Alpha-Fetoprotein (AFP) Test System
Product Code: 1925-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Alpha-Fetoprotein (AFP) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Alpha-Fetoprotein (AFP) is a glycoprotein with a molecular weight of 70 kDA. AFP is normally produced during fetal development by the hepatocytes, yolk sac and, to a lesser extent, the gastrointestinal tract. Serum concentrations reach a peak level of up to 10 mg/ml at twelve weeks of gestation.[1] This peak level gradually decreases to less than 25 ng/ml after one year of postpartum. Thereafter, the levels reduce further to less than 10 ng/ml.

Elevated levels of AFP are found in patients with primary heptatoma and yolk sac-derived germ tumors. AFP is the most useful marker for the diagnosis and management of hepatocellular carcinoma.[2] AFP is also elevated in pregnant women. Presence of abnormally high AFP concentrations in pregnant women provides a risk marker for Down syndrome.[3]

In this method, AFP calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated and enzyme labeled monoclonal antibodies (directed against distinct and different epitopes of AFP) are added and the reactants mixed. Reaction between the various AFP antibodies and native AFP forms a sandwich complex that binds with the streptavidin coated to the well. After the completion of the required incubation period, the enzyme-AFP antibody bound conjugate is separated from the unbound enzyme-AFP 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 alpha-fetoprotein (AFP) 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 AFP concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay 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-AFP 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:

$$^{Enz}Ab + Ag_{AFP} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{AFP} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{AFP} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{AFP} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{AFP} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

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:

A. AFP Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of references AFP antigen at levels of 0 (**A**), 5 (**B**), 25 (**C**), 50 (**D**), 250 (**E**) and 500 (**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 WHO 1st IRP # 72/225.

B. AFP Enzyme Reagent – 13ml/vial – Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Microplate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial – Icon 💧
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: 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:
1. Pipette(s) capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. 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.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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 (50µl) 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

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** - Stable for one (1) year
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.

Note 1: 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 reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, 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.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the AFP Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Mix **(See Note)** the microplate for 20-30 seconds until homogenous.
5. Swirl the microplate gently for 20-30 seconds to mix and cover.
6. Incubate 60 minutes at room temperature.
7. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
8. Add 0.350ml (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.**
9. Add 0.100ml (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
10. Incubate at room temperature for fifteen (15) minutes.
11. 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.**
12. 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.**

Note: Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycle.

10.0 CALCULATION OF RESULTS

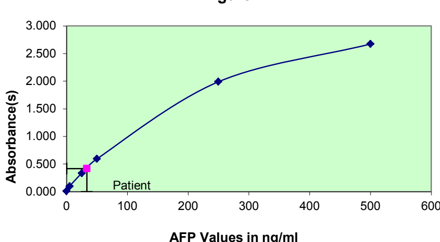
A dose response curve is used to ascertain the concentration of AFP in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding AFP concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of AFP 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 (0.420) intersects the dose response curve at 33.2 ng/ml AFP 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	0.012	0.011	0
	B1	0.011		
Cal B	C1	0.100	0.098	5
	D1	0.097		
Cal C	E1	0.336	0.335	25
	F1	0.333		
Cal D	G1	0.612	0.594	50
	H1	0.577		
Cal E	A2	2.005	1.990	250
	B2	1.975		
Cal F	C2	2.664	2.672	500
	D2	2.680		
Patient	E2	0.427	0.420	33.2
	F2	0.413		

Figure 1



*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.

11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be $\geq$ 1.3.
2. The absorbance (OD) of calibrator A should be $\leq$ 0.035.
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 are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with AFP concentrations above 500 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (AFP < 10 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (x10).
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.
11. 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.
12. 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.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. The reagents for the test system procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin.Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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.
6. 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.
7. AFP has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **AFP value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. AFP levels are known to be elevated in a number of benign diseases and conditions including pregnancy and non-malignant liver diseases such as hepatitis and cirrhosis.

13.0 EXPECTED RANGE OF VALUES

Approximately 97-98% of the normal healthy population has AFP levels less than 8.5ng/ml.[4] In high-risk patients, AFP values between 100-350 ng/ml suggest hepatocellular carcinoma. Concentrations over 350 ng/ml usually indicate the disease.

TABLE I
Expected Values for the AFP AccuBind® ELISA Test System

Male and Female	<8.5ng/ml (97-98%)

Values for AFP for a normal, healthy population and pregnant women, during gestation cycle, are given in Table 2. The values depicted below represent limited in house studies in concordance with published literature.[8,9,10]

TABLE 2
Median Values during Gestation.

Gestation (Week)	AFP (ng/ml)
15	40.14
16	42.91
17	52.34
18	61.50
19	75.57
20	83.31
21	90.46

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 precision of the AFP 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 3 and Table 4.

TABLE 3
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	24	14.71	0.67	4.6
Level 2	24	71.89	2.68	3.7
Level 3	24	148.62	7.24	4.9

TABLE 4
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	30	16.20	1.41	8.7
Level 2	30	88.26	7.47	8.5
Level 3	30	188.43	11.92	6.3

*As measured in thirty experiments in duplicate.

14.2 Sensitivity

The AFP AccuBind® ELISA Test System has a sensitivity of 0.01 ng. This is equivalent to a sample containing 0.44 ng/ml AFP concentration. The sensitivity (detection limit) was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The AFP AccuBind® ELISA Test System was compared with a reference method. Biological specimens with concentrations ranging from 1.0 to 41 ng/ml were assayed. The total number of such specimens was 42. The least square regression equation and the correlation coefficient were computed for the AFP procedure in comparison with the reference method. The data obtained is displayed in Table 5.

TABLE 5

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (Y)	5.27	y = 0.746(x) + 1.0007	0.973
Reference (X)	5.72		

Only slight amounts of bias between the AFP AccuBind® ELSIA Test System 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 AFP AccuBind® ELISA Test System upon addition of massive amounts of the following substances to a human serum pool.

SUBSTANCE	Cross Reactivity	Concentration
Acetylsalicylic Acid	ND	100 µg/ml
Amethopterin	ND	100 µg/ml
Ascorbic Acid	ND	100 µg/ml
Atropine	ND	100 µg/ml
Caffeine	ND	100 µg/ml
CEA	ND	10 µg/ml
PSA	ND	1.0 µg/ml
CA-125	ND	10,000 U/ml
hCG	ND	1000 IU/ml
hLH	ND	10 IU/ml
hTSH	ND	100 mIU/ml
hPRL	ND	100 µg/ml

14.5 Linearity & Hook Effect:

Three different lot preparations of the AFP AccuBind® ELISA test system reagents were used to assess the linearity and hook effect. Massive concentrations of AFP (> 100,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 10,000 ng/ml and a with a dose recovery of 86.1 to 113.6%.

15.0 REFERENCES

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Effective Date: 2021-Sep-23 Rev. 8 DCO: 1509
MP1925 Product Code: 1925-300

	Size	96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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Glossary of Symbols
(EN 980/ISO 15223)

In Vitro - Diagnostic Medical Device

Temperature Limitation Storage Condition (2-8° C)

Consult Instructions for Use

Catalogue Number

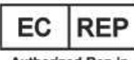
Contains Sufficient Test for Σ

Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer


Authorized Rep in European Country

European Conformity

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
Diagnostic Bioprobes Srl
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(Milano) - Italy
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Fax +39 02 44386771
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 immunosorbent assays (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 hIgG&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 H_2SO_4 O.3 M
1x32ml/bottle. It contains 0.3 M H_2SO_4 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 RECOMMANDATIONS

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° 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 +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated 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 ±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 ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the 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°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 100µl 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 100µl 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 100µl 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 Samples Assay Diluent (DILAS)	200 ul 200ul dil.+10ul 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/H2O2	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 = 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 or 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	3rd 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	3rd 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	3rd 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	3rd 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	3rd 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	3rd 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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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:
Dia.Pro Diagnostic Bioprobes Srl.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy

0318

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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REF CVAB.CE
96/192/480/960 pruebas

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 imnunoabsorción (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 proteinas funcionales involucradas en la replicación viral y la síntesis de proteinas. 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 antigénicos 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 substrato/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 proteinas 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 proteinas del suero de cabra, anticuerpos humanos anti-HCV, tampón Citrato sódico10mM 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-WI, tampón Citrato sódico10mM 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 (H_2O_2) 0.02% así como dimetilsulfóxido 4%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

8. Diluente 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: H_2SO_4 O.3 M
1x32ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Diluente de muestras DILSPE
2x50ml. Contiene una solución tamponada citrato sódico 10 mM pH 6.0 +/- 0.1, 1% de proteinas 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 37ºC.
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 substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 ºC, 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 2-8ºC. 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 quimicamente.

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 quimicamente.
Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

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 solucion prestar mucha atencion 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 8ºC.*

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. Diluente 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. Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

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

1. 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 periodicamente los residuos de los componentes del equipo.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) 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.
3. 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.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
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 $\leq$ 10 nm; b) Rango de absorbancia de 0 a $\geq$ 2,0; c) Linealidad $\geq$ 2,0; d) Reproducibilidad $\geq$ 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.
6. 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 seccione "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 automaticos, en el caso en que los contenedores para los frascos del in strumento no sean adecuados a los frascos del kit, transferir la solucion en ellos contenida en frascos idoneos al instrumento y etiquetarlos con la misma etiqueta utilizada en el frasco original. Esta operacion 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 37ºC 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 Diluente 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 Diluente 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 Diluente de muestras, después de adicionada la misma, cambia de verde a verde azul oscuro.*

4. Dispensar 50 ul de Diluente 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 diluída, segun según se indica (section I.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 Muestras Diluente de ensayo (DILAS)	200 µl 200µl dil.+10µl 50 µl
1ra incubación	**45 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2da incubación	**45 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ra 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:

Valor de corte = 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 diluído 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 diluídas 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	1ra corrida	2da corrida	3ra 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	1ra corrida	2da corrida	3ra 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	1ra corrida	2da corrida	3ra 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	1ra corrida	2da corrida	3ra 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	1ra corrida	2da corrida	3ra 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	1ra corrida	2da corrida	3ra 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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Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni
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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 -

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Diagnostic Bioprobes Srl
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REF SAG1ULTRA.CE
96/192/480/960 Tests

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 0.045% ProClin 300 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 0.045% ProClin 300 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 0.045% 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 Diluent CONJ DIL
2x16ml/vial. Ready to use and pink/red color coded regent. It contains 10 mM Tris buffer pH 6.8+/-0.1, 1% normal mouse serum, 5% BSA, 0.045% ProClin 300 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, 0.045% ProClin 300 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 (H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid H_2SO_4 0.3 M
1x25ml/bottle. It contains 0.3 M H_2SO_4 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. 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. 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.

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 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 sample should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8u 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 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.

5. **Incubation times** have a tolerance of +5%.
6. The **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 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; (d) repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
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°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°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.
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 150µl 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°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, mandatory), blanking the instrument on A1.

Important general notes:

1. *Ensure that no fingerprints or dust are present on the external bottom of the microwell before reading. They could generate false positive results on reading.*
2. *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.*
3. *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 ul of controls, calibrator and samples*
   - *incubate 60 min at +37°C on shaking*
   - *wash according to instructions (section I.4)*
   - *dispense 100 ul diluted enzyme tracer*
   - *incubate 30 min at +37°C on shaking*
   - *wash*
   - *dispense 100 ul 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 Diluted Enzyme Conjugate	150 ul 100 ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	200ul
2nd incubation	**30 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

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 ≥ 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.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 11.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of a cut-off value determined on the mean OD450nm/620-630nm value of the negative control (NC) with the following formula:

NC + 0.050 = 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/620-630nm (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:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 u 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.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *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 (data obtained proceeding as the the reading step described in the section M, point 11):

The following data must not be used instead or 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	1st 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 Total n = 144	Negative Sample	Calibrator 0.5 IU/ml
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, crioglobulins, 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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0318

HBsAg one

Versión ULTRA

Ensayo inmunoenzimático de cuarta generación (ELISA) para la determinación de antígeno de superficie de la hepatitis B o HBsAg en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF SAG1ULTRA.CE
96/192/480/960 pruebas

HBsAg One versión ULTRA

A. OBJETIVO DEL EQUIPO.

Ensayo inmunoenzimático de cuarta generación (ELISA) para la determinación en un paso del antígeno de superficie de la hepatitis B o HBsAg en plasma y suero humanos.
El equipo está diseñado para el cribado en unidades de sangre, es capaz de detectar mutantes de HBsAg y puede aplicarse al seguimiento de pacientes infectados con HBV.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

La Organización Mundial de la Salud (OMS) define la infección con virus de hepatitis B del siguiente modo:

"La Hepatitis B es una de las enfermedades más importantes que aquejan a la humanidad y constituye un problema de salud pública global. El término hepatitis significa inflamación del hígado y la causa más común es la infección por uno de los cinco virus, denominados A, B, C, D y E. Estos virus pueden causar una enfermedad aguda cuyos síntomas persisten por varias semanas, se caracterizan por el color amarillo de la piel y los ojos (ictericia); orina oscura; fatiga extrema; náuseas; vómitos y dolor abdominal. La recuperación puede tardar de varios meses a un año. Los virus de la Hepatitis son causantes de infecciones crónicas en las que el paciente nunca se libera del virus e incluso, años más tarde, desarrolla cirrosis hepática o cáncer de hígado.

El tipo más serio de hepatitis viral es la causada por el HBV, siendo el único tipo, de los que provocan infección crónica, para el cual existe una vacuna disponible. El virus de la Hepatitis B se transmite por contacto con sangre o fluidos corporales de personas infectadas, de la misma forma que el virus de la inmunodeficiencia humana (HIV), agente causal del SIDA. Sin embargo, el HBV es entre 50 y 100 veces más infeccioso que el HIV. Las principales vías de transmisión del HBV son: (a) vía perinatal (transmisión de madre a hijo durante el parto); (b) de niño a niño; (c) mediante inyecciones y transfusiones inseguras (d) por contacto sexual.

A nivel mundial, la mayor parte de las infecciones ocurre de madre infectada a hijo, de niño a niño en hogares y por la reutilización de agujas y jeringuillas sin previa esterilización. En muchos países en desarrollo, casi todos los niños se infectan con el virus. En muchos países desarrollados (Europa Occidental y Norteamérica), el patrón de transmisión es diferente. En estos países, la transmisión de madre a hijo y de niño a niño representaban cerca de un tercio de las infecciones crónicas antes de que se implantara el programa de vacunación infantil. Sin embargo, la mayoría de las infecciones en estos países se adquiere por la actividad sexual durante la adolescencia, y por el consumo de drogas inyectables. Por otra parte, el virus de la hepatitis B constituye el principal riesgo en el trabajo, dentro del colectivo de los profesionales de la salud, motivo por el cual se ha aplicado la vacunación para la protección de los mismos.

El virus de la hepatitis B no se trasmite por la comida o agua contaminadas, ni por contactos casuales en el ámbito laboral. En zonas del Este y Centro de Europa se han encontrado tasas elevadas de infección crónica por HBV. En el Asia Central y en regiones de la India, aproximadamente el 5% de la población está infectada de forma crónica, mientras que en Europa Occidental y Norteamérica, los índices son menores del 1%.

Los niños infectados con HBV, constituyen el grupo más susceptible a la infección crónica. Aproximadamente el 90% de los niños infectados durante el primer año de vida y entre el 30 y el 50% de los niños infectados entre 1 y 4 años, desarrollan este tipo de infección. La mortalidad por cáncer de hígado o cirrosis asociados al HBV es cerca del 25%, entre las personas que han adquirido la infección crónica en la niñez. En ciertos pacientes, la hepatitis B crónica es tratada con interferones o lamivudinas, lo cual puede ayudar en ocasiones. En algunos casos de cirrosis se han realizado trasplantes de hígado, pero el resultado ha sido variable. La prevención de esta enfermedad a través de la vacunación constituye la mejor opción.

La vacuna contra la Hepatitis B tiene índices de seguridad y eficacia demostrados. A partir de 1982, han sido administradas mundialmente alrededor de mil millones de dosis. Se aplica por vía intramuscular en series de tres dosis. Los estudios realizados demuestran un 95% de eficacia en la prevención de la infección crónica en niños y adultos sin infección previa. En muchos países donde el índice de infección crónica en niños oscila entre 8% y 15%, se ha observado una reducción a menos del 1% en grupos de niños inmunizados. Desde 1991, la OMS ha hecho un llamamiento para la introducción de la vacuna contra la hepatitis B en todos los programas nacionales de vacunación."

El antígeno de superficie de la hepatitis B o HBsAg es la proteína más importante de la envoltura del virus, responsable de las hepatitis virales agudas y crónicas.
Contiene el determinante "a", común a todos los subtipos virales conocidos, dividido inmunológicamente en dos subgrupos distintos (ay y ad).
En los últimos años la posibilidad de detectar el HBsAg mediante inmunoensayos altamente sensibles, ha permitido comprender su distribución y epidemiología en el mundo así como la gran disminución del riesgo de infección por transfusiones.

C. PRINCIPIOS DEL ENSAYO.

La superficie de los pocillos está recubierta con una mezcla de anticuerpos monoclonales de ratón específicos para los determinantes "a", "d" e "y" de HBsAg. El suero/plasma del paciente se adiciona al pocillo conjuntamente a una segunda mezcla de anticuerpos monoclonales de ratón conjugada con peroxidasa (HRP) y dirigido contra un epitopo diferente del determinante "a" y contra "preS".
El inmunocomplejo específico, formado en presencia del HBsAg de la muestra, queda capturado en la fase sólida.
Terminada la incubación de un solo paso, los pocillos son lavados para eliminar las proteínas séricas no ligadas y el conjugado HRP.
Después se añade el substrato/ cromogénico, que en presencia del inmunocomplejo de HBsAg capturado, el substrato incoloro es hidrolizado por el conjugado HRP unido, generando un producto final coloreado. Después de bloquear la reacción enzimática, su densidad óptica se mide en un lector ELISA.
La intensidad del color es proporcional a la cantidad de HBsAg presente en la muestra.
La versión ULTRA es especialmente idónea para cribados automatizados y es capaz de detectar mutantes "s".

D. COMPONENTES.

La configuración estándar contiene reactivos suficientes para realizar 192 pruebas y está formada por los siguientes componentes:

1. Microplaca MICROPLATE

n° 2. 12 tiras de 8 pocillos rompibles recubiertos con anticuerpos monoclonales de ratón, purificados por afinidad, anti HBsAg, específicos para determinantes "a", "y" y "d" en una bolsa sellada con desecante.

2. Control negativo CONTROL -

1x4.0 ml/vial. Listo para el uso. Contiene suero de cabra, tampón fosfato 10 mM pH 7.4 +/- 0.1, así como azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control negativo está codificado con color amarillo pálido.

3. Control positivo CONTROL +

1x4.0 ml/vial. Listo para el uso. Contiene suero de cabra, HBsAg recombinante, no infeccioso, tampón fosfato 10 mM pH 7.4 +/- 0.1, además de sulfato de gentamicina 0.02% y ProClin 300 0.045% como conservantes. El control positivo está codificado con el color verde.

4. Calibrador CAL ….

n° 2 viales. Calibrador liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, HBsAg recombinante no infeccioso a 0.5 IU/mL (2° Estándar internacional O.M.S. para HBsAg, NIBSC código

00/588), tampón fosfato 10 mM pH 7.4 +/- 0.1, así como sulfato de gentamicina 0.02% 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. Solución de lavado concentrada 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. Diluente de conjugado CONJ DIL
2x16ml/vial. Listo para el uso y reactivo codificado con color rosa/rojo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 1% de suero de ratón normal, 5% de BSA, además 0.02% de sulfato de gentamicina y ProClin al 300 0.045% como conservantes. La solución es normalmente opalescente.

7. Conjugado CONJ 20X
2x1ml/vial. Reactivo concentrado 20X. Contiene anticuerpos monoclonales de ratón anti HBsAg marcados con peroxidasa (HRP), determinante "a" y preS", tampón Tris 10 mM a pH 6.8+/-0.1, 5% BSA, ProClin 300 al 0.045% y sulfato de gentamicina 0.02% como conservantes.

