Standard code 99/588-003-WI, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Note: 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 .

5. Wash buffer concentrate **WASHBUF 20X**

2x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate **CONJ**

2x16ml/vial. Ready to use and pink/red colour coded reagent. It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate **SUBS TMB**

2x16ml/vial. Ready-to-use component. It contains 50 mM citrate-phosphate 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.

8. Assay Diluent **DILAS**

1x15ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference.

9. Sulphuric Acid **H₂SO₄ 0.3 M**

1x32ml/bottle. It contains 0.3 M H₂SO₄ solution. Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Sample Diluent: **DILSPE**

2x50ml/bottle. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.

Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.

11. Plate sealing foils n° 4

12. Package insert n° 1

Important note: Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below:

1. Microplate	n°1	n°5	n°10
2.NegativeControl	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
3.PositiveControl	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
4.Calibrator	n° 1 vial	n° 5 vials	n° 10 vials
5.Wash buff conc	1x60ml/bottle	5x60ml/bottles	4x150ml/bottles
6.Enz. Conjugate	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
7.Chromog/Subs	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
8.Assay Diluent	1x8ml/vial	1x40ml/bottle	1x80ml/bottle
9.Sulphuric Acid	1x15ml/vial	2x40ml/bottle	2x80ml/bottles
10.SampleDiluent	1x50ml/vial	5x50ml/bottles	4x125ml/bottles
11.Plate seal foils	n° 2	n° 10	n° 20
12. Pack. insert	n° 1	n° 1	n° 1
Number of tests	96	480	960
Code	CVAB.CE.96	CVAB.CE.480	CVAB.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. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
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 an 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 venipuncture 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 in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, 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, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

1. Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of manufacturing. In this case call Dia.Pro'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 till the indicator of humidity inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Positive Control:

Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

4. Calibrator:

Dissolve carefully the content of the lyophilised vial with the volume of EIA grade water reported on its label. Mix well on vortex before use.

Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

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

5. 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.

Note: Once diluted, the wash solution is stable for 1 week at $+2..8^{\circ}\text{C}$.

6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

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

If this component has to be transferred use only plastic, possibly sterile disposable containers.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex 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.

8. Assay Diluent:

Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

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.

10. Sample Diluent:

Ready to use. Mix well on vortex 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 $\pm 2\%$. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at $+37^{\circ}\text{C}$ (tolerance of $\pm 0.5^{\circ}\text{C}$) 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 in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution. The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested). 5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing. An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
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 of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq 10\text{ nm}$; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 section O "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 $2..8^{\circ}\text{C}$, 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 as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. 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 reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. 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.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 200 ul Sample Diluent and then 10 ul sample.

All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

Do not dilute controls/calibrator as they are ready to use.

Dispense 200 ul controls/calibrator in the appropriate control/calibration wells.

Important Note: Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the colour of dispensed samples has turned to dark bluish-green while the colour of the negative control has remained olive green.

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.

Manual assay:

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 ul of Negative Control in triplicate, 200 ul Calibrator in duplicate and 200 ul Positive Control in single in proper wells. Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 ul of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 ul sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: Check that the colour of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.

4. Dispense 50 ul Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.
5. Incubate the microplate for **45 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.

6. Wash the microplate with an automatic washer by delivering and aspirating 350ul/well of diluted washing solution as reported previously (section I.3).
7. Pipette 100ul Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this pink/red coloured component has been dispensed in all the wells, except A1.

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

8. Incubate the microplate for **45 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100ul Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

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

11. Pipette 100ul Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.
12. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. Ensure that no finger prints are present on the bottom of the microwell before reading. 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 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.
3. Shaking at 350 ±150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.
4. 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
Controls & Calibrator	200 ul
Samples	200ul dil.+10ul
Assay Diluent (DILAS)	50 ul
1st incubation	45 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 ul
2nd incubation	45 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂	100 ul
3rd incubation	15 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control 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)	< 0.050 mean OD450nm value after blanking
Calibrator	S/Co > 1.1
Positive Control	> 1.000 OD450nm value

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) > 0.050 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.
Calibrator S/Co < 1.1	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead of control serum) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.000 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 > 0.150, 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.

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):

$$NC + 0.350 = \text{Cut-Off (Co)}$$

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.

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
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HCV or that the blood unit may be transfused.

Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.

A positive result is indicative of HCV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

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. Any positive result should be confirmed by an alternative method capable to detect IgG and IgM antibodies (confirmation test) before a diagnosis of viral hepatitis is formulated.
3. As proved in the Performance Evaluation of the product, the assay is able to detect seroconversion to anti HCV core antibodies **earlier** than some other commercial kits. Therefore a positive result, not confirmed with these commercial kits, does not have to be ruled out as a false positive result ! The sample has to be anyway submitted to a confirmation test (supplied upon request by DiaPro srl, code CCONF).
4. As long as the assay is able to detect also IgM antibodies some discrepant results with other commercial products for the detection of anti HCV antibodies - lacking anti hIgM conjugate in the formulation of the enzyme tracer and therefore missing IgM reactivity - may be present. The real positivity of the sample for antibodies to HCV should be then confirmed by examining also IgM reactivity, important for the diagnosis of HCV infection.
5. When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.
6. Diagnosis of viral hepatitis 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 of real figures obtained by the user.

Negative Control: 0.019 – 0.020 – 0.021 OD450nm

Mean Value: 0.020 OD450nm

Lower than 0.050 – Accepted

Positive Control: 2.189 OD450nm

Higher than 1.000 – Accepted

Cut-Off = 0.020+0.350 = 0.370

Calibrator: 0.550 - 0.530 OD450nm

Mean value: 0.540 OD450nm S/Co = 1.4

S/Co higher than 1.1 – Accepted

Sample 1: 0.070 OD450nm

Sample 2: 1.690 OD450nm

Sample 1 S/Co < 0.9 = negative

Sample 2 S/Co > 1.1 = 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).

1. LIMIT OF DETECTION

The limit of detection of the assay has been calculated by means of the British Working Standard for anti-HCV, NIBSC code 99/588-003-WI. The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined.

Dilution	Lot # 1	Lot # 2
Factor	S/Co	S/Co
1 X	2.0	2.0
2 X	1.1	1.2
4 X	0.7	0.8
8 X	0.5	0.5
Negative plasma	0.3	0.3

In addition the sample coded Accurun 1 – series 3000 - supplied by Boston Biomedica Inc., USA, has been evaluated "in toto" showing the results below:

CVAB.CE Lot ID	Accurun 1 Series	S/Co
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

In addition, n° 7 samples, tested positive for HCV Ab with Ortho HCV 3.0 SAvE, code 930820, lot. # EXE065-1, were diluted in HCV Ab negative plasma in order to generate limiting dilutions and then tested again on CVAB.CE, lot. # 1202, and Ortho.

The following table reports the data obtained.

Sample n°	Limit Dilution	CVAB.CE S/Co	Ortho 3.0 S/Co
1	256 X	1.9	1.3
2	256 X	1.9	0.7
3	256 X	2.4	1.0
4	128 X	2.5	3.2
5	85 X	3.3	1.4
6	128 X	2.2	0.8
7	135 X	3.2	2.2

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 5000 samples.

2.1 Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5043 unselected blood donors, (including 1st time donors), 210 hospitalized patients and 162 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 examined, the diagnostic specificity was recently assessed by testing a total of 2876 negative blood donors on six different lots. A value of specificity of 100% was found.

