is rec controls positive reporte	Doc.;
is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "internal Quality Control". Regular calibration of the volumes delivered and Regular calibration of the volumes delivered and	INS SAGIULTRA.
assay racterized check to r 'Internal o	BEER
with the dinegalive a negalive almatch the valuality Control delivered and delivered and the control delivered and the con	EEng Page
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according to the manufacturers instructions, number of washing cycles as found in the valinationment for its use with the kit. Check that the ELISA reader has been turned mountes before reaction	5 of 9 Rev.:
manufacti g cycles a g cytles the se with the	44
urers instructions as found in the weektt	Date:

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When using ELISA automated workstations, all critical

steps (dispensation, incubation, washing, reading shaking, data handling, etc.) have to be casefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Cuality 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 fault handling part of the station (dispensation and washing) has to evalidated 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 confamiliation 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 tasted which services and the stated of the carry over the extension of the carry over effect must be screening and when the number of samples to be tasted when the number of samples to be tasted or who have the controlled for blood screening and when the number of samples to be tasted or who have the carries and the samples to be tasted or who have the carries and the samples to be tasted to the carries of the carry over the carries of the carry over effect must be considered to the carries of the carries of the carry over effect must be sufficient to the carries of the carr

When using automatic devices, in case the vial holder of the instrument does not if with the vials supplied in the kit, transfer the solution into appropriate condainers 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 lest over, tretum the secondary labeled containers to 2,8°C, over, tretum the secondary labeled containers to 2,8°C,

φ firmly capped:

Dia-Pro Support to the user in the serior 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.

- 2

- maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

 Incubation times have a tolerance of ±5%.
 Incubation times have a tolerance of ±5%.
 In microplate reader has to be equipped with a reading filter of 450m and with a second filter (620-630m, strongly recommended) for blanking purposes, its standard performances should be (a) bandwidth < 10 mm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 1%. Blanking is cerried 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 incurrence.

L PRE ASSAY CONTROLS AND OPERATIONS 1. Check the expiration date of the kit printed o

- Check the expiration date of the kit printed on the external label of the kit box, Do not use if expired.
- Check that the liquid components are not contaminated by naked-by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or lade blue. Check that no breakage occurred in transportation and no spilage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or pouch, containing the microplate, is not punctured or
- Dilute all the content of the 20x concentrated Wash Solution
- as described above.

 Dilute the 20X concentrated Enzyme Conjugate with Diluent as reported ij
- თთ
- Dissolve the Calibrator as described above.

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

 Set the EUSA incubator at +37°C and prepare the EUSA.
- When the first incubation is over previously described (section I.4)

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

Automated assay; In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 ut controls & calibrator, then all the samples and finally 100 ut

diluted Enzyme Conjugate, For the pre-washing step (point 1 of the assay procedure) and all the next operations follow the operative instructions reported

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 inconsideration by delaying the first washing operation accordingly.

- 2. Leave the A1 well empty for blanking purposes.

 3. Pipette 150µd of the Negative Control in Inflicate, 150µd of the Calibrator in duplicate and then 150µd of the Positive Control in single followed by 150µ 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 450520nm, (samples show OD values higher than 0,100).

 5. Dispense 100µl diluted Enzymatic Conjugate in all wells,

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

Important notes:

- Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips
- wash the microwells

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- ed on at least 20
- 11. Check that all the other equipment is available and ready

Manual Assay:

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!

- except for A1; used for blanking operations

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.

- when using ELISA automatic instrume
- If the procedure is carried out on shaking, be sure to deliver the rpm reported for in Section 13 as otherwise intra-well

the validation of the	uctions, Set
tion of th	t the right
Б	14
	c)o

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

below for the Manual Assay.

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.

N. ASSAY SCHEME

Operations	Procedure
re-Washing step	n" 1 cycle
Controls&Calibrator&samples	150 ul
Diluted Enzyme Conjugate	100 ul
incubation	120 min
Temperature	+37°C
Washing steps	nº 4-5
Chromogen/Substrate	200ut
2 nd incubation	30 min
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm

nelided	Pipette 200	
	T	
	Chromogen/Substrate	
	into	
	≗	
	ine	
	wells,	
	ž	
section.	An example	

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

- calibrator and positive samples will turn from clear to blue.
 10. Pipette 100 µl Sulphuric Acid into all the wells to stop the Incubate the microplate protected from light at 18-24°C for 30 min. Wells dispensed with the positive control, 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
- 11. Measure the color intensity of the solution in each well, as described in section 1,6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly nded), blanking the instrument on A1. (background subtraction,

- Important general notes:

 1. If the second filter is not available, ensure that no imgeprints or dust are present on the external buttom of the microwell before reading at 450nm. They could generate
- false positive results on reading.
 Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen
- can occur leading to a higher background.
 When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended as long as it is fair less sensitive to nerrormended as long as it is fair less sensitive to inderferences due to hemolysis, hyporipaemia, backerial 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

- dispense 100 ul TMB&H2O2 mix
- stop and read incubate 30 min at r.t. on shaking

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.

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

e of dispensation scheme is reponed in the following

Microplate

	Þ	(0)	O	0	m	n	9	I
1	뮍	N C	NO	NO	CAL	CAL	PC	cs.
K	\$2	S3	52	SS	88	\$7	88	98
G	ì							ľ.
4	à							
ij								
σ								
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α			Ť			Ì	23	ij
Œ			1					
10								
7.7								
71	Ĩ							

Elbrador	BLK -	
PC - Pushing	K = Blunk	
re Control S	NC - Negative (
- Sample	¿ Control	

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 CD450nm or S/Co values have been matched in the analysis,

Ensure that the following results are met

a the management at	The section of the se
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after
	blanking
Calibrator 0.5 (U/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

Problem	Check
Blank well	1. that the Chromogen/Substrate solution
> 0_100	has not become contaminated during the
OD450nm	assay
Negative	1. that the washing procedure and the
Control (NC)	washer settings are as validated in the
> 0.050	pre qualification study;
OD450nm after	2, that the proper washing solution has
blanking	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 micropipeltes have not become
	contaminated with positive samples or
	with the enzyme conjugate
	6, that the washer needles are not
	hiorked or nadially obstructed

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Calibrator S/Co < 2	that the procedure has been correctly performed: that no microke has postured during the land.		positive, the sample has to be test before a diagnosts of viral t	npie has to be gnosis of viral t
	 that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of calibrator) 	<u>د</u> - د د د	When lest results are transmanother department, altention	Its are transm ment, attention
	3 that the washing procedure and the washer settings are as validated in the	4	Diagnosis of viral hepatitis inf	al hepatitis inf
	pre qualification study; 4 that no external contamination of the		doctor.	
Positive Control	1. that the procedure has been correctly	1	Con exemple of calculation is tehorie	andin s repone
< 1,000	performed.	The	The following data must not be	must not be u
OD450nm	that no mistake has occurred during the distribution of the control	obtai	obtained by the user.	••
	of nostly control in the case the	Nega	Negative Control: (0.012 ~ 0.008 -
		Lowe	0.050 -	Accepted
	value > 0.050).	Posit	Positive Control: 2,489 OD450nr	2,489 OD450nr
	washer settings are as validated in the	Cul-c	Cut-Off = 0.010+0.050 = 0.060	0.000
	pre qualification study;	Calib	Calibrator: (0.350 - 0.370 0
	4. that no external contamination of the	Mear	Mean value:	0.360 OD450nr
	positive control has occurred.	S/Co	S/Co higher than 2.0 – Accepted	- Accepted
		Sam	Sample 1: 0,028 OD450nm	450nm

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

P. CALCULATION OF THE CUT-OFF

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

NC + 0.050 = Cut-Off (Co)

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

Important note: When the calculation of results is performed by the operating system of an EUSA automated work station, easure that the proper formulation is used to calculate the curoff value and generate the correct interpretation of results.

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

v	0.9 - 1.1	< 0.9	SICo
Positive	Equivocal	Negative	Interpretation

A positive result is indicative of HBV infection and therefore the patient should be treated accordingly on the blood unit should be 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 mittal sample; the blood unit should not be transfused.

Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of
- judgment errors and mismiterpretations.

 Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0, 8 utilities to remove any microparticles unergenere. Then, it still to remove any microparticles unergenere.

hepatitis is released, nifted from the laboratory to on must be paid to avoid

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fection has to be taken and suitably qualified medical suitably qualified

ed below,

used instead or real figures

Cantrol: le: 1 0.050 – 5ntrol: 1 1.000 – 1 0.010+0.0
igher than 1,000 – Accepted ut-Off = 0,010+0,050 = 0,060
'alibrator: 0,350 - 0,370 OD450nm
fean value: 0.360 OD450nm S/Co = 6,0 VCo higher than 2,0 = Accepted
ample 1: 0,028 OD450nm
ample 2: 1.690 OD450nm
ample 1 S/Co < 0,9 = negative
ample 2 S/Co > 1,1 = positive

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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 sequivatent 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 2rd WHO international standard, NIBSC code 0/S98. In the following abile, results are given for three lots (P1, P2 and P3) of the version ULTRA in comparison with the reference device (Ref.):

1000	0.025	0.05	0.1	0.2	0.4	IM/UI OHW
0.2	0.6	8.0	1.4	2.3	4.8	Lot # P1 S/Co
0.0	0,6	8.0	1.4	2.4	4.8	Lot # P2 S/Co
0	9.0	1.0	1.5	2.4	4,6	Lot # P3 S/Co
-	-	-	_	-		Ref. S/Co

The assay shows an Analytical Sensitivity better than 0,1 WHO IU/ml of H8sAg.

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

Panel EFS Ag HBs HB1-HB6 iot nº 04

Sample ID	Characteristics	ng/md
HB1	diluent	,
HB2	adw2+ayw3	0.05
HB3	adw2+ayw3	0.1
HB4	adw2+ayw3	0.2
HBS	adw2+ayw3	0.3
HB6	adw2+ayw3	0.5

Sensitivity panel
SFTS,
France,
Ą
HBS
2005

189	188	187	186	185	184	183	182	181	180	179	178	177	176	175	174	173	172	171	Sample ID
Ave	Ayw4	Ayw3	Ayw3	Ayw2	Ayw1	Adr	Adw4	Adw2	Adw2 + ayw3	Adv/2 + ayv/3	Characteristics								
05_10	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0	0.03 + 0.01	0.06 + 0.01	0.11 + 0.02	0.25 + 0.02	0.39 + 0.02	0.49 + 0.03	0.64 + 0.04	1.02 + 0.05	1,18 ± 0,10	2.21 + 0.15	ng/ml
6.4	6,9	CT CO	7,3	6,4	5,1	4.5	3,6	4.7	0,8	0,9	14	2,0	2,6	3,4	4.0	di di	8.7	15.4	S/Co

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

21	20	19	18	17	6	15	14	13	12	11	10	80	80	07	90	65	94	03	02	01	Sample 10
negative	ay	ay	ay	ay	ау	ay	ay	ay	ay.	ye	ad	Be	8.	ad	ad	ad	ad	ad	ad	26.	Characteristics
1	0.13	0,23	0,33	0,42	0.48	0,63	0.77	0.97	1,26	2.51	0,23	0,30	0.37	0,41	0,50	0,69	0.96	1,02	1,17	2,49	ng/ml
3.0		.es	1.8	2,0	12,4	2.0	3,7	4.	5,9	11,2	7.0	1,2	1.3	j.	2,2	2,9	3,8	26,23	4,5	19,2	S/Co

2. Diagnostic Sensitivity.

The diagnostic sensitivity was tested according to what required by Common Trechnical Specifications (CTS) of the directive 987/9EC on IVD for HSAS testing.