8. Cromógeno/substrato SUBS TMB
2x25ml/botella. Contiene solución tamponada citrato-fosfato 50 mM a pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

9. Ácido sulfúrico H_2SO_4 O.3 M
1x25ml/botella. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Sellador adhesivo, n° 4

11. Manual de instrucciones

Nota importante:
A solicitud del cliente, Dia.Pro puede suministrar reactivos para 96, 480, 960 pruebas, como se describe a continuación**:**

Microplacas	N°1	N°5	N°10
Control negativo	1x2ml/vial	1x10ml/vial	1x20 ml/vial
Control Positivo	1x2ml/vial	1x10ml/vial	1x20 ml/vial
Calibrador	N° 1 vial	Viales n.º 5	Viales n.º 10
Solución de lavado concentrada	1x60ml/vial	5x60ml/vial	4x150ml/vial
Conjugado	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Diluente de conjugado	1x16ml/vial	2x40ml/vial	2x80ml/vial
Cromógeno/substrato	1x25 ml/vial	3x42ml/vial	2x125ml/vial
Ácido Sulfúrico	1x15 ml/vial	2x40ml/vial	2x80ml/vial
Sellador adhesivo	N° 2	N° 10	N° 20
Manual de instrucciones	N° 1	N° 1	N° 1
Número de pruebas	96	480	960
Código SAG1ULTRA.CE	.96	.480	.960

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (150, 100 y 50 µl) y puntas de plástico 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 (en seco o húmedo), capaz de agitar a 1300 rpm+/-150, ajustado 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. Cuando el equipo se utiliza para el cribado de unidades de sangre y componentes sanguíneos, debe utilizarse en un laboratorio certificado y homologado por la autoridad nacional en ese campo (Ministerio de Sanidad o entidad similar) para realizar dicho tipo de análisis.
3. Todo el personal que participe en la realización de los ensayos deberá llevar la indumentaria protectora adecuada de laboratorio, guantes sin talco 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 entorno del laboratorio para evitar la contaminación por polvo o agentes microbianos en el aire al abrir los viales del equipo y las microplacas, así como durante la realización del ensayo. Evitar la exposición del cromógeno (TMB) a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
7. No intercambiar componentes de diferentes lotes ni tampoco de diferentes equipos del mismo lote.
8. Comprobar que los reactivos no contengan 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 vencimiento indicada en el equipo e internamente en los reactivos. En un estudio realizado con un equipo abierto no se ha detectado pérdida de actividad relevante utilizándolo hasta 6 veces y durante 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 cruzadas.
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. La solución de parada 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 la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar la adición de conservantes a las muestras, en particular azida sódica, ya que podría afectar a la actividad enzimática del conjugado, generando resultados falsos negativos.
3. Las muestras deben ser 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 y la lectura electrónica.
4. Las muestras hemolizadas (color rojo) o lipémicas (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 porque pueden dar lugar a fasos positivos. Las muestras con una vía de coagulación alterada, que presentan partículas tras la extracción y preparación de suero/plasma y las que proceden de pacientes hemodializados, pueden originar resultados falsos positivos.
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 almenos 12 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 algo de turbidez o se sospecha de la presencia de micropartículas tras descongelar, filtrar la muestra en un filtro de 0.2-0.8u desechable para limpiarla para las pruebas o usar el método alternativo de dos pasos.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Estudios de estabilidad realizados en equipos en uso no han arrojado pérdida de actividad significativa 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 color verde, lo que indicaría un defecto de conservación.
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 2-8ºC. Cuando se abre por primera vez, las tiras sobrantes se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambia de amarillo a verde.

2. Control negativo:
Listo para el uso. Mezclar bien con un vórtex antes de usar.

3. Control positivo:
Listo para el uso. Mezclar bien con un vórtex antes de usar. El control positivo no contiene ningún HBV infeccioso ya que se compone de HBsAg recombinante sintético.

4. Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta; dejar disolver completamente y después mezclar cuidadosamente con el vórtex antes de usar. Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.

5. Solución de lavado concentrada:
La solución concentrada 20x debe diluirse con agua de calidad EIA hasta 1200 ml y mezclarse suavemente antes del uso. Dado que pueden existir algunos cristales de sal en el vial, debe prestarse atención a que todo el contenido quede disuelto al preparar la solución.
Durante la preparación hay que evitar la formación de espuma y burbujas, que podrían reducir la eficiencia de lavado.
Nota: Una vez diluida, la solución es estable durante una semana a temperaturas entre +2 y 8ºC.

6. Conjugado:
La solución de trabajo se prepara diluyendo el reactivo concentrado 20X con el Diluente de Conjugado.
Mezclar cuidadosamente con el vórtex antes de usar.
Evitar la contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables únicamente.
***Nota importante**: La solución de trabajo no es estable. Preparar solamente el volumen necesario para el trabajo del día. Como un ejemplo cuando el equipo se utiliza en combinación con otros instrumentos o manualmente, diluir 0.1 ml de Conjugado 20X con 1.9 ml de Diluente de Conjugado en un vial de plástico desechable y mezclar cuidadosamente antes de usar.*

7. Cromógeno/ Substrato:
Listo para el uso. Mezclar bien volteando.
Evitar la 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 volteando.
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.

1. **Las micropipetas** deben estar 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%, desinfectantes de calidad hospitalaria). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La **incubadora ELISA** debe ser ajustada a 37ºC (+/- 1ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadores secos o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. En caso de **agitación** durante las incubaciones, el instrumento debe garantizar 350 rpm ±150. La amplitud de la agitación es muy importante ya que si es errónea pueden producirse salpicaduras y por lo tanto falsos positivos.
4. 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.
5. Los tiempos de incubación deben tener un margen de ±5%.
6. El **lector de microplacas** debe estar provisto de un filtro de lectura de 450 nm 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 <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=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.
7. En caso de usar **sistemas automatizados ELISA**, los pasos críticos (dispensado, incubación, lavado, lectura, agitación, procesamiento de datos, etc.) 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 comprobando la plena coincidencia de los rendimientos declarados del equipo. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente, prestando particular atención a evitar el arrastre por las agujas de dispensación de muestras y de lavado. Debe estudiarse y controlarse el efecto de arrastre a fin de minimizar la posibilidad de contaminación de pocillos adyacentes debido a muestras muy reactivas, lo que provocaría resultados falsos positivos. Se recomienda el uso de sistemas automatizados de Elisa para el cribado en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por serie.
8. Cuando se utilizan instrumentos automáticos, en el caso en que los contenedores para viales del instrumento no se ajusten a los viales del equipo, debe transferirse la solución a contenedores adecuados y marcarlos con la misma etiqueta despegada del vial original. Esta operación es importante para evitar la falta de coincidencia de los contenidos de los viales al transferirlos. Cuando la prueba termine, guardar los contenedores secundarios etiquetados a 2-8°C, firmemente cerrados.
9. 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 esenciales del ensayo. También se ofrece apoyo para la instalación de nuevos instrumentos a usar en combinación con el equipo.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Comprobar la fecha de caducidad indicada en la etiqueta externa de la caja del equipo. No usar si ha caducado.
2. Comprobar que los componentes líquidos no están contaminados con partículas ni agregados observables a simple vista. Comprobar que el cromógeno/substrato es incoloro o azul pálido. Comprobar que no se han producido roturas ni derrames de líquido dentro de la caja 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. Diluir el conjugado concentrado 20X con su diluente, tal y como se describe.
5. Disolver el calibrador como se ha descrito anteriormente.
6. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego según se describe.
7. Ajustar la incubadora de ELISA a 37ºC y alimentar 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.
8. Comprobar que el lector ELISA ha sido encendido al menos 20 minutos antes de realizar la lectura.
9. Si se utiliza un sistema automatizado, encenderlo y comprobar que los protocolos estén correctamente programados.
10. Comprobar que las micropipetas estén fijadas en el volumen requerido.
11. Asegurarse de que el resto de equipamiento esté disponible y listo para el uso.
12. 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.

Ensayo automatizado:

En caso de que el ensayo se realice automáticamente con un sistema ELISA, se recomienda que el instrumento dispense primero 150 ul de controles y calibrador, después todas las muestras y finalmente 100 ul de conjugado diluido.
Para el paso de pre-lavado (primer punto del procedimiento del ensayo) y 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 y hacer un ciclo de lavado para hidratar los pocillos. Identificar cuidadosamente los pocillos de controles, calibradores y muestras.

Nota importante: El prelavado (1 ciclo: dispensación de 350 ul de solución de lavado por pocillo además de aspiración) es fundamental para obtener resultados confiables y específicos tanto en el procedimiento automático como en el manual. ¡No omitir!

2. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 150µl del Control Negativo, por triplicado, 150µl de Calibrador por duplicado y 150 µl del Control Positivo. Posteriormente, añadir 150 µl de cada muestra.
4. Comprobar la presencia de las muestras en los pocillos a simple vista (existe una marcada diferencia de color entre los llenos y los vacíos) o por lectura a 450/620nm. (la densidad óptica de las muestras es superior a 0.100).
5. Dispensar 100 µl del Conjugado diluido en todos los pocillos, excepto en el A1 que se utiliza para operaciones de blanco.

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.*

6. Después de la adición del conjugado comprobar que las muestras han cambiado de color amarillo a rosa/rojo y después incubar la microplaca por **120 min a +37°C**.

Notas importantes:

c. *Las tiras se deben sellar con el adhesivo suministrado sólo cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*
d. *Si el proceso es efectuado agitando, asegúrese de tener las mismas rpm de la sección I.3. De lo contrario se podría verificar contaminación dentro el pocillo.*

7. Tras la primera incubación, lavar los pocillos como se ha descrito previamente (sección I.4).
8. Dispensar 200 µl de cromógeno/substrato en todos los pocillos, incluido el A1.

Nota importante: *No exponer directamente a fuerte iluminación, de lo contrario se puede generar un fondo excesivo.*

9. Incubar la microplaca protegida de la luz a **18-24°C durante 30 minutos.** Los pocillos con control positivo, calibrador y muestras positivas deben pasar de un tono claro a azul.
10. Dispensar 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática usando la misma secuencia que en el paso 8. La adición de la solución de ácido cambiará el color del control positivo, el calibrador y las muestras positivas de azul a amarillo/ marrón.
11. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.6, 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 generales importantes:

1. *Asegurarse de que no hay impresiones digitales ni polvo en el fondo externo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debería hacerse inmediatamente después de añadir la solución de ácido y, en cualquier caso, nunca transcurridos más de 20 minutos de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *Cuando las muestras que se van a analizar no sean seguramente limpias o hayan estado congeladas, se recomienda seguir el procedimiento abajo descrito en cuanto es menos susceptible a la interferencia de la hemólisis, hiperlipemia, contaminación bacteriana y micropartículas de fibrina. El ensayo se realiza en dos pasos a +37°C con agitación a* 350 rpm ±150 *como sigue:*
   a. *dispensar 100 µl de controles, calibradores y muestras*
   b. *incubar 60 min a +37°C con agitación*
   c. *lavar según las instrucciones (sección I.4)*
   d. *dispensar 100 ul de trazador enzimático diluido*
   e. *incubar 30 min a +37°C con agitación*
   f. *lavar*
   g. *dispensar 100 ul de mezcla TMB y H2O2*
   h. *incubar 30 min a t.amb. con agitación*
   i. *parar y leer*

   En este procedimiento se puede omitir el prelavado. Este método muestra un rendimiento similar al método estándar por lo cual puede ser utilizado como alternativa.
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.

Operaciones	Procedimiento
Paso de pre-lavado	ciclo n° 1
Controles&Calibradores& muestras Conjugado diluido	150 µl 100 µl
1ra incubación	**120 min**
Temperatura	+37°C
Pasos de lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Cromógeno/substrato	200ul
2da incubación	**30 min**
Temperatura	temperatura ambiente
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm/620-630nm

En la sección siguiente 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	M1	M9										

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

O. CONTROL DE CALIDAD INTERNO.
Se realiza una comprobación en los controles/calibrador cada vez que se usa el equipo para verificar si los valores DO450nm / 620-630 nm o M/Co son los esperados en el análisis.

Asegurar el cumplimiento de los siguientes resultados:

Parámetro	Exigencia
Pocillo blanco	valor < 0.100 DO450nm
Control negativo (CN)	Valor medio < 0.050 de DO450nm después de leer el blanco
Calibrador 0.5 IU/ml	M/Co > 2
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 > 0.100 DO450nm	1. 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 alimentado 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 < 2	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en su distribución (por ejemplo, dispensar control negativo en lugar de 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 tendrá un valor de DO450nm > 0.050). 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.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 11.

P. CÁLCULO DEL VALOR DE CORTE.
Los resultados de las pruebas se calculan a partir de un valor de corte determinado con la fórmula siguiente sobre el valor medio de DO450nm/620-630nm del control negativo (CN):

CN + 0.050 = Valor de corte (Co)

El valor encontrado en la prueba es utilizado 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 relación entre el valor de DO450nm/620-630nm de la muestra (M) y el valor de corte (Co), matemáticamente 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 HBV 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 recogida 1 o 2 semanas después de la inicial. En este caso la unidad de la sangre no debe ser transfundida.
Un resultado positivo es indicativo de infección por HBV y por consiguiente el paciente debe ser tratado adecuadamente o 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. *Cualquier resultado positivo debe confirmarse antes repitiendo el ensayo sobre la muestra, después de haberla filtrado en un filtro de 0.2-0.8 u para eliminar la interferencia de las micropartículas. Después, si todavía es positivo, la muestra debe someterse a una prueba de confirmación antes de emitir un diagnóstico de hepatitis viral.*
3. *Cuando se transmiten los resultados de la prueba, del laboratorio a otro departamento, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
4. *El diagnóstico de infección con un virus de la hepatitis debe ser realizado y comunicado al paciente por un médico cualificado.*

A continuación se describe un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 11).

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

***Control negativo**: 0.012 – 0.008 – 0.010 DO450nm*
Valor medio: 0.010 DO450nm
Menor de 0.050 – Válido

***Control positivo**: 2.489 DO450nm*
Mayor de 1.000 – Válido
Valor de corte = 0.010+0.050 = 0.060
Calibrador: 0.350 - 0.370 DO 450nm
Valor medio: 0.360 DO450nm M/Co = 6.0
M/Co Mayor de 2.0 – Válido
Muestra 1: 0.028 DO450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 0.9 = negativa
Muestra 2 M/Co > 1.1 = positiva

R. CARACTERÍSTICAS DEL RENDIMIENTO.

La evaluación del rendimiento ha sido realizada según lo establecido en las Especificaciones Técnicas Comunes (ETC) (Art. 5, Capítulo 3 de la Directiva IVD 98/79/CE). La versión ULTRA ha demostrado ser al menos equivalente al diseño original en un estudio realizado para la validación de la nueva versión.

1. Sensibilidad analítica

El límite de detección del ensayo se ha calculado sobre el 2° estándar internacional O.M.S., NIBSC código 00/588.
En la siguiente tabla se muestran los resultados de tres lotes (P1, P2 y P3) de la versión ULTRA en comparación con el dispositivo de referencia (Ref.):

O.M.S. IU/ml	Lote P1 M/Co	Lote P2 M/Co	Lote P3 M/Co	Ref. M/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
SFB (CN)	0.3	0.2	0.3	0.1

El ensayo mostró una sensibilidad analítica mejor a 0.1 O.M.S. IU/ml de HBsAg.

Además se probaron dos paneles de sensibilidad suministrados por EFS, Francia, y por SFTS, Francia, y se obtuvieron los siguientes resultados en condiciones óptimas:

Panel EFS Ag HBs HB1-HB6 lote n° 04

ID muestra	Características	ng/ml	M/Co
HB1	diluente	/	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**

Panel de sensibilidad SFTS, Francia, Ag HBs 2005

ID muestra	Características	ng/ml	M/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	diluente	/	0,6

El panel n.º 808, suministrado por Boston Biomedical Inc., Estados Unidos, también se probó para definir el límite de sensibilidad.
Los resultados en condiciones óptimas son los siguientes:

BBI panel PHA 808

ID muestra	Características	ng/ml	M/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	negativo	/	0,6

2. Sensibilidad Diagnóstica:

La sensibilidad diagnóstica ha sido probada según lo establecido en las Especificaciones Técnicas Comunes (ETC) de la Directiva 98/79/CE en IVD para pruebas HBsAg.
Las muestras positivas, incluidos los subtipos de HBsAg y un panel de mutantes "s" de las mutaciones más frecuentes, se recogieron de distintas patologías de HBV (hepatitis B aguda, asintomática y crónica) o producidas sintéticamente, y se detectaron positivas en el ensayo.
Todos los subtipos conocidos de HBsAg, "ay" y "ad", y los isoformes "w" y "r", suministrados por CNTS, Francia, se probaron en el ensayo y el equipo los determinó positivos según lo previsto.
Se ha hallado un valor global de 100% en un estudio realizado sobre un número total de más de 400 muestras positivas con la referencia original IVD código SAG1.CE, marca CE.
Se estudiaron 30 sero-conversiones en total, la mayoría producidas por Boston Biomedica Inc., EE.UU.
Los resultados obtenidos al examinar ocho paneles suministrados por Boston Biomedica Inc., EE.UU., se indican abajo para la versión ULTRA en comparación con el dispositivo de referencia código SAG1.CE.

Panel ID	1ª muestra positiva	HBsAg subtipo	HBsAg ng/ml	Versión ULTRA M/Co	Ref. dispositivo M/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. Especificidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico. Además del primer estudio, donde se examinaron más de 5000 muestras negativas de donantes de sangre (dos centros de donación) clasificadas como negativas con un dispositivo con marca CE en uso en el laboratorio de recogida, la especificidad diagnóstica se evaluó recientemente examinando un total de 2288 muestras de donantes de sangre negativas en siete 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 para determinar la especificidad.
No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.
Las muestras congeladas también se han probado para comprobar si la congelación interfiere con el rendimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.
Se examinaron muestras procedentes de pacientes afectados por hepatitis víricas (HCV, HVA) y patologías no víricas del hígado, que pudieran provocar interferencia en el ensayo. No se detectó reacción cruzada.

4. Precisión:
Ha sido calculada para la versión ULTRA en dos muestras examinadas en 16 réplicas en 3 series diferentes para tres lotes.
Los resultados se indican en la siguiente tabla:

Valores promedio Total n = 144	Negativo Muestra	Calibrador 0.5 IU/ml
DO450nm	0.026	0.332
Desviación estándar	0.004	0.027
CV %	16%	8%

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

S. LIMITACIONES.
Se evaluaron resultados falso positivo repetibles en muestras recién recogidas en menos del 0.1% de la población normal, debido principalmente a altos títulos de anticuerpo anti ratón heterofílicos (HAMA).
También se observaron interferencias en muestras frescas cuando no estaban libres de partículas o se recogieron incorrectamente (ver capítulo G).
Las muestras antiguas o congeladas, con coágulos de fibrina, crioglobulinas, micelas que contienen lípidos o micropartículas después de almacenar o descongelar, pueden generar 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 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 -

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REF. BCAB.CE
96 Tests

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 0.045% ProClin 300 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 0.045% ProClin 300 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 0.045% ProClin 300 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 0.045% 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 mouse monoclonal antibody to HBcAg in presence of 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300. 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 (H_2O_2) 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 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference. The component is blue colour coded.

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

9. Sulphuric Acid H_2SO_4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

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. 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 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 RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
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 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 (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 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 ±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 ≥ 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 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 (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.
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 performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

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. Dispense 50 ul Specimen Diluent into all the control and sample wells.
4. Pipette 50 µl of the Negative Control in triplicate, 50 ul of the Calibrator in duplicate and then 50 ul of the Positive Control in single. Then dispense 50 ul of each of the samples.
5. 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.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. 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.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. 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.*

10. 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).
11. 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.
12. 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 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

Specimen Diluent	50 ul
Controls&calibrator and samples	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash	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	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	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
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/620-630nm 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.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. RESULTS

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

Cut-Off = (NC + 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 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 (data obtained proceeding as the the reading step described in the section M, point 12):

The following data must not be used instead or 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

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. In addition to the first study, where a total of 5179 unselected donors, including 1st time donors, 206 samples from hospitalized patients and 164 potentially interfering specimen were examined, the diagnostic specificity was recently assessed by testing a total of 1498 negative samples on seven different lots. A value of specificity of 100% was observed. In addition to the above population, 189 potentially interfering samples (other liver diseases,pregnant women, hemolized, lipemic, RF positives) have been tested and found negative, confirming a 100% of specificity of the device.
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.
In addition to the first Performance Evaluation Study, in order to further evaluate the diagnostic sensitivity of the device, a total of 262 positive samples were recently evaluated. The respective results, collected from seven different lots of the device show a diagnostic sensitivity of 100%.

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	3rd 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	3rd 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	3rd 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	3rd 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	3rd 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	3rd run	Average value
OD 450nm	0.193	0.190	0.199	0.134
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.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

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.