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.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

The diagnostic sensitivity has been assessed externally on a total number of 359 specimens; a diagnostic sensitivity of 100% was found. Internally more than other 50 positive samples were tested, providing a value of diagnostic sensitivity of again 100%. Positive samples from infections carried out by different genotypes of HCV were tested as well.

Furthermore, most of seroconversion panels available from Boston Biomedica Inc., USA, (PHV) and Zeptometrix, USA, (HCV) have been studied.

Results are reported below for some of them.

Panel	N° samples	DiaPro*	Ortho* **
PHV 901	11	9	9
PHV 904	7	2	4
PHV 905	9	3	4
PHV 906	7	7	7
PHV 907	7	3	2
PHV 908	13	10	8
PHV 909	3	2	2
PHV 910	5	3	3
PHV 911	5	3	3
PHV 912	3	1	1
PHV 913	4	2	2
PHV 914	9	5	5
PHV 915	4	3	0
PHV 916	8	4	3
PHV 917	10	6	6
PHV 918	8	2	0
PHV 919	7	3	3
PHV 920	10	6	6
HCV 10039	5	2	0
HCV 6212	9	6	7
HCV 10165	9	5	4

Note: * Positive samples detected

** HCV v.3.0

Finally the Product has been tested on the panel EFS Ac HCV, lot n° 01/08.03.22C/01/A, supplied by the Etablissement Francais Du Sang (EFS), France, with the following results:

EFS Panel Ac HCV

Sample	Lot # 1 S/Co	Lot # 2 S/Co	Lot # 2 S/Co	Results expected
HCV 1	2.2	2.4	2.6	positive
HCV 2	1.6	2.0	2.1	positive
HCV 3	1.5	1.7	1.6	positive
HCV 4	5.2	6.5	5.5	positive
HCV 5	1.6	1.8	1.6	positive
HCV 6	0.4	0.4	0.4	negative

3. PRECISION:

It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. Results are reported as follows:

Lot # 1202

Negative Sample (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.094	0.099	0.096	0.096
Std.Deviation	0.008	0.007	0.008	0.007
CV %	8.7	6.6	7.9	7.7

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.396	0.403	0.418	0.406
Std.Deviation	0.023	0.029	0.027	0.026
CV %	5.9	7.1	6.4	6.5
S/Co	1.1	1.1	1.2	1.1

Lot # 0602

Negative Sample (N = 16)

Mean values	1st run	2nd run	3 rd run	Average
OD 450nm	0.097	0.096	0.094	0.096
Std.Deviation	0.009	0.010	0.008	0.009
CV %	8.9	10.1	8.4	9.1

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.400	0.395	0.393	0.396
Std.Deviation	0.021	0.025	0.026	0.024
CV %	5.4	6.2	6.6	6.1
S/Co	1.2	1.2	1.1	1.2

Lot # 0602/2

Negative Sample (N = 16)

Mean values	1st run	2nd run	3 rd run	Average
OD 450nm	0.087	0.091	0.088	0.089
Std.Deviation	0.009	0.007	0.008	0.008
CV %	10.0	8.2	8.6	8.9

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3 rd run	Average
OD 450nm	0.386	0.390	0.391	0.389
Std.Deviation	0.023	0.021	0.023	0.022
CV %	6.0	5.3	5.8	5.7
S/Co	1.1	1.2	1.2	1.2

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS

Repeatable false positive results, not confirmed by RIBA or similar confirmation techniques, were assessed as less than 0.1% of the normal population. Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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HCV Ab

**Versión 4.0 del Ensayo
Inmunoenzimático para la determinación
de anticuerpos frente Virus de la
Hepatitis C
en plasma y suero humanos.**

Uso exclusivo para diagnóstico "in vitro"



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HCV Ab

A. OBJETIVO DEL EQUIPO.

Versión 4.0 del Ensayo Inmunoenzimático (ELISA) para la determinación de anticuerpos al virus de la Hepatitis C en plasma y suero humanos.

El equipo está diseñado para el cribado en unidades de sangre así como para el seguimiento de pacientes infectados con HCV. Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

La Organización Mundial de la Salud (OMS) define la infección por el virus de la Hepatitis C como:

"La Hepatitis C es una infección viral del hígado, definida como hepatitis de transmisión parenteral "no A no B" hasta el descubrimiento del agente causal en 1989. El descubrimiento y la caracterización del virus de la hepatitis C (HCV) ha permitido comprender su papel primario en la hepatitis post-transfusional y su tendencia a inducir la infección persistente. El virus de la hepatitis C es la causa principal de hepatitis aguda y enfermedad hepática crónica, incluyendo cirrosis y cáncer de hígado. A nivel mundial se estima que 170 millones de personas estén infectadas de forma crónica con HCV y que de 3 a 4 millones se infecten cada año.

El virus se transmite por contacto directo con sangre humana. Las causas principales de infección por HCV en el mundo son las transfusiones sanguíneas no controladas y la reutilización de jeringuillas y agujas sin una correcta esterilización previa. En la actualidad aún no existe una vacuna eficaz contra el virus y el tratamiento para la hepatitis C crónica es demasiado costoso para la mayoría de las personas en países en vías de desarrollo. Desde una perspectiva global, el mayor impacto contra la hepatitis C puede lograrse a través de esfuerzos orientados hacia la prevención y el control de la transmisión por exposiciones nosocomiales (como las transfusiones sanguíneas y las prácticas invasoras inseguras) y los comportamientos que conllevan alto riesgo (como el consumo de drogas inyectables).

El virus de la hepatitis C aparece en la mayoría de los casos de hepatitis viral. Es un virus RNA envuelto, perteneciente a la familia Flaviviridae y que parece tener un estrecho margen de huéspedes. Humanos y chimpancés son las únicas especies susceptibles conocidas y ambas desarrollan una enfermedad similar. Una característica importante del virus es su variabilidad genómica, la cual pudiera estar relacionada a su elevada capacidad (80%) de inducir infección crónica. El HCV ha sido agrupado por genotipos, lo cual puede ser útil para determinar la gravedad de la enfermedad y la respuesta al tratamiento.

El periodo de incubación varía desde 15 hasta 150 días. En la infección aguda los síntomas más comunes son fatiga e ictericia, sin embargo la mayoría de los casos (entre el 60% y el 70%), incluso aquellos que desarrollan la infección crónica, son asintomáticos. Cerca del 80% de los nuevos pacientes infectados progresan a la infección crónica. Del 10 al 20% de las personas con infección crónica desarrollan cirrosis, mientras que el cáncer de hígado lo presentan entre el 1 y el 5% de las personas con este tipo de infección, en un periodo de 20 a 30 años. Muchos pacientes que padecen cáncer de hígado y no están infectados por el virus de la hepatitis B, presentan evidencias de infección por el virus de la hepatitis C. Los mecanismos que relacionan la infección por HCV y el desarrollo de cáncer hepático no han sido aún esclarecidos. La hepatitis C puede exacerbar la gravedad de una enfermedad subyacente

del hígado cuando coexiste con otras disfunciones hepáticas; particularmente la enfermedad progresa más rápidamente en personas alcohólicas e infectadas por HCV. Las formas de transmisión más frecuentes son a través de transfusiones sanguíneas sin controlar y por la reutilización de agujas, jeringuillas y material médico contaminados. La transmisión sexual y perinatal puede suceder aunque es menos frecuente. Determinadas prácticas y comportamientos sociales y culturales (perforaciones en orejas y otras partes del cuerpo (piercing), circuncisiones y tatuajes) pueden constituir modos de transmisión si existe una inadecuada esterilización de los instrumentos usados. El HCV no se transmite por estornudos, tos, abrazos, agua o alimentos, estrechar la mano, compartir cubiertos o en general por contactos casuales. Tanto en países desarrollados como en aquellos en vías de desarrollo, los grupos de alto riesgo incluyen drogadictos, receptores de transfusiones sin analizar, hemofílicos, pacientes sometidos a diálisis y personas con actividad sexual promiscua y sin la debida protección. En los países desarrollados, se ha estimado que el 90% de las personas con infección crónica por HCV son o han sido drogadictos o han recibido donaciones de sangre o hemoderivados contaminados. En muchos países en vías de desarrollo, donde aún se utilizan transfusiones o hemoderivados sin analizar, los principales medios de transmisión son los instrumentos para inyecciones y las transfusiones sin analizar.