Positive samples, including HBASq subtypes and a panel of 's' mutants from most frequent mutations, were collected from different HBV pathologies (soute, asymptomatic and chronic hapatitis 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 lested 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 servicenversions were studied, most of them produced by Boston Biomedica Inc., USA.

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

Panel	PHM 906	PHM 907 (M)	506 WHd	PHM 914	PHM 918	PHM 923	PHM 925	PHM 934
sample positive								
subtype	be	ay	ad.	be	86	ye	bul	20.
ng/ml	0.5	1.0	0,3	0.5	0,1	< 0.2	n,d	nd
ULTRA S/Co	3.7	4.4	1.2	1.	1.8	2.2	1,4	10
device S/Co	1.4	2.9	0.8	1.1	0.5	1.2	6.0	0.8
		Ц					Ш	

3. Diagnostic Specificity:

It is defined as the probability of the assay of sconing negative in the desired of specific analyte. In addition to the first study, where more than 5000 negative samples from blood donors (two blood realies), 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 lobs. A value of specificity of 100% was found.

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

No false reactivity due to the method of specimen preparation the service of the se

0,6

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 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.

If 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:

22%	Std.Deviation	OD450nm	Average values Total n = 144
16%	0.004	0.026	Negative Sample
B%	0.027	0.332	Calibrator 0.5 IU/ml

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

Repealable 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

interferences in fresh samples were also observed when they were not particles free or were badly collected see charpe of old or frazen samples, presenting fibrin clots, programmers, projectomaining micelles or microparticles after storage or thaving 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:
Dia.Pro Diagnostic Bioprobes S.r.i.
Via G. Carducci n* 27 – Sesto San Giovanni (Mi) – Italy

0318

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

for "in vitro" diagnostic use only -



DIA.PRO

Via G. Carducci nº 27 Diagnostic Bioprobes Srl (Milano) - Italy 20099 Sesto San Giovanni

e-mail: info@diapro.it Fax +39 02 26007726 Phone +39 02 27007161

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HBs Ab

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called interferon or lamivadine, which can help some patients. Patients with circhosis are sometimes given liver transplants, with varying smootess. It is preferable to prevent this disease with vaccine than to try and cure it.

offectiveness, 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

Hepatitis B vaccine has an outstanding record of safety and

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

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

Chronic hepatitis B in some patients is treated with drugs

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

infection as follows:

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 ALSC,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yullowing of the skin and eyes (jaundice); dark urine; extreme futigue; months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the circum and many years later develops cirrhosis of the liver or "Hepatitis B is one of the major diseases of mankind and is a vomiting and abdominal pain. It can take several

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) perinarial (from mether to bely at the hinth: (b) shill: to child transmission: (c) unsafe injections and transfusions: (d) sexual contact HBV is the most serious type of viral hepatitis and the only

against the "a' determinant.

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

The detection of HBs/h has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBs/sq.

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

Hepatitis B surface Antigen (HBsAg) is the major structural polypoptide of the envelope of the Hapatitis B Virus (HBV). This antigen is composed mainly of the type common determinant "a and the type specific determinants" and of the type specific determinants."

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 with the virus. In many industrialized countries (Western Europe and North America), the pattern Worldwide, most infections occur from infacted muther 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 programmes were implemented. However, the majority of of chronic infections before childhood hepatitis B vaccination transmission is different. In these countries, mother to infant and child-to-child transmission accounted for up to one third workers have received hepatitis B vaccine countries (e.g.

water, and cannot be spread casually in the workplace. High radies 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 Hepatitis B virus is not spread by contaminated food or imerica, 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%, it offs, of children infected hetween 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus

to become chronically infacted with HBV, the rate of chronic infaction has been reduced to less than 1% in immunized groups of children. Since 1991. WHO has called five all countries to add hepatitis B vaccine into their national

immunization programmes.

C. PRINCIPLE OF THE TEST

(C. PRINCIPLE OF THE TEST)

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

After washing, captured antibodies are detected by an HBsAg. After washing captured antibodies are detected by an HBsAg. The provided only the provides of the Principle of the provides of the Principle of

second available binding site of these ambiodies. The enzyme specifically bound to wells, by acting on the substrate/chronogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader. The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference

preparation.
Samples are pre-treated in the well with an specimen diluent

able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Micropiate: MICROPLATE

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

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

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3. Wash buffer concentrate: WASHBUF 20X

as preservatives. Standards are blue coloured.

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

4. Enzyme conjugate: CONJ
1x16.0 ml/vial, Ready-to-use solution and

It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml genlamicine sulphate and 0.1% Kathon GC as red color coded

5. Chromogen/Substrate: SUBS TMB 1x16m/Visial. Conlains a 50 mM citrate-phosphate buffered solution at pH 3,5-3,8, 4% dimethy/subproxide, 0,03% tetramethy-benddine (TMB) and 0,02% hydrogen peroxide (H2O2), Note: To be stored protected from light as sensitive to strong illumination

1x15ml/vial_Contains 0.3 M HzSO₄ s Attention: Irritant (H215 ...

1x15ml/vial_Contains 0,3 M HzSO₄ solution, Atlention; Irrilant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P388, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
1x8ml, 10 mM Tris Buffered solution ph 7,4
be used in the follow up of vaccination,
sodium azide as preservatives. ph 7.4 +/-0.1 suggested to sination. If contains 0.09%

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

9. Plate sealing foil n° 2

10_Package insert nº 1

et m MATERIALS REQUIRED BUT NOT PROVIDED Calibrated Micropipettes (100ul and 50ul) and c

- 2 plastic tips EIA grade water (double distilled or deionised, charcoal disposable
- treated to remove oxidizing cludisinfectants).
 Timer with 60 minute range or higher. chemicals used
- w 4 ru Absorbent paper tissues, Calibrated ELISA microplate thermostatic incubator (dry or cellbrated ELISA microplate thermostatic incubator (dry or wet), set at +37° (1+1°C tolerance).

 Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended)

ch

- Calibrated ELISA microplate washer Vortex or similar mixing loois

 The kit has to be used by skilled and property trained technical personnel only, under the supervision of a medical coctor responsible of the laboratory. F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by ski technical personnel only, under the

2. All the personnel involved in performing the assay fieve to wear protective laboratory citches, latc-free gloves and glasses. The use of any sharp (needless) or cutting (bades) pervices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Allaria, U.S. and reported in the National Institute of Health's publication: : "Biosafety in Microbiological and

safe and effective 4. The laborate The laboratory environment should be controlled so as

replacement

Avoid cross-contamination between kit reagents by using osable lips and changing them between the use of each

discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the wasting 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 has or freat inactivation by autocave at 121°C for 20 min.

(example: tips used for samples and controls, used micropiates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

i. blood is drawn aseptically by verbipurcure 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. SPECIMEN: PREPARATION AND WARNINGS Blood is drawn aseptically by venipuncture an

Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available

avoid contaminants such as dust or air-born microbial agents, when opening kit valis and microbiates and when performing the test. Protect hie Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken, 5. Upon receipt, store he kit at 2_8°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different lots of the kits, It is recommended that components between two kits

of the same lot should not be interchanged.

Check that the reagents are clear and do not contain visible heavy paticles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit

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

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

11. Treat all specimens as potentially infective. All human serum specimens should be handled at Blosafety Level 2, as for compliance with what reported in the Institutes of Health's publication. "Stosafety in Microbiological and Stomedical"

publication. Blosafety in Microbiological and Sigmedical Laborations, ed. 1984.

12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

cross contamination,

13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws

adsorbed with paper issues scaked with household bleach and then with water, Tissues should then be discarded in proper containers designated for laboratory/hospital waste, 15. The Sulphuric Acid is an irritant, In case of spills, wash the

5. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals,
or microbes. If this component has to be transferred, use

only

Ready to use, Mix well on vortex before use. plastic, and if possible, sterile disposable containers.

Specimen Diluent

surface with plenty of water 16. Other waste materials generated from the use of the kit

electronic reading is strongly recom Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and

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could give rise to false results. have to be discarded as they could generate laise results. Samples containing residues of librin or heavy particles or microbial filaments and bodies should be discarded as they ("red") and visibly hyperlipemic ("milky") samples scarded as they could generate false results.

4. Sera and plasma can be stored at +2"...8°C for up to five days

after collection. For longer storage periods, samples can be stored forzer at -20°C for several months. Any forzer samples should not be freezed/haved more than once as this may generate particles that could affect the test result.

5. If particles are present, contrilings at 2,000 pm for 20 min or filter using 0,20,00 filters to clean up the sample for testing.

6. Samples whose anti-HSA4g anticlory concentration is expected to be higher than 250 mll/lim, Ishould be diffued before use either 1:10 or 1:100 in the Calibrator on III/Im. Diffuen have to be done in clean disposable tubes by disting 50 ut of each specimen with 450 ut of Cali 0 (1:10). Then 50 ut of the cali 0 (1:10), Mix ubes thoroughly on vortex when preparing the diluted samples.

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

protective gloves/protective clothing/eye

protection face protection,
P302 + P352 – If ON SKIN: Wash with plenty of soap and
water,
P313 – If skin irritation occurs: Get medical
advice/site-informatic particles and state of soap and
p305 + P351 + P338 – If IN EVES: Rinse cautiously with water
for several minutes. Remove contact lenses, if present and easy
to do Continue irrising,
p307 + P313 – If eye irritation persists: Get medical
advire/altention,
P362 + P363 – Take off contaminated clothing and wash it
before reuse.

INSTRUMENTS AND TOOLS USED IN COMBINATION

WITH THE KIT

WITH THE KIT

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

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

Allow the microplate to reach norm temperature (about 1 hy) 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.

H. PREPARATION OF COMPONENTS AND WARNINGS

Interfess of 1,2% of the set at 43°C (loterance of 1.4°C) and regularly checked to ensure the correct of 1.4°C) and regularly checked to ensure the correct temperature is maintained. Both dy incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routhne aboratory tests. Usually 4.5° washing cycles (aspiration + dispensation of 350,u/well of washing solution = 1 cycle) are sufficient to resture that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested, in order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and necletive settings are and other? In controls/calibrator and well-characterized negative and procedure settings are and other? 4 10

The whole content of the concentrated solution has to be diffued 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbies could impact on the efficiency of the washing cycles. Note: Once diffused, the wash solution is stable for 1 week at +2..8° C.

Wash buffer concentrate:

aliquots at -20°C

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

Note: The control after dissolution is not stable. Store frozen in

Ready to use. Mix well on vortex before use

positive reference samples, and check to make the values reported below in the sections "Validation of I feet and "Assay Performances," Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

In the ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes, its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to < 2.0; (c) Innearity to < 2.0; as the proposed of the 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 instruments."