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0318

HBcAb

Ensayo inmunoenzimático competitivo (ELISA) para la determinación de anticuerpos frente al Antígeno *core* del virus de la Hepatitis B en plasma y suero humanos

Uso exclusivo para diagnóstico "in vitro"

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REF. BCAB.CE
96 pruebas

HBcAb

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático competitivo (ELISA) para la determinación de anticuerpos frente al antígeno *core* del virus de la Hepatitis B en plasma y suero.
El equipo está diseñado para el cribado en unidades de sangre y para el seguimiento de los pacientes infectados con HBV.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
La Organización Mundial de la Salud (OMS) define la infección por el virus de la Hepatitis B como:

"La Hepatitis B es una de las enfermedades más importantes que aquejan a la humanidad y constituye un problema de salud pública global. El término hepatitis significa inflamación del hígado, y la causa más común es la infección por uno de los cinco virus, denominados A, B, C, D y E. Estos virus pueden causar una enfermedad aguda cuyos síntomas persisten por varias semanas, se caracterizan por el color amarillo de la piel y los ojos (ictericia); orina oscura; fatiga extrema; náuseas; vómitos y dolor abdominal. La recuperación puede tardar de varios meses a un año. Los virus de la Hepatitis son causantes de infecciones crónicas en las que el paciente nunca se libera del virus e incluso, años más tarde, desarrolla cirrosis hepática o cáncer de hígado.
El tipo más serio de hepatitis viral es la causada por el HBV, siendo el único tipo, de los que provocan infección crónica, para el cual existe una vacuna disponible. El virus de la Hepatitis B se transmite por contacto con sangre o fluidos corporales de personas infectadas, de la misma forma que el virus de la inmunodeficiencia humana (HIV), agente causal del SIDA. Sin embargo, el HBV es entre 50 y 100 veces más infeccioso que el HIV. Las principales vías de transmisión del HBV son: (a) vía perinatal (transmisión de madre a hijo durante el parto); (b) de niño a niño; (c) mediante inyecciones y transfusiones inseguras (d) por contacto sexual.
A nivel mundial, la mayor parte de las infecciones ocurre de madre infectada a hijo, de niño a niño en hogares infantiles y por la reutilización de agujas y jeringuillas sin previa esterilización. En muchos países desarrollados (Europa Occidental y Norteamérica), el patrón de transmisión es diferente. En estos países, la transmisión de madre a hijo y de niño a niño representaban cerca de un tercio de las infecciones crónicas antes de que se implantara el programa de vacunación infantil. Sin embargo, la mayoría de las infecciones en estos países se adquiere por la actividad sexual durante la adolescencia, y por el consumo de drogas inyectables. Por otra parte, el virus de la hepatitis B constituye el principal riesgo en el trabajo, dentro del colectivo de los profesionales de la salud, motivo por el cual se ha aplicado la vacunación para la protección de los mismos.
El virus de la hepatitis B no se trasmite por la comida o agua contaminadas, ni por contactos casuales en el ámbito laboral. En zonas del Este y Centro de Europa se han encontrado tasas elevadas de infección crónica por HBV. En el Asia Central y en regiones de la India, aproximadamente el 5% de la población está infectada de forma crónica, mientras que en Europa Occidental y Norteamérica, los índices son menores del 1%.
Los niños infectados con HBV, constituyen el grupo más susceptible a la infección crónica. Aproximadamente el 90% de los niños infectados durante el primer año de vida y entre el 30 y el 50% de los niños infectados entre 1 y 4 años, desarrollan este tipo de infección. La mortalidad por cáncer de hígado o cirrosis asociados al HBV es cerca del 25%, entre las personas que han adquirido la infección crónica en la niñez. En determinado grupo de pacientes, la hepatitis B crónica es tratada con interferones o lamivudinas, lo cual puede ayudar en ocasiones. En algunos casos de cirrosis se han realizado trasplantes de hígado, pero el resultado ha sido variable.
La prevención de esta enfermedad a través de la vacunación, constituye la mejor opción. La vacuna contra la Hepatitis B tiene índices de seguridad y eficacia demostrados. A partir de 1982, han sido administradas mundialmente, alrededor de un billón de dosis. Se aplica por vía intramuscular en series de tres dosis. Los estudios realizados demuestran un 95% de eficacia en la prevención de la infección crónica en niños y adultos sin infección previa. En muchos países donde el índice de infección crónica en niños oscila entre 8% y 15%, se ha observado una reducción a menos del 1% en grupos de niños inmunizados. Desde 1991, la OMS ha hecho un llamamiento para la introducción de la vacuna contra la hepatitis B en todos los programas nacionales de vacunación."
El antígeno core *del virus de la Hepatitis B (HBcAg) es el elemento principal del núcleo viral. Está compuesto por un polipéptido simple de 17 kD, el cual es liberado en el proceso de disgregación de la partícula viral. Este antígeno contiene al menos un determinante inmunogénico. Durante la infección primaria, los anticuerpos anti- HbcAg, son unos de los primeros marcadores del HBV que aparecen en el suero, poco antes de que aparezca el antígeno de superficie (HBsAg). Los títulos de anticuerpos producidos contra HBcAg son altos y pueden ser detectados incluso varios años después de la infección. Debido a su presencia en bolsas de sangre de donantes se ha implementado esta técnica para el cribado en las unidades de sangre.*

La determinación de HBcAb es de gran importancia para la clasificación del agente viral en suero y plasma, conjuntamente con la detección del resto de los marcadores de la infección por HBV.

C. PRINCIPIOS DEL ENSAYO.
El ensayo es de tipo competitivo, donde los anticuerpos de la muestra compiten con un anticuerpo monoclonal por el antígeno de la fase sólida.
Los pocillos de la placa están recubiertos con el antígeno *core* del virus de la hepatitis B, obtenido por vía recombinante y purificado.
El suero/plasma de los pacientes se añade a los pocillos conjuntamente a una solución capaz de bloquear interferencias que puedan deberse a la naturaleza de la muestra.
A continuación, previo lavado que elimina los componentes no fijados de la muestra, se adiciona un anticuerpo monoclonal anti-HBcAg conjugado con Peroxidasa (HRP), el cual se une a cualquier traza de antígeno remanente en la placa.
Después de una segunda incubación, los pocillos son lavados para eliminar el conjugado en exceso, luego se añade el substrato cromogénico, que en presencia de la peroxidasa es hidrolizado a un producto final con color.
La intensidad del color es inversamente proporcional a la presencia de anticuerpos al HBcAg presentes en la muestra.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca MICROPLATE
12 tiras de 8 pocillos recubiertos con HBcAg recombinante, 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 2-8ºC.

2. Control Negativo CONTROL -
1x1.0ml/vial. Listo para el uso. Contiene 5% de albúmina de suero bovino, tampón fosfato 10 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 +

1x1.0ml/vial. Listo para el uso. Contiene 5% de albúmina de suero bovino, anticuerpos anti HBcAg a una concentración aproximada de 10 PEI U/ml, (Calibrado según PEI HBc Reference Material 82), tampón fosfato 10 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 ...

vial nº 1. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, anticuerpos humanos al HBcAg en una concentración de 2 PEI U/ml +/- 10% (Calibrado según PEI HBc Reference Material 82) y ProClin 300 0.045% como conservante.

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 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 monoclonal de ratón anti-HBcAg conjugado con peroxidasa (HPR) en presencia de 0.3 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes. El conjugado está codificado con el color rojo.

7. Cromógeno/Substrato SUBS TMB

1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.6 +/- 0.1, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido 4%.

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

8. Diluente de muestras DILSPE

4x3ml/vial. Contiene una solución tamponada Tris 10 mM pH 8.0 +/- 0.1 y ProClin 300 0.045% para el pretratamiento de las muestras y controles en la placa, así como para bloquear inespecificidades. El componente está codificado con el color azul.

Note: Use todo el contenido del vial antes de abrir un segundo. El reactivo es sensible a oxidación.

9. Ácido Sulfúrico: H_2SO_4 0.3 M

1x15ml/vial. Contiene solución de H_2SO_4 0.3M

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

10. Sellador adhesivo, n° 2

11. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (100µl y 50µl) 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 37ºC.
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. 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 substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 ºC, 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.
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.
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.
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 almenos 12 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, en uso por 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 conservación. 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 2-8ºC. 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:

Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.

Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

5. Solución de Lavado Concentrada:

Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada 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 8ºC.*

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. Diluente de muestras :

Solución lista para el uso. Mezclar bien con un vórtex antes de usar. Use todo el contenido del vial antes de abrir un segundo. El reactivo es sensible a oxidación.

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.

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

1. 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 (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%.

2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) 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.
3. 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.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=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 la correcta medición de la densidad óptica, según las normas del fabricante.
6. 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 cribado en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. 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 usando un vórtex.
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 37ºC 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.

1. Poner el número necesario de tiras en el soporte plástico e identificar cuidadosamente los pocillos para los controles, calibrador y muestras.
2. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 50µl de Diluente de Muestras en todos los pocillos para muestras y controles & calibrador.
4. Dispensar 50µl del Control Negativo, por triplicado, 50µl de Calibrador, por duplicado, y 50µl del Control Positivo. Posteriormente, añadir 50µl de cada muestra.
5. Incubar la microplaca durante **60 minutos a +37ºC.**

Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Después de la primera incubación, lavar los pocillos según lo descrito previamente (sección I.3).
7. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1; 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.*

8. Después de la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
9. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1.

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

10. Incubar la microplaca protegida de la luz a **temperatura ambiente (18-24°C) durante 20 minutos**. Los pocillos con Control Negativo y muestras negativas deben pasar de un tono claro a azul, (método competitivo).

11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición de la solución de parada cambia el color del Control Negativo y las muestras negativas de azul a amarillo.
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, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

1. *Asegurarse de que no hay impresiones digitales 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. *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.

Diluente de Muestras	50 ul
Controles&Calibrador y muestras	50 ul
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 ul
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H_2O_2	100 ul
3ra incubación	**20 min**
Temperatura	t.a.*
Acido Sulfúrico	100 ul
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

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	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/calibrador cada vez que se usa el equipo para verificar si los valores DO450nm / 620-630 nm o Co/M son los esperados.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	Valor < 0.050 DO450nm
Control Negativo (CN)	Valor > 1.000 DO450nm después de leer el blanco Coeficiente de variación < 20%
Calibrador (aprox. 2 PEI U/ml)	Co/M > 1
Control Positivo	Valor< 0.200 DO450nm

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 > 0.050 DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) < 1.000DO450nm después de leer el blanco Coeficiente de variación > 20%	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 Co/M < 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 > 0.200DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 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 12.

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

Valor de corte = (CN + 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 el Valor de corte y las DO a 450nm / 620-630nm de las muestras (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 noestáinfectado por HBV.
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 la sangre no debe ser transfundida.
Un resultado positivo es indicativo de infección por HBV y por consiguiente el paciente debe ser tratado adecuadamente. La unidad de la 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. *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, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

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

Control Negativo: 2.000 – 2.200 – 2.000 DO 450nm
Valor medio: 2.100 DO 450nm
Mayor de 1.000 – Válido

Control Positivo: 0.100 DO 450nm
Menor de 0.200 – Válido

Valor de corte = (2.100 + 0.100) / 5 = 0.440

Calibrador: 0.400-0.360 DO 450nm
Valor medio: 0.380 DO 450nm
Co/M>1 – Válido
Muestra 1: 0.028 DO 450nm
Muestra 2: 1.890 DO 450nm
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.
La sensibilidad del ensayo ha sido calculada por medio de una preparación estándar de referencia para HBcAb suministrada por el Instituto Paul Erlich (PEI HBc Reference Material 82). El ensayo muestra una sensibilidad de aproximadamente 1.25 PEI U/ml.

La siguiente tabla muestra los valores de Co/M para PEI estándar diluido, como se sugiere por el fabricante, para construir la curva de dilución límite en suero fetal bovino (SFB).

PEI U/ml	Lote 1001	Lote 0702	Lote 0702/2	Lote 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

Se evaluaron además, paneles Accurun 1 – series 3000 – suministrados por Boston Biomedical Inc., Estados Unidos, a fin de determinar sus valores Co/M. Los resultados obtenidos se muestran a continuación:

Accurun 1 – series 3000

Valor	Lote 1001	Lote 0702	Lote 1202
Co/M	2.9	2.3	2.2

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS.
La evaluación del procedimiento diagnóstico se realizó mediante un ensayo con más de 6000 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 5179 muestras de donantes no seleccionados, incluyendo donantes por 1ª vez, 206 muestras de pacientes hospitalizados y 164 muestras que pudieran provocar interferencia, la especificidad diagnóstica se evaluó recientemente examinando un total de 1498 muestras negativas en siete lotes distintos. Se observó un valor de especificidad de 100%. Además de la población anterior, se examinaron 189 muestras que pudieran provocar interferencia (pacientes con otras enfermedades hepáticas, mujeres embarazadas, hemolizadas, lipémicas, RF positivas) y se encontraron negativas, confirmando un 100% de especificidad del dispositivo.
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.

2.2 Sensibilidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
Además del primer estudio de evaluación del rendimiento, para evaluar adicionalmente la sensibilidad diagnóstica del producto, se examinaron recientemente un total de 262 muestras positivas. Los resultados correspondientes, obtenidos de siete lotes distintos del dispositivo, muestran una sensibilidad diagnóstica de 100%.

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

BCAB.CE: lote # 1202

Control Negativo (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	1.943	1.939	1.924	1.935
Desviación estándar	0.081	0.078	0.103	0.087
CV %	4.2	4.0	5.3	4.5

Calibrador (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.143	0.147	0.148	0.146
Desviación estándar	0.014	0.017	0.018	0.016
CV %	9.8	11.4	12.1	11.1
Co/M	2.8	2.7	2.6	2.7

BCAB.CE: lote # 0702

Control Negativo (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.163	2.110	2.106	2.126
Desviación estándar	0.105	0.088	0.139	0.111
CV %	4.9	4.2	6.6	5.2

Calibrador (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.182	0.193	0.195	0.190
Desviación estándar	0.018	0.023	0.019	0.020
CV %	10.0	12.0	9.9	10.6
Co/M	2.5	2.2	2.3	2.3

BCAB.CE: lote# 0702/2

Control Negativo (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.278	2.098	2.130	2.169
Desviación estándar	0.135	0.126	0.159	0.140
CV %	5.9	6.0	7.5	6.5

Calibrador (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.193	0.190	0.199	0.134
Desviación estándar	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/M	2.4	2.2	2.2	2.3

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 12.

S. LIMITACIONES DEL PROCEDIMIENTO.
La contaminación bacteriana de las muestras o la inactivaciòn 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

Carcinoembryonic Antigen Next Generation (CEA-Next Generation) Test System

Product Code: 4625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Carcinoembryonic Antigen (CEA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 kDA. CEA is the first of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freeman.[1] CEA is the most widely used marker for gastrointestinal cancer.

Although CEA is primarily associated with colorectal cancers, other malignancies that can cause elevated levels of CEA include breast, lung, stomach, pancreas, ovary and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver cancer.[2,3] Heavy smokers, as a group, have higher than normal baseline concentration of CEA.

In this method, CEA 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 CEA) are added and the reactants mixed. Reaction between the various CEA antibodies and native CEA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CEA antibody bound conjugate is separated from the unbound enzyme-CEA 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 carcinoembryonic antigen (CEA) 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 CEA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay 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-CEA 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:

$$^{Enz}Ab + Ag_{CEA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{CEA} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$ + $Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

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:

A. CEA Next Generation Calibrators – 1ml/vial Icons A-F
Six (6) vials of references CEA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 100(**E**) and 250(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
Note: The standards, human serum based, were calibrated using a reference preparation, which was assayed against the 1st International Reference Preparation (IRP# 73/601).

B. CEA Next Generation Enzyme Reagent -13ml/vial -Icon E
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, red dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See “Reagent Preparation.”

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See “Reagent Preparation.”

G. Stop Solution – 8ml/vial - Icon STOP
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

H. 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:
1. Pipette(s) capable of delivering 0.025 & 0.050ml (25µl & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. 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.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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 (50µl) 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

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one (1) year
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.

Note 1: 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 reference calibrators and controls to room temperature (20 -27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates’ wells for each serum reference calibrator, 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.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CEA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (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.**
8. 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

9. Incubate at room temperature for fifteen (15) minutes.
10. 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.**
11. 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 Carcinoembryonic antigen in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CEA 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 0.320 ng/ml intersects the dose response curve at 18.1 ng/ml CEA concentration (see Figure 1).

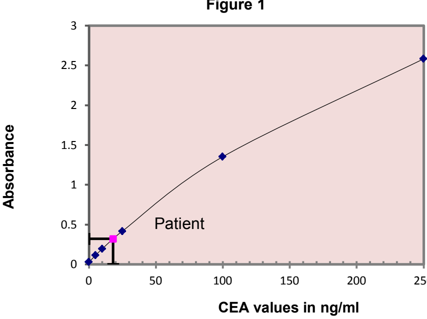
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.028	0.027	0
	B1	0.026		
Cal B	C1	0.115	0.115	5
	D1	0.114		
Cal C	E1	0.196	0.196	10
	F1	0.196		
Cal D	G1	0.432	0.418	25
	H1	0.404		
Cal E	A2	1.403	1.353	100
	B2	1.303		
Cal F	C2	2.580	2.558	250
	D2	2.535		
Patient	E2	0.302	0.320	**18.1**
	F2	0.337		

*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:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. 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 are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CEA concentrations above 250 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (CEA < 5 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
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.
11. 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.
12. 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.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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.
6. 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.
7. CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CEA value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

13.0 EXPECTED RANGES OF VALUES

Nearly 99% of non-smokers have CEA concentrations less than 5ng/ml. Similarly 99% of smokers have concentrations less than 10ng/ml.[4]

TABLE I
Expected Values for the CEA Next Generation AccuBind® ELISA Test System

Non-smokers	<5ng/ml
Smokers	<10ng/ml

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 CEA Next Generation AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) 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	σ	C.V.
Level 1	20	2.6	0.25	9.6%
Level 2	20	12.5	1.01	8.1%
Level 3	20	24.1	1.35	5.6%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	2.8	0.30	10.7%
Level 2	10	12.8	1.18	9.2%
Level 3	10	23.5	1.85	7.8%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CEA Next Generation AccuBind® ELISA test system has a sensitivity of 0.025 ng. This is equivalent to a sample containing 1 ng/ml CEA concentration. The sensitivity was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CEA Next Generation AccuBind® ELISA method was compared with a reference method. Biological specimens from normal and elevated concentrations were assayed. The total number of such specimens was 64. The values ranged from 0.4 – 128ng/ml. The least square regression equation and the correlation coefficient were computed for the CEA Next Generation AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (X)	10.01	y = 1.17+0.977x	0.995
Reference (Y)	9.04		

E. Specificity:

Highly specific antibodies to CEA molecules have been used in the CEA Next Generation AccuBind® ELISA test system. No interference was detected with the performance of CEA Next Generation AccuBind® ELISA upon addition of massive amounts of the following substances to a human serum pool.

Substance	Concentration
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
AFP	10 µg/ml
PSA	1.0 µg/ml
CA-125	10,000 U/ml
hCG	1000 IU/ml
hLH	10 IU/ml
hTSH	100 mIU/ml
hPRL	100 µg/ml

14.5 Linearity & Hook Effect:

Three different lot preparations of the CEA Next Generation AccuBind® ELISA reagents were used to assess the linearity and hook effect. Massive concentrations of CEA (> 60,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 60,000 ng/ml and a within dose recovery of 92.0 to 111.4%.

15.0 REFERENCES

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Revision: 3 Date: 2019-Jul-16 DCO: 1353
MP4625 Product Code: *4625-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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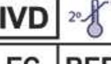

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Glossary of Symbols
(EN 980/ISO 15223)

In Vitro - Diagnostic Medical Device

Temperature Limitation Storage Condition (2-8° C)

Consult Instructions for Use

Catalogue Number

Contains Sufficient Test for Σ

Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

Authorized Rep in European Country

European Conformity

IgA общий-ИФА-БЕСТ

A-8666

Набор реагентов
для иммуноферментного определения
концентрации общего иммуноглобулина
класса А в сыворотке крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 14.08.2018

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного определения концентрации общего иммуноглобулина класса А в сыворотке крови «IgA общий-ИФА-БЕСТ» (далее по тексту – набор) предназначен для определения концентрации общего иммуноглобулина класса А ($IgA_{общ}$) в сыворотке крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение анализа в дублях 41 неизвестного, 6 калибровочных и 1 контрольного образцов (всего 96 определений при использовании всех стрипов планшета).

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения основан на двухстадийном «сэндвич»-варианте твердофазного иммуноферментного анализа с применением моноклональных антител к IgA.

На первой стадии калибровочные образцы с известной концентрацией $IgA_{общ}$ и анализируемые образцы инкубируются в лунках стрипированного планшета с иммобилизованными моноклональными антителами (МКАТ) к альфа-цепям IgA. На второй стадии связавшийся в лунках IgAобщ обрабатывают конъюгатом МКАТ к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой.

Образовавшиеся иммунные комплексы «иммобилизованные МКАТ – IgA – конъюгат» выявляют ферментативной реакцией с раствором тетраметилбензидина. Степень окрашивания пропорциональна концентрации $IgA_{общ}$ в анализируемом образце. После измерения величины оптической плотности раствора в лунках на основании калибровочного графика рассчитывается концентрация $IgA_{общ}$ в анализируемых образцах.

2.2. Состав набора

В состав набора входят:

– планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к альфа-цепям IgA человека, готовый для использования – 1 шт.;
– калибровочные образцы, содержащие известные количества $IgA_{общ}$ – 0; 17,5; 35; 75; 150; 300 Ед/мл (0; 0,25; 0,49; 1,05; 2,1 и 4,2 мг/мл); аттестованные относительно WHO International Standard Immunoglobulins G, A and M, human serum, NIBSC 67/086; концентрации $IgA_{общ}$ в калибровочных образцах могут несколько отличаться от указанных величин, точные величины указаны на этикетках флаконов, готовые для использования – 6 флаконов (по 0,5 мл);
– контрольный образец на основе инактивированной сыворотки крови человека с известным содержанием $IgA_{общ}$, аттестованный относительно WHO International Standard Immunoglobulins G, A and

M, human serum, NIBSC 67/086; готовый для использования – 1 флакон (0,5 мл);
- конъюгат моноклональных антител к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
- раствор для разведения сывороток (РРС), концентрат – 1 флакон (28 мл);
- концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
- раствор тетраметилбензидина плюс (раствор ТМБ плюс), готовый для использования – 1 флакон (13 мл);
- стоп-реагент, готовый для использования – 1 флакон (12 мл).
- пленка для заклеивания планшета – 2 шт.;
- ванночка для реагента – 2 шт.;
- наконечники для пипетки на 5–200 мкл – 16 шт.;
- планшет для предварительного разведения исследуемых образцов – 1 шт.

Принадлежности:
- трафарет для построения калибровочного графика – 1 шт.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. В наборе «IgA общий-ИФА-БЕСТ» используются моноклональные антитела, обладающие высокой специфичностью к альфа-цепям IgA. Перекрестного связывания с IgG, IgM, IgE или альбумином в физиологических концентрациях не наблюдалось.

3.2. «Хук»-эффект при использовании набора реагентов не зафиксирован. Оптическая плотность образцов сыворотки крови с концентрацией $IgA_{общ}$ до 1000 Ед/мл всегда превышала оптическую плотность калибровочного образца с максимальной концентрацией $IgA_{общ}$.

3.3.* Воспроизводимость. Коэффициент вариации результатов определения концентрации $IgA_{общ}$ в лунках, содержащих контрольный образец, не превышает 8%.

3.4.* Линейность. Данный аналитический параметр проверяется тестом на «линейность» - отклонение от расчетной величины концентрации $IgA_{общ}$ при разведении калибровочных образцов, содержащих 300, 150, 75, 35 Ед/мл в 2 раза. Процент «линейности» составляет: 90–110 %.