La OMS estima que cerca de 170 millones de personas, es decir el 3% de la población mundial, están infectadas por el HCV y bajo riesgo de desarrollar cirrosis y/o cáncer hepático. La prevalencia de la infección por HCV en países de África, el Mediterráneo oriental, Sudeste Asiático y el Pacífico Occidental es alta, comparada con países de Norteamérica y Europa.

Las pruebas de diagnóstico para el HCV contribuyen a prevenir la infección mediante el cribado de la sangre y plasma del donante, son útiles para establecer un diagnóstico clínico y en el seguimiento de los pacientes. Las pruebas de diagnóstico comerciales disponibles en la actualidad, se basan en ensayos enzimáticos de inmunoabsorción (EIA) para la detección de anticuerpos específicos contra HCV. Estos métodos pueden detectar más del 95% de los pacientes con infección crónica, pero solo entre el 50 y el 70% de las infecciones agudas. Para confirmar los resultados positivos por EIA se usa frecuentemente el sistema inmunoblot recombinante (RIBA), el cual identifica anticuerpos contra los antígenos individuales del HCV. Por otra parte, algunas técnicas de biología molecular (amplificación de ácidos nucleicos: Reacción en Cadena de la Polimerasa (PCR) y DNA ramificado) han sido utilizadas para confirmar los resultados serológicos así como para determinar la efectividad de la terapia antiviral. Un resultado positivo indica la presencia de una infección activa, de una fuente potencial de transmisión y/o del desarrollo de una enfermedad hepática crónica.

Para el tratamiento de personas con hepatitis C crónica se emplean fármacos antivirales como el interferón (administrado solo o en combinación con la ribavirina), pero el costo del tratamiento es elevado. Si se emplea solo el tratamiento con interferón, la eficacia en los pacientes es de 10 a 20%, mientras que en combinación con la ribavirina es eficaz en cerca del 30-50% de los casos. El tratamiento solo con ribavirina no parece ser efectivo.

No existe en la actualidad una vacuna contra HCV, debido en parte, a la alta frecuencia de mutaciones del virus. El escaso conocimiento de la respuesta inmune protectora que sigue a la infección por HCV ha dificultado el desarrollo de la vacuna. No se conoce tampoco acerca de los mecanismos del sistema inmune para la eliminación del virus. Algunos estudios, sin embargo, han demostrado la aparición de anticuerpos neutralizantes en pacientes con infección HCV. En ausencia de la vacuna, es conveniente tomar todas las medidas posibles para prevenir la infección (a) cribado y análisis de sangre y órganos de donantes; (b) inactivación del virus en productos derivados del plasma; (c) implementación y mantenimiento de las prácticas para el control de la infección incluyendo la

esterilización del material médico y dental; (d) promover cambios en la conducta entre el público en general y el personal sanitario para evitar las prácticas incorrectas y (e) vigilancia de los grupos de riesgo (personas con promiscuidad sexual y drogadictos)."

El genoma codifica para componentes estructurales: una proteína de la nucleocápside y dos glicoproteínas de la envoltura, así como para proteínas funcionales involucradas en la replicación viral y la síntesis de proteínas. La región que codifica para la nucleocápside parece estar altamente conservada entre los aislamientos obtenidos en todo el mundo.

C. PRINCIPIOS DEL ENSAYO.

Las microplacas están recubiertas con antígenos específicos del HCV correspondientes a las regiones del "core" y "ns" que codifican para determinantes antígenicos inmunodominantes y conservados (péptido del core y péptidos recombinantes NS3, NS4 y NS5).

Se añade la muestra diluida y los anticuerpos contra HCV, presentes en la muestra, son capturados por los antígenos de la fase sólida.

Después del lavado, en la 2ª incubación, los anticuerpos IgG e IgM son detectados mediante anticuerpos policlonales específicos anti-IgG/IgM humanos, conjugados con Peroxidasa (HPR).

La enzima capturada en la fase sólida, combinada con la mezcla sustrato/cromógeno, genera una señal óptica proporcional a la cantidad de anticuerpos anti-HCV presentes en la muestra. Posteriormente, mediante un valor de corte calculado, las densidades ópticas pueden interpretarse como resultados negativos o positivos a la presencia de anticuerpos al HCV.

D. COMPONENTES.

Cada equipo (Código CVAB.CE) contiene reactivos suficientes para realizar 192 pruebas.

1. Microplaca: MICROPLATE

n° 2 microplacas

12 tiras de 8 pocillos recubiertos con péptidos recombinantes para el "core" y para NS3, NS4 y NS5. Las placas están empaquetadas en bolsas selladas con desecante.

2. Control Negativo: CONTROL -

1x4.0ml/vial

Listo para el uso. Contiene 1% de proteínas del suero de cabra, tampón Citrato sódico 10mM pH 6.0 +/-0.1, 0.5% de Tween 20, además de azida sódica 0.09% y ProClin 300 al 0,045% como conservantes. El control negativo está codificado con el color verde olivo.

3. Control Positivo: CONTROL +

1x4.0ml/vial

Listo para el uso. Contiene 1% de proteínas del suero de cabra, anticuerpos humanos anti-HCV, tampón Citrato sódico 10mM pH 6.0 +/-0.1, 0.5% de Tween 20, así como azida sódica 0.09% y ProClin 300 al 0,045% como conservantes. El control positivo está codificado con el color azul.

4. Calibrador CAL

n° 2 viales

Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, anticuerpos humanos al HCV, calibrados según el código Estándar de Trabajo de NIBSC 99/588-003-VI, tampón Citrato sódico 10mM pH 6.0 +/-0.1, además de sulfato de gentamicina 0.3 mg/ml y ProClin 300 al 0,045% como conservantes.

Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Tampón de Lavado Concentrado: WASHBUF 20X

2x60ml/botella. Solución concentrada 20x.

Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0,045%.

6. Conjugado CONJ

2x16ml/vial. Solución lista para el uso. Contiene 5% de albúmina de suero bovino, tampón Tris 10mM a pH 6.8 +/- 0.1, anticuerpo policlonal de cabra anti-IgM/IgG humanos conjugado con peroxidasa (HPR) en presencia de 0.2 % de sulfato de gentamicina y ProClin 300 al 0,045% como conservantes. El conjugado está codificado con el color rosa/rojo.

7. Cromógeno/Substrato SUBS TMB

2x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H₂O₂) 0.02% así como dimetilsulfóxido 4%.

Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

8. Diluyente de ensayo: DILAS

1x15ml/vial. Contiene una solución tamponada Tris 10 mM pH 8.0 +/- 0.1 y 0.1% de ProClin 300 al 0,045% para el pre-tratamiento de muestras y controles, bloquea posibles interferencias.

Nota: Usar todo el contenido del vial antes de abrir un segundo. El reactivo es sensible a oxidación.