σ When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data hamiding) have to be carefully set, calibrated controlled and regularly serviced in order to match the

8. Sulphuric Acid

Ready to use: Mix well on vortex before

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

driven dust or microbes. Do not expose to strong light, oxidising

Avoid contamination of the liquid with exidising chemicals, air-

Ready to use. Mix well on vortex before use

agents and metallic surfaces. If this component has to be

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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 addispent wells of the to strong samples.

adjacent wells due to strongly reachive samples, leading to laise 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 Pros's customer service offers support to the user in the setting and checking of instruments used in combinate 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
 L Check the expiration date of the kit printed on the external Check the expiration vacuum. It is able of the kit box, Do not use if expired, label of the kit box, Do not use if expired, Check that the figure components are not confaminated by Check that the individual of the confidence or aggregates, Check that the
- Ν 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 micropiate, is not punctured or damaged
- ω as described above.

 Dissolve the Control Serum as described above. Dilute all the content of the 20x concentrated Wash Solution
- 4 10
- တ Allow all the other components to reach room temperature (about 1 hr) and then mix as described.

 Set the ELISA incubator at 1-37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing opties as found in the validation of the
- 7. instrument for its use with the kit.

 Check that the ELISA reader has been turned on at least 20 minutes before reading
- œ If using an automated workstation, turn it on, check settings
- and be sure to use the right assay protocol.

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

 10. Check that all the other equipments are available and ready

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

The assay has to be carried out according to what reported below, laking care to maintain the same incubation time for all

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

M.1 Quantitative analysis

1. Place the required under of strps in the microplate holder, taken e.A. and B1 wells empty for the operation of blanking.

Store the other strips into the bag in presence of the desircant at 2.8°C, sealed.

A1 and B1, 50µl of the Specimen Diluent.

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

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

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

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

Wash the microplate as reported in section I.3.

- Important note:

 1) Be craft not to touch the inner surface of the well with the fiperfite tip, when dispensing the Enzyme Conjugate, Contamination might occur.

 2) Mix thoroughly the Enzyme Conjugate on vortex before use.

- 5. Wash the microplate as described.
 6. Pipette 100µT MRJRH-Oo mixture in such well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at room temperature for 20
- important note: Do not expose to strong direct light as a high background might be generated.
- into each well and using the same pipeting sequence as in step 6. Then measure the colour intensity with a micropiate reader at 450nm (reading) and at 670-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells,

Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2,,8°C, sealed,

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

5. Wash the microplate as described.
6. Pipette 100µl TMBH202 mixture in sech well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at room temperature for 20

In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added, Incubate the microplate at +37°C for 60 minutes.

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder

2. Dispense 50 ul Specimen Diluani in all the wells, except for the blank A1. Then pipette 100µi of the Calibrator 0 mU/mi in duplicate, 100µi of the Calibrator 10 mU/mi in duplicate, 100µi of the Calibrator 250 mU/mi in single, and then 100µi of the Calibrator 250 mU/mi in single, and then 100µi of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at +37°C for 60

- Important note:

 3) Be careful not to louch the inner surface of the well with the piperte tip when dispensing the Enzyme Conjugate. Contamination might occur.

 4) Mix thoroughly the Enzyme Conjugate on vortex before use.

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

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7. Stop the enzymatic roadion by pipette 100µl Sujphuric Acid pinto each well and using the same pipeting sequence as in set in Then measure the colour intensity with a micropiate reader at 450nm (reading) and all 620-630nm (blanking, strongly recommended), blanking the instrument on Al and B1 wells.

- Important general notes:

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

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

N. ASSAY SCHEME (standard procedure)

450nm & 620nm	Reading OD
100 ul	Sulphuric Acid
T.	Temperature
20 min	3" incubation
100 ut	TMB/H2O2 mix
4-5 cycles	Wash step
+37°C	Temperature
60 min	2" incubation
100 ul	Enzyme Conjugate
4-5 cycles	Wash step
+37°C	Temperature
60 min	1 st incubation
100 ul	Samples
100 ut	Cantrol Serum
100 山	Calibrators
50 ul	Specimen Diluent

reported below An example of dispensation scheme in quantitative assays is

		22	į.	4	4	0	7	6	œ	10	N.
Þ	BLK	CAL4	ES			1					
87	ELK.	CAL4	\$4								
O	CALT	CALS	38	П							
D	CAL	CAL5	88								
m	CAL2	CS	57								
19	CAL2	Ca	SB.								
G	CAL3	US.	88								
ij	CAL3	S2	\$10								

An example of dispensation scheme in qualitative assays is

_	+		7		n	0 10	2 7	7	7 0	7 0 0	7 0 0
L	4	2	Ģ	h	G.	œ	8 7	6 7 8	7	7 8	7 8 9
Þ	BLK	() ()									
æ	CALT	84									
C	CALT	55									
D	CAL2	8.8									
m	CAL2	57									
71)	CALS	US SS		Ú							
G	U)	E 23	S 17								
Ŧ	3	510									

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

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

Danasa	Control
1	hat
	the t
	ollowing
	data are r
	are
	matche
	ed:

Parameters	Requirements
Blank well	< 0,100 OD450nm
Calibrator D WHO mill/mil	< 9.200 OD450nm after Manking
Calibrator	OD450nm higher than the OD450nm of the
Calibrator 250 WHD mlU/ml	> 1,500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mlU/ml ± 10%
Coefficient of	< 30% for the Calibrator 0 mill/mi

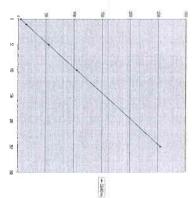
If the results of the test match the requirements stated above, proceed to the next section.

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

A that no contamination of the Cal 0 mlU/ml or	variation > 30% before use; 3, that no mistake ha		50nm
or our wells wilder it was dispersed tips	before use; 3, that no mistake has been done in the assey	 Inal time washing procedure and the washer settings are as validated in the pre qualification study; It hat the proper washing solution has been used and the washer has been primed with it before use. 	that the Chromogen/Substrate solution has not become contaminated during the assay

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8	
the problem to the Superv	
test to match the expected value +/-10%	
a) a value up to */-20% is obtained; the overall Precision of the laboratory might not enable the	
follows:	
If no mistake has been found, proceed as	
of this error.	
to be repeated after eliminating the	
If a mistake has been pointed out, the assay	
the right volume reported on the label	
has occurred.	
4 no external contamination of the standard	
3 the washing procedure and the washer	
sample):	and agreed agreement
slake has occurred durin	expected rates
1 the procedure has been correctly performed;	
First verify that	Control Serum
inderd has occurred	
4 that no external contamination of the	
study;	
3, that the washing procedure and the washer	
distribution;	
2. Ihat no mistake has occurred during its	< 1,500 OD450nm
rformed:	INTIME.
1, that the procedure has been correctly	Calibrator 250
s accurred.	
4 that no external contamination of the	
settings are as validated in the pre qualification	
3 that the washing procedure and the washer	
tribution (ex. dispensation of a wro	< Cal 0 + 0,100
2 that no mistake has occurred during its	OD450nm
	mIU/mI
the side proposed that peet collective	



Important Note:

Do not use the calibration curve above to make calculations

P_2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 mIU/mI and then check that the assay is valid.

Example of calculation:

The following data must not be used or real tigures obtained by

Lower than 0,200 - Accepted

Calibrator 0 mlU/ml; 0.020 = 0.024 OD450nm Mean Value: 0.022 OD450nm

Calibrator 10 mtU/mt: 0,250 – 0,270 OD450nm Mean Value: 0,260 OD450nm Higher than Cal 0 + 0,100 – Accepted

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

P.1 Quantitative method P. RESULTS

An example of Calibration curve is reported in the next page. interpolation is suggested).
Then on the calibration curve calculate the concentration of anti-

HBsAg antibody in samples

Calibrator 250 mlU/ml: 2,845 QD450nm Higher than 1,500 — Accepted

Samples with a concentration higher than 10 WHO mtUlml are considered positive for anti H8sAg antibody. The followup of vaccination recipients, however, the value of 20 WHO mtUlmi is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against H8V infection. Q.INTERPRETATION OF RESULTS
Samples with a concentration lower than 10 WHO mIUml are considered negative for anti HBsAg antibody by most of the international medical literature.

- Important notes:

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

Example of Calibration Curve :

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R_PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC),

1. LIMIT OF DETECTION

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

	HOJUH	SAB,CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
0.219 0.192	50	0.933	0.812	0.846
380.0	10	0.219	0.192	0.194
00000	5	0.110	0.096	0.104
0.057 0.058	2,5	0.057	0.058	0.067
Std 0 0.021 0.015 0.023	Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY A Performance Evaluation has been conducted number of more than 700 samples. conducted

the absence of specific analyte.

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

A diagnostic specificity of 98.8% was assessed. 2.1 Diagnostic Specificity
It is defined as the probability of the assay of scoring negative in

Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases,

has been observed.

5

2.2 Diagnostic Sensitivity
It defined as the probability of the assay of scoring positive in
the presence of specific analyte,
106 vaccinated patients were evaluated providing a diagnostic

Std.Deviation 0.4	OD 450nm 0.	Mean values 1st	Calibrator 10 mill/ml (N = 16)	CV% 8	Std.Deviation 0.1	OD 450nm 0.0	Mean values 1st	Calibrator 0 mlU/ml (N =
	0.250 0.243	strun 2nd run	= 16)	8.8 9.5	0.003 0.004	0.038 0.038	fstrun 2nd run	= 15)
5,017	0.244	3" run		11.8	0.005	0.039	3 ^{or} run	
0.020	0.246	Average		10.0	0.004	0.039	Average	

on a lotal

patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemoticzed, lipenic, etc.) against the European company. A value of specificity of 100% was assessed. Finally, both human plasma, derived with different standard fichiliques of preparation (citrate, EDTA and hepatin), and human sera have been used to determine the specificity. No laise reactivity due to the method of specimen preparation.

sensitivity of 100%.