3.5.* Точность. Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации $IgA_{общ}$ расчетному значению в пробе, полученной путем смешивания равных объемов контрольного образца и калибровочного образца с концентрацией $IgA_{общ}$ 35 Ед/мл (0,49 мг/мл). Процент «открытия» составляет 90–110%.

3.6.* Чувствительность. Минимально определяемая концентрация $IgA_{общ}$, рассчитанная на основании среднего арифметического значения оптической плотности калибровочного

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* по ГОСТ Р 51352-2013.

образца B_0 (с концентрацией $IgA_{общ}$ 0 Ед/мл) плюс 2σ (σ – среднее квадратичное отклонение от среднего арифметического значения B_0) не превышает 1,5 Ед/мл (0,021 мг/мл).

3.7. Клиническая проверка. Концентрация $IgA_{общ}$, измеренная в сыворотке крови, у условно здоровых доноров, находилась в диапазоне 20÷285 Ед/мл (см. также с. 29).

3.8. Рекомендуется в каждой лаборатории при использовании набора уточнить значения концентрации $IgA_{общ}$, соответствующие нормальным для данного региона у обследуемого контингента людей.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2а (Приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболе-

ваний при работе в клинико-диагностических лабораториях лечебно-профилактических учреждений», утвержденной Минздравом СССР 17 января 1991 г. и в методических указаниях МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998.

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфекционные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Лабораторная посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе

ЧАС, спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

– спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение только при длине волны 450 нм;
– шейкер термостатируемый орбитального типа, позволяющий производить встряхивание при температуре 37±1°С и 400–800 об/мин;
– микроцентрифуга, позволяющая центрифугировать при 1500–2000 об/мин;
– промывочное устройство для планшетов;
– холодильник бытовой;

- пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл;
- пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 до 350 мкл;
- флаконы стеклянные вместимостью 15 мл;
- цилиндр мерный вместимостью 1000 мл;
- вода дистиллированная;
- перчатки медицинские диагностические одноразовые;
- бумага фильтровальная лабораторная;
- дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизованную, мутную сыворотку крови.

6.2. Образцы сыворотки крови можно хранить при температуре от 2 до 8°С не более 48 часов, при температуре минус 20°С (и ниже) не более 3 месяцев. Повторное замораживание и размораживание образцов сыворотки крови не допускается. После размораживания образцы следует тщательно перемешать.

6.3. Образцы сывороток крови, содержащие осадок, необходимо очистить центрифугированием при 1500 об/мин в течение 5 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед проведением анализа компоненты набора и исследуемые образцы следует выдержать при температуре от 18 до 25°C не менее 30 мин.

7.2. Подготовка планшета

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Использовать в течение 1 часа после установки. Оставшиеся стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок.

Хранить при температуре от 2 до 8°C в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

Раствор готовится из концентрата фосфатно-солевого буферного раствора. При выпадении осадка солей в концентрате необходимо прогреть его при температуре 30–40°C до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

7.4. Приготовление рабочего раствора для разведения сывороток

Приготовить за 30 мин до начала постановки анализа.

При выпадении осадка солей в концентрате РРС необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата раствора для разведения сывороток и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут.

7.5. Приготовление калибровочных образцов и контрольного образца

Калибровочные образцы и контрольный образец готовы к использованию и не требуют дополнительного разведения. Перед использованием флаконы встряхнуть или центрифугировать на микроцентрифуге так, чтобы капли растворов со стенок и крышки опустились на дно. Затем содержимое флаконов тщательно

Таблица расхода компонентов набора реагентов

Кол-во одновремено используемых стрипов	Промывочный раствор		Рабочий раствор для разведения сывороток		Коньюгат, мл	Раствор ТМБ плюс, мл
	ФСБ-Т×25, мл	Дистиллированная вода, мл	РРС, концентрат, мл	Дистиллированная вода, мл		
2	4,0	до 100	4,0	до 100	2,0	2,0
3	6,0	до 150	6,0	до 150	3,0	3,0
4	8,0	до 200	8,0	до 200	4,0	4,0
5	10,0	до 250	10,0	до 250	5,0	5,0
6	12,0	до 300	12,0	до 300	6,0	6,0
7	14,0	до 350	14,0	до 350	7,0	7,0
8	16,0	до 400	16,0	до 400	8,0	8,0
9	18,0	до 450	18,0	до 450	9,0	9,0
10	20,0	до 500	20,0	до 500	10,0	10,0
11	22,0	до 550	22,0	до 550	11,0	11,0
12	24,0	до 600	24,0	до 600	12,0	12,0

перемешать на вортексе или пипетированием, избегая образования пены.

Калибровочные образцы и контрольный образец после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Приготовление рабочего разведения анализируемых образцов сыворотки крови

Готовится в стеклянных заранее промаркированных флаконах за 5–10 мин до начала постановки анализа.

В чистый флакон с 10 мл рабочего раствора для разведения сывороток (см. п. 7.4) добавить 10 мкл исследуемой сыворотки и тщательно перемешать. Таким образом, рабочее разведение сыворотки составляет 1000 раз*.

Использовать в течение 30 мин после приготовления.

7.7. Подготовка конъюгата.

Конъюгат готов к использованию.

Необходимое количество конъюгата отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА конъюгат утилизировать ***(не сливать во флакон с исходным конъюгатом).***

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Конъюгат после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.8. Подготовка раствора тетраметилбензидина плюс.

* См. также раздел «Дополнительная информация для потребителей», п. 2

Раствор ТМБ плюс готов к использованию.

Необходимое количество раствора ТМБ плюс отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА раствор ТМБ плюс утилизировать ***(не сливать во флакон с исходным раствором ТМБ плюс).***

Необходимо исключить воздействие прямого света на раствор ТМБ плюс.

Раствор ТМБ плюс после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

7.9. Стоп-реагент готов к использованию.

После вскрытия стоп-реагент можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.10. Внести во все лунки по 100 мкл рабочего раствора для разведения сывороток (см п. 7.4).

Внести в соответствующие лунки в дублях, начиная с верхних лунок первых двух стрипов, по 20 мкл каждого калибровочного образца. В следующую пару лунок внести по 20 мкл контрольного образца. В остальные лунки внести в дублях по 20 мкл анализируемых образцов сы-

воротки крови в рабочем разведении (см п. 7.6), каждый раз меняя наконечник.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.11. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.12. По окончании инкубации снять липкую пленку и удалить ее в сосуд с дезинфицирующим раствором. Содержимое лунок удалить отсасыванием в сосуд с дезинфицирующим раствором и промыть, добавляя во все лунки по 350 мкл промывочного раствора. Процесс промывки повторить еще 4 раза. Общее количество отмывок равно 5. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо следить за полным опорожнением лунок после каждого цикла отмывки. Затем удалить остатки жидкости из лунок, постукивая планшетом в перевернутом положении по фильтровальной бумаге.

7.13. Внести во все лунки планшета по 100 мкл конъюгата (см п. 7.7).

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на

термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.15. По окончании инкубации удалить содержимое лунок и промыть планшет, как это указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл раствора ТМБ плюс (см п. 7.8) и инкубировать в защищенном от света месте в течение 15 мин при температуре от 18 до 25°С.

Для внесения раствора ТМБ плюс использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор ТМБ плюс, по 100 мкл стоп-реагента, при этом содержимое лунок окрашивается в желтый цвет.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Измерить величину оптической плотности растворов в лунках стрипов на спектрофотометре вертикального сканирования в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–655 нм; допускается измерение только с фильтром 450 нм. Измерение проводить через 2–3 мин после остановки реакции.

Время между остановкой реакции и измерением оптической плотности не должно превышать 10 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ

9.1. Вычислить средние арифметические значения оптической плотности для каждой пары лунок, содержащих калибровочные образцы.

9.2. Построить в линейных координатах калибровочный график зависимости среднего арифметического значения оптической плотности (ед. опт. плотн.) от концентрации $IgA_{общ}$ в калибровочных образцах (Ед/мл или мг/мл).

9.3. Определить концентрацию $IgA_{общ}$ в контрольном образце и анализируемых образцах по калибровочному графику. Вычислить среднее арифметическое значение концентрации для каждой пары лунок, содержащих анализируемые образцы.

9.4. Если при проведении анализа использовали разведение сыворотки в 1000 раз (базовое разведение для данного набора), то найденное по графику количество $IgA_{общ}$ соответствует концентрации $IgA_{общ}$ в анализируемом образце в Ед/мл (мг/мл). Если использовали другое разведение образца, то найденное по графику количество $IgA_{общ}$ пересчитывают с учетом дополнительного разведения, также получая в результате концентрацию $IgA_{общ}$ в Ед/мл (мг/мл).

Если значение оптической плотности анализируемого образца превышает значение ОП для калибровочного образца 300 Ед/мл (4,2 мг/мл), то данный образец анализируют повторно после до-

полнительного разведения в 2 раза, полученный результат умножить на 2.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности.

В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности набора;
– калибровочные образцы, контрольный образец и конъюгат после вскрытия можно хранить в плот-

но закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;

– концентрат фосфатно-солевого буферного раствора с твином, концентрат раствора для разведения сывороток; раствор ТМБ плюс и стоп-реагент после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
– рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут;
– промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Построение калибровочного графика необходимо проводить для каждого независимого эксперимента, рекомендуется также каждый раз определять концентрацию $IgA_{общ}$ в контрольном образце.

10.6. Для перевода результатов измерений концентрации общего иммуноглобулина класса А из Ед/мл в мг/мл следует использовать коэффициент пересчета 0,014 (1 Ед/мл $IgA_{общ}$ = 0,014 мг/мл $IgA_{общ}$).

10.7. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, раствор ТМБ плюс, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

10.8. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «IgA общий-ИФА-БЕСТ»,

следует обращаться в АО «Вектор-Бест» по адресу:

630559, Новосибирская область,
Новосибирский район,
п. Кольцово, а/я 121,
тел. (383) 363-20-60, 227-75-43,
тел./факс (383) 363-35-55,
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации IgA в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут влиять на ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– рабочие поверхности столов, оборудования обрабатывайте 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона с ТМБ;

– если допущена ошибка при внесении анализируемого образца, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.

– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.

– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Рекомендации по подготовке анализируемых образцов

Вместо одноступенчатого (п. 7.6.) допустимо проводить двухступенчатое разведение сывороток

с использованием планшета для предварительного разведения исследуемых образцов. Для этого, в каждую лунку планшета для предварительного разведения внести по 310 мкл рабочего раствора для разведения сывороток. Далее в одну из лунок, например, А-1, добавить 10 мкл исследуемой сыворотки, сменить использованный наконечник пипетки на новый и затем с его помощью тщательно перемешать содержимое лунки (5–6 круговых движений, во время которых следует 3–4 раза набрать и опорожнить наконечник), избегая образования пены. После этого из лунки отобрать 10 мкл, внести в соседнюю лунку, например, А-2, и таким же образом тщательно перемешать (для этой операции также желательно использовать новый чистый наконечник пипетки). В лунке А-2 получаем рабочее разведение сыворотки 1000 раз. Аналогично развести и другие исследуемые сыворотки (например, в лунках В-1 и В-2, С-1 и т.д.):

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл исследуемого образца → предварительное разведение образца в 32 раза;

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл образца после предварительного разведения → рабочее разведение образца в 1000 раз.

Внимание! *Точность приготовления разведений определяет качество постановки теста!*

При исследовании не сыворотки, а других биологических жидкостей, степень разведения исследуемых образцов следует заранее подобрать

Таблица 2

Абсолютные значения уровней содержания иммуноглобулинов в различных биологических жидкостях у здоровых лиц (M±δ)

(Тотолян А.А. Современные подходы к диагностике иммунопатологических состояний. Мед.иммунология, 1999 ,т.1 №1-2, с. 75-108)

Биологические жидкости	Содержание иммуноглобулинов классов:				
	IgA, г/л	IgM, г/л	IgG, г/л	sIgA, г/л	IgE, кЕ/л
ЦСЖ	0,006±0,0013	0,0049±0,001	0,037±0,004	н/опр	0±0
Слюна	0,069±0,028	0,055±0,011	0,042±0,017	0,768±0,275	н/опр
Назальный смыв	0,014±0,006	0,025±0,017	0,042±0,017	0,071±0,022	0±0
Ларингеальный секрет	0,071±0,022	0,063±0,044	0,085±0,044	1,31±1,87	н/опр
Слезная жидкость	0,165±0,02	0,038±0,008	0,185±0,06	н/опр	н/опр
Эякулят	1,01±0,67	0,9±0,46	0,51±0,2	2,21±1,01	0±0
Сыворотка крови	2,15±0,85	1,63±0,46	12,3±2,97	0,79±0,22	50,0±12,5

Примечание: *н/опр – данный показатель не определяли.*

Приведенные показатели можно использовать только как ориентировочные, и в каждой лаборатории рекомендуется вычислить собственные границы нормальных значений концентрации общего IgA в сыворотке крови.

опытным путем, используя как ориентир данные таблицы 2.

3. Условия правильности работы набора

Результаты анализа исследуемых образцов учитывать, если будут выполнены следующие условия:

– соотношение оптических плотностей калибровочных образцов: $ОП_0 < ОП_{17,5} < ОП_{35} < ОП_{75} < ОП_{150} < ОП_{300}$;

– $ОП_{300} \geq 1,0$ ед. опт. плотн. (о.е.);

– вычисленное по калибровочному графику значение концентрации $IgA_{общ}$ в контрольном образце попадает в пределы, указанные на этикетке флакона.

$ОП_0$, $ОП_{17,5}$, $ОП_{35}$, $ОП_{75}$, $ОП_{150}$ и $ОП_{300}$ – среднее значение оптической плотности калибровочных образов, содержащих 0; 17,5; 35; 75; 150 и 300 Ед/мл $IgA_{общ}$ соответственно.

4. Расчет результатов анализа

По результатам измерения вычислить среднее арифметическое значение оптической плотности (ОП) в лунках с анализируемыми образцами.

Построить в линейных координатах калибровочный график зависимости оптической плотности (ось ординат) от концентрации $IgA_{общ}$ (ось абсцисс) в калибровочных образцах. Для этого на прилагаемом трафарете для построения графика против концентрации каждого

калибровочного образца отложить соответствующее ей среднее значение оптической плотности. Последовательно соединить полученные точки отрезками прямых линий.

Пример калибровочного графика представлен на рисунке.

Определить содержание $IgA_{общ}$ в контрольном образце и в анализируемых образцах по калибровочному графику. Для этого на оси ординат отметить значение ОП анализируемого образца. Провести прямую линию, параллельно оси абсцисс, до пересечения с калибровочным графиком. От точки пересечения опустить

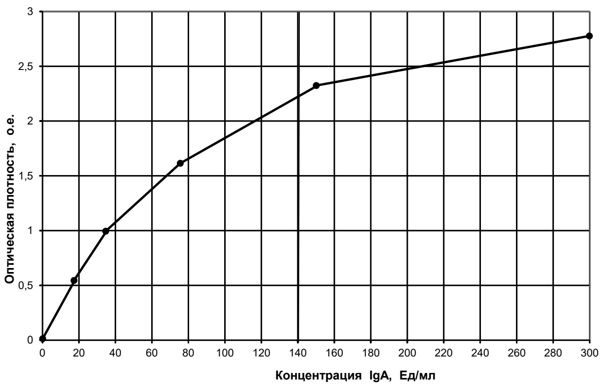

Рис. Зависимость оптической плотности от концентрации $IgA_{общ}$ в калибровочных образцах.

перпендикуляр на ось абсцисс. По полученной точке пересечения определить значение концентрации $IgA_{общ}$ в образце.

При использовании для расчетов концентраций компьютерного или встроенного в спектрофотометр программного обеспечения в настройках выбрать метод, соответствующий кусочно-линейной аппроксимации.

5. Диагностическая значимость

IgA, как и другие иммуноглобулины, относится к гуморальным факторам иммунитета. Карта гуморального иммунитета довольно индивидуальна, тем не менее, пределы нормальных физиологических концентраций достаточно хорошо очерчены.

По нашим данным, концентрация $IgA_{общ}$ в сыворотке крови клинически здоровых доноров (Новосибирская область, Алтайский край) в возрасте от 20 до 50 лет находится в пределах диапазона 57–285 Ед/мл (0,8–4,0мг/мл). У детей этого же региона в возрасте 1–15 лет концентрация $IgA_{общ}$ составила 20–200 Ед/мл. Эти значения в целом близки нормальным значениям концентрации IgA, приводимым в работе (Тотолян А.А., Марфичева Н.А., Тотолян Н.А. «Иммуноглобулины в клинической лабораторной диагностике», С-Пб, 1999.). Однако их можно использовать только как ориентировочные, поскольку диапазоны нормальных концентраций

IgA могут довольно существенно отличаться в зависимости от региона, возраста и некоторых др. причин. Известно также, что для использования в диагностике важнее знать не абсолютное значение концентрации общего иммуноглобулина, а его относительное отклонение от нормального местного, возрастного или, например, профессионального уровня. **В идеале, нормальные региональные уровни и по взрослым и по детям должны определяться каждой лабораторией самостоятельно!**

Уровень концентрации общего IgA в сыворотке крови новорожденных составляет около 1% от уровня взрослых. В возрасте 1–3 месяцев он обычно достигает 14%, 4–5 месяцев – 28%, 8–24 месяцев – 40%, 6 лет – 65%, 9 лет – 75%, 12–13 лет – 90–100% от уровня взрослого человека (15–45 лет).

Результаты определения концентрации общего сывороточного IgA могут быть с успехом использованы для дифференциальной диагностики целого ряда заболеваний (см. иммунограмму).

Более полную картину способно дать параллельное определение всех трех основных классов иммуноглобулинов – G, M и A, а также иммуноглобулина E.

Иммунограмма при некоторых заболеваниях

	IgG	IgA	IgM	IgE
Заболевания печени				
Острый инфекционный гепатит	+	N/+	N/++	N
Хронический персистирующий гепатит	N/+	N	N/+	N/+
Хронический агрессивный гепатит	++	+	N/++	N/+
Постгепатитный криптогенный цирроз	++	+	+	N/+
Первичный билиарный цирроз	N/+	N	+/++	N
Алкогольный цирроз	N/+	++	N/+	N
Болезни почек				
Острый пиелонефрит	N	N	+/++	N
Хронический пиелонефрит	+/++	N	+/++	N/+
Нефротический синдром	—	—	N/—	N/—
Инфекционные заболевания				
Острая инфекция	N	N	+/++	N
Хроническая инфекция	+/++	N/+	N/+	N/+
Системные ревматические заболевания				
Ревматоидный артрит	N/++	N/++	N/+	+/++
Системная красная волчанка	+	N	N/+	N/+
Склеродермия	N	N	N	N/+
Смешанные системные заболевания	N/+	N/+	N	N/+
Атопия, аллергические заболевания	N/+	N	N/—	+/++
Гельминтозы и др. паразитарные заболевания	N/+	N/+	N/+	+/++

N – нормальная регионально-возрастная концентрация иммуноглобулина (в пределах нормального диапазона от Nmin до Nmax)

\+ – повышенная концентрация иммуноглобулина (от Nmax до 1,3Nmax)

++ – сильно повышенная концентрация иммуно**гло**булина (более 1,3Nmax)

– – пониженная концентрация иммуноглобулина (ниже Nmin)

6. Краткая схема проведения ИФА для набора реагентов «IgA общий-ИФА-БЕСТ»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл рабочего раствора для разведения сывороток;
	по 20 мкл калибровочных и контрольного образцов в дублях в контрольные лунки;
	по 20 мкл разведенных анализируемых образцов в дублях в лунки для исследуемых образцов.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл раствора ТМБ плюс.
Инкубировать:	15 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

7. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …	i	Обратитесь к инструкции по применению
!	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-05-97.

12.11.18.

АКЦИОНЕРНОЕ ОБЩЕСТВО
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Immunoglobulin E (IgE) Test System
Product Code: 2525-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Immunoglobulin E (IgE) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Allergic reactions, which are becoming more widespread, are usually diagnosed on the basis of medical history and clinical symptoms. In vitro and in vivo testing, however, play a key role in confirming clinical suspicions and tailoring treatment. The measurement of immunoglobulin E (**IgE**) in serum is widely used in the diagnosis of allergic reactions and parasitic infections. Many allergies are caused by the immunoglobulins of subclass IgE acting as point of contact between the allergen and specialized cells. The IgE molecules (MW 200,000) bind to the surface of the mast cells and basophillic granulocytes. Subsequently the binding of allergen to cell-bound IgE causes these cells to release histamines and other vasoactive substances. The release of histamines in the body results initiates what is commonly known as an allergic reaction.

Before making any therapeutic determination it is important, however, to know whether the allergic reaction is IgE mediated or non-IgE mediated. Measurement of total IgE in serum sample, along with other supporting diagnostic information, can help to make that determination. Measurement of total circulating IgE may also be of value in the early detection of allergy in infants and as a means of predicting future atopic manifestations. Before deciding on any therapy it is important to take into consideration all the relevant clinical information as well as information supplied by specific allergy testing.

IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, the total IgE levels increase with the allergies a person has and the number of times of exposure to the relevant allergens. Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonay aspergillosis, and during the active stages of parasitic infections.