9. Ácido Sulfúrico: H₂SO₄ 0.3M

1x32ml/vial. Contiene solución de H₂SO₄ 0.3M

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Diluyente de muestras DILSPE

2x50ml. Contiene una solución tamponada citrato sódico 10 mM pH 6.0 +/- 0.1, 1% de proteínas del suero de cabra, 0.5% de Tween 20, azida sódica 0.09% y ProClin 300 al 0,045% como conservantes. Se usa para diluir las muestras.

11. Sellador adhesivo, n° 4

12. Manual de instrucciones, n° 1

Nota importante: A solicitud del cliente, Dia.Pro puede suministrar reactivos para realizar 96, 480 ó 960 pruebas, según se reporta a continuación:

1.Microplaca	n°1	n°5	n°10
2.ControlNegativo	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
3.ControlPositivo	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
4.Calibrador	n° 1 vial	n° 5 vials	n° 10 vials
5.Soluc. Lav. conc	1x60ml/bot.	5x60ml/frasc.	4x150ml/frasc.
6.Conjugado	1x16ml/vial	2x40ml/frasc.	4x40ml/frasc.
7.Cromóg/Subs	1x16ml/vial	2x40ml/frasc.	4x40ml/frasc.
8.Diluent. ensayo	1x8ml/vial	1x40ml/ frasc.	1x80ml/frasc.
9.Acido Sulfúrico	1x15ml/vial	2x40ml/ frasc.	2x80ml/frasc.
10.Diluent.muestr.	1x50ml/vial	5x50ml/frasc.	4x125ml/frasc.
11.Sellador adhes.	n° 2	n° 10	n° 20
12.Manual de instrucciones	n° 1	n° 1	n° 1
Número de pruebas	96	480	960
Código	CVAB.CE.96	CVAB.CE.480	CVAB.CE.960

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (200µl y 10µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37°C.
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de filtros de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Cuando el equipo es usado para cribado en unidades de sangre, el laboratorio debe estar certificado y calificado para realizar este tipo de análisis (Ministerio de Salud o entidad similar).
3. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
4. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
5. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 °C, en un refrigerador con temperatura regulada o en cámara fría.
7. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
8. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente y reemplazar el equipo.
9. Evitar contaminación cruzada entre muestras de suero/plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
11. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 6 meses.
12. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
13. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.

14. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
15. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
16. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
17. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar el uso de conservantes, en particular azida sódica, ya que pudiera afectar la actividad enzimática del conjugado, generando resultados falsos negativos.
3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para el cribado en unidades de sangre, se recomienda el uso del código de barras.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a -20°C durante varios meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, utilizados hasta 6 veces, en un período de hasta 6 meses.

1. Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el

desecante a 2-8°C. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Control Negativo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Control Positivo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar. Manipule este reactivo como potencialmente infeccioso, aunque las partículas virales presentes en el control han sido inactivadas químicamente.

4. Calibrador:

Disolver cuidadosamente el contenido del vial en el volumen de agua de calidad EIA indicado en la etiqueta. Mezclar bien con el vórtex antes de usar.

Manipule este reactivo como potencialmente infeccioso, aunque las partículas virales presentes en el control han sido inactivadas químicamente.

Nota: Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a -20°C.

5. Solución de Lavado Concentrada:

Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada fino a 1200 ml y mezclarse suavemente antes de usarse.

Por que en los frascos pueden estar presente los cristales, cuando se prepara la solución prestar mucha atención en diluir todo el contenido. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.

Nota: Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8°C.

6. Conjugado:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

7. Cromógeno/ Substrato:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Diluyente de ensayo:

Listo para el uso. Mezclar bien con un vórtex antes de usar.

9. Ácido Sulfúrico:

Listo para el uso. Mezclar bien con un vórtex antes de usar.

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**

H315 – Provoca irritación cutánea.

H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**

P280 – Llevar guantes/prendas/gafas/máscara de protección.

P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.

P332 + P313 – En caso de irritación cutánea: Consultar a un médico.

P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.

P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.

P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

10. Diluyente de muestras :

Listo para el uso. Mezclar bien con un vórtex antes de usar.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

- Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periódicamente los residuos de los componentes del equipo.
- La incubadora de ELISA debe ser ajustada a 37°C (+/- 0.5°C) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
- El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
- Los tiempos de incubación deben tener un margen de ±5%.
- El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm, obligatorio para el blanco. El procedimiento estándar debe contemplar: a) Ancho de banda ≤ 10 nm; b) Rango de absorbancia de 0 a ≥ 2,0; c) Linealidad ≥ 2,0; d) Reproducibilidad ≥ 1%. El blanco se prueba en el pocillo indicado en la sección "Procedimiento del ensayo". El sistema óptico del lector debe calibrarse periódicamente para garantizar que se mide la densidad óptica correcta. Periódicamente se debe proceder al mantenimiento según las instrucciones del fabricante.
- En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en la sección "Control interno de calidad". El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular

atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el cribado en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.

7. Cuando se utilizan instrumentos automáticos, en el caso en que los contenedores para los frascos del instrumento no sean adecuados a los frascos del kit, transferir la solución en ellos contenida en frascos idóneos al instrumento y etiquetarlos con la misma etiqueta utilizada en el frasco original. Esta operación es importante para evitar el cambio del contenido de los frascos durante el transferimiento. Cuando el test a terminado colocar los contenedores secundarios etiquetados y tapados a 2..8°C.
8. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37°C y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

Ensayos Automatizados.

En el caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar al equipo para aspirar 200µl de Diluyente de Muestras, y posteriormente 10µl de muestra.

La mezcla debe ser dispensada cuidadosamente en los pocillos correspondientes a cada muestra. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras.

No diluir el Calibrador ni los controles ya que están listos para el uso.

Dispensar 200µl de controles/Calibrador en los pocillos correspondientes.

Nota importante: Controle a simple vista que las muestras han sido diluidas y dispensadas en los pocillos adecuados, para lo cual el color de las muestras dispensadas debe ser verde azul oscuro, mientras que el del control negativo debe permanecer verde olivo.

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

Ensayo Manual.

1. Poner el número de tiras necesarias en el soporte de plástico. Dejar el primer pocillo vacío para el blanco.
2. Dispensar 200µl del Control Negativo, por triplicado, 200µl de Calibrador por duplicado y 200µl del Control Positivo. No diluir el Calibrador ni los controles ya que están listos para el uso!
3. Dispensar 200µl del Diluyente de muestras (DILSPE) a todos los pocillos de muestras, después dispensar 10 µl de cada muestra en su pocillo correspondiente. Resuspender suavemente evitando la formación de espuma y la contaminación de los pocillos adyacentes.

Nota importante: Comprobar que el color del Diluyente de muestras, después de adicionada la misma, cambia de verde a verde azul oscuro.

4. Dispensar 50 µl de Diluyente de ensayo (DILAS) en los pocillos de los controles/Calibrador y muestras. Compruebe que el color de las muestras sea azul oscuro.
5. Incubar la microplaca **45 min a +37°C**.

Nota importante: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el test manualmente. No sellar cuando se emplean equipos automatizados de ELISA.

6. Lavar la microplaca con el lavador automático dispensando y aspirando 350 µl/pocillo de solución de lavado diluida, según según se indica (sección 1.3).
7. Dispensar 100µl del Conjugado en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rosa/rojo ha sido añadido en todos los pocillos excepto el A1.

Nota importante: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.

8. Incubar la microplaca **45 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) durante 15 minutos**.

Nota importante: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.

11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color del Control Positivo y las muestras positivas de azul a amarillo/marrón.

12. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y, otro de 620-630 nm (substracción del fondo), calibrando el instrumento con el pocillo A1 (blanco, obligatorio).

Notas importantes:

1. Asegurarse de que no hay impresiones digitales ni polvo en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.
2. La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.
3. Se ha probado que la agitación a 350 +/- 150 rpm, durante la incubación, aumenta en un 20% la sensibilidad del ensayo.
4. El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.

N. ESQUEMA DEL ENSAYO.

Método	Operaciones
Controles & Calibrador	200 µl
Muestras	200µl dil.+10µl
Diluyente de ensayo (DILAS)	50 µl
1ª incubación	45 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2ª incubación	45 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ª incubación	15 min
Temperatura	18-24°C
Acido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

A continuación se describe un ejemplo del esquema de dispensado.

Microplaca												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M2										
B	CN	M3										
C	CN	M4										
D	CN	M5										
E	CAL	M6										
F	CAL	M7										
G	CP	M8										
H	M 1	M9										

Leyenda: BL = Blanco CN = Control Negativo CAL = Calibrador CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles/calibrador cada vez que se usa el equipo para verificar si los valores DO450nm son los esperados.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	Valor < 0.100 DO450nm
Control Negativo (CN)	Valor medio < 0.050 DO450nm después de leer el blanco
Calibrador	M/Co > 1.1
Control Positivo	Valor > 1.000 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.050 DO450nm después de leer el blanco	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador M/Co < 1.1	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Control Positivo < 1.000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). En este caso el control negativo debe tener un valor de DO450nm > 0.150. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, después de comprobar, informe al responsable para tomar las medidas pertinentes.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

$$\text{Valor de corte} = \text{CN medio DO450nm} + 0.350$$

El valor encontrado para el ensayo se usa para la interpretación de los resultados, según se describe a continuación:

Nota Importante: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.

Q. INTERPRETACIÓN DE LOS RESULTADOS.

La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm de las muestras y el Valor de corte (M/Co).

Los resultados se interpretan según la siguiente tabla:

(M/Co)	Interpretación
< 0.9	Negativo
0.9 – 1.1	Equívoco
> 1.1	Positivo

Un resultado negativo indica que el paciente no está infectado por HCV y la unidad de sangre se puede transfundir. Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial. En este caso la unidad de sangre no debe ser transfundida. Un resultado positivo es indicativo de infección por HCV y por consiguiente el paciente debe ser tratado adecuadamente. La unidad de sangre debe ser descartada.

Notas importantes:

1. La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.
2. Antes de formular un diagnóstico de hepatitis viral, los resultados positivos deben comprobarse a través de un método alternativo, capaz de detectar anticuerpos IgG e IgM (prueba confirmatoria).
3. Según se demuestra en la Evaluación del Performance del producto, el ensayo es capaz de detectar los anticuerpos anti HCV core, en etapas más tempranas en comparación con otros equipos comerciales. Sin embargo, un resultado positivo, no confirmado con estos equipos comerciales, no debe necesariamente considerarse como falso positivo! Es necesario realizar una prueba de confirmación (suministrada, bajo solicitud del cliente, por Dia.pro srl. Codificada CCONF).
4. Como el ensayo es capaz de detectar además anticuerpos IgM, pueden presentarse resultados discrepantes (pérdida de reactividad IgM) con respecto a otros productos comerciales para la detección de anticuerpos anti-HCV. La positividad real de una muestra debe confirmarse probando la reactividad IgM, lo cual resulta muy importante para el diagnóstico de infección por HCV.
5. Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
6. El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.

A continuación, un ejemplo de los cálculos a realizar:

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 0.019 – 0.020 – 0.021 DO450nm
 Valor medio: 0.020 DO450nm
 Menor de 0.050 – Válido

Control Positivo: 2.189 DO450nm
 Mayor de 1.000 – Válido
 Valor de corte = 0.020+0.350 = 0.370

Calibrador: 0.550 - 0.530 DO450nm
 Valor medio: 0.540 DO450nm M/Co = 1.4
 M/Co Mayor de 1.1 – Válido

Muestra 1: 0.070 DO450nm
 Muestra 2: 1.690 DO450nm
 Muestra 1 M/Co < 0.9 = negativa
 Muestra 2 M/Co > 1.1 = positiva

R. FUNCIONAMIENTO.

La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. LÍMITE DE DETECCIÓN.

El límite de detección ha sido calculado por medio del estándar de trabajo británico anti-HCV NIBSC, código 99/558-003-WI). La siguiente tabla muestra los valores medios de DO450nm de este estándar diluido en plasma negativo y examinado:

Dilución	Lote # 1	Lote # 2
Factor	M/Co	M/Co
1 X	2.0	2.0
2 X	1.1	1.2
4 X	0.7	0.8
8 X	0.5	0.5
Plasma Negativo	0.3	0.3

Se evaluó además la muestra Accurun 1 –serie 3000– suministrado por Boston Biomedica Inc., Estados Unidos.

Los resultados son los siguientes:

CVAB.CE Lote ID	Accurun 1 Serie	M/Co
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

Por otra parte, un total de 7 muestras, positivas para HCVAb según Ortho HCV 3.0 SAVe, código 930820, lote # EXE065-1, fueron diluidas en plasma negativo a HCVAb con el fin de obtener diluciones limitantes y luego fueron probadas nuevamente en CVAB.CE, lote # 1202, y Ortho.

Las tablas siguientes reflejan los resultados obtenidos:

Muestra n°	Dilución Límite	CVAB.CE M/Co	Ortho 3.0 M/Co
1	256 X	1.9	1.3
2	256 X	1.9	0.7
3	256 X	2.4	1.0
4	128 X	2.5	3.2
5	85 X	3.3	1.4
6	128 X	2.2	0.8
7	135 X	3.2	2.2

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS.

La evaluación del procedimiento diagnóstico se realizó mediante un ensayo con más de 5000 muestras.

2.1 Especificidad Diagnóstica:

Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.

Además del primer estudio, donde se examinaron en total 5043 muestras de donantes de sangre no seleccionados, (incluyendo donantes por 1ª vez), 210 muestras de pacientes hospitalizados y 162 muestras que pudieran provocar interferencia (otras enfermedades infecciosas, positivas para anticuerpos de E. coli, pacientes con enfermedades hepáticas no virales, pacientes en diálisis, mujeres embarazadas, hemolizadas, lipémicas, etc.), la especificidad diagnóstica se evaluó recientemente examinando un total de 2876 muestras de donantes de sangre negativas en seis lotes distintos. Se observó un valor de especificidad de 100%.

Se emplearon además, plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Por último se analizaron muestras congeladas, para determinar posibles interferencias debidas a la toma de muestra y al almacenamiento. No se observaron interferencias.

2.2 Sensibilidad Diagnóstica.

Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.

La sensibilidad diagnóstica ha sido estimada de forma externa en un total de 359 muestras, el valor obtenido fue de 100%. Más de 50 muestras positivas fueron probadas de forma interna, en este caso el resultado fue también de 100%.

Se evaluaron además, muestras positivas producto de infecciones por diferentes genotipos de HCV, así como también se estudió gran parte de los paneles de seroconversión de Boston Biomedica Inc (PHV) y Zeptometrix, USA (HCV), disponibles.