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

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

SAB.CE: lot # 1202

OD 450nm 0.250 0.243	Mean values 1st run 2nd run	Calibrator 10 mil/mi (N = 16)	CV% 88 95	Std.Deviation 0.003 0.004	OD 450nm 0,036 0.038	Mean values 1st run 2nd run	ł
D 244	3" sun		11.8	0.005	0.039	3" run	
0.246	Average		10.0	0.004	0.039	Average value	

Mean values 1strun OD 450nm 2.998 3,000 2nd run 3" run 3.259

Calibrator 0 mlU/ml (N = 16) SAB,CE: lot # 1002

CV %	Std,Deviation	OD 450nm	Mean values
9.4	0.005	0.048	tetrun
8.4	0.004	0.048	2nd run
11.5	0.006	0.050	3" run
9.8	0,005	0.049	Average

Mean values	Calibrator 250 mil	CV %	Std.Deviation	00 450nm		Mean values	Calibrator 10 mlU/ml (N = 16)
"Si zun	IU/ml (N = 16)	8.3	0.021	0.249		Tel fun	mi (N = 16)
2nd run		7.9	0.020	0.252	Sent	200 000	
3""		9,6	0.023	0.242	100	3" 710	
Average		8.5	0,021	0.248	ante	Average	

3,544 0,153

SAB CE: lot # 1002/2

Calibrator 0 mill/s	nl (N = 16)			
Mean values	Jet cim	2nd nun	3"run	Awerage
OD 450nm	0.050	0.051	0.050	0.050
Std Deviation	0,005	0.006	900.0	200.0
CV %	10.0	10.9	11.0	5.01

Calibrator 250 mi	CV%	Std. Deviation	OD 450nm	Мерп илдер	Collected to THICKER IN - 10
U/ml (N = 16	6,5	0.015	0.226	1st run	ATTEN - NO. INSTANTA
	7.0	0.017	0.238	2nd run	
	7.5	810,0	0.239	3" nun	
	7.0	0.016	0.234	Average	
	Calibrator 250 miU/mi (N = 16)	6.5 7.0 7.5 ntU/mt (N = 16)	N/% 6.5 7.0 7.5 stor 250 miU/mi (N = 16)	450nm 0.226 0.239	1 kenkluss 1 st rum 2 nd rum 450nm 0.226 0.238 Javiation 0.015 0.017 7/ % 6.5 7.0

misclassification,	The variability shown in the lables did not result in sample	CV %	Std.Deviation	OD 450nm	Mean values
7	shown in	3.9	0.137	3.526	UNU ISE
	the lables	4.1	0.143	3,457	2nd run
	did not resul	4.6	0.162	3,499	3 run
	It in sample	4.2	0.147	3.494	Average

4. ACCURACY
A COURACY
The assay accuracy has been checked by the dilution and recovery tests, Any 'hook effect', underestimation likely to happen at high closes of analyte, was ruled out up to 10,000 mill/mil.

S, LIMITATIONS OF THE PROCEDURE Bacterial contamination or heat inactive

Bacterial confamination 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 product one.

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,

- REFERENCES

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 6. Baker L.F., Dodd R.J., Sander S.G., In 'viral Hepatitis'.
 6. Babratoury and Clinical Science's Epothward J. Deinhardt J. D

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 (Mf) - Italy

"Capture" Enzyme ImmunoAssay (ELISA) determination of IgM class antibody to Hepatitis B Virus core Antigen for the quantitative/qualitative in human plasma and sera

for "in vitro" diagnostic use only



DIA.PRO

20099 Sesto San Giovanni (Milano) - Italy Via G. Carducci nº 27 Diagnostic Bioprobes Srl

Fax +39 02 26007726 e-mail: info@diapro.it

Phone +39 02 27007161

REF BCM.CE 96 Tests

HBc IgM

the follow-up of chronic patients under therapy, For "in vitro" diagnostic use only,

colour coded.

phase coated with anti higM antibody,

particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of recombinant HBcAg, labelled with a monoclonal antibody conjugated with peroxidase (HRP).

After incubation, microwells are washed to remove unbound

U/mI /I CAL 5 = 50 U/mI /I CAL 6 = 100 U/mI

infectious,

3. Wash buffer concentrate: WASHBUF 20X 1x60m/bottle, 20x concentrated solution, Once dituted, the wash solution contains 10 mM phosphate buffer pH 7,04-0.2, 0,05% Tween 20 and 0,1% Kathon GC,

4. Enzyme Conjugate (Immunocomplex) : CONJ

S. Specimen Diluent: DILSPE 2x60.0 ml/vial. Buffered solution for the dilution of samples; it contains 100 mM fris buffer H 7.4+/-0.1, 0.5% Tween 20, 2% Casein, 0.1% Kathon GC and 0.09% sodium azide as

preservatives. The component is blue color coded.

1. 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.

Important Note: Even if plasma has been chemically activated, handle this component as potentially

Important Notes preservatives

conjugate and then the chromogen/substrate is added.

In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of ight antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests

Infeceptate: [MICROPLATE]
812. microwell strips coated with purified anti human IgM
822. microwell strips coated with post-coaled with bovine
specific mouse monoclonal antibody, post-coaled with bovine
serum proteins and sealed into a bag with desiceant. Allow the
microplate to reach room temperature before opening; reseal
unused strips in the bag with desiceant and store at 4°C.

 Calibration Curve: CAL N°...
 RS.2.0 ml/vial. Ready to use and color coded standard curve calibrated on the +RicigM reference preparation supplied by Paul Erisch Institute (+Ric-Referenceseum-H)M 84), ranging: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 <u></u>5 ω 4 Ν

In contains chemical inactivated HadyM positive human plasma. 100 mM Tits buffer pH 7.4+0.1, 0.5% Tween 20, 0.05% sodium azide and 0.1% Kalhon GC as preservatives. The Calibration Curve is coded with blue alimentary dye, important Note: Even if Jasama has been chemically inactivated, handle this component as potentially

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A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the
quantilative/qualitative determination of IgM class antibodies
to Hepatitis B Virus core Antigen in human plasma and sera with the "capture" system.
The kit is inlended for the classification of the viral agent and for

IX16.0 mlWiai. Ready.to-use solution. Contains an immunocomplex formed by a specific mouse monoclonal antibody, labelled with HRP, and a purified recombinant HBCAq. The reagent is dissolved into a buffer solution 10 mM Tris buffer pH 6.8-4.01, 2% BSA, 0.1% Kalbnn GC and 0.22% gentamicine sulphele as preservatives. The component is red

Hepatilis B core Antigen (or HBcAq) is the major component of the core particles of Hepatilis B winds (or HBV). Particles have a size of 27nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBcAq, HBcAq is composed of a single polyperatide of about 17 KD that is released upon disaggregation of the core particles; the antigen contains at least one immunological determinant, Upon primary infection, and HBcAq lgtM antibodes are one of the first markers of HBV hepatilis appearing in the serum of the patient together or slightly later than HBsAq, the viral surface and contains the same of the

antigen.

Anti HBCAg IgM titers, very high during the acute phase, Anti HBCAg IgM titers, as IgG antibodies appear, down to undetacable levels in convalisseent patients.

In chronic hepatitis, however, spiles of anti HBCAg IgM synthesis are present, confirming reactivation of HBV in

hepalocities and giving origin to permanent (gM low titers. The determination of ant! HBcAg gM antibodies has become very important for the fast classification of the virus, of the phase of the liness and for the monitoring of patients under treatment

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid

After washing out all the other components of the sample and in

7. Chromogen/Substrate : SUBS TMB 1x16ml/viel. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% cimethylsubpoxide, 0.03%, letramethyl-benzidine or TMB and 0.02% hydrogen peroxide or the contains the substrate of the contains the c

H2O2.

Note: To be stored protected from light as sensitive

8. Sulphuric Acid: H2SO4 0.3 M 1x15ml/vial. Contains 0.3 M H2SO4 solution.

strong illumination.

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

10. Package insert: n° 1

Plate sealing foils: n° 2

E. MATERIALS REQUIRED BUT NOT PROVIDED 1._ Calibrated Micropipettes (150ul, 100ul and the calibrated Micropipettes).

Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.

EIA grade water (double distilled or deionised, charcoal

2 remove axidizing chemicals used as

Timer with 60 minute range or higher

Absorbent paper tissues.
Calibrated ELISA microplete thermostatic incubator (dry or wet) set at +57°C.
Calibrated ELISA microplete reader with 450nm (reading) and with 520-630mm (blainling) filters.
Calibrated ELISA microplate washer.

Vortex or similar mixing tools

æ .v

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F. WARNINGS AND PRECAUTIONS

 The kit has to be used by skilled and properly train technical personnel only, under the supervision of a medic octor responsible of the laboratory.
 All the personnel involved in performing the assay have properly trained sion of a medica

wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (reedles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosative procedures, as recommended by the Center for Disease Control, National, U.S. and reported in the National Institute of Health's publication. "Biosafety in Microbiological and institute of Health's publication." Biosafety in Microbiological and

Biomedical Laboratories ed. 1984

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

sale and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or all-born microbial agents, when opening kit vals and microbiates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is

Upon receipt, store the kit at 2-8°C into a temperature

6. Do not interchange components between different tots of the kits. It is recommended that components between two kits of the same tot should not be interchanged.

7. Check that the resonance.

samples by using disposable tips and changing them after each visible heavy particles or aggregates. If not advis laboratory supervisor to initiate the necessary procedures.

8. Avoid cross-contamination habitance. 7. Check that the reagents are clear and do visible heavy particles or aggregates... If not and do not contain If not, advise the serum/plasma

sample.

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

10. Do not use the kit after the expiration date stated on external (primary container) and internal (valis) abots.

11. "Treat all specimens as opticately infective. All human serum specimens should be handled at Biosalety Level 2, as recommended by the Center for Disease Control, Atlanta, US. in compliance with what reported in the Institutes of Health's publication. "Biosafety in Microbiological and Biomedical publication."

Laboratories, ed. 1984.
Laboratories, ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstalions, in

order to avoid contamination.

3. 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 used in the substances of the su

laboratory/hospital waste.

15. The Stop Solution is an irritant, in case of spills, wash the

surface with plenty of water
16. Other waste materials generated from the use of the kil
16. Other waste materials generated from the use of the kil
(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

6. SPECIMEN: PREPARATION AND RECOMMANDATIONS
1. Blood is drawn aseptically by venepuncture and plasma on serum is prepared using slandard techniques of preparation or samples for clinical laboratory analysis.—No influence has been

observed in the preparation of the sample with citrate,

Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results,
3. Samples have to be clearly identified with codes or names in

4. Haemolysed and visibly hyperlipemic ("miliy") samples have to be discarded as they could generate false results, Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give

rise to false results.

5. Sera and plasma can be stored at +2°...8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freeze/thewed more than once as this

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3

green,

asn

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200ml 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°

Control Serum

Dissolve the content of the vial with EIA grade water as re in the label. Mix well on vortex before use. The dissolved water as reported

stable.

Chromogen/Substrate:
Ready to use. Mixwell on vortex before use.
Acold contamination of the figuid with oxidizing chemicals, airarriven 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

EDT/

order to avoid misinterpretation of results

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.

Microplate

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

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

Calibration Curve:

Peady to use, Mix well on vortex before

plastic, Enzyme conjugate:
Ready to use. Mix well on vortex before use,
Avoid contamination of the liquid with oxidizing chemicals,
or microtes. If this component has to be transferred, use and if possible, sterile disposable containers dust

Specimen Diluent

Ready to use. Mix on vortex before use

serum is ready to use.

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

Sulphuric Acid: Ready to use, Mix well on vortex before use. Attention: Intrant (1915, H319, P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363),

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Legenda:

Warning H statements H315 – Causes skin irri

H319 – Causes serious eye Irrilation skin irritation

advice/attention,
P362 + P363 - Take off contaminated clothing and wash it P337 + P313 - If for several minutes. P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water water, P332 + P313 - If protection/face protection.
P302 + P352 - IF ON SKIN: Wash with plenty of soap and Precautionary P statements: Wear protective Remove contact lenses, if present and easy skin eye irritation persists: Get irritation gloves/protective occurs: Get clathing/eye medical medical

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kill components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trainers of 12%. The ELISA incubator has to be set at 43°C (lokerance of +1.05°C) and regularly checked to ensure the cornect temperature is maintained. Both dry incubators and water hastly are subable for the noutrainors, provided that the instrument is validated for the incubation of ELISA tests. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household atchold, 10% solution of bleach, hospital grade disinfectants) of those parts that

instrument is validated for the incubation of ELISA tests.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully relievence and correctly optimized using the kit or outline labrationy reference panels, before using the kit for outline labrations. ensure that the assay performs as expected, A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an washing cycles (aspiration + dispensation of of washing solution = 1 cycle) are sufficient to

assay with the kit controls and well characterized negative and positive reterence samples, and check to match the values reported below in the sections valuation of Test and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be examined out according to the instructions of the manufacturer.