In this method, IgE calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for IgE) is added and the reactants mixed. Reaction between the IgE antibodies and native IgE forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to IgE is added to the wells. The enzyme labeled antibody binds to the IgE already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the IgE in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay 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-IgE antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(IgE)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(IgE)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(IgE)}$ = Native Antigen (Variable Quantity)
$Ag_{(IgE)} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(IgE)} - {}^{Btn}Ab_{(m)}$ + <u>Streptavidin</u>$_{C.W.}$ ⇒ <u>Immobilized complex</u> (IC)
<u>Streptavidin</u>$_{C.W.}$ = Streptavidin immobilized on well
<u>Immobilized complex (IC)</u> = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}IgE)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}IgE)} - IC$$

${}^{Enz}Ab_{(x\text{-}IgE)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(x\text{-}IgE)} - IC$ = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. IgE Calibrators – 1.0 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 5 (**B**), 25 **(C),** 50 (**D**), 150 (**E**) and 400 (**F**) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The Calibrators are standardized against WHO's 2ndIRP 75/502 for IgE

B. IgE Biotin Reagent – 13 ml/vial – Icon ∇
One (1) vial containing biotinylated anti-human IgE mIgG reagent presented in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. IgE Enzyme Reagent – 13 ml/vial - Icon Ⓔ
One (1) vial containing anti-human IgE-HRP incorporated complex in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7.0ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

G. Substrate B – 7.0ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in acetate buffer. Store at 2-8°C.

H. Stop Solution – 8.0ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. 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 label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 and 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. 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 for samples. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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 (50µl) 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

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Storediluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
Pour the contents of vial labeled Solution 'A' into the vial labeled Solution 'B'. Place the yellow cap on the mixed reagent for easy identification. Mix and label accordingly. Store at 2-8 °C.

Note 1: 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 reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, 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.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the IgE Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (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.**
8. Add 0.100 ml (100µl) of the IgE Enzyme Reagent labeled antibody to each well.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (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.**
12. 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.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. 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 IgE in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding IgE concentration in IU/ml on linear graph

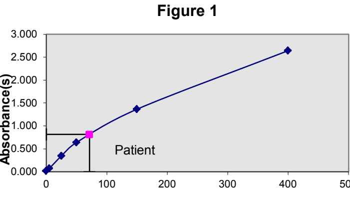
paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of IgE for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/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 (1.323) intersects the dose response curve at 142 IU/ml IgE 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	Abs	Mean Abs (B)	Conc
Cal A	A1	0.014	0.015	0
	B1	0.016		
Cal B	C1	0.072	0.073	5
	D1	0.074		
Cal C	E1	0.364	0.345	25
	F1	0.326		
Cal D	G1	0.663	0.639	50
	H1	0.614		
Cal E	A2	1.340	1.364	150
	B2	1.388		
Cal F	C2	2.601	2.641	400
	D2	2.682		
Ctrl 1	E2	2.575	2.562	***375.3***
	F2	2.549		
Ctrl 2	G2	0.818	0.813	***71.2***
	H2	0.807		
Patient 1	A3	1.322	1.323	***142.0***
	B3	1.324		

Figure 1



IgE Values in IU/ml

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'A' should be $\leq$ 0.05
2. The absorbance (OD) of calibrator 'F' should be $\geq$ 1.3
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 are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. 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.
11. 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.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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.
6. 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.
7. Serum IgE concentration is dependent upon a multiplicity of factors: including if the patient is sensitized, how many times the patient has been exposed to a specific allergen etc. Total IgE concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.
8. Since all atopic reactions are not IgE mediated, all relevant clinical information should be taken into consideration before making any determination for patients who may be in the normal range.

13.0 EXPECTED RANGES OF VALUES

A study of population from different age groups was conducted to evaluate the IgE AccuBind® ELISA test system. The results are presented in Table 1:

TABLE 1
Expected Values for the IgE (In IU/ml)

Age (Yrs)	Number (n)	Median	Absolute Range
0-3	31	6.4	ND - 46
3-16	43	25.0	ND – 280
Adult	145	43	0 - 200

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 precision of the IgE AccuBind® ELISA Test System were determined by analyses on three different levels of pool 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
Intra-Assay Precision (in IU/ml)

SAMPLE	N	X	σ	C.V.%
Low	20	48.9	2.87	5.87
Medium	20	160.5	6.47	4.03
High	20	297.6	5.81	1.95

TABLE 3
Inter Assay Precision (in IU/ml)

SAMPLE	N	X	σ	C.V.%
Low	10	46.3	3.9	8.42
Medium	10	157.0	7.3	4.64
High	10	301.0	10.6	3.52

14.2 Sensitivity

The IgE AccuBind® ELISA test system has a sensitivity of 0.125 IU/ml. The sensitivity was ascertained by determining the variability of the 0 IU/ml serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The IgE AccuBind® ELISA test system was compared with a reference method. Biological specimens with IgE levels in the low, medium and high ranges were used. The values ranged from 0.8 to 3100 IIU/ml. The total number of such specimens was 219. The least square regression equation and the correlation coefficient were computed for this IgE AccuBind® ELISA method in comparison with the predicate method (Table 4):

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (X)	179	x= -12.9 + 1.21(Y)	0.967
Predicate (Y)	157		

Only slight amounts of bias between this 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

The specificity of the IgE AccuBind® ELISA test system, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix. No cross-reaction between the antibodies used and the related molecules was detected.

14.5. High Dose Effect

Since the assay is sequential in design, high concentrations of IgE do not show the hook effect. Myeloma IgE patient samples with concentrations over 8 million IU/ml demonstrated extremely high levels of absorbance.

14.6 Linearity

Two patient pools were assayed diluted (in 'A' Calibrator) and undiluted with the IgE AccuBind® ELISA test system. The observed and expected values are listed below in Table 5:

TABLE 5

Sample	Observed (O) (IU/ml)	Expected (E) (IU/ml)	% Recovery (O/E)
Pool 1	106.8	-	-
Pool 1/2	50.8	53.4	95.1
Pool 1/4	25.3	26.7	94.8
Pool 1/8	13.4	13.3	100.6
Pool 1/16	6.6	6.7	98.5
Pool 2	395.9	-	-
Pool 2/2	189.5	197.9	95.8
Pool 2/4	106.1	98.9	107.2
Pool 2/8	48.0	49.5	96.9
Pool 2/16	25.8	24.7	104.2

14.7 Recovery

Two patient pools were spiked with known amounts of IgE and assayed with the IgE AccuBind® ELISA test system. The observed and expected values are listed below in Table 6.

TABLE 6

Sample	Observed (O) (IU/ml)	Expected (E) (IU/ml)	% Recovery (O/E)
Pool 1	25.7	-	-
Pool 1+ 25	50.7	50.7	100.0
Pool 1+ 50	74.8	75.7	101.2
Pool 1+ 100	122.7	125.7	97.6
Pool 1+ 200	232.0	225.7	102.7
Pool 2	12.3	-	-
Pool 2 + 25	41.7	37.3	111.2
Pool 2+ 50	62.6	62.3	100.6
Pool 2+ 100	109.4	112.3	97.4
Pool 2+ 200	197.2	212.3	92.8

15.0 REFERENCES

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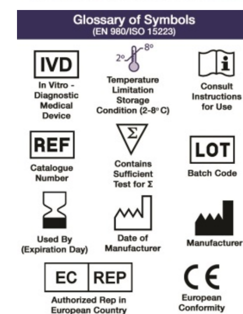

IgG общий-ИФА-БЕСТ

А-8662

Набор реагентов
для иммуноферментного определения
концентрации общего иммуноглобулина
класса G в сыворотке крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 14.08.2018

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного определения концентрации общего иммуноглобулина класса G в сыворотке крови «IgG общий-ИФА-БЕСТ» (далее по тексту – набор) предназначен для определения концентрации общего иммуноглобулина класса G ($IgG_{общ}$) в сыворотке крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение анализа в дублях 41 неизвестного, 6 калибровочных и 1 контрольного образцов (всего 96 определений при использовании всех стрипов планшета).

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения основан на двухстадийном «сэндвич»-варианте твердофазного иммуно-ферментного анализа с применением моноклональных антител к IgG.

На первой стадии калибровочные образцы с известной концентрацией $IgG_{общ}$ и анализируемые образцы инкубируются в лунках стрипированного планшета с иммобилизованными моноклональными антителами (МКАТ) к гамма-цепям IgG. На второй стадии связавшийся в лунках IgG обрабатывают конъюгатом МКАТ к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой.

Образовавшиеся иммунные комплексы «иммобилизованные МКАТ – IgG – конъюгат» выявляют ферментативной реакцией с раствором тетраметилбензидина. Степень окрашивания пропорциональна концентрации $IgG_{общ}$ в анализируемом образце. После измерения величины оптической плотности раствора в лунках на основании калибровочного графика рассчитывается концентрация $IgG_{общ}$ в анализируемых образцах.

2.2. Состав набора

В состав набора входят:

– планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к гамма-цепям IgG человека, готовый для использования – 1 шт.;
– калибровочные образцы, содержащие известные количества $IgG_{общ}$ – 0; 17,5; 35; 75; 150; 300 Ед/мл (0; 1,4; 2,8; 6; 12 и 24 мг/мл), аттестованные относительно WHO International Standard Immunoglobulins G, A and M, human serum, NIBSC 67/086; концентрации IgGобщ в калибровочных образцах могут несколько отличаться от указанных величин, точные величины указаны на этикетках флаконов, готовые для использования – 6 флаконов (по 0,5 мл);
– контрольный образец на основе инактивированной сыворотки крови человека с известным содержанием $IgG_{общ}$, аттестованный относительно WHO International Standard Immunoglobulins G, A and

M, human serum, NIBSC 67/086; готовый для использования – 1 флакон (0,5 мл);

– конъюгат моноклональных антител к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
– раствор для разведения сывороток (РРС), концентрат – 1 флакон (28 мл);
– концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
– раствор тетраметилбензидина плюс (раствор ТМБ плюс), готовый для использования – 1 флакон (13 мл);
– стоп-реагент, готовый для использования – 1 флакон (12 мл);
– пленка для заклеивания планшета – 2 шт.;
– ванночка для реагентов – 2 шт.;
– наконечники для пипеток на 5–200 мкл – 16 шт.;
– планшет для предварительного разведения исследуемых образцов – 1 шт.

Принадлежности:

– трафарет для построения калибровочного графика – 1 шт.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. В наборе «IgG общий – ИФА – БЕСТ» используются моноклональные антитела, обладающие высокой специфичностью к гамма-цепям IgG. Перекрестного связывания с IgM, IgA, IgE или альбумином в физиологических концентрациях не наблюдалось.

3.2. «Хук»-эффект при использовании набора реагентов не зафиксирован. Оптическая плотность образцов сыворотки крови с концентрацией $IgG_{общ}$ до 1000 Ед/мл всегда превышала оптическую плотность калибровочного образца с максимальной концентрацией $IgG_{общ}$.

3.3. *Воспроизводимость. Коэффициент вариации результатов определения концентрации $IgG_{общ}$ в лунках, содержащих контрольный образец, не превышает 8%.

3.4. *Линейность. Данный аналитический параметр проверяется тестом на «линейность» – отклонение от расчетной величины концентрации $IgG_{общ}$ при разведении калибровочных образцов, содержащих 300, 150, 75, 35 Ед/мл в 2 раза. Процент «линейности» составляет: 90–110 %.

3.5. *Точность. Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации $IgG_{общ}$ расчетному значению в пробе, полученной путем смешивания равных объемов контрольного образца и калибровочного образца с концентрацией $IgG_{общ}$ 35 Ед/мл. Процент «открытия» составляет 90–110%.

3.6. *Чувствительность. Минимально определяемая концентрация $IgG_{общ}$, рассчитанная на основании среднего арифметического значения оптической плотности калибровочного образца B_0 (с концентрацией $IgG_{общ}$ 0 Ед/мл) плюс 2σ (σ - среднее квадратичное отклонение

от среднего арифметического значения B_0) не превышает 2,5 Ед/мл (0,2 мг/мл).

3.7. Клиническая проверка. Концентрация $IgG_{общ}$, измеренная в сыворотке крови условно здоровых доноров находилась в диапазоне 37–200 Ед/мл (см. стр. 29).

3.8. Рекомендуется в каждой лаборатории при использовании набора уточнить значения концентрации $IgG_{общ}$, соответствующие нормальным для данного региона у обследуемого контингента людей.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2а (Приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболеваний при работе в клинико-диагностических лабораториях лечебно-профилактических уч-

реждений», утвержденной Минздравом СССР 17 января 1991 г. и в методических указаниях МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998.

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфекционные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Лабораторная посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС,

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* по ГОСТ Р 51352-2013.

спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ДЛЯ РАБОТЫ С НАБОРОМ:

– Спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620 – 655 нм; допускается измерение только при длине волны 450 нм;
– шейкер термостатируемый орбитального типа, позволяющий производить встряхивание при температуре 37±1°С и 400–800 об/мин;
– микроцентрифуга, позволяющая центрифугировать при 1500–2000 об/мин;
– промывочное устройство для планшетов;
– холодильник бытовой;

- пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл;
- пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 до 350 мкл;
- флаконы стеклянные вместимостью 15 мл;
- цилиндр мерный вместимостью 1000 мл;
- вода дистиллированная;
- перчатки медицинские диагностические одноразовые;
- бумага фильтровальная лабораторная;
- дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизованную, мутную сыворотку крови.

6.2. Образцы сыворотки крови можно хранить при температуре от 2 до 8°С не более 48 часов, при температуре минус 20°С (и ниже) не более 3 месяцев. Повторное замораживание и размораживание образцов сыворотки крови не допускается. После размораживания образцы следует тщательно перемешать.

6.3. Образцы сывороток крови, содержащие осадок, необходимо очистить центрифугированием при 1500 об/мин в течение 5 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед проведением анализа компоненты набора и исследуемые образцы следует выдержать при температуре от 18 до 25°С не менее 30 мин.

7.2. Подготовка планшета

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Использовать в течение 1 часа после установки. Оставшиеся стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок.

Хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

Раствор готовится из концентрата фосфатно-солевого буферного раствора. При выпадении осадка солей в концентрате необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

7.4. Приготовление рабочего раствора для разведения сывороток

Приготовить за 30 мин до начала постановки анализа.

При выпадении осадка солей в концентрате РРС необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата раствора для разведения сывороток и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут.

7.5. Подготовка калибровочных образцов и контрольного образца

Калибровочные образцы и контрольный образец готовы к использованию и не требуют дополнительного разведения. Перед использованием флаконы встряхнуть или центрифугировать на микроцентрифуге так, чтобы капли

растворов со стенок и крышки опустились на дно. Затем содержимое флаконов тщательно перемешать на вортексе или пипетированием, избегая образования пены.

Калибровочные образцы и контрольный образец после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Приготовление рабочего разведения анализируемых образцов сыворотки крови

Готовится в стеклянных заранее промаркированных флаконах за 5–10 мин до начала постановки анализа.

В чистый флакон с 10 мл рабочего раствора для разведения сывороток (см. п. 7.4) добавить 10 мкл исследуемой сыворотки и тщательно перемешать. Таким образом, рабочее разведение сыворотки составляет 1000 раз*.

Использовать в течение 30 мин после приготовления.

7.7. Подготовка конъюгата.

Конъюгат готов к использованию.

Необходимое количество конъюгата отобрать в чистый флакон или ванночку для реагента.

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* См. также раздел «Дополнительная информация для потребителей», п. 2

Оставшийся после проведения ИФА конъюгат утилизировать ***(не сливать во флакон с исходным конъюгатом).***

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Конъюгат после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.8. Подготовка раствора тетраметилбензидина плюс.

Раствор ТМБ плюс готов к использованию.

Необходимое количество раствора ТМБ плюс отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА раствор ТМБ плюс утилизировать ***(не сливать во флакон с исходным раствором ТМБ плюс).***

Необходимо исключить воздействие прямого света на раствор ТМБ плюс.

Раствор ТМБ плюс после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

7.9. Стоп-реагент готов к использованию.

После первого вскрытия стоп-реагент можно хранить в плотно закрытом флаконе

Таблица 1

Кол-во используемых стрипов	Промывочный раствор		Рабочий раствор для разведения сывороток		Конъюгат, мл	Раствор ТМБ плюс, мл
	ФСБ-Т×25, концентрат, мл	Дистил. вода, мл	PPC, концентрат, мл	Дистил. вода, мл		
2	4,0	до 100	4,0	до 100	2,0	2,0
3	6,0	до 150	6,0	до 150	3,0	3,0
4	8,0	до 200	8,0	до 200	4,0	4,0
5	10,0	до 250	10,0	до 250	5,0	5,0
6	12,0	до 300	12,0	до 300	6,0	6,0
7	14,0	до 350	14,0	до 350	7,0	7,0
8	16,0	до 400	16,0	до 400	8,0	8,0
9	18,0	до 450	18,0	до 450	9,0	9,0
10	20,0	до 500	20,0	до 500	10,0	10,0
11	22,0	до 550	22,0	до 550	11,0	11,0
12	24,0	до 600	24,0	до 600	12,0	12,0

при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.10. Внести во все лунки по 100 мкл рабочего раствора для разведения сывороток (см п. 7.4).

Внести в соответствующие лунки в дублях, начиная с верхних лунок первых двух стрипов, по 20 мкл каждого калибровочного образца. В следующую пару лунок внести по 20 мкл контрольного образца. В остальные лунки внести

в дублях по 20 мкл анализируемых образцов сыворотки крови в рабочем разведении (см п. 7.6), каждый раз меняя наконечник.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.11. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.12. По окончании инкубации снять липкую пленку и удалить ее в сосуд с дезинфицирующим раствором. Содержимое лунок удалить отсасыванием в сосуд с дезинфицирующим раствором и промыть, добавляя во все лунки по 350 мкл промывочного раствора. Процесс промывки повторить еще 4 раза. Общее количество отмывок равно 5. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо следить за полным опорожнением лунок после каждого цикла отмывки. Затем удалить остатки жидкости из лунок, постукивая планшетом в перевернутом положении по фильтровальной бумаге.

7.13. Внести во все лунки планшета по 100 мкл конъюгата (см п. 7.7).

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на

термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.15. По окончании инкубации удалить содержимое лунок и промыть планшет, как это указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл раствора ТМБ плюс (см п. 7.8) и инкубировать в защищенном от света месте в течение 15 мин при температуре от 18 до 25°С.

Для внесения раствора ТМБ плюс использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор ТМБ плюс, по 100 мкл стоп-реагента, при этом содержимое лунок окрашивается в желтый цвет.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Измерить величину оптической плотности растворов в лунках стрипов на спектрофотометре вертикального сканирования в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–655 нм; допускается измерение только с фильтром 450 нм. Измерение проводить через 2–3 мин после остановки реакции.

Время между остановкой реакции и измерением оптической плотности не должно превышать 10 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ

9.1. Вычислить средние арифметические значения оптической плотности для каждой пары лунок, содержащих калибровочные образцы.

9.2. Построить в линейных координатах калибровочный график зависимости среднего арифметического значения оптической плотности (ед. опт. плотн.) от концентрации $IgG_{общ}$ в калибровочных образцах (Ед/мл или мг/мл).

9.3. Определить концентрацию $IgG_{общ}$ в контрольном образце и анализируемых образцах по калибровочному графику. Вычислить среднее арифметическое значение концентрации для каждой пары лунок, содержащих анализируемые образцы.

9.4. Если при проведении анализа использовали разведение сыворотки в 1000 раз (базовое разведение для данного набора), то найденное по графику количество $IgG_{общ}$ соответствует концентрации $IgG_{общ}$ в анализируемом образце в Ед/мл (мг/мл). Если использовали другое разведение образца, то найденное по графику количество $IgG_{общ}$ пересчитывают с учетом дополнительного разведения, также получая в результате концентрацию $IgG_{общ}$ в Ед/мл (мг/мл).

Если значение оптической плотности анализируемого образца превышает значение ОП для калибровочного образца 300 Ед/мл (24 мг/мл), то данный образец анализируют повторно после дополнительного разведения в 2 раза, полученный результат умножают на 2.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности.

В случае дробного использования набора:

- неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности набора;
- калибровочные образцы, контрольный образец и конъюгат после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
- концентрат фосфатно-солевого буферного раствора с твином, концентрат раствора для разведения

сывороток; раствор ТМБ плюс и стоп-реагент после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;

– рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут;
– промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Построение калибровочного графика необходимо проводить для каждого независимого эксперимента, рекомендуется также каждый раз определять концентрацию $IgG_{общ}$ в контрольном образце.

10.6. Для перевода результатов измерений концентрации общего иммуноглобулина класса G из Ед/мл в мг/мл следует использовать коэффициент пересчета 0,08 (1 Ед/мл $IgG_{общ}$ = 0,08 мг/мл $IgG_{общ}$).

10.7. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, раствор ТМБ плюс, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

10.8. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «IgG общий-ИФА-БЕСТ», следует обращаться в АО «Вектор-БЕСТ» по адресу:

630559, Новосибирская область,
Новосибирский р-н,
р.п. Кольцово, а/я 121,
тел. (383) 363-20-60, 227-75-43,
тел./факс (383) 363-35-55.
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации $IgG_{общ}$ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут влиять на ферментативную активность конъюгатов;
– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;
– рабочие поверхности столов, оборудования обрабатывайте 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);
– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;
– перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона с ТМБ;
– если допущена ошибка при внесении анализируемого образца, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.
– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.
– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.
– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Рекомендации по подготовке анализируемых образцов

Вместо одноступенчатого (п. 7.6.) допустимо проводить двухступенчатое разведение сывороток с использованием планшета для предварительного разведения исследуемых образцов. Для этого, в каждую лунку планшета для предварительного разведения внести по 310 мкл рабочего раствора для разведения сывороток. Далее в одну из лунок, например, А-1, добавить 10 мкл исследуемой сыворотки, сменить исполь-

зованный наконечник пипетки на новый и затем с его помощью тщательно перемешать содержимое лунки (5–6 круговых движений, во время которых следует 3–4 раза набрать и опорожнить наконечник), избегая образования пены. После этого из лунки отобрать 10 мкл, внести в соседнюю лунку, например, А-2, и таким же образом тщательно перемешать (для этой операции также желательно использовать новый чистый наконечник пипетки). В лунке А-2 получаем рабочее разведение сыворотки 1000 раз. Аналогично развести и другие исследуемые сыворотки (например, в лунках В-1 и В-2, С-1 и т.д.):

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл исследуемого образца → предварительное разведение образца в 32 раза;

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл образца после предварительного разведения → рабочее разведение образца в 1000 раз.