Los resultados para algunos de ellos se describen a continuación:

Panel	N° samples	DiaPro*	Ortho* **
PHV 901	11	9	9
PHV 904	7	2	4
PHV 905	9	3	4
PHV 906	7	7	7
PHV 907	7	3	2
PHV 908	13	10	8
PHV 909	3	2	2
PHV 910	5	3	3
PHV 911	5	3	3
PHV 912	3	1	1
PHV 913	4	2	2
PHV 914	9	5	5
PHV 915	4	3	0
PHV 916	8	4	3
PHV 917	10	6	6
PHV 918	8	2	0
PHV 919	7	3	3
PHV 920	10	6	6
HCV 10039	5	2	0
HCV 6212	9	6	7
HCV 10165	9	5	4

Note: * Positive samples detected

** HCV v.3.0

Por último, el producto ha sido probado contra el panel EFS Ac HCV, lote n° 01/08.03.22C/01/A, suministrado por Etablissement Francais Du Sang (EFS), Francia, obteniéndose los siguientes resultados:

EFS Panel Ac HCV

Muestra	Lote # 1 M/Co	Lote # 2 M/Co	Lote# 3 M/Co	Resultados esperados
HCV 1	2.2	2.4	2.6	positivo
HCV 2	1.6	2.0	2.1	positivo
HCV 3	1.5	1.7	1.6	positivo
HCV 4	5.2	6.5	5.5	positivo
HCV 5	1.6	1.8	1.6	positivo
HCV 6	0.4	0.4	0.4	negativo

3. PRECISIÓN.

Ha sido calculada utilizando dos muestras, una negativa y una débil positiva, examinadas en 16 réplicas en tres corridas separadas.

Los resultados se muestran a continuación:

Lote # 1202

Muestra Negativa (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.094	0.099	0.096	0.096
Desviación estándar	0.008	0.007	0.008	0.007
CV %	8.7	6.6	7.9	7.7

Cal # 2 – 7K (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.396	0.403	0.418	0.406
Desviación estándar	0.023	0.029	0.027	0.026
CV %	5.9	7.1	6.4	6.5
M/Co	1.1	1.1	1.2	1.1

Lote # 0602

Muestra Negativa (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.097	0.096	0.094	0.096
Desviación estándar	0.009	0.010	0.008	0.009
CV %	8.9	10.1	8.4	9.1

Cal # 2 – 7K (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.400	0.395	0.393	0.396
Desviación estándar	0.021	0.025	0.026	0.024
CV %	5.4	6.2	6.6	6.1
M/Co	1.2	1.2	1.1	1.2

Lote # 0602/2

Muestra Negativa (N = 16)

Valores medios	1 ^{ra} corrida	2 ^{da} corrida	3 ^{ra} corrida	Valor Promedio
DO 450nm	0.087	0.091	0.088	0.089
Desviación estándar	0.009	0.007	0.008	0.008
CV %	10.0	8.2	8.6	8.9

Cal # 2 – 7K (N = 16)

Valores medios	1 ^{ra} corrida	2 ^{da} corrida	3 ^{ra} corrida	Valor Promedio
DO 450nm	0.386	0.390	0.391	0.389
Desviación estándar	0.023	0.021	0.023	0.022
CV %	6.0	5.3	5.8	5.7
M/Co	1.1	1.2	1.2	1.2

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

S. LIMITACIONES.

Los falsos positivos repetibles, no confirmados por RIBA o similares técnicas de confirmación, fueron estimados como menos del 0.1% de la población normal.

Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el mercado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni
(Milán) – Italia



0318



Dia.Pro
Diagnostic
Bio***Probes***

Letter of Authorization

We, "Dia.Pro Diagnostic Bioprobes S.r.l." located at Via G. Carducci, Nr. 27 – Sesto San Giovanni (Milan) 20099, Italy, authorize

GLOBAL BIOMARKETING GROUP – MOLDOVA SRL
Str. Tighina 65, Oficiu 607
MD-2001 CHISINAU
REP. MOLDOVA

as our **exclusive distributor for the territory of the Republic of Moldova**, to participate in various tenders with **Dia.Pro** ELISA products.

We, Dia.Pro Diagnostic Bioprobes S.r.l shall supply our distributor GLOBAL BIOMARKETING GROUP – MOLDOVA SRL with all products in strict compliance with the existing "Distribution Agreement" rev.0121 valid until 31-Dec-2023, with possibility of renewal upon agreement between both parties for an additional period.

Dia.Pro Diagnostic Bioprobes S.r.l will grant the supply of all awarded tenders until their natural expiry, of which a documental proof has to be provided to Dia.Pro by the distributor GLOBAL BIOMARKETING GROUP – MOLDOVA SRL.

Sincerely yours,

Date: **Milan, 04-February-2021**

Dia.Pro Diagnostic Bioprobes S.r.l.

DIA. PRO.
DIAGNOSTIC BIOPROBES S.r.l.

Dr.ssa Fiorenza Scozzesi

Legal Representative

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DECLARATION OF CONFORMITY

1) Manufacturer (Name, department): **Monobind Inc.**

Address: **100 North Pointe, LAKE FOREST, CA 92630. UNITED STATES**

and

2) European authorized representative: **CEpartner4U BV,**

Address: **ESDOORNLAAN 13, 3951DB MAARN, THE NETHERLANDS;**

(on product labels printed as:

CEpartner4U , ESDOORNLAAN 13, 3951DB MAARN, THE NETHERLANDS Tel.: +31 (0)6 516 536 26;

or as: CEpartner4U, 3951DB; 13. NL tel: +31 (0)6 – 516.536.26)

3) Product(s) (name, type or model/batch number, etc.):

Immunoassay products;

ELISA,

CLIA,

Control,

Instruments

(see appendix)

4) The product(s) described above is in conformity with:

<u>Document No.</u>	<u>Title</u>	<u>Edition / Date of issue</u>
L 331; 98/79/EC	In-Vitro-Diagnostic Directive	1998-10-27

5) Additional information (conformity procedure, Notified Body, CE certificate, etc.):

Conformity assessment procedure for CE marking: IVD Directive, Annex III

Lake Forest, USA; 2011-09-27



Tony Shatola; QA Director, Monobind Inc.

(Place & date of issue (yyyy-mm-dd))

(name, function and signature of manufacturer)

Maarn, NL; 2011-09-27



Olga Teirlinck; Consultant, CEpartner4U BV

(Place & date of issue (yyyy-mm-dd))

(name; function and signature of authorized representative)