Incubation times have a lolerance of ±5%.
The ELLSA reacter has to be equipped with a reading filter of 450nm, and with a second filter (620-630nm, strongly recommended) for blanking purposes Blanking is carried out on the west literatified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured it should be regularly maintained according to the manufacturer 's establishment's and the section "Assay Procedure". 4 10 regularly maintained instructions.

o When using an ELISA automated work station, steps (dispensation, incubation, washing, readispensation, incubation, washing, reading, data have to be carefully set calibrated, controlled and serviced in order to match the values reported in ւ, all critical ading, data

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 and for washing. This must be suited and controlled to minimize the passibility of confamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run. 7. Dia Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure comprisione with the equirements described. Support is also provided for the instruments used in combination with the kit. setting and checking of instruments used in combinal with the kit, in order to assure compliance with requirements described. Support is also provided for institution of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.

Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Suschale (TMB+HO2O) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipelte, 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 Check that no breakage occurred in transportation

microplate, is not punctured or damaged.

Dilute all the content of the 20x concentrated Wash Solution

Dissolve the Control Serum as described above and gently

Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid

reagents.

Set the ELISA incubator at +37°C and prepare the ELISA washer by primiting with the diluted washing solution, according to the manufacturers instructions, Set the right number of washing guptes as found in the validation of the instrument for its use with the kit.

Check that the ELISA reader is turned on or ensure it will be

turned on at least 20 minutes before reading. If using an automated work station, turn on, check settings

and be sure to use the right assay protocol.

Check that the micropipettes are set to the required volume.

Check that all the other equipment is available and read;

× case of problems, do not proceed nd advise the supervisor. further with the test

M. ASSAY PROCEDURE

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

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

M.1 Quantitative analysis1. Place the required number of strips in the plastic holder and

2 carefully identify the wells for standards and samples.
Dilute samples 1:101 dispensing 1 ml Sample Diluent into a disposable tube and then 10 ut sample; mix on vortex before use. Do not dilute the Calibrators and the dissolved Control

ω 4 Serum as they are ready/douse.
Leave the A1-61 wells empty for blanking purposes.
Pipette 100 µl of the Calibrators in duplicate, 100 µl of the Calibrators in duplicate, 100 µl of the Calibrate followed-by-00-µl-of-dibuced samples. The Control Serum is used to verify that

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the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been

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 EUSA automatic instruments,

- When the first incubation is finished, wash the microwells as
- previously described (section I.3)
 7. In all the wells except A1+B1, pipette 100 μl Enzyme Conjugate, Incubate the microplate for 60 min at +37°C.

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

- When the second incubation is finished, wash the
- Pipette 100 µl Chromogen/Substrate into all the wells, A1+B1 included. microwells as previously described (section I,3)

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

- with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.

 11. Picette 100 µll Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction. Addition of the stop solution will turn the positive 10. Incubate the micropiate protected from light at room temperature (18-24°C) for 20 minutes. Wells dispensed
- control and positive 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, strongly recommended), blanking the instrument on A1 or B1 or both.

- M.2 Qualifative analysis

 Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.

 Dittle samples: 1:01 disspensing 1 in Sample Dittent into a disposable bube and then 10 ul sample; mix on vortex before use. Do not diffue the Calibrators as they are ready-to-use.
- ω 4 Leave the A1 well empty for blanking purposes.

 Pipette 100 µl Calibrator 0 Ulml in duplicate, 100 µl Calibrator 100 mi in duplicate and 100 µl Calibrator 100 Ulml in single. Then dispense 100 µl diluted samples in proper sample wells. Check that Calibrators and samples
- Incubate the microplate for 60 min at +37°C. have been correctly added.

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

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

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

- When the second incubation is finished, wash the microwells as previously described (section 1.3). Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

- Important note: Do not expose to strong direct light, as a high background might be generated.
- In incubate the microplate protected from light at room temperature (18-24°C) for 20 minutes, Wells dispensed with pasitive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.

 11 Pipette 100 µ Sulphuric Acid into all the wells using the same pipetting satuance as in step 9 to block the enzymatic reaction. Addition of the step solution will hum the positive control and positive samples from blue to yellow.
- Measure the colour intensity of the solution in each well, as described in section 1.5 using a 450nm filter (reading) and a 520-530nm filter (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

- Important notes:

 If the second filter is not evaliable, ensure that no finger pints are present on the bottom of the microwell before reading at 450nm. Finger pints could generate false positive exalts on reading to performed immediately after the addition of the Stop Sobulion but definitely no diograph and 20 minutes afterwards. Some self oxidation of the
- chromogen can occur leading to a higher background.
 The Control Serum (CS) does not affect the calculation and therefore the test results calculation. Control Serum may be used only when a laboratory internal quality control is required by the management

N. ASSAY SCHEME

The assay protocol can be summarized in the table below:

Reading OD	Sulphuric Acid	emperature	incubation	hromogen/Substrate	Vashing steps	emperature	incubation	nzyme Conjugate	Washing steps	emperature	incubation	& dissolved Control Serum	Campianors of minten
450nm	100 u	room	20 mir	100ul	nº 4-5	+37°C	60 mir	100 ul	п° 4-5	+37°C	60 min		1000

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

enda I	CAL3	CAL3	CAL2	CAL2	CALI	CALT	BLX	BLK	- 10	
BLK = Stank // CAL = Calibrators	CS	CS	CALE	CAL6	CALS	CALS	CAL4	CALA	2	
nk // C	SS	\$7	Se	SS	S4	S3	S2	S	Cal	
AT ×	İ								4	MIC
Callb									¢5	Micropiate
rator	Ì								6	ate
									7	
	1								œ	
									9	
									10	
									11	
									53	

\$ = 0 m m 0 0 m >

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

	Þ	æ	0	O	m	ক	ഗ	I
	BLX	CAL1	CAL1	CAL3	CAL3	CAL6	S i	82
2	S3	0	St	co co	87	58	6.5	S 10
3	511		S 13		S 15	S 16	S 17	818
4								
5					ľ			
6								
7								
œ								
9								
10								
-								
12								

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are as qualified.
Control that the following data are matched: O. INTERNAL QUALITY CONTROL
A validation check is carried out on the controls any time the used in order to verify whether the performances of the a

	Calibrator > 1,0 100 PEI U/ml	10 PEI U/mi	Calibrator OD4 5 PEI U/ml anyw	Calibrator < 0.150 0 PEI U/ml coefficient of < 30% variation	Blank well < 0.1	Parameter	
OD450nm = OD450nm of the Calibrator 20 LUml + 10%	> 1,000 OD450nm	OD450nm > OD450nm Cal 0 U/ml + 0,200	OD450nm > OD450nm Cal 0 U/ml + 55D and anyway > OD450nm Cal 0 U/ml + 0,100	< 0.150 OD450nm after blanking < 30%	< 0.100 QD450mm	Requirements	

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

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

Problem	Chica
0.100 OD450nm	 that the Chromogen/Substrale solution has not become contaminated during the assay.
Calibrator 0 Umi - 0. 150 OD450nm atter denking	that the washing procedure and the washing settings are as validated in the pre-qualification study. that the proper washing solution has been made and the washing solution has been made and the washing solution.
cefficient of variation >	3. That no metakar has been drine in the assay procedure (dispondance of positive cubinduse invalidation of Casi 0). 4. But no consummation of the Cali 0, or of the walls, where this work of the procedure constraint with protein the procedure consummation of the Calif 0, or of the walls. 5. But investigate there and become unsummated with proteins cample on with the receives consupale. with proteins cample on earth the receives consupale of the calif rnia of t

1 122 CAL 0 + SSO of break and the washing procedure and the washing procedure and the washing procedure and the washing procedure and the calebrater as comment. Calibration 19 Units. Link in ordered conformation of the calebrater as the washing procedure and the washing before as the calebrater and the washing breather and the washing breather and the washing breather as the calebrater as the calebrater as the calebrater as the calebrater and the washing breather as the calebrater as the calebrate		Calibrator 5 Umd	that the procedure has been correctly performed; That no mistake has occurred during its distribution;
Calibrator 10 Until Calibrator 100 Until Calibrator 100 Until 4 1,000 Obs50mm Cartinal Serum Different from aspected value	1 12	« CAL 0 + 5SD or « CAL 0 + 0.100	3 that the washing procedure and the washer setting are as validated in the pre qualification study. 4, that no external confamination of the calibrator b.
Calibrater 100 Ums 1.000 OD450mm 1.000 OD450mm Control Serum Odfanost from aspected value	1	Calibrator 10 Wml	that the procedure has been correctly performed; that no mistake has occurred during its distribution;
Calibrates 188 Umd < 1,000 Obs50mm Control Serum Different from uspected value		≪ CAL 0 + 0,200	3 that the washing procedure and the washer setting are as validated in the pre-qualification study;
Californate fill Limit 1.000 ODESOnm Control Serium Different from uspected value	İ		4, that no external contamination of the calibrator ha
Control Serum Offerent from appended value		Calibrator 100 U/ml < 1,000 OD450nm	that the procedure has been correctly performed. that no existake has occurred during the distribute of the calibrator;
Control Serum Officered from appended value			 that the washing procedure and the washer setting are as validated in the pre-gualification study; that no external contamination of the calibrator had occurred.
Cifigered from a specified value		Control Serum	First verify that:
value		Different from expected	the procedure has been correctly performed; no mistake has occurred during its distribution (c)
		value	dispensation of a wrong sample): 3. the washing procedure and the washer settings a
	ne kit		correct
5. (the Control Serum has larner discovered with the registry values reported on the labert.) values reported on the labert. values reported on the labert profession of the service of	assay		
If a mission was been pointed out, the assay was to be represented after elimination the extense of list error. If no mission to see says of list error, if no mission to see says of list error, and a value or up -40% is obtained the extense of the laboratory might not exable the text so metals here of the laboratory might not extend the mission metals not expected value -40%, Report the problem on the Soperiorist for completing or location of this result to a was of the completing or location of the laboratory might not not complete or location of this case in the data the DePhr's to comprese service on the laboratory might not not complete the complete or location of the laboratory complete service of the laboratory in the case in the laboratory of the laboratory in the laboratory complete service of the laboratory in the labor			5_ the Control Serum has been dissolved with the rig volume reported on the label.
In peeded aller eliminating the eason of this error, if no nisible ites bench hand, proceeds as fellows: If no nisible ites bench hand, proceeds as fellows: If no expected value up to -1/2% is obtained; the over Procession of the leabarapy might, not expected value +1/10%. Report the problem that has Committed to expected value +1/10%. Report the problem of the Supervisor to coupsigner; or thesis of this research to the Supervisor that on page 14 out -1/20% is obtained in this case the back its needed such the Debrit's souther section.			d out the assay has
a) a value up to +(20% is oblamed the over Processor of the laboratory might not readle the test- metch the expected value +(10%, Report the proble- metch the expected value +(10%, Report the proble- to the Supervision for acceptance or rectal of this research to be supervised the acceptance of the control of the total or laborator of the Charles or such control or the first out it is revised and the Charles or such control or the first out it is revised and the Charles or such control or the			If no mistake has been found proceed as follows:
Procision of the laboratory might not evable the test. Procision of the laboratory might not evable the test. Install the operated value 4-10%, Report the probled in the result to the September for acceptance or relegated this result to the September to obtained in this result in relating the procision of the control			a) a value up to -1-20% is obtained the over
match the expected value 4-10%, Report the problet to the Supervisor for acceptance or results of this result b) a value higher than 4-20% is o biamod, in this case the test is invalid and the Dielfor's customer service h.			Precision of the laboratory might not enable the test
b) a value higher than +/-20% is obtained, in this case the test is invalid and the DiePro's customer service h.			match the expected value */-10% Report the proble to the Supervisor for acceptance or refusal of this results.
the test is invalid and the DiaPro's customer service ha			b) a volue higher than +/-20% is obtained in this car
			the test is invalid and the DiePro's customer service ha

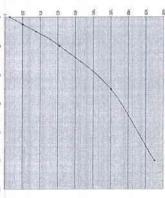
if any of the above problems have occurred, report the problem to the supervisor for further actions.