Внимание! *Точность приготовления разведений определяет качество постановки теста!*

При исследовании не сыворотки, а других биологических жидкостей, степень разведения исследуемых образцов следует заранее подобрать опытным путем, используя как ориентир данные таблицы 2.

3. Условия правильности работы набора

Результаты анализа исследуемых образцов

Таблица 2

Абсолютные значения уровней содержания иммуноглобулинов в различных биологических жидкостях у здоровых лиц (M±δ)

(Тотолян А.А. Современные подходы к диагностике иммунопатологических состояний. Мед. иммунология, 1999 ,т.1 №1-2, с. 75-108)

Биологические жидкости	Содержание иммуноглобулинов классов:				
	***IgA*, г/л**	***IgM*, г/л**	***IgG*, г/л**	***sIgA*, г/л**	***IgE*, кЕ/л**
ЦСЖ	0,006±0,0013	0,0049±0,001	0,037±0,004	н/опр	0±0
Слюна	0,069±0,028	0,055±0,011	0,042±0,017	0,768±0,275	н/опр
Назальный смыв	0,014±0,006	0,025±0,017	0,042±0,017	0,071±0,022	0±0
Ларингеальный секрет	0,071±0,022	0,063±0,044	0,085±0,044	1,31±1,87	н/опр
Слезная жидкость	0,165±0,02	0,038±0,008	0,185±0,06	н/опр	н/опр
Эякулят	1,01±0,67	0,9±0,46	0,51±0,2	2,21±1,01	0±0
Сыворотка крови	2,15±0,85	1,63±0,46	12,3±2,97	0,79±0,22	50,0±12,5

Примечание: н/опр – данный показатель не определяли.

Приведенные показатели можно использовать только как ориентировочные, и в каждой лаборатории рекомендуется вычислить собственные границы нормальных значений концентрации общего IgG в сыворотке крови.

учитывать, если будут выполнены следующие условия:

– соотношение оптических плотностей калибровочных образцов: $ОП_0 < ОП_{17,5} < ОП_{35} < ОП_{75} < ОП_{150} < ОП_{300}$;

– $ОП_{300} \geq 1{,}0$ ед. опт. плотн. (о.е.);

– вычисленное по калибровочному графику значение концентрации $IgG_{общ}$ в контрольном образце попадает в пределы, указанные на этикетке флакона.

$ОП_0$, $ОП_{17,5}$, $ОП_{35}$, $ОП_{75}$, $ОП_{150}$ и $ОП_{300}$ – среднее значение оптической плотности калибровочных образов, содержащих 0; 17,5; 35; 75; 150 и 300 Ед/мл $IgG_{общ}$ соответственно.

4. Расчет результатов анализа

По результатам измерения вычислить среднее арифметическое значение оптической плотности (ОП) в лунках с анализируемыми образцами.

Построить в линейных координатах калибровочный график зависимости оптической плотности (ось ординат) от концентрации $IgG_{общ}$ (ось абсцисс) в калибровочных образцах. Для этого на прилагаемом трафарете для построения графика против концентрации каждого калибровочного образца отложить соответствующее ей среднее значение оптической плотности. Последовательно соединить полученные точки отрезками прямых линий.

Пример калибровочного графика представлен на рисунке.

Определить содержание $IgG_{общ}$ в контрольном и в анализируемых образцах по калибровочному графику. Для этого на оси ординат отметить значение ОП анализируемого образца. Провести прямую линию параллельно оси абсцисс до пересечения с калибровочным графиком. От точки пересечения опустить перпендикуляр на ось абсцисс. По полученной точке пересечения определить значение концентрации $IgG_{общ}$ в образце.

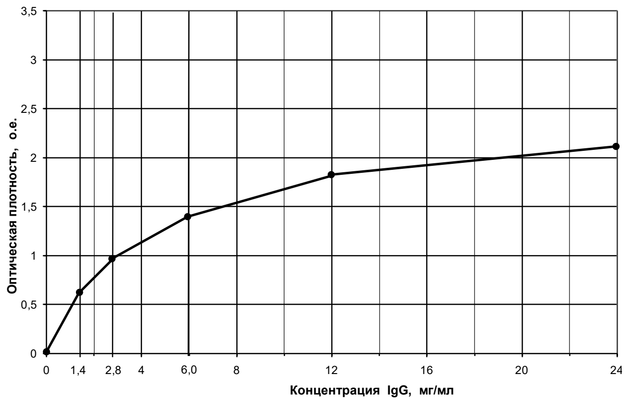

Рисунок. Зависимость оптической плотности от концентрации IgG в калибровочных пробах.

При использовании для расчетов концентраций компьютерного или встроенного в спектрофотометр программного обеспечения в настройках выбрать метод, соответствующий кусочно-линейной аппроксимации.

5. Диагностическая значимость

IgG, как и другие иммуноглобулины, относится к гуморальным факторам иммунитета. Карта гуморального иммунитета довольно индивидуальна, тем не менее пределы нормальных физиологических концентраций достаточно хорошо очерчены.

Диапазоны концентрации IgG в сыворотке крови здоровых доноров, начиная с 12 лет, составляют 5,3–16,5 мг/мл (Тотолян А.А., Марфичева Н.А., Тотолян Н.А. «Иммуноглобулины в клинической лабораторной диагностике», С-Пб, 1996).

По нашим собственным данным, концентрация $IgG_{общ.}$ в сыворотке крови условно здоровых мужчин и женщин из юго-восточного региона Западной Сибири в возрасте 20–50 лет (n=107) в основном находится в диапазоне 60–200 Ед/мл (4,8–16,0 мг/мл); а у детей из этого же региона в возрасте 1–12 лет – соответственно в диапазоне 37–194 Ед/мл (2,96–15,52 мг/мл). Следует, однако, учитывать, что нормальные значения концентраций $IgG_{общ.}$ могут довольно существенно отличаться в зависимости от реги-

она, возраста, экологических и многих других причин. Известно также, что для использования в диагностике часто бывает важнее знать не абсолютное значение концентрации общего иммуноглобулина, а его относительное отклонение от нормального местного, возрастного или, например, профессионального уровня. Поэтому нормальные региональные уровни общих иммуноглобулинов должны определяться каждой лабораторией самостоятельно.

У новорожденных детей уровень концентрации IgG в сыворотке крови часто такой же как у взрослых, однако уже через 1–2 месяца он уменьшается до 30–40% от исходного. Затем он медленно увеличивается, достигая к 6 месяцам 45% (в среднем), к 8–10 месяцам – 62%, к 6 годам – 90%, и только в 9–12 лет он, наконец, становится равным уровню взрослого человека. Отклонение концентрации IgG от нормы отражает состояние иммунной системы и может свидетельствовать о серьезном заболевании.

Результаты определения общего сывороточного иммуноглобулина G могут быть с успехом использованы для дифференциальной диагностики целого ряда заболеваний (см. Иммунограмму).

Во всех случаях более полную картину способно дать параллельное определение трех основных классов иммуноглобулинов – G, M и A, а также иммуноглобулина E (см. иммунограмму).

Иммунограмма при некоторых заболеваниях

	IgG	IgA	IgM	IgE
Заболевания печени				
Острый инфекционный гепатит	+	N/+	N/++	N
Хронический персистирующий гепатит	N/+	N	N/+	N/+
Хронический агрессивный гепатит	++	+	N/++	N/+
Постгепатитный криптогенный цирроз	++	+	+	N/+
Первичный билиарный цирроз	N/+	N	+/++	N
Алкогольный цирроз	N/+	++	N/+	N
Болезни почек				
Острый пиелонефрит	N	N	+/++	N
Хронический пиелонефрит	+/++	N	+/++	N/+
Нефротический синдром	—	—	N/—	N/—
Инфекционные заболевания				
Острая инфекция	N	N	+/++	N
Хроническая инфекция	+/++	N/+	N/+	N/+
Системные ревматические заболевания				
Ревматоидный артрит	N/++	N/++	N/+	+/++
Системная красная волчанка	+	N	N/+	N/+
Склеродермия	N	N	N	N/+
Смешанные системные заболевания	N/+	N/+	N	N/+
Атопия, аллергические заболевания	N/+	N	N/—	+/++
Гельминтозы и др. паразитарные заболевания	N/+	N/+	N/+	+/++

N – нормальная регионально-возрастная концентрация иммуноглобулина (в пределах нормального диапазона от Nmin до Nmax)

\+ – повышенная концентрация иммуноглобулина (от Nmax до 1,3Nmax)

++ – сильно повышенная концентрация иммуноглобулина (более 1,3Nmax)

– – пониженная концентрация иммуноглобулина (ниже Nmin)

6. Краткая схема проведения ИФА для набора реагентов «IgG общий-ИФА-БЕСТ»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл рабочего раствора для разведения сывороток; по 20 мкл калибровочных и контрольного образцов в дублях в контрольные лунки; по 20 мкл разведенных анализируемых образцов в дублях в лунки для исследуемых образцов.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл раствора ТМБ плюс.
Инкубировать:	15 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

7. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …	i	Обратитесь к инструкции по применению
⚠	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-05-97.

12.11.18

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492
Тел./факс: (383) 227-73-60 (многоканальный)
Тел.: (383) 332-37-10, 332-37-58, 332-36-34,
332-67-49, 332-67-52
E-mail: vbmarket@vector-best.ru

www.vector-best.ru

IgM общий-ИФА-БЕСТ

A-8664

Набор реагентов
для иммуноферментного определения
концентрации общего иммуноглобулина
класса М в сыворотке крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 14.08.18

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного определения концентрации общего иммуноглобулина класса М в сыворотке крови «IgM общий-ИФА-БЕСТ» (далее по тексту – набор) предназначен для определения концентрации общего иммуноглобулина класса М ($IgM_{общ}$) в сыворотке крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение анализа в дублях 41 неизвестного, 6 калибровочных и 1 контрольного образцов (всего 96 определений при использовании всех стрипов планшета).

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения основан на двухстадийном «сэндвич» – варианте твердофазного иммуноферментного анализа с применением моноклональных антител к IgM.

На первой стадии калибровочные образцы с известной концентрацией $IgM_{общ}$ и анализируемые образцы инкубируются в лунках стрипированного планшета с иммобилизованными моноклональными антителами (МКАТ) к мю-цепям IgM. На второй стадии связавшийся в лунках IgM обрабатывают конъюгатом МКАТ к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой.

Образовавшиеся иммунные комплексы «иммобилизованные МКАТ – IgM – конъюгат» выявляют ферментативной реакцией с раствором тетраметилбензидина. Степень окрашивания пропорциональна концентрации $IgM_{общ}$ в анализируемом образце. После измерения величины оптической плотности раствора в лунках на основании калибровочного графика рассчитывается концентрация $IgM_{общ}$ в анализируемых образцах.

2.2. Состав набора

В состав набора входят:

– планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к мю-цепям IgM человека, готовый для использования – 1 шт.;
– калибровочные образцы, содержащие известные количества $IgM_{общ}$ – 0, 20, 40, 100, 200 и 400 Ед/мл (0; 0,16; 0,32; 0,8; 1,6 и 3,2 мг/мл), аттестованные относительно WHO International Standard Immunoglobulins G, A and M, human serum, NIBSC 67/086; концентрации $IgM_{общ}$ в калибровочных образцах могут несколько отличаться от указанных величин, точные величины указаны на этикетках флаконов, готовые для использования – 6 флаконов (по 0,5 мл);
– контрольный образец на основе инактивированной сыворотки крови человека с известным содержанием $IgM_{общ}$, аттестованный относительно WHO International Standard Immunoglobulins G, A and

M, human serum, NIBSC 67/086; готовый для использования – 1 флакон (0,5 мл);
- конъюгат моноклональных антител к легким цепям (лямбда и каппа) иммуноглобулинов человека с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
- раствор для разведения сывороток (РРС), концентрат – 1 флакон (28 мл);
- концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
- раствор тетраметилбензидина плюс (раствор ТМБ плюс), готовый для использования – 1 флакон (13 мл);
- стоп-реагент, готовый для использования – 1 флакон (12 мл);
- пленка для заклеивания планшета – 2 шт.;
- ванночка для реагента – 2 шт.;
- наконечники для пипетки на 5–200 мкл – 16 шт.

Принадлежности:
- трафарет для построения калибровочного графика – 1 шт.;
- планшет для предварительного разведения исследуемых образцов – 1 шт.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. В наборе «IgM общий-ИФА-БЕСТ» используются моноклональные антитела, обладающие высокой специфичностью к мю-цепям IgM. Перекрестного связывания с IgG, IgA, IgE или альбумином в физиологических концентрациях не наблюдалось.

3.2. «Хук-эффект» при использовании набора реагентов не зафиксирован. Оптическая плотность образцов сыворотки крови с концентрацией $IgM_{общ}$ до 1000 Ед/мл всегда превышала оптическую плотность калибровочного образца с максимальной концентрацией $IgM_{общ}$.

3.3.* Воспроизводимость. Коэффициент вариации результатов определения концентрации $IgM_{общ}$ в лунках, содержащих контрольный образец, не превышает 8%.

3.4.* Линейность. Данный аналитический параметр проверяется тестом на «линейность» – отклонение от расчетной величины концентрации $IgM_{общ}$ при разведении калибровочных образцов, содержащих 400, 200, 100 и 40 Ед/мл, в 2 раза. Процент «линейности» составляет 90–110%.

3.5.* Точность. Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации $IgM_{общ}$ расчетному значению в пробе, полученной путем смешивания равных объемов контрольного образца и калибровочного образца с концентрацией $IgM_{общ}$ 40 Ед/мл. Процент «открытия» составляет 90–110%.

3.6.* Чувствительность. Минимально определяемая концентрация $IgM_{общ}$, рассчитанная на основании среднего арифметического значения оптической плотности калибровочного

* по ГОСТ Р 51352-2013.

образца B_0 (с концентрацией $IgM_{общ}$ 0 Ед/мл) плюс 2σ (σ-среднее квадратичное отклонение от среднего арифметического значения B_0), не превышает 4 Ед/мл (0,032 мг/мл).

3.7. Клиническая проверка. Концентрация $IgM_{общ}$, измеренная в сыворотке крови условно здоровых доноров находилась в диапазоне 60÷270 Ед/мл (см. также стр. 28).

3.8. Рекомендуется в каждой лаборатории при использовании набора уточнить значения концентрации $IgM_{общ}$, соответствующие нормальным для данного региона, у обследуемого контингента людей.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2а (приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболе-

ваний при работе в клинико-диагностических лабораториях лечебно-профилактических учреждений», утвержденной Минздравом СССР 17 января 1991 г и в методических указаниях МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998.

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфицированные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Лабораторная посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС,

спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ДЛЯ РАБОТЫ С НАБОРОМ:

– спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение только при длине волны 450 нм;
– шейкер термостатируемый орбитального типа, позволяющий производить встряхивание при температуре 37±1°С и 400–800 об/мин;
– микроцентрифуга, позволяющая центрифугировать при 1500–2000 об/мин;
– промывочное устройство для планшетов;
– холодильник бытовой;

- пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл;
- пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 до 350 мкл;
- флаконы стеклянные вместимостью 15 мл;
- цилиндр мерный вместимостью 1000 мл;
- вода дистиллированная;
- перчатки медицинские диагностические одноразовые;
- бумага фильтровальная лабораторная;
- дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизованную, мутную сыворотку крови.

6.2. Образцы сыворотки крови можно хранить при температуре от 2 до 8°С не более 48 часов или при температуре минус 20°С (и ниже) не более 3 мес. Повторное замораживание и размораживание образцов сыворотки крови не допускается. После размораживания образцы следует тщательно перемешать.

6.3. Образцы сывороток крови, содержащие осадок, необходимо очистить центрифугированием при 1500 об/мин в течение 10 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед проведением анализа компоненты набора и исследуемые образцы сыворотки крови следует выдержать при температуре от 18 до 25°С не менее 30 мин.

7.2. Подготовка планшета.

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Использовать в течение 1 часа после установки. Оставшиеся стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок.

Хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

Раствор готовится из концентрата фосфатно-солевого буферного раствора. При выпадении осадка солей в концентрате необходимо прогреть его при температуре от 30 до 40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

7.4. Приготовление рабочего раствора для разведения сывороток.

Приготовить за 30 мин до начала постановки анализа.

При выпадении осадка солей в концентрате РРС необходимо прогреть его при температуре от 30 до 40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата раствора для разведения сывороток и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут.

7.5. Подготовка калибровочных образцов и контрольного образца.

Калибровочные образцы и контрольный образец готовы к использованию и не требуют дополнительного разведения. Перед использованием флаконы встряхнуть или центрифугировать на микроцентрифуге так, чтобы капли растворов со стенок и крышки опустились на

дно. Затем содержимое флаконов тщательно перемешать на вортексе или пипетированием, избегая образования пены.

Калибровочные образцы и контрольный образец после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Приготовление рабочего разведения анализируемых образцов сыворотки крови.

Готовятся в стеклянных заранее промаркированных флаконах за 5–10 мин до начала постановки анализа.

Таблица 1

Кол-во используемых стрипов	Промывочный раствор		Рабочий раствор для разведения сывороток		Конъюгат, мл	Раствор ТМБ плюс, мл
	ФСБ-Т×25, концентрат, мл	Дистил. вода, мл	РРС, концентрат, мл	Дистил. вода, мл		
2	4,0	до 100	4,0	до 100	2,0	2,0
3	6,0	до 150	6,0	до 150	3,0	3,0
4	8,0	до 200	8,0	до 200	4,0	4,0
5	10,0	до 250	10,0	до 250	5,0	5,0
6	12,0	до 300	12,0	до 300	6,0	6,0
7	14,0	до 350	14,0	до 350	7,0	7,0
8	16,0	до 400	16,0	до 400	8,0	8,0
9	18,0	до 450	18,0	до 450	9,0	9,0
10	20,0	до 500	20,0	до 500	10,0	10,0
11	22,0	до 550	22,0	до 550	11,0	11,0
12	24,0	до 600	24,0	до 600	12,0	12,0

В чистый флакон с 10 мл рабочего раствора для разведения сывороток (см п. 7.4) добавить 10 мкл исследуемой сыворотки и тщательно перемешать. Таким образом, рабочее разведение сыворотки составит 1000 раз*.

Использовать в течение 30 мин после приготовления.

7.7. Подготовка конъюгата.

Конъюгат готов к использованию.

Необходимое количество конъюгата отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА конъюгат утилизировать ***(не сливать во флакон с исходным конъюгатом).***

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Конъюгат после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.8. Подготовка раствора тетраметилбензидина плюс.

Раствор ТМБ плюс готов к использованию.

Необходимое количество раствора ТМБ плюс отобрать в чистый флакон или ванночку для реагента.

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* См. также раздел «Дополнительная информация для потребителей», п. 2

Оставшийся после проведения ИФА раствор ТМБ плюс утилизировать ***(не сливать во флакон с исходным раствором ТМБ плюс).***

Необходимо исключить воздействие прямого света на раствор тетраметилбензидина плюс.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Раствор ТМБ плюс после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.9. Стоп-реагент готов к использованию.

После вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.10. Внести во все лунки по 100 мкл рабочего раствора для разведения сывороток (см п. 7.4).

Внести в соответствующие лунки в дублях, начиная с верхних лунок первых двух стрипов, по 20 мкл каждого калибровочного образца. В следующую пару лунок внести по 20 мкл контрольного образца. В остальные лунки внести в дублях по 20 мкл анализируемых образцов сыворотки крови в рабочем разведении (см п. 7.6.), каждый раз меняя наконечник.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.11. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.12. По окончании инкубации снять пленку и удалить ее в сосуд с дезинфицирующим раствором. Содержимое лунок удалить отсасыванием в сосуд с дезинфицирующим раствором и промыть, добавляя во все лунки по 350 мкл промывочного раствора (см п. 7.3.). Процесс промывки повторить еще 4 раза. Общее количество отмывок равно 5. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо следить за полным опорожнением лунок после каждого цикла отмывки. Затем удалить остатки жидкости из лунок, постукивая планшетом в перевернутом положении по фильтровальной бумаге.

7.13. Внести во все лунки планшета по 100 мкл конъюгата (см п. 7.7.).

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.15. По окончании инкубации удалить содержимое лунок и промыть планшет, как это указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл раствора тетраметилбензидина плюс (см п. 7.8.) и инкубировать в защищенном от света месте в течение 15 мин при температуре от 18 до 25°С.

Для внесения раствора тетраметилбензидина плюс использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор тетраметилбензидина плюс, по 100 мкл стоп-реагента; при этом содержимое лунок окрашивается в желтый цвет.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Измерить величину оптической плотности растворов в лунках стрипов на спектрофотометре вертикального сканирования в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–655 нм; допускается измерение только с фильтром 450 нм. Измерение проводить через 2–3 мин после остановки реакции.