Appendix

Date: 2011-09-26

Device types	Item# ELISA	Item# CLIA	Item# Control	Item# Instrument	EDMS code	Risk Class	Certificate #	First date of CE-marking
Thyroid								
T3 – Triiodothyronine	125-300	175-300			12.04.01.05.00	Low		2005-11-11
ft3 – Free Triiodothyronine	1325-300	1375-300			12.04.01.01.00	Low		2005-11-11
T4 – Thyroxine	225-300	275-300			12.04.01.07.00	Low		2005-11-11
ft4 – Free Thyroxine	1225-300	1275-300			12.04.01.02.00	Low		2005-11-11
TSH – Thyrotropin	325-300	375-300			12.04.01.11.00	Low		2005-11-11
Rapid TSH – Rapid Thyrotropin	6025-300	6075-300			12.04.01.11.00	Low		2010-06-29
T3U – Triiodothyronine Uptake	525-300	575-300			12.04.01.06.00	Low		2005-11-11
TBG – Thyroxine-Binding Globulin	3525-300	3575-300			12.04.01.09.00	Low		2005-11-11
Tg – Thyroglobulin	2225-300	2275-300			12.04.01.08.00	Low		2005-11-11
T3, T4 & TSH – Triiodothyronine, Thyroxine & Thyrotropin Combo (VAST)	8025-300	8075-300			12.04.01.01.00	Low		2005-11-11
T3 – Triiodothyronine (SBS)	8125-300	8175-300			12.04.01.01.00	Low		2010-06-29
T4- Thyroxine (SBS)	8225-300	8275-300			12.04.01.01.00	Low		2010-06-29
ft3, ft4 & TSH – Free Triiodothyronine, Free Thyroxine & Thyrotropin Combo (VAST)	7025-300	7075-300			12.04.01.01.00	Low		2010-06-29
Neonatal Thyroid & Genetics								
NTSH – Neonatal Thyrotropin	3425-300	3475-300			12.04.01.90.00	Low		2005-11-11
NT4 – Neonatal Thyroxine	2625-300	2675-300			12.04.01.12.00	Low		2005-11-11
N 17OHP – Neonatal 17 OH Progesterone	5525-300				12.05.01.07	Low		2008-02-01
Biotinidase	8825-300				12 07 02 90 00	Low		2011-09-26
Autoimmune Thyroid								
Anti-Tg – Anti-Thyroglobulin Antigen	1025-300	1075-300			12.10.03.04.00	Low		2005-11-11
Anti-TPO – Anti-Thyropoxidase Antigen	1125-300	1175-300			12.10.03.01.00	Low		2005-11-11
Fertility & Prenatal								
LH – Lutropin	625-300	675-300			12.05.01.05.00	Low		2005-11-11
FSH – Folitropin	425-300	475-300			12.05.01.04.00	Low		2005-11-11
PRL – Prolactin	725-300	775-300			12.05.01.08.00	Low		2005-11-11
PRL – Prolactin Sequential	6025-300	6075-300			12.05.01.08.00	Low		2005-11-11
hCG – Human Chorionic Gonadotropin	825-300	875-300			12.05.02.05.00	Low		2005-11-11
Rapid hCG – Rapid Human Chorionic Gonadotropin	3325-300				12.05.02.05.00	Low		2005-11-11
FSH, LH, hCG, sPRL Combo (VAST)	8325-300	8375-300			12.05.01.90.00	Low		2006-08-24
AFP, hCG, uE3 Combo (VAST)	8525-300	8575-300			12.05.01.90.00	Low		2010-06-29
Steroid								
Cortisol	3625-300	3675-300			12.06.02.04.00	Low		2005-11-11
DHEA-S – Dehydroepiandrosterone sulfate	5125-300	5175-300			12.05.01.02.00	Low		2010-06-29
DHEA - Dehydroepiandrosterone	7425-300	7475-300			12.05.01.02.00	Low		2011-09-26

<i>Device types</i>	<i>Item# ELISA</i>	<i>Item# CLIA</i>	<i>Item# Control</i>	<i>Item# Instrument</i>	<i>EDMS code</i>	<i>Risk Class</i>	<i>Certificate #</i>	<i>First date of CE-marking</i>
E2 – Estradiol	4925-300	4975-300			12.05.01.03.00	Low		2010-06-29
uE3 – Estriol, Unconjugated	5025-300	5075-300			12.05.02.02.00	Low		2010-06-29
Progesterone	4825-300	4875-300			12.05.01.06.00	Low		2010-06-29
Testosterone	3725-300	3775-300			12.05.01.10.00	Low		2007-11-01
Free Testosterone	5325-300	5375-300			12.05.01.10.00	Low		2010-06-29
17OHP - 17-Hydroxyprogesterone	5225-300	5275-300			12.05.01.07.00	Low		2010-06-29
17OHP - 17-Hydroxyprogesterone Ext. Range	9925-300	9975-300			12.05.01.07.00	Low		2010-10-18
Vitamin D3 – 25-Hydroxyvitamin D3	7725-300	7775-300			12.06.03.10.00	Low		2011-09-26
Growth & Bone Metabolism								
hGH - Human Growth Hormone	1725-300	1775-300			12.06.04.02.00	Low		2005-11-11
PTH - Parathyroid Hormone	7825-300	7875-300			12.06.03.13.00	Low		2011-09-26
Diabetes								
Insulin	2425-300	2475-300			12.06.01.03.00	Low		2005-11-11
Insulin Rapid	5825-300				12.06.01.03.00	Low		2010-06-29
C-peptide	2725-300	2775-300			12.06.01.01.00	Low		2005-11-11
Insulin & C-peptide Combo (VAST)	7325-300	7375-300			12.06.01.03.00	Low		2005-11-11
Cardiac Markers								
CKMB – Circulating Creatine Kinase (MB)	2925-300	2975-300			12.13.01.02.00	Low		2005-11-11
CTnI – Troponin I	3825-300	3875-300			12.13.01.07.00	Low		2005-11-11
DIG – Digoxin	925-300	975-300			12.08.01.01.00	Low		2005-11-11
HS-CRP – High Sensitivity C- Reactive Protein	3125-300	3175-300			12.13.01.90.00	Low		2005-11-11
Myoglobin	3225-300	3275-300			12.13.01.05.00	Low		2005-11-11
Infectious Diseases								
IgG – Anti/H. Pylori	1425-300	1475-300			15.01.04.03.00	Low		2005-11-11
IgM – Anti/H. Pylori	1525-300	1575-300			15.01.04.03.00	Low		2005-11-11
IgA – Anti/H. Pylori	1625-300	1675-300			15.01.04.03.00	Low		2005-11-11
Cancer Markers								
AFP – Alpha-Fetoprotein	1925-300	1975-300			12.03.90.01.00	Low		2005-11-11
CA 125 Ovarian Cancer Antigen	3025-300	3075-300			12.03.01.06.00	Low		2005-11-11
CA 15-3 Breast Cancer Antigen	5625-300	5675-300			12.03.01.02.00	Low		2010-06-29
CA 19-9 - Pancreatic Cancer Antigen	3925-300	3975-300			12.03.01.03.00	Low		2005-11-11
CEA – Carcinoembryonic Antigen	1825-300	1875-300			12.03.01.31.00	Low		2005-11-11
CEA - Carcinoembryonic Antigen Next Generation	4625-300	4675-300			12.03.01.31.00	Low		2010-06-29
fβhCG – Free Beta Human Chorionic Gonadotropin	2025-300	2075-300			12.03.01.90.00	Low		2005-11-11
Allergy & Anemia								
Ferritin	2825-300	2875-300			12.07.01.02.00	Low		2005-11-11
Folate	7525-300	7575-300			12.07.01.03.00	Low		2010-06-29
IgE – Immunoglobulin E	2525-300	2575-300			12.02.01.02.00	Low		2005-11-11
sTfR - Transferrin Soluble Receptor	8625-300	8675-300			12.07.01.06.00	Low		2010-06-29
Vitamin B12	7625-300	7675-300			12.07.02.04.00	Low		2011-09-26