P. RESULTS

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested),
Then on the calibration curve calculate the concentration of anti-P.1 Quantitative method

HBc IgM antibody in samples

An example of Calibration curve is reported below.



calculations on samples. Important Note: Do not use this example to make

P 2 Qualitative method in the qualitative method, of for the Calibrators 0 and 10 is valid. calculate the mean OD450nm
 10 U/ml and then check that the values

Example of calculation

Calibrator 0 U/ml obtained by the user. The following data must not 0.020 - 0.024 OD450nm be used instead or real figures

Constraint of Contract	0.000
Mean Value:	0.022 OD450nm
Lower than 0,150 - Accepted	
Calibrator 10 U/ml:	0.350 - 0.330 OD450nm
Mean Value:	0.340 OD450nm
Higher than Cal 0 + 0.200 - Accepted	Accepted
Calibrator 100 U/ml:	2,845 OD450nm
Higher than 1,000 - Accepted	ď

Q, INTERPRETATION OF RESULTS

Q.1 Qualitative results For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of HBc IgM > 10 PEI Ulml. Test results are therefore interpreted as a ratio of the sample OD450nm and the OD450nm of the Cal 10 PEI \cup /ml (or S/Co)

Negative	< 0.9
Interpretation	S/Co

>1.1

Positive

according to the following table:

O.2 Quantitative results

The calibration curve is used to determine the concentration of IgN enibodies to HBcAg in samples.

Samples with a concentration lower than 5 PEI Ulml are considered negative for HBcIght.

Samples with a concentration between 5 and 10 PEI Ulml are considered in a gray-zone.

In the follow up of chronic hapatitis, however, values higher of 5 PEI Ulml may be considered positive for HBcIght, when in presence of other clinical signs.

considered positive for HBclgM. Samples with a concentration higher than 10 PEI U/ml are

Important general notes:

- 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 produce the calibration curve, calculate sample concentration and generate the correct interpretation of results.

 Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of

ما N

- 4 judgement errors and misinterpretations;
 A positive result is indicative of HBV infection and therefore the patient should be treated accordingly.
 When test results are treasmitted from the laboratory to another facility attention must be paid to avoid erroreous
- ţh data transfer.

 Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical

R. PERFORMANCES
Evaluation of Performances has been conducted to what reported in the Common Technical Sp CTS (ant. 5, Chapter 3 of IVD Directive 98/79/EC).

means of : 1; Limit of detection
The limit of detection of the assay has been calculated

- 1.1 the HBclgM reference preparation supplied by Paul Erlich Institute, Germany (HBc-Referenzserum-IgM 84), on
- Institute Germany (HBc-Referenzserum-IgM 64), on which the Standard Curve has been calibrated.
 Accurun 113 (cal. N° A113-5001) supplied by Boston Biomedica Inc., USA

12

Results of Quality Control for three lots are given in the following tables:

The state of the s	1.25	25	ch	10	20	50	100	PEI U/ml C	BCM.CE
0000	0.084	0.155	0.310	0.544	0.980	1,917	2.752	OD450nm	Lot#
	0.3	0.5	1.0	.00	3.2	5.3	8.9	S/Co	0103
250.0	0.084	0.149	0.296	0.513	0.914	1.972	2.883	OD450nm	Lot #
	0.3	0.5	1.0	1.7	3,1	5.7	9,7	S/Co	0103/2
D OAA	0.093	0.161	0.321	0.592	1.095	2,053	2911	00459nm	Lot#
	0.3	0.5	1.0	1.00	3.4	6,4	9,1	S/Co	0303

| BCMC/E | Log# | D032 | Log# | D032 | Log# | D032 | | BBI 113 | OD445/mm | SiGo | OD455/mm | SiGo | OD456/mm | Sigo | O

Moreover the BBI's panel # PHE 102 was also examined in three lots of product; data are reported below with reference to a European kit (BBI's results),

BBI - Panel code PHE 102

15	4.4	13	12	11	10	60	80	07	06	05	04	03	02	01	Mamber		
12.4	7.0	11.6	12,7	5.9	11.7	12.2	9.2	0.1	12.1	11.3	5.8	9,5	11.3	6.7	S/Ca	Lot#0103	
11.5	6.3	11.0	11,4	5.8	10.2	11.7	8.5	0.1	11.6	11.4	3.4	7.2	10.0	6.3	S/Co	Lot# 0103/2	COL - Laurel Code : tic lot
11.8	5.6	11,3	11,7	5.8	10,8	11.9	8.8	0.1	11.8	11.2	4.1	8,4	10.7	6,5	SICo	Lot # 0303	AC
4,5	23	3,6	5,2	2.1	2.8	4.2	2.3	0.2	4.1	3,1	2.1	3,0	6.1	2.0	S/Co	Sorin EIA	

Diagnostic Sensitivity:
 It is defined as the probability of the assay of scoring positive in
the presence of the specific analyte.
 The diagnostic sensitivity has been tested internally and
externally in a qualified Clinical Laboratory on panels of samples
classified positive by a US FDA approved kit.
 Positive samples were collected from different patients and from
different HBV pathologies (acute and chronic hepatitis).

ances has been conducted in accordance he Common Technical Specifications or

BCM.CE PEI U/ml	Lot# OD450nm	0 543	Lot# OD450nm	50 01	0103/2 S/Co	0
100	2.752		2.883	_	9,7	Н
50	1,917	5.2	1.972	-	5.7	
20	0.980	3.2	0.914		3,1	3,1 1,095
10	0.544	1.8	0.513		1.7	
ch	0.310	1.0	0.296	_	1.0	1.0 0.321
25	0.155	0.5	0.149	-	0.5	
1.25	0.084	0.3	0.084		0.3	0.3 0.093
regative	0.040		0.035			0,044

No interference was observed in the study.

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

4. Precision: It has been calculated on three samples examined in 16 replicate in three different runs, carried out on three different lots. The values found were as follows:

pooled ones,

BCM.CE: lot # 0103 Cal 0 U/ml (N = 16)

Maan values OD 450nm Std.Deviation	0.055 0.005	2nd run 0.053 0.006	0.051 0.005	Average 0.050 0.050
OD 450nm	0.055	0.053	0.051	
% AO	9.8	12.3	10.7	П
Cal 5 U/ml (N = 16)	2			
Mean values	unitst	2nd run	3 ^{rr} run	
00 450nm	0.324	0.308	0.321	0.318
Std.Deviation	0.022	0.018	0.024	0.021

An overall value > 8% has been found in the study conducted on a total number of more than 2.00 samples. A Seroconversion panel produced by BBI, USA, code # PHM 35A, have also been studied; results are reported below with reference to two commercial kits (BBI's results).

OD 45/mm 2,109 2,048 2,052 Std.Deviation 0,101 0,088 0,135 CV % 4,8 4,3 0,7

120

2nd run

3" run

Average value 2,070 0,109 5.2

3CMLCE: lot # 0103/2

unuss

2nd run

3st run

	Lot Panel	Abbott	DiaSorin
	# 0103		EIA
Member #		SICo	SICo
10	0,2	0,1	0.1
02		0.1	0.1
03	0.2	0,1	0.1
D4		0.1	0.1
05		0.1	0.1
90		0.1	0.1
70		0.1	0.1
08	1,0	0	1.0
90		0.1	0.1
10		0.1	0.1
11	0.2	0.1	0.1
122	0.2	0.1	0.1
13	2.8	3.7	5.7
34	5.0	6.4	0.9
iń.	> 12	6.2	4.5
6	> 12	57,60	4.5
17	> 12	5.5	4.3
8	>12	4.00	4.3
19	> 12	> 6.6	4.4
20	> 12	>6.6	5.2

Mean values

1st run

2nd run

3" run

OD 450nm Std Deviation CV %

0.053 0.005 9.0

0.054

Average value 0.055 0.004 8.2

OD 450nm Std.Deviation

0.332

0.322

BCM.CE: lot # 0303 Cal 0 U/ml (N = 16) Mean values

fistrun

בויגו זערו

3" run

Mean values

uri 35 L

2nd run

3" run

Average

0.110

2.208 0.090 4.1

2,212 0,095 4,3

2,244 0.098

Col 5 Urnd (N = 16)
Mean values

fist run

2nd run

34 1110

0.023

D314 0026 8.2

Average value 0.320 0.024 7.5

0.043 0.004 10.3

0.042

Average value 0.042 0.004 10.6

3. Diagnostic Specificity:

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

The diagnostic specificity has been determined internally and externally in a qualified Clinical Laboratory on panels of negative examples from normal individuals and blood domors, classified negative with a US FDA approved kit.

A total number of more than 400 negative specimens were tested. A diagnostic specificity > 98% has been found, but the diagnostic specificity sea assessed by testing more than 50 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, diseases, patients, affected by non viral hepatic diseases, dispiss patients, pregnant women, hemolized, lipernic, etc.).

Cal 50 U/ml (N = 16) Mean values Std. Devit

Tat nan

Znd run

3" 1110

Average

OD 450nm 2,150 2,163 Std.Deviation 0,057 0,067 CV % 2,6 3,1

2,092 0,076 3,6

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

S. LIMITATIONS

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not generate false positive results Frozen samples containing fibrin particles or aggregates

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.

- REFERENCES

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Rev. 3	
2015/10	

BCA

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

- for "in vitro" diagnostic use only -



DIA.PRO

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REF. BCAB.CE

HBcAb

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06/2014

A. INTENDED USE

human plasma and sera.

The kit is intended for the screening of blood units and the follow-up of HBV-infected patients. Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis B core Antigen in

For "in vitro" diagnostic use only

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B as

serious global public health problem, Hepatitis means inflammation of the liver, and the most cummon cause is inflection with one of 5 viruses, called hopothis A.B.C.D, and E. All of these viruses can cause an acute disease with nonths 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. nausea; vomiting and abdominal pain, It can take several skin and eyes (jaundice); dark urine; symptoms lasting several weeks including yellowing of the "Hepatitis B is one of the major diseases of mankind and is a extreme fatigue;

AIDS. However, HBV is 50 to 100 times more influeinous than AID, The main ways of getting infected with HBV artic (a) perinstal from nother to haby at the hitch: (b) child trensmission (c) unsafe injections and transfusions: (d) 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 fittids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes 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 programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting dung use. In addition, hepatitis B virus is the major infectious 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 developing countries, almost all children become infected with the virus. In many industrialized countries (e.g., Western Europe and North America), the pattern of workers have received hepatitis B vaccine. occupational hazard of health workers, and most health care

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 chro

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 Young children who become infected with HBV are the most

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

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. Chronic hepatitis B in some patients is treated with called interferon or lumivadine, which can help

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 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 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 immunization programmes series of three intramuscular doses. Studies have shown that

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, and HEAva antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patent, eightly later than HEAva, the viral surface antigen.

Anti HBAva antibodies are produced usually at high titers and their presence is detectable even years ster infection, isolated the HBAva, in a visate even years ster infection, isolated in infected blood units, suggesting the use of this test for screening HBV, in addition of HBAva, in a distinct of HBAva.

The determination of HBAva base become important for the classification of the viral agent, together with the detection of the roll agent.

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 affixed amount of antigen on the solid phase. A purified recombinant HBcAg is coated to the microwell together with an additive able to block interferences present in the

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

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 HBc/sg present in the sample. plastic.
After incubation, microwells are washed to remove any unbound

D. COMPONENTS

fficient reagents to perform 96 tests



Microplate MICROPLATE
 Bx12 microwell strips coated with recombinant HBcAg and sealed into a bag with desicciant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2,8°C.

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2. Negative Control CONTROL.

71. Unifusal, Ready to use, Contains 5% bovine serum altumin, 10 m/M phosphase buffer pl 1, 4-4-0, 1, 0,09% sodium azide and 0,1% Kathon CC as preservatives. The negative control is pale

3. Positive Control CONTROL—1

X1.0ml/Mai, Ready to use, Contains 5% bovine serum albumin, anti-HBcAg antibodies at a concentration of about 10 PEI Ulmi, calibrated on PEI HBC Reference Material 82), 10 mM phosphate buffer pH 7.4 +4-0.1, 0.09% sodium acide and 0.1% Kathon GC as preservatives. The positive control is green color

4. Calibrator CAL.....

17 viel, Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains letal bovine serum, human antibodies to HBcAg at a concentration of 2 PEI Ulrinl +7-10% (calibrated on PEI HBc Reference Material 82) and 0.1% Kathon

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.

cn

5. Wash buffer concentrate [MASHBUF 20X]
1x6Dml/bottle, 20x concentrated solution,
Once diluted, the wash solution contains 10 mM phosphate
buffer pH 7.0+/-0.2, 0,05% Tween 20 and 0,1% Kathon GC,

6. Enzyme Conjugate CONJ

Antismilivial. Ready-lo-use solution, Contains 5% bovine serum albumine, 10 mM tris buffer, pH 6.8 #4.0.1, Horseradish peroxidase conjugated mouse monoclonal antibody to HBcAg in presence of 0.3 mg/mi gentamicine sulphate and 0.1% Kathon GC as preservatives, The component is red colour coded.

7. Chromogen/Substrate SUBS_TMB
x16mi/val. Contains a 50 mM citrate-phosphate buffered solution at pH 36 +0.1.0.03% tetra-methyl-benzidine (TMB).
0.02% hydrogen perxide (H2O2) and 4% dimethylsulphoxide Note. To be stored protected from light as sensitive to

8. Specimen Diluent <u>DILSPB</u>
4.X3ml/val. 10 mM ris buffered solution pH 8.0 +/-0,1 containing 0.1% Kathon GC for the pre-treatment of samples and controls in the plate, blocking interfarence.

Note: Use all the content of one vial before opening a Note: Use all the content of one vial before opening a

second one. The reagent is sensitive to oxidation

9. Sulphuric Acid H2SO4 O.3 M Attention: Irritant (Xi R36/38; S2/26/30) 0.3 M HzSO₄ solution

10. Plate sealing foil n°2

11. Instruction manual n°1

E MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipelles (100ul and 50ul) and disposable

Ν EIA grade water (double distilled or deionised, charcoal to remove oxidizing chemicals used as plastic tips.

Timer with 60 minute range or higher

ω 4 ro Calibrated ELISA micropiate thermostatic incubator (dry pr Absorbent paper tissues

wet) sellat +37°C.
Calibrated ELISA microwell reader with 450nm (reading)

თ 00 -1 and with 620-530nm (blanking) lifter Calibrated-ELISA-micropiate-washe -530nm (blanking) filters

Vodex or similar mixing

F. WARNINGS AND PRECAUTIONS

rechnical personnel only, under the supervision of a medical doctor responsible of the laboratory, 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

U.S. and reported in the National Institute of Health's publication. Biosafely in Microbiological and Biomedical wear protective laboratory clothes, taic-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved analysis,

All the personnel involved in performing the assay have to wear protective jaboratory clothes, talc-free gloves and ended by the Cenler for Disease trained biosafety procedures. Control, Atlanta

б

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

each one

4

external (primary container) and internal (valas) habets.

Treat all speciments as potentially infective. All human serum securin speciments should be handled at Biosalety Level 2, as recommended by the Center for Disease Control Allania, U.S. in compliance with what reported in the Institutes of Health's publication. Biosafety in Microbiological and Biomedical Laboratories', ed. 1994.

13. The use of Gaposable plastic-ware is recommended in the preparation of the washing soution or in transferring components into other containers of automated workstations, in order to avoid contamination.

14. Waste produced during the use of the kin has to be discarded in compliance with national directives and laws concerning aboratory 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 irrealment with a 10% final concentration of household bleach for 16.18 hrs or heat inactivation by autoclave at 15°C for 20 min. 14

5 Accidental spills have to be adsorbed with paper tissues casted with brusschold blosch and then with water Tissues should then be discarded in proper containers designated for laboratory/hospilal waste.

The Sulphuric Acid is an inflant, in case of spills, wash the

6

The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a

Laboratories", ed. 1984.
All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are

available, safe and effective.

The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and micropiates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid wibration 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. Don ont interchange components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits, it is recommended that components between the kits of the same lost should not be interchanged.

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. Avoid cross-contamination between serumiplasma

10 samples by using disposable tips and changing them after

Do not use the kit after the expiration date stated on

ü

surface with plenty of water

17, Other waste materials generated from the use of the kit (example: tips used for samples and controls, used

disposed according to na concerning laboratory wastes

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.

Avoid any addition of preservatives to samples; especially sodium actide as this chemical would affect the enzymatic status of the sample with the solid control of the samples. SPECIMEN: PREPARATION AND RECOMMANDATIONS
Blood is drawn aseptically by venepuncture and plasma of

activity of the conjugate.

Samples have to be clearly identified with codes or names.

When the kit

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. Haenmolysed (red) and visibly hyperligentic (milky) samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filiaments and bondles should be discarded as they could give rise to false results. See a fall plasma can be stored at +2,8°C for up to five days after collection. For longer storage periods, samples can be stored frozen al +20°C for several months. Any frozen samples should not be frozenthawed more than once as this may generate particles that could affect the test

If particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0,2-0.8v 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

Aflow the micropiate to reach room temperature (about 1 hr) before opening the container. Check that the desicuant 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°,3°C. After first opening, remaring strips are stable until the humidity indicator inside the desiccant bag turns from yellow to

Negative Control

to use. Mix on vortex before use.

Ready to use. Mix well on vortex before use.

Note: The dissolved calibrator is not stable. Store it frozen aliquots at -20°C. the lyophilised powder; let fully dissolve and then gently mix Add the volume of ELISA grade water, reported on the label. 9 5

5. Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidstitled 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.

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Swel

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

agents and metallic surfaces.
If this component has to be transferred use only plastic, and if Ready to use, Mix well on vortex before use, Avoid contamination of the Ilquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing

9. Sulphuric Acid: Ready to use Mix well on Attention: Imfant (Xi R36/ reagent is sensitive to oxidation.

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

Specimen Diluent

Legenda: R 36/38 = Irritation to eyes and skin, S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice,

R36/38; S2/26/30)

use

I. INSTRUMENTS AND TOOLS USED IN COMBINATION

1. Micropipette Micropipetts have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% chann). Thus soulion of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample of the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a runners of 42%.

Trueness of ±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 dy incubators and water balts are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kill controls/calibration and reference panels, before using the kill controls/calibration and reference panels, before using the kill controls/calibration and reference panels, before using the kill controls/calibration and reference panels, before using the kill controls/calibration of 530 divised of washing solution. To cycle) are sufficient to insure that the assay performs as expected. A seaking time of 20-30 seconds between cycles is suggested, in order to set correctly bein rumber; it is recommended to run an assay with the kit control/cathbrar and well characterized negative and positive reference samples, and check to match the values reported below in the sections. Validation of frest and Assay Performances: Regular cathbration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

un 4 strougly recommended for blanking purposes. Its standard performances should be (a) bandwidn ½ 10 nm, (b) absorbance range from 0 to 2.20; (c) linearity to 2.20; reseability 2 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The oblical 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 Incubation times have a tolerance of ±5%.

The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm

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6. When using an ELISA automated work station, all critical steps (dispensation, incubation, wasning, reading, shaking data handing) 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 waster 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 faste 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. Check that the fluid 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 found is present inside the box (primary containing). Check that the aluminium pouch, containing the microplate, is not
- punctured or damaged.

 Dilute all the content of the 20x concentrated Wash Solution as described above.
- 0 reagents.

 Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diffued washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the Dissolve the Calibrator as described above and gently mix.

 Allow all the other components to reach room lemperature (about 1 hr) and then mix gently on vortex all liquid
- instrument for its use with the kit.

 7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol. Check that the micropipettes are set to the required volume.
 Check that all the other equipment is available and ready
- 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.

- Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and
- Dispense 50 ul Specimen Diluent into all the control and Leave the A1 well empty for blanking purposes
- 4 sample wells.

 Pipette 50 µi of the Negative Control in Iriplicate, 50 µi of the Calibrator in duplicate and then 50 µi of the Positive Control in single. Then dispense 50 µi of each of the
- incubate the microplate for 60 min at +37°C

un

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

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

 Pipette 100 µl Enzyme Conjugate in all the wells, except

 A1; incubate the microplate for 60 min at +37%.

- When the second incubation is finished, wash
- microwells as previously described (section L3)
 Pipette 100 µl Chromogen/Substrate into all the wells, A1 듄

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

- 10.
- 12
- recommended), blanking the instrument on A1.

N. ASSAY SCHEME

Reading OD	Sulphuric Acid	Temperature	3 rd incubation	TMB/H2O2 mix		emperature	2 ^{rs} incubation	Enzyme Conjugate	Wash	Temperature	1" incubation	Controls&calibrator and samples	Charles and and and and and and and and and and
450nm	100 ul	1,7	20 min	100 ul	n°4-5	+37°C	60 min	100 ul	n°45	+37%	60 min	50 u	50

An example of dispensation scheme is reported below

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

- 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 µ1. 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 furn the negative control and negative samples from the to yellow.

 2. Measure the colour intensity of the solution in each well, as the scale of the solution in each well, as a 20-830m. filler (background subtraction, strongly higher than the solution of the solution in section 15 using a 450mm filler (teading) and a 262-830m.
- If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before the microwell before the prints are present as a senior prints could generate laise
- reading at 450nm. Finger prints could generate false positive results on reading.

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

> 0.200 OD450nm	Co/S < 1	Negative Control (Negative Control (Negative Control (Negative) Negative Description Coefficient of variation > 20%	≥ 0.050 OD450nm	Problem
I that the procedure has been correctly performed 2 that no missible has occurred uniting the distribution of the context (disponantion of negative context in of positive context). 3. that the washing procedure and the washer set		I had the worker and the worker and the worker and an advantage of the worker and the proper working squalien has been used to all the proper working squalien has been done in the all the worker has been done in the a square prompt depth before use. 3. That for commanders of the negative control or or been a square control. 4. That no continues measured was depended has occur waits written the control was depended has occur to pushine samples, to a wide or an the error of the positive samples or with the uncommanders of the positive samples or with the uncommanders. 5. The positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples of the positive samples or with the positive samples or with the uncommanders of the positive samples or with the positive samples or with the positive samples or with the positive samples or with the positive samples or with the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples or with the uncommanders of the positive samples of the p	that the Chromogen/Substrate solution has not bee contaminated during the assay	Check

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

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		3		4	63 P	Microplate	ate	5	20	0	0	0
100	-	10	(L)	4	¢5	6	7	8	9	10		11
	BLK	52										
	NO	S3										
	NC.	S4							Ö			
2	NC	SS										
362	CAL	88										
300	CAL	\$7										
23	PC	88										
L	2	20						Ī			_	

GAL = Blank CAL = Calibrator NC = Negative Control S = Sample

O, INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or Co/S values have been matched in the analysis, Ensure that the following parameters are mel:

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control	> 1,000 OD450nm after
(NC)	blanking
	coefficient of variation < 20%
Calibrator	Co/S > 1
(about 2 PEI U/ml)	
Pasitive Control	< 0.200 OD450nm

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

If they do not, do not proceed any further arid perform the

Problem	Check
Blank well	that the Chromogen/Substrate solution has not become

Problem	Check
Hank well > 0,050 OD450nm	that the Chromogen/Substrate solution has not become confaminated during the assay
Negative Control (NC)	1. That the washing procedure and the washer settings are as validated in the pre-qualification study:
< 1,000 OD450nm after blanking	2. That the proper washing solution has been used and the washer has been primed with it before use: 3. That has mistake has been done in the assay.
coefficient of variation > 20%	ocedure (dispensation of positive control instigative control; gative control; that no contamination of the negative control or this where the control was dispensed has or to loositive samples, to spills or to the e
	conjugate: 5. hat meropipelies have not become contaminated with positive samples or with the enzyme conjugate b. hat the washer needles are not blocked or partially obstitutied.
Calibrator Co/S < 1	I filed the propositive less there correctly performed 2. Insid no middlick hos occurred during its distribution (e.e. disspersation of regaline control harbed 2. Insid the washing procedure and the washer sellings are as validated in the price qualification study; 4. Insid no external contamination of the calibrator has propositive.
≥ 0.200 OD450nm	I that the imposition has been connectly performed 2. Inch on missible has occurred during the distribution of the control (disponantion of negative control in of positive control). 3. Inch line washing baccolure and the washer settings are as validated in the pre-qualification subor. 4. Inch line certains contamenation of the positive control

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

Cut-Off = (NC + PC) / 5

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

Q. INTERPRETATION OF RESULTS Results are interpreted as ratio between the cut-off value and

the sample OD450nm or Co/S,

Results are interpreted according to the following table:

Positive	v 1
Equivocal	0.9 - 1.1
Negative	< 0.9
interpretation	Co/S

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:

- Interpretation of results should be done under the supervision of the faboratory supervisor to reduce the risk of judgement errors and misinterpretations.

 When less results are transmitted from the laboratory to
- another facility, attention must be paid to avoid emoneous
- data transfer.

 Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical

An example of calculation is reported below.

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

Negative Control: Mean Value: 2.000 - 2.200 - 2.000 OD450nm 2.100 OD450nm

Higher than 1:000 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440 Lower than 0.200 - Accepted

Positive Control

0.100 OD450nm

Co/S>1 - Accepted Mean value: 0.380 OD450nm 0.400-0.360 OD450nm

Sample 1: 0.028 OD450nm Sample 2: 1.890 OD450nm Sample 1 Co/S > 1:1 Sample 2 Co/S < 0.9

positive negative

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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 H50-b supplied by Paul Erich Institute (PEI H50-Reference Material 82). The assay shows a sensitivity of about 1.25 PEI Urm.

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 FeIal Calf Serum (FCS).

0.625	1.25	2.5	tri	PEI U/ml
0.4	1.1	8.0	22.6	Lot 1001
0.4	1.3	5.5	18.0	Lot 0702
0,4	1.0	5.4	19.0	Lot 0702/2
0,4	1.0	5.0	17.7	Lot 1202

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

Accurun 1 - series 3000

Co/S 2.9	Lot 1001
23	Lot 0702
2.2	Lot 1202

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY....
The Performance Evaluation of the device was carried out in a Irial conducted on more than total 6000 samples.

In a first sludy 2023 samples were tested against a US company as reference. A specificity of 99.5% was found. In a second study 1588 samples were examined against a European company. A specificity of 99.7%, was found. In the last study 1565 samples were assayed against the same US company, a value of 99.8% was found. 2.1 Diagnostic Specificity
It is defined as the probability of the assay of scoring negative in the absence of specific enalyte. A total of more 5000 unselected donors, including 1st time donors, were examined.

in addition to the above population, 206 samples from nospitalized patients were tested against the European company, A value of 93.3% specificity was found. Moreover, diagnostic specificity was assessed by testing 164 potentially interfering specimens (other infectious diseases, patients affected by non-viral hepatic diseases, dalaysis patients, pregnant women, hemolized, lipentic, etc.) against the European company. A value of specificity of 100% was assessed. A value of specificity of 100% was assessed. Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and hepami), and human sets have been used to determine the specificity.

No false reactivity due to the method of specimen preparation

2.2 Diagnostic Sensitivity
It defined as the probability of the assay of scoring positive in

the presence of specific analyte.

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

Mean values	Unit 181	Zod run	3" 1141	Average
OD 450nm	1 943	1.939	1.924	1.935
Std. Deviation	0.061	0.078	0.103	0.087
CV %	4.2	40	5.3	4.5

san values	1st run	2nd run	3 7110	Average
D 450nm	0.143	0.147	0.148	0.146
L'Daviation	0.014	0.017	0.018	0.016
CV %	8.8	17.4	12.1	11.1
Co/S	2.8	2.7	2.6	2.7

Mean values	unu (Si	2nd run	3" 1141	Average
				value
CD 450nm	2.163	2110	2.106	2.126
td.Deviation	0.105	0.088	0,139	0.111
CV.%	4.9	4.2	6,6	5.2

Mean values	fst run	2nd run	3" गमा	Average
CD 450nm	2.163	2110	2.106	2.126
Std.Deviation	0.105	0.088	0.139	0 111
CV:%	4.9	4.2	6.6	5.2
Mean villues	_			
The second second	1st run	2nd run	3 140	Average
OD 450nm		2nd run 0.193	0.195	Average value 0.190
OD 450nm Std Deviation		2nd run 0.193 0.023	0.195 0.019	Average value 0.190 0.020
OD 450nm Std Deviation CV %		2nd run 0.193 0.023 12.0	0.195 0.019 9.9	Average value 0.190 0.020

BCAB_CE: lot # 0702/2

Mean values	um 181	2nd nun	3"04	Averag
OD 450mm	2.275	2.098	2.130	2.159
Std.Deviation	0.135	0.126	0.159	0.140
CV %	6.8	6.0	7.5	85

Mean values	tstrun	2nd run	3 ^m run	Average
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	2.5	23

[] Σ

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

S. LIMITATIONS OF THE PROCEDURE Baclerial contamination or heat inactive

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 contamination or heat inactivation of the specimen

PRECISION
 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

sames .	fall run	2od run	3" 040	enter Senervé
450nm	1.943	1.939	1.924	1.935
eviation	0.061	0.076	0.103	0.087
CV %	4.2	40	5,3	45

values	1st run	2nd run	onu ge	aren
450nm	0.143	0.147	0.148	0.146
aviation	0.014	0.017	0.018	0.016
1 % V	8.8	11.4	12.1	11.1
D/S	2.8	2.7	2.6	2.7

BCAB.CE: |o1 # 0702

sauley near	Tel run	2nd run	3 लामा	Average
				Value
CD 450nm	2.163	2,110	2.106	2.126
td.Deviation	0.105	0.088	0.139	0.111
CV.%	4.9	4.2	6,6	5.2

rator (N = 16					
SQUARY ORS	nn ist	2nd run	35000	Average	
D 450nm	0.182	0.193	0.195	0.190	
Deviation	0.018	0.023	610.0	0.020	48
CV%	10.0	12.0	6.6	10.6	
Co/S	2.5	2.2	2.3	2.3	4
					ı

Std.Deviation	OD 450nm	Mean values
0.135	2.275	1st run
0.126	2.098	2nd run
0.159	2.130	3" (14)
0.140	2169	Average

Mean values.	Istrun	2nd run	3" (1)	Average
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	2.5	23

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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

Dia Pro Diagnostic Bioprobes Srl Via G. Carducci nº 27 – Sesto San Giovanni (MI) – Italy

HBe Ag&Ab

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

for "in vitro" diagnostic use only -



DIA.PRO

20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl

(Milano) - Italy e-mail: into@diapro.i Fax +39 02 26007726 Phone +39 02 27007161

REF, HBE,CE 96 Tests

HBe Ag&Ab

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A. INTENDED USE

For 'in vitro" diagnostic use only Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy. Enzyme ImmunoAssay (ELISA) for the determination of Hepatilis B "" or dissolved with ElA mechanis recombinant HBeAg at 1 PEI Ulml +10%, 02% gentamicing suphate and 0,1% kathon GC as preservatives.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5, Antigen Calibrator: CALAG ...ml
n° 1 vial. Lyophilised calibrator for HBeAg. To be dissolved with
n° 2 vial. The Calibrator of HBEAg. To be dissolved with
grade water as reported in the label, it contains felal boxin

The positive control is yellow color coded. preservatives. The label is red colored.

4, Antibody Positive Control: CONTROL + Ab TX,10ml/val Ready to use control, it comiains 2% bovine serum albumin, human anti Hab4y positive plasma al about 10 PEI Uriml, 100 mM tris buffer pH 7,44/-0,1,0,09% sodium adde and