Время между остановкой реакции и измерением оптической плотности не должно превышать 10 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ

9.1. Вычислить средние арифметические значения оптической плотности для каждой пары лунок, содержащих калибровочные образцы.

9.2. Построить в линейных координатах калибровочный график зависимости среднего арифметического значения оптической плотности (ед. опт. плотн.) от концентрации $IgM_{общ}$ в калибровочных образцах (Ед/мл или мг/мл).

9.3. Определить концентрацию $IgM_{общ}$ в контрольном образце и анализируемых образцах по калибровочному графику. Вычислить среднее арифметическое значение концентрации для каждой пары лунок, содержащих анализируемые образцы.

9.4. Если при проведении анализа использовали разведение сыворотки в 1000 раз (базовое разведение для данного набора), то найденное по графику количество $IgM_{общ}$ соответствует концентрации $IgM_{общ}$ в анализируемом образце в Ед/мл (мг/мл). Если использовали другое разведение образца, то найденное по графику количество $IgM_{общ}$ пересчитывают с учетом дополнительного разведения, также получая в результате концентрацию $IgM_{общ}$ в Ед/мл (мг/мл).

Если значение оптической плотности анализируемого образца превышает значение ОП для калибровочного образца 400 Ед/мл (3,2 мг/мл), то данный образец анализируют повторно после дополнительного разведения в 2 раза, полученный результат умножают на 2.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности. В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности;
– калибровочные образцы, контрольный образец и конъюгат после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
– концентрат фосфатно-солевого буферного раствора с твином, концентрат раствора для разведения сыво-

роток, раствор ТМБ плюс и стоп-реагент после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
- рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут;
- промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Построение калибровочного графика необходимо проводить для каждого независимого эксперимента, рекомендуется также каждый раз определять концентрацию $IgM_{общ}$ в контрольном образце.

10.6. Для перевода результатов измерений концентрации иммуноглобулина М из Ед/мл в мг/мл следует использовать коэффициент пересчета 0,008 (1 Ед/мл IgM = 0,008 мг/мл IgM).

10.7. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (раствор ТМБ плюс, ФСБ-Т×25, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

10.8. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «IgM общий-ИФА-БЕСТ», следует обращаться в АО «Вектор-БЕСТ» по адресу:

630559, Новосибирская область,
Новосибирский район, п. Кольцово, а/я 121,
тел. (383) 363-20-60, 227-75-43,
тел./факс (383) 363-35-55.
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ.

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации $IgM_{общ}$ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут влиять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– рабочие поверхности столов, оборудования обрабатывайте 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона с ТМБ;

– если допущена ошибка при внесении в лунку анализируемого образца, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки используйте автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.

– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.

– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Рекомендации по подготовке анализируемых образцов

Вместо одноступенчатого (п. 7.6.) допустимо проводить двухступенчатое разведение сывороток с использованием планшета для предварительного разведения исследуемых образцов. Для этого, в каждую лунку планшета для предварительного разведения внести по 310 мкл рабочего раствора для разведения сывороток. Далее в одну из лунок, например, А-1, добавить 10 мкл исследуемой сыворотки, сменить использованный наконечник пипетки на новый и затем с его помощью тщательно перемешать содержимое лунки (5–6 круговых движений, во время которых следует 3–4 раза набрать и опорожнить наконечник), избегая образования пены. После

этого из лунки отобрать 10 мкл, внести в соседнюю лунку, например, А-2, и таким же образом тщательно перемешать (для этой операции также желательно использовать новый чистый наконечник пипетки). В лунке А-2 получаем рабочее разведение сыворотки 1000 раз. Аналогично развести и другие исследуемые сыворотки (например, в лунках В-1 и В-2, С-1 и т.д.):

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл исследуемого образца → предварительное разведение образца в 32 раза;

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл образца после предварительного разведения → рабочее разведение образца в 1000 раз.

Внимание! *Точность приготовления разведений определяет качество постановки теста!*

При исследовании не сыворотки, а других биологических жидкостей, степень разведения исследуемых образцов следует заранее подобрать опытным путем, используя как ориентир данные таблицы 2.

3. Условия правильности работы набора

Результаты анализа исследуемых образцов учитывать, если будут выполнены следующие условия:

– соотношение оптических плотностей калибровочных образцов:

$ОП_0 < ОП_{20} < ОП_{40} < ОП_{100} < ОП_{200} < ОП_{400}$;

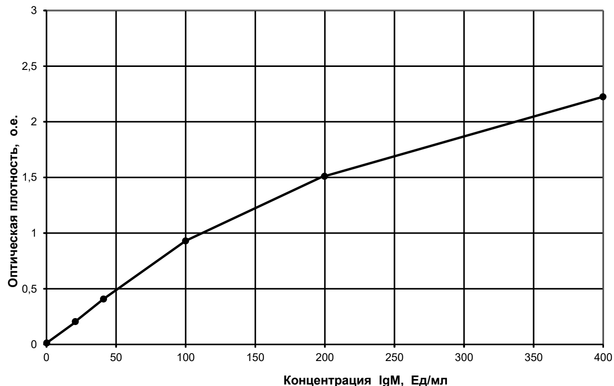

Рисунок. Зависимость оптической плотности от концентрации $IgM_{общ}$ в калибровочных образцах.

– $ОП_{400} \geq 1{,}0$ ед. опт. плотн. (о.е.);

– вычисленное по калибровочному графику значение концентрации $IgM_{общ}$ в контрольном образце попадает в пределы, указанные на этикетке флакона.

$ОП_0$, $ОП_{20}$, $ОП_{40}$, $ОП_{100}$, $ОП_{200}$ и $ОП_{400}$ – среднее значение оптической плотности калибровочных образцов 0; 20; 40; 100; 200 и 400 мг/мл соответственно.

4. Расчет результатов анализа

По результатам измерения вычислить среднее арифметическое значение оптической плотности (ОП) в лунках с анализируемыми образцами.

Построить в линейных координатах калибровочный график зависимости оптической плотности (ось ординат) от концентрации $IgM_{общ}$ (ось абсцисс) в калибровочных образцах. Для этого на прилагаемом трафарете для построения графика против концентрации каждого калибровочного образца отложить соответствующее ей среднее значение оптической плотности. Последовательно соединить полученные точки отрезками прямых линий.

Пример калибровочного графика представлен на рисунке.

Определить содержание $IgM_{общ}$ в контрольном и в анализируемых образцах по калибровочному графику. Для этого на оси ординат отметить значение ОП анализируемого образца. Провести прямую линию параллельно оси абсцисс до пересечения с калибровочным графиком. От точки пересечения опустить перпендикуляр на ось абсцисс. По полученной точке пересечения определить значение концентрации $IgM_{общ}$ в образце.

При использовании для расчетов концентраций компьютерного или встроенного в спектрофотометр программного обеспечения в настройках выбрать метод, соответствующий кусочно-линейной аппроксимации.

5. Диагностическая значимость

IgM, как и другие иммуноглобулины, относится к гуморальным факторам иммунитета.

Карта гуморального иммунитета довольно индивидуальна, тем не менее, пределы нормальных физиологических концентраций достаточно хорошо очерчены.

По нашим данным, концентрация $IgM_{общ}$ в сыворотке крови клинически здоровых доноров (Новосибирская область, Алтайский край) в возрасте от 20 до 50 лет находится в пределах диапазона 60–250 Ед/мл (0,48–2,0 мг/мл). У детей того же региона в возрасте 1–15 лет концентрация $IgM_{общ}$ составила 60–270 Ед/мл (0,48–2,16 мг/мл). Эти значения в целом близки нормальным значениям концентрации IgM (0,5–2,0 мг/мл), приводимым в работе Тотолян А.А., Марфичева Н.А., Тотолян Н.А. «Иммуноглобулины в клинической лабораторной диагностике», С-Пб, 1999. Однако их можно использовать только как ориентировочные, поскольку диапазоны нормальных концентраций IgM могут довольно существенно отличаться в зависимости от региона, возраста и некоторых др. причин. Известно также, что для использования в диагностике важнее знать не абсолютное значение концентрации общего иммуноглобулина, а его относительное отклонение от нормального местного, возрастного или, например, профессионального уровня. **В идеале, нормальные региональные уровни и по взрослым и по детям должны определяться каждой лабораторией самостоятельно!**

Таблица 2

Абсолютные значения уровней содержания иммуноглобулинов в различных биологических жидкостях у здоровых лиц (М±δ)

(Тотолян А.А. Современные подходы к диагностике иммунопатологических состояний. Мед. иммунология, 1999 ,т.1 №1-2, с. 75-108)

Биологические жидкости	Содержание иммуноглобулинов классов:				
	***IgA*, г/л**	***IgM*, г/л**	***IgG*, г/л**	***sIgA*, г/л**	***IgE*, кЕ/л**
ЦСЖ	0,006±0,0013	0,0049±0,001	0,037±0,004	н/опр	0±0
Слюна	0,069±0,028	0,055±0,011	0,042±0,017	0,768±0,275	н/опр
Назальный смыв	0,014±0,006	0,025±0,017	0,042±0,017	0,071±0,022	0±0
Ларингеальный секрет	0,071±0,022	0,063±0,044	0,085±0,044	1,31±1,87	н/опр
Слезная жидкость	0,165±0,02	0,038±0,008	0,185±0,06	н/опр	н/опр
Эякулят	1,01±0,67	0,9±0,46	0,51±0,2	2,21±1,01	0±0
Сыворотка крови	2,15±0,85	1,63±0,46	12,3±2,97	0,79±0,22	50,0±12,5

Примечание: н/опр – данный показатель не определяли.

Приведенные показатели можно использовать только как ориентировочные, и в каждой лаборатории рекомендуется вычислить собственные границы нормальных значений концентрации общего IgM в сыворотке крови.

Уровень концентрации общего IgM в сыворотке крови новорожденных составляет около 10% от уровня взрослых. Через 1–3 месяца он увеличивается до 60-65%, а еще через 1–2 года нередко уже достигает уровня взрослого человека. Однако в этом возрасте возможны его значительные колебания. У подавляющего же большинства здоровых детей уровень концентрации общего IgM стабилизируется и становится равным уровню взрослых в возрасте 6–8 лет.

Результаты определения концентрации общего сывороточного IgM могут быть с успехом использованы для дифференциальной диагностики целого ряда заболеваний (см. иммунограмму).

Более полную картину способно дать параллельное определение всех трех основных классов иммуноглобулинов – G, M и A, а также иммуноглобулина E.

Иммунограмма при некоторых заболеваниях

	IgG	IgA	IgM	IgE
Заболевания печени				
Острый инфекционный гепатит	+	N/+	N/++	N
Хронический персистирующий гепатит	N/+	N	N/+	N/+
Хронический агрессивный гепатит	++	+	N/++	N/+
Постгепатитный криптогенный цирроз	++	+	+	N/+
Первичный билиарный цирроз	N/+	N	+/++	N
Алкогольный цирроз	N/+	++	N/+	N
Болезни почек				
Острый пиелонефрит	N	N	+/++	N
Хронический пиелонефрит	+/++	N	+/++	N/+
Нефротический синдром	—	—	N/—	N/—
Инфекционные заболевания				
Острая инфекция	N	N	+/++	N
Хроническая инфекция	+/++	N/+	N/+	N/+
Системные ревматические заболевания				
Ревматоидный артрит	N/++	N/++	N/+	+/++
Системная красная волчанка	+	N	N/+	N/+
Склеродермия	N	N	N	N/+
Смешанные системные заболевания	N/+	N/+	N	N/+
Атопия, аллергические заболевания	N/+	N	N/—	+/++
Гельминтозы и др. паразитарные заболевания	N/+	N/+	N/+	+/++

N – нормальная регионально-возрастная концентрация иммуноглобулина (в пределах нормального диапазона от Nmin до Nmax)

\+ – повышенная концентрация иммуноглобулина (от Nmax до 1,3Nmax)

++ – сильно повышенная концентрация иммуноглобулина (более 1,3Nmax)

– – пониженная концентрация иммуноглобулина (ниже Nmin)

6. Краткая схема проведения ИФА для набора реагентов «IgM общий-ИФА-БЕСТ»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл рабочего раствора для разведения сывороток; по 20 мкл калибровочных и контрольного образцов в дублях в контрольные лунки; по 20 мкл разведенных анализируемых образцов в дублях в лунки для исследуемых образцов.
Инкубировать:	20 мин, 37°C, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	20 мин, 37°C, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл раствора ТМБ плюс.
Инкубировать:	15 мин, 18–25°C, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

7. Графические символы

Символ	Значение	Символ	Значение
REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …	i	Обратитесь к инструкции по применению
!	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-05-97.

12.11.18

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Total Prostate Specific Antigen (PSA) Test System

Product Code: 2125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (PSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

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 to be 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 total 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

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay 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:

$$^{Enz}Ab_{(p)} + Ag_{PSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ =Biotinylated Antibody (Excess Quantity)
Ag_{PSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$ +Streptavidin$_{C.W.}$ ⇒Immobilized complex
Streptavidin$_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = complex bound to the solid surface

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:

A. PSA Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of serum references PSA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50(**E**) and 100(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.

B. PSA Enzyme Reagent – 13 ml/vial – Icon E
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C. (see Reagent Preparation Section).

E. Substrate A – 7 ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7 ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. (see Reagent Preparation Section).

G. Stop Solution – 8 ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. 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 96-well microplate. For other kit configurations, refer to the table at the end of this insert.

4.1 Required But Not Provided:

1. Pipette(s) capable of delivering 0.025, 0.050 & 0.100 ml (25, 50, & 100 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. 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.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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, 0.050 ml (50 µl) 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

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one year
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 reference calibrators and controls to room temperature (20 - 27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, 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.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (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.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (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.**
8. Add 0.100ml (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

9. Incubate at room temperature for fifteen (15) minutes.
10. 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.**
11. 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.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. 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).
3. Draw the best-fit curve through the plotted points.
4. 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.

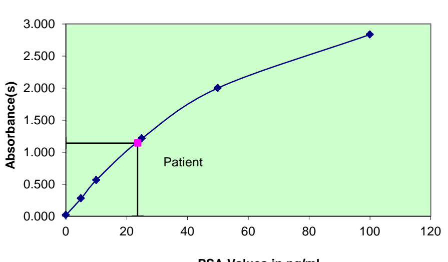
EXAMPLE 1

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	E2	1.186	1.142	23.6

	F2	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:

1. The absorbance (OD) of calibrator F should be $\geq$ 1.3.
2. 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 are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. 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).
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.
11. 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.
12. 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.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem.* 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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.
6. 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.
7. PSA is elevated in benign prostate hypertrophy (BPH). Clinically, an elevated **PSA value alone is not of diagnostic value as a specific test for cance**r 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 prostate cancer conditions.[5]
8. Due to the variation in the calibration used in PSA/ fPSA test kits and differences in epitopic recognition of different antibodies, it is always suggested that the patient sample should be tested with PSA/ fPSA tests made by the same manufacturer**. (*Monobind Inc. offers a fPSA ELISA test that should be used for consistency reasons, when needed.*)**

13.0 PERFORMANCE CHARACTERISTICS

Healthy males are expected to have values below 4 ng/ml.[4]

TABLE I
Expected Values for PSA AccuBind® ELISA Test System

Healthy Males	<4 ng/ml

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 PSA 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	σ	C.V.
Level 1	20	1.06	0.06	5.2%
Level 2	20	3.56	0.18	5.1%
Level 3	20	23.07	0.88	3.8%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.98	0.08	8.5%
Level 2	20	3.35	0.19	5.7%
Level 3	20	23.17	0.95	4.1%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The PSA AccuBind® ELISA test system has a sensitivity of 0.0003 ng/well. This is equivalent to a sample containing 0.013 ng/ml PSA concentration.

14.3 Accuracy

The PSA AccuBind® ELISA test system 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 PSA AccuBind® ELISA test method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square 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 PSA AccuBind® ELISA test system 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 PSA AccuBind® ELISA test system upon addition of massive amounts of the following substances to a human serum pool.

Substance	Concentration
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

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Revision: 6 Date: 2022-MAY-01 DCO: 1557
MP2125 Product Code: *2125-300*

	Size	96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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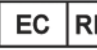

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Glossary of Symbols
(EN 980/ISO 15223)

In Vitro - Diagnostic Medical Device

Temperature Limitation Storage Condition (2-8° C)

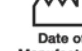
Consult Instructions for Use

Catalogue Number

Contains Sufficient Test for Σ

Batch Code

Used By (Expiration Day)


Date of Manufacturer

Manufacturer

Authorized Rep in European Country

European Conformity

Total Triiodothyronine (T3) Test System
Product Code: 125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Triiodothyronine Concentration in T3 Calibrators or Plasma by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassays (1,2).

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native triiodothyronine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound T3-enzyme conjugate is separated from the unbound T3-enzyme 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 triiodothyronine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with T3 concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5):

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites.

The interaction is illustrated by the following equation:

$$^{Enz}Ag + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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 inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. T3 Calibrators References – 1ml/vial - Icons A-F
Six (6) vials of serum reference for triiodothyronine at concentrations of 0 (**A**), 0.5 (**B**), 1.0 (**C**), 2.5 (**D**), 5.0(**E**) and 7.5(**F**) ng/ml. Store at 2-8°C. A preservative has been added. **For SI units: ng/ml x 1.536 = nmol/L**

B. T3 Enzyme Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of T3-horseradish peroxidase (HRP) conjugate in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C

C. T3/T4 Conjugate Buffer – 13ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T3 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with Sheep anti-T3 serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7 ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7 ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon (STOP)
One (1) bottle of stop solution containing a strong acid (1N HCL). Store at 2-30°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **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 96-well microplate. For other kit configurations, see table at end of IFU.

4.1 Materials Required But Not Provided:

1. Pipettes capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate preparation.
4. Microplate washers or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for preparation of enzyme conjugate and substrate A plus B.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. 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 required tests. Since no known test can offer complete assurance that infectious agents are absent, all T3 Calibrators 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 or plasma 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 (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma 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, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay external controls at levels in the hypothyroid, euthyroid and hyperthyroid 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. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. 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

1. **Working Reagent A - T3-enzyme Conjugate Solution**
Dilute the T3-enzyme conjugate 1:11 with T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
General Formula:
Amount of Buffer required = Number of wells ∗ 0.1
Quantity of T3-Enzyme necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 Conjugate Buffer
16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate

2. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.

3. **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*****

1. 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.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent A, T3 Enzyme Reagent to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. 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.**
8. 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

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. 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.**

Note: For re-assaying specimens with concentrations greater than 7.5ng/ml, pipette 25µl of the specimen and 25µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T3 concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis (y-axis) of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis (X-axis) of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.130) intersects the dose response curve at 1.95ng/ml T3 concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may be used for the data reduction. If such

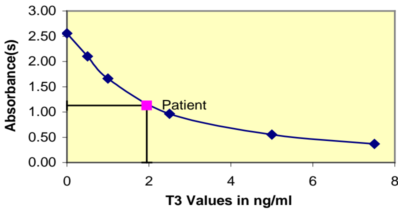
software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.604	2.556	0
	B1	2.507		
Cal B	C1	2.073	2.101	0.5
	D1	2.128		
Cal C	E1	1.678	1.662	1.0
	F1	1.646		
Cal D	G1	0.964	0.966	2.5
	H1	0.969		
Cal E	A2	0.550	0.551	5.0
	B2	0.551		
Cal F	C2	0.372	0.370	7.5
	D2	0.369		
Ctrl 1	E2	1.701	1.726	***0.92***
	F2	1.638		
Ctrl 2	G2	0.755	0.734	***3.58***
	H2	0.791		
Patient	A3	1.145	1.130	***1.95***
	B3	1.115		

*The data presented in Example 1 and Figure 1 are 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:

1. The absorbance (OD) of calibrator 0 ng/ml should be $\geq$ 1.3.
2. 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

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with T3 concentrations above 7.5 ng/mL may be diluted ½ with '0' serum reference. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
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.
11. 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.
12. 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.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. 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.
5. 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.
6. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG (3, 4). **Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.**
7. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total triiodothyronine values, has been compiled by the Journal of the American Association of Clinical Chemists[3].

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T3 AccuBind™ ELISA Test System. The mean (R) values standard deviations (σ) and expected ranges (±2 σ) are presented in Table 1. The total number of samples was 105.

TABLE I
Expected Values for the T3 ELISA Test System (in ng/ml)

Mean (X)	**1.184**
Standard Deviation (σ)	0.334
Expected Ranges (±2 σ)	0.52 – 1.85

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 T3 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) 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	σ	C.V.
Low	16	0.78	0.06	7.9%
Normal	16	1.92	0.10	5.4%
High	16	3.55	0.14	3.9 %

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	0.76	0.07	8.9%
Normal	10	1.85	0.13	6.7%
High	10	3.43	0.16	4.5%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The T3 AccuBind™ ELISA test system has a sensitivity of 0.04 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The T3 AccuBind™ ELISA method was compared with a reference radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.15ng/ml – 8.0ng/ml). The total number of such specimens was 120. The least square regression equation (y= mx+b) and the correlation coefficient were computed for the T3 AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	1.62	y = 3.8 + 0.947(x)	0.987
Reference	1.68		

Only slight amounts of bias between this 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

The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of conjugate.

Substance	Cross Reactivity	Concentration
l-Triiodothyronine	1.0000	-
l-Thyroxine	< 0.0002	10µg/ml
Iodothyrosine	< 0.0001	10µg/ml
Diiodothyrosine	< 0.0001	10µg/ml
Diiodothyronine	< 0.0001	10µg/ml
Phenylbutazone	< 0.0001	10µg/ml
Sodium Salicylate	< 0.0001	10µg/ml

15.0 REFERENCES

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Revision: 4 2022-May-01 DCO: 1557
Cat #: *125-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

For Orders and Inquiries, please contact

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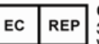

CEpartner4U, Esdoornlaan 13, 3951DB Maarn, The Netherlands
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Total Thyroxine (T4) Test System
Product Code: 225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Thyroxine Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum thyroxine concentration is generally regarded as an important *in-vitro* diagnostic test for assessing thyroid function. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last three decades. This procedural evolution can be traced from the empirical protein bound iodine (PBI) test (1) to the theoretically sophisticated radioimmunoassay (2).

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine 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 thyroxine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5)
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the equation in the following below.

$$^{Enz}Ag + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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 inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

A. T4 **Calibrators – 1ml/vial - Icons A-F**
Six (6) vials of serum reference for thyroxine at concentrations of 0 (**A**), 2.0 (**B**), 5.0 (**C**), 10.0 (**D**), 15.0 (**E**) and 25.0 (**F**) µg/dl. Store at 2-8°C. A preservative has been added. **For SI units: µg/dl x 12.9 = nmol/L**

B. T4-Enzyme Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3/T4 Conjugate Buffer – 13 ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T4 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with sheep anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon STOP
One (1) bottle containing a strong acid (1.0N HCl). Store at 2-8°C.

I. Product Insert.

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 96-well microplate. For other kit configurations, see table at the end of this IFU.

4.1 Required But Not Provided:
1. Pipette capable of delivering 25µl & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate preparation
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for preparation of enzyme conjugate.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. 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 required tests. 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 PEPARATION

The specimens shall be blood; serum or plasma 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 (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma 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 hypothyroid, euthyroid and hyperthyroid 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. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. 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

1. **Working Reagent A = T4-Enzyme Conjugate Solution**
   Dilute the T4-enzyme conjugate 1:11 with Total T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
   General Formula:
   Amount of Buffer required = Number of wells ∗ 0.1
   Quantity of T4 Enzyme necessary = # of wells ∗ 0.01
   i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 conjugate buffer
   16 x 0.01 = 0.16ml (160µl) for T4 enzyme conjugate
2. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
3. **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*****

1. Format the microplate's 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.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent A, T4 Enzyme Reagent to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. 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.**
8. 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
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. 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.**

Note: For reassaying specimens with concentrations greater than 25 µg/dl, pipet 12.5µl of the specimen and 12.5µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the thyroxine concentration.

10.0 CALCULATION OF RESULTS

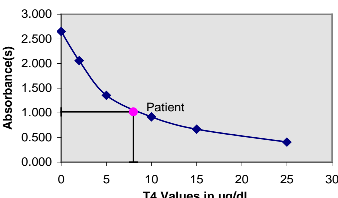
A dose response curve is used to ascertain the concentration of thyroxine in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T4 concentration in µg/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of T4 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 µg/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.022) intersects the standard curve at (8 µg/dl) T4 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.

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (μg/dl)
Cal A	A1	2.648	2.650	0
	B1	2.652		
Cal B	C1	2.090	2.060	2
	D1	2.031		
Cal C	E1	1.344	1.355	5
	F1	1.366		
Cal D	G1	0.897	0.918	10
	H1	0.939		
Cal E	A2	0.676	0.668	15
	B2	0.659		
Cal F	C2	0.408	0.406	25
	D2	0.404		
Ctrl 1	E2	1.425	1.435	**4.6**
	F2	1.383		
Ctrl 2	G2	0.611	0.613	**16.3**
	H2	0.608		
Patient	A3	0.984	1.022	**8.0**
	B3	1.060		

EXAMPLE 1

Figure 1



The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 μg/dl should be $\geq$ 1.3.
2. 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

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with T4 concentrations greater than 35 μg/dl may be diluted ½ with the '0' serum reference into the sample well; pipet 12.5μl of the specimen and 12.5μl of the '0' serum reference in the sample well to maintain a uniform protein concentration. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
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.
11. 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.
12. 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.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. 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.
5. 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.

2. Total serum thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG (3, 4). **Thus, total thyroxine concentration alone is not sufficient to assess clinical status.**
3. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated T4 is caused by TBG variation.
4. A decrease in total thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T4 AccuBind™ ELISA Test System. The mean (X) values, standard deviations (σ) and expected ranges (±2 σ) are presented in Table 1.

TABLE 1
Expected Values for the T4 ELISA Test System (in μg/dl)

	Male	**Female ***
Number of Specimens	42	58
Mean (X)	7.6	8.2
Std.Dev (σ)	1.6	1.7
Expected Ranges (±2 σ)	4.4 – 10.8	4.8 – 11.6

*Normal patients with high TBG levels were **not** excluded except if pregnant.

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 T4 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in μg/dl)

Sample	N	X	σ	C.V.%
Low	20	6.87	0.16	2.3
Normal	20	9.95	0.16	1.6
High	20	13.13	0.17	1.3

TABLE 3
Between Assay Precision (Values in μg/dl)

Sample	N	X	σ	C.V.%
Low	20	5.76	0.37	6.3
Normal	20	9.41	0.57	6.1
High	20	16.18	1.21	7.5

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The T4 AccuBind™ ELISA test system has a sensitivity of 3.2ng/well. This is equivalent to a sample containing a concentration of 0.128 μg/dl. The sensitivity was ascertained by determining the variability of the 0 μg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The tT4 AccuBind™ ELISA method was compared with a coated tube radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.8μg/dl – 25μg/dl). The total number of such specimens was 131. The least square regression equation and the correlation coefficient were computed for the T4 AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	8.07	y = 0.39+0.952(x)	0.934
Reference	8.06		

Only slight amounts of bias between this 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

The cross-reactivity of the thyroxine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyroxine needed to displace the same amount of conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	-
d-Thyroxine	0.9800	10μg/dl
d-Triiodothyronine	0.0150	100μg/dl
l–Triiodothyronine	0.0300	100μg/dl
Iodothyrosine	0.0001	100μg/ml
Diiodothyrosine	0.0001	100μg/ml
Diiodothyronine	0.0001	100μg/ml

15.0 REFERENCES

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Revision: 4 **Date: 2022-May-01** **DCO: 1557**
Cat #: ***225-300***

	Size	96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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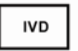


CEpartner4U, Esdoornlaan 13, 3951DB Maarn, The Netherlands
www.cepartner4u.eu

Thyrotropin (TSH) Test System
Product Code: 325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyrotropin Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 Daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism.[1,2] The structure of human TSH is similar to that of the pituitary and placental gonadotropins, consisting of an 89-amino acid α-subunit which is similar or identical between these hormones and a 115-amino acid β-subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with apparent excess production of the α-subunit. The TSH molecule has a linear structure consisting of the protein core with carbohydrate side chains; the latter accounts for 16% of the molecular weight.

TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provides the means for discrimination in the hyperthyroid-euthyroid range. The functional sensitivity (<20% between assay CV) of the one-hour procedure is 0.195 µIU/ml while the two-hour procedure has a functional sensitivity of 0.095µIU/ml.[3]

In this method, TSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from the unbound enzyme-thyrotropin 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 thyrotropin levels permits 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 thyrotropin concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated 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-TSH 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:

$$^{Enz}Ab_{(p)} + Ag_{TSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{TSH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme -Polyclonal Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the well surface

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:

A. **TSH Calibrators – 1ml/vial - Icons A-G**
Seven (7) vials of references for TSH Antigen at levels of 0(**A**), 0.5(**B**), 2.5(**C**), 5.0(**D**), 10(**E**), 20(**F**) and 40(**G**) µIU/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 WHO 2nd IRP 80/558.

B. **TSH Enzyme Reagent – 13ml/vial - Icon Ⓔ**
One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. **Streptavidin Coated Plate – 96 wells – Icon ⇓**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. **Wash Solution Concentrate – 20 ml/ml - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. **Substrate A – 7ml/vial - Icon S^A**
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. **Substrate B – 7ml/vial - Icon S^B**
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. **Stop Solution – 8ml/vial - Icon STOP**
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. **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:

1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. 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 required tests. 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.

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 gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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, (100µl) 0.100 ml 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. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. 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

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or de-ionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one year
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 reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, 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.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the TSH Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature. **
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (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.**
8. 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

9. Incubate at room temperature for fifteen (15) minutes.
10. 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.**
11. 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.**

** For better low-end sensitivity (< 0.5µIU/ml), incubate 120 minutes at room temperature. The 40µIU/ml calibrator should be excluded since absorbance over 3.0 units will be experienced. Follow the remaining steps.

Note: Dilute samples reading over 40 µIU/ml by 1:5 and 1:10 with TSH '0' Calibrator. Multiply the results by the dilution factor to obtain accurate results.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1
2. Plot the absorbance for each duplicate serum reference versus the corresponding TSH concentration in µIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

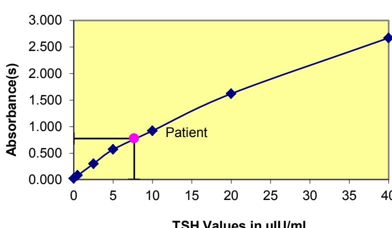
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.775) intersects the dose response curve at (7.66 µIU/ml) TSH concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assay 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	Mean Abs	Value (µIU/ml)
Cal A	A1	0.018	0.019	0
	B1	0.021		
Cal B	C1	0.076	0.079	0.5
	D1	0.082		
Cal C	E1	0.302	0.298	2.5
	F1	0.293		
Cal D	G1	0.556	0.567	5.0
	H1	0.577		
Cal E	A2	0.926	0.921	10
	B2	0.916		
Cal F	C2	1.610	1.619	20
	D2	1.629		
Cal G	E2	2.694	2.671	40
	F2	2.647		
Control	G2	0.800	0.775	**7.66**
	H2	0.751		
Patient	A3	1. 391	1.383	**16.65**
	B3	1.375		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance of calibrator 'G' (40 µIU/ml) should be $\geq$ 1.3.
2. 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

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. Patient specimens with TSH concentrations over 40µIU/ml may be diluted (1:5 or 1:10) with the '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
11. 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.
12. 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.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurement and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. 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.
5. 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.
6. Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. **Thus, thyrotropin concentration alone is not sufficient to assess clinical status.**
7. Serum TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.
8. A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine.[4]
9. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the TSH AccuBind® ELISA Test System. The number and determined range are given in Table 1. A nonparametric method (95% Percentile Estimate) was used.

TABLE I
Expected Values for the TSH ELISA Test System (in µIU/ml)

Number	139	2.5 Percentile–70% Conf Int	
Low Normal	0.39	Low Range	0.28 – 0.53
High Normal	6.16	High Range	5.60 – 6.82

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 TSH AccuBind® test system were determined by analyses on three different levels of pool control sera. The number (N), mean (X) value, standard deviation **(σ)** and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	24	0.37	0.03	8.1%
Pool 2	24	6.75	0.43	6.4%
Pool 3	24	29.30	1.94	6.6%

TABLE 3
Between Assay Precision* (Values in µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	0.43	0.04	9.3%
Pool 2	10	6.80	0.54	7.9%
Pool 3	10	28.40	1.67	5.9%

*As measured in ten experiments in duplicate over seven days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:

For I hr incubation = 0.078 µIU/ml
For 2 hr incubation = 0.027 µIU/ml

14.3 Accuracy

The TSH AccuBind® ELISA test system was compared with a reference immunochemiluminescence assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01µIU/ml – 61µIU/ml). The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the TSH AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	4.54	y = 0.47 + 0.968 (x)	0.995
Reference	4.21		

Only slight amounts of bias between the TSH 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

The cross-reactivity of the TSH AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyrotropin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Follitropin (hFSH)	< 0.0001	1000ng/ml
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin(hCG)	< 0.0001	1000ng/ml

14.5 Correlation between 1 hr and 2 hr incubation

The one- (1) hr and two (2) hr (optional) incubation procedures were compared. Thirty (30) biological specimens (ranging from 0.1 – 18.5 µIU/ml) were used The least square regression equation and the correlation coefficient were computed for the 2 hr procedure (y) in comparison with the 1 hr method (x). Excellent agreement is evidenced by the correlation coefficient, slope and intercept: Y = 0.986 (x) + 0.119 Regression Correlation =0.998

15.0 REFERENCES

1. Hopton MR, & Harrap JJ, "Immunoradiometric assay of thyrotropin as a first line thyroid function test in the routine laboratory", *Clinical Chemistry*, 32, 691 (1986).
2. Caldwell, G et al, "A new strategy for thyroid function testing", *Lancet*, I, 1117 (1985).
3. Young DS, Pestaner LC, and Gilberman U, "Effects of Drugs on Clinical Laboratory Tests", *Clinical Chemistry,* 21, 3660 (1975).
4. Spencer, CA, et al, "Interlaboratory/Intermethod differences in Functional Sensitivity of Immunometric Assays of Thyrotropin (TSH) and Impact on Reliability of Measurement of Subnormal Concentrations of TSH", *Clinical Chemistry,* 41, 367 (1995).
5. Beck-Peccoz P, Persani L, "Variable biological activity of thyroid stimulating hormone", *Eur J Endocrinol,* 131, 331-340 (1994).
6. Bravermann, LE, "Evaluation of thyroid status in patients with thyrotoxicosis", *Clin Chem*, 42, 174-181 (1996).
7. Fisher, DA, "Physiological variations in thyroid hormones. Physiological and pathophysiological considerations", *Clin Chem*, 42, 135-139 (1996).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP325 Product Code: *325-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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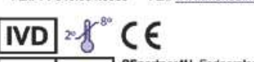

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Glossary of Symbols
(EN 980/ISO 15223)

In Vitro - Diagnostic Medical Device

Temperature Limitation Storage Condition (2-8° C)

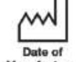
Consult Instructions for Use

Catalogue Number

Contains Sufficient Test for Σ

Batch Code

Used By (Expiration Day)


Date of Manufacturer

Manufacturer

Authorized Rep in European Country

European Conformity

Free Thyroxine (Free T4) Test System

Product Code: 1225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Thyroxine Concentration in Human Serum by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroxine, the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main carrier is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate better with clinical status than total thyroxine levels.

The increase in total thyroxine associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the free thyroxine concentration remains in the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration. The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result. The free thyroxine concentration can help in uncovering the patient's actual clinical status.

In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate (analog method) is added and the reactants are mixed. A competition reaction results between the enzyme conjugate and the free thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate via a wash step. 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 free thyroxine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with free thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay, Analog Method for Free-T4 (TYPE 5):

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
K_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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 inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. **Free T4 Calibrators – 1 ml/vial - Icons A-F**
Six (6) vials of human serum based reference calibrators for free thyroxine at **approximate*** concentrations of 0 (**A**), 0.40 (**B**), 1.25 (**C**), 2.10 (**D**), 5.00 (**E**) and 7.40 (**F**) ng/dl. Store at 2-8°C. A preservative has been added. For SI units use the conversion factor 12.9 to convert ng/dl to pmol/L.
* Exact levels are given on the labels on a lot specific basis.

B. **fT4- Enzyme Reagent – 13 ml/vial - Icon** Ⓔ
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. **Free T4 Antibody Coated Plate – 96 wells - Icon**
One 96-well microplate coated with anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. **Wash Solution Concentrate – 20ml - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. **Substrate A – 7 ml/vial - Icon S^A**
One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

F. **Substrate B – 7 ml/vial - Icon S^B**
One (1) bottle containing hydrogen peroxide (H_2O_2) in acetate buffer. Store at 2-8°C.

G. **Stop Solution – 8 ml/vial - Icon** (STOP)
One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

H. **Product Instructions.**

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **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 96-well microplate. For other kit configurations, please refer to the table at the end of this IFU.

4.1 Materials Required But Not Provided:

1. Pipette capable of delivering 50µl & 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. 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, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid 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:

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
2. **Working Substrate Solution**
Pour the contents of the plastic 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*****

1. Format the microplate 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**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT4 Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. 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.**
8. 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
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. 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.**

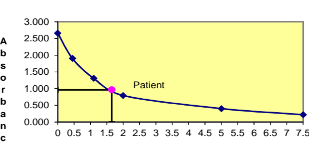
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of free T4 in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free T4 concentration in ng/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Free T4 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/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.964) intersects the dose response curve at (1.65ng/dl) free T4 concentration (See Figure 1).

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay. **Assigned values for calibrators are lot specific.**

Figure 1



EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value* (ng/dl)
Cal A	A1	2.658	2.612	0.00
	B1	2.566		
Cal B	C1	1.919	1.900	0.45
	D1	1.880		
Cal C	E1	1.339	1.306	1.10
	F1	1.273		

Cal D	G1	0.769	0.790	2.00
	H1	0.811		
Cal E	A2	0.396	0.400	5.00
	B2	0.404		
Cal F	C2	0.215	0.217	7.40
	D2	0.219		
Ctrl 1	E2	1.827	1.835	**0.50**
	F2	1.843		
Ctrl 2	G2	0.541	0.557	**2.70**
	H2	0.573		
Patient	A3	0.951	0.964	**1.65**
	B3	0.976		

Note 1: 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.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/dl should be $\geq$ 1.3.
2. 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

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. 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.
10. 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.
11. 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.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. 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.
5. 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.
6. If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 7.4 ng/dl). ***Do not try to dilute the sample. TBG variations in different matrices will not allow Free T4 hormone to dilute serially.***
7. Serum free-thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, Thyroxine binding globulin (TBG) concentration, and the binding of Thyroxine to TBG (3, 4). Thus, free-Thyroxine concentration alone is not sufficient to assess the clinical status.
8. Serum free-thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
9. A decrease in free thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect free Thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.
10. The interpretation of Free T4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere in its metabolism to T3. In severe non-thyroidal illness (NTI) the assessment of thyroid becomes especially difficult. Since the patients in this category may suffer from concomitant primary hypothyroidism or from compensatory secondary hypothyroidism. In cases like these a sensitive TSH evaluation of the patient may be recommended. Please see Monobind Cat# 325-300.
11. In rare conditions associated with extreme variations in albumin binding capacity for T4- such as familial dysalbuminemic hyperthyroxinemia (FDH) – direct assessment of Free T4 may be misleading.
12. Circulating antibodies to T4 and hormone binding inhibitors may interfere in the performance of the assay.
13. Heparin is reported to have in vivo and in vitro effects on free T4 levels. Samples from patients undergoing heparin therapy should be collected well before the administration of the anticoagulant.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the Free T4 AccuBind® ELISA test system. The mean (X) values, standard deviations (σ) and expected ranges ($\pm 2\sigma$) are presented in Table 1.

TABLE 1
Expected Values for Free T4 ELISA Test System (in ng/dl)

	Adult	Pregnancy
Number of Specimens	89	31
Mean (X)	1.40	1.50
Standard Deviation (σ)	0.30	0.37
Expected Ranges ($\pm 2\ \sigma$)	0.8 – 2.0	0.76 – 2.24

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 *inter* and *intra* assay precisions of the Free T4 AccuBind® ELISA test system were determined by analyses on three different levels of pooled patient sera. The number (n), mean values (x), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

In order to validate the *intra-assay* precision of the Free T4 AccuBind® ELISA test system, twenty replicates of each of three pooled sera (low medium and high ranges of the dose response curve) were assayed in the same assay. An intra-assay precision of 3.25 to 10.98% was obtained.

TABLE 2
Intra-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	20	0.550	0.061	10.98%
Medium	20	1.740	0.074	4.26%
High	20	3.250	0.106	3.25%

In order to validate the *inter-assay* precision of fT4 AccuBind® ELISA test system, one duplicate of each of three pooled sera (low medium and high ranges of the dose response curve) was assayed in 10 assays done over a period of six months that involved five different sets of reagents and three different technicians. An inter-assay precision of 6.01 to 10.81% was obtained.

TABLE 3
Inter-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	10	0.480	0.052	10.81%
Medium	10	1.410	0.085	6.01%
High	10	3.490	0.279	7.90%

14.2 Sensitivity

The Free T4 AccuBind® ELISA test system has a sensitivity of 0.162 ng/dl. The sensitivity was ascertained by determining the variability of the 0 ng/dl serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The Free T4 AccuBind® ELISA test system was compared with a coated tube radioimmunoassay (**RIA**) method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1ng/dl – 8ng/dl). The total number of such specimens was 197. The least square regression equation and the correlation coefficient were computed for this Free T4 AccuBind® ELISA method in comparison with the predicate method (Table 4).

TABLE 4
Linear Regression Analysis

Method	Mean (x)	Equation	Correlation Coefficient
Monobind EIA "X"	1.56	y = 0.1034 + 0.9525x	0.920
Predicate RIA "Y"	1.59		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values.

14.4 Specificity:

The cross-reactivity of the thyroxine antibody used for Free T4 AccuBind® ELISA to selected substances was evaluated by adding massive amounts of the interfering substance to a serum matrix. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of the conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	----
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodotyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml
TBG	N/D	40 µg/ml
Albumin	N/D	40 mg/ml
Phenylbutazone	N/D	10 µg/ml
Phenytoin	N/D	40 µg/ml
Salicylates	N/D	500 µg/ml

15.0 REFERENCES

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Revision: 6 Date: 2022-MAY-01 DCO: 1557
Cat #: ***1225-300***

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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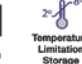
Glossary of Symbols
(EN 980/ISO 15223)

IVD
In Vitro - Diagnostic Medical Device


Temperature Limitation Storage Condition (2-8°C)

Consult Instructions for Use

Catalogue Number

Contains Sufficient Test for Σ

Batch Code

Used By (Expiration Day)

Date of Manufacture

Manufacturer

Authorized Rep in European Country

European Conformity