Miscellaneous Controls								
Anti-Tg & Anti-TPO – Positive & Negative - Anti-Thyroglobulin, Anti-Thyroperoxidase			AIT-101		12.50.01.16.00	Low		2010-06-29
High Level Fertility Control – Single Level – Progesterone, Estradiol, Human Chorionic Gonadotropin			FC-300		12.50.01.16.00	Low		2010-06-29
Maternal Control – Tri Level - Human Chorionic Gonadotropin, Free Beta Human Chorionic Gonadotropin Subunit, Alpha Feta Protein, Estriol			MC-300		12.50.01.16.00	Low		2010-06-29
Thyroglobulin Control – Tri Level			TG-300		12.50.01.16.00	Low		2010-06-29
H. Pylori IgG Control – Positive & Negative			HPy-IgG-300		12.50.01.16.00	Low		2010-06-29
Miscellaneous Instruments								
IC hardware + dedicated accessories + software – Autoplex ELISA Analyzer & CLIA Processor				IN006	21.02.10.01	Low		2010-06-29
IC hardware + dedicated accessories + software – Lumax Chemiluminescence Strip Reader				IN001	21.02.10.01	Low		2006-08-24
IC hardware + dedicated accessories + software – Neo-Lumax Chemiluminescence Strip Reader				IN010	21.02.10.01	Low		2011-09-26
IC hardware + dedicated accessories + software – Impulse 2 Chemiluminescence Strip Reader				IN005	21.02.10.01	Low		2006-08-24
IC hardware + dedicated accessories + software – Impulse 3 Chemiluminescence Strip Reader				IN007	21.02.10.01	Low		2010-06-29
IC hardware + dedicated accessories + software – Lumax96 Chemiluminescence Plate Reader				IN004	21.02.10.01	Low		2007-03-01
IC hardware + dedicated accessories + software – LuMatic Chemiluminescence Plate Reader				IN008	21.02.10.01	Low		2011-09-26
IC hardware + dedicated accessories + software – Eldex 3.8 ELISA Strip Reader				IN003	21.02.10.01	Low		2007-09-10
IC hardware + dedicated accessories + software – Neo-Eldex ELISA Strip Reader				IN009	21.02.10.01	Low		2011-09-26
IC hardware + dedicated accessories + software – Mircoplate Washer				IN002	21.02.10.01	Low		2010-06-29



Total Prostate Specific Antigen (tPSA) Test System

Product Code: 2125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (tPSA) Concentration in Human Serum by a Microplate Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity (1,2). The protein is a single chain glycoprotein with a molecular weight of 28.4 kDa (3). PSA derives its name from the observation that it is a normal antigen of the prostate but is not found in any other normal or malignant tissue.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found too more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity (4).

In this method, PSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PSA) are added and the reactants mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-PSA antibody bound conjugate is separated from the unbound enzyme-PSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

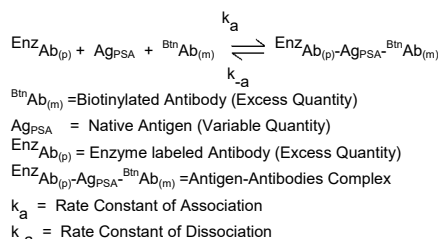
The employment of several serum references of known prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with PSA concentration.

3.0 PRINCIPLE

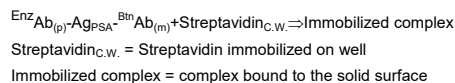
Immunoassay (TYPE 3):

The essential reagents required for an immunoassay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on

the well and exogenously added biotinylated monoclonal anti-PSA antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:



Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:



After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

- Prostate Specific antigen (PSA) 1ml/vial – Icons A-F**
Six (6) vials of references PSA Antigen at levels of 0(A), 5(B), 10(C), 25(D), 50(E) and 100(F) ng/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.
- PSA Enzyme Reagent – 13ml/vial - Icon E**
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
- Streptavidin Coated Plate – 96 wells - Icon J**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
- Wash Solution Concentrate – 20 ml - Icon K**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.
- Substrate A – 7ml/vial - Icon S^A**
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
- Substrate B – 7ml/vial - Icon S^B**
One (1) bottle containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.
- Stop Solution – 8ml/vial - Icon L**
One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.
- Product Instructions.**

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**

Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:

- Pipette(s) capable of delivering 25 & 50µl volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.
- Quality control materials

5.0 PRECAUTIONS

**For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals**

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION:

- Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.
- Working Substrate Solution**
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
Note1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

- Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
- Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
- Add 0.100 ml (100µl) of the PSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
- Swirl the microplate gently for 20-30 seconds to mix and cover.
- Incubate 30 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
- Incubate at room temperature for fifteen (15) minutes.
- Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding PSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
- Draw the best-fit curve through the plotted points.
- To determine the concentration of PSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.142) intersects the dose response curve at (23.6 ng/ml) PSA concentration (See Figure 1).

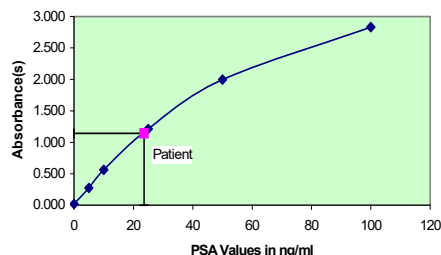
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.019	0.019	0
	B1	0.019		
Cal B	C1	0.279	0.276	5
	D1	0.273		
Cal C	E1	0.567	0.563	10
	F1	0.559		
Cal D	G1	1.248	1.213	25
	H1	1.179		
Cal E	A2	2.051	1.999	50
	B2	1.947		
Cal F	C2	2.892	2.833	100
	D2	2.775		
Patient	E3	1.186	1.142	23.6
	F3	1.099		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

- The absorbance (OD) of calibrator F should be ≥ 1.3 .
- Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- Patient specimens with PSA concentrations above 100 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
- Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
- All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
- It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
- Risk Analysis- as required by CE Mark IVD Directive ISO 14971:2009 - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, **Monobind shall have no liability.**
- If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
- PSA is elevated in benign prostrate hypertrophy (BPH). Clinically an elevated **PSA value alone is not of diagnostic value as a specific test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in regard to the discrimination of BPH and prostrate cancer conditions (5).

13.0 EXPECTED RANGES OF VALUES

Healthy males are expected to have values below 4 ng/ml (4).

TABLE I

Expected Values for the PSA Elisa Test System

Healthy Males	<4 ng/ml
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It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by

the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the tPSA AccuBind™ ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2

Within Assay Precision (Values in ng/ml)

Sample	N	X	S.D.	C.V.
Level 1	20	0.7	0.05	7.1%
Level 2	20	4.5	0.20	4.4%
Level 3	20	28.3	1.07	3.7%

TABLE 3

Between Assay Precision* (Values in ng/ml)

Sample	N	X	S.D.	C.V.
Level 1	10	0.8	0.09	11.3%
Level 2	10	4.3	0.25	5.8%
Level 3	10	27.5	1.42	5.2%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The tPSA AccuBind™ ELISA test system has a sensitivity of 0.012 ng. This is equivalent to a sample containing 0.5 ng/ml tPSA concentration.

14.3 Accuracy

The tPSA AccuBind™ ELISA method was compared with a reference Elisa method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the tPSA AccuBind™ ELISA test method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Least Square

Method	Mean	Regression Analysis	Correlation Coefficient
This Method (X)	5.62	$y = -0.0598 + 0.98(X)$	0.987
Reference (Y)	5.57		

Only slight amounts of bias between the tPSA AccuBind™ ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

No interference was detected with the performance of tPSA AccuBind™ ELISA test system upon addition of massive amounts of the following substances to a human serum pool.

Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
CEA	10 µg/ml
AFP	10 µg/ml
CA-125	10,000 U/ml
hCG	1000 IU/ml
hLH	10 IU/ml
hTSH	100 mIU/ml
hPRL	100 µg/ml

15.0 REFERENCES

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Revision: 3 Date: 081511 DCO: 0524

Cat #: 2125-300

Size	96(A)	192(B)
Reagent (fill)	A)	1ml set
	B)	1 (13ml)
	C)	1 plate
	D)	1 (20ml)
	E)	1 (7ml)
	F)	1 (7ml)
	G)	1 (8ml)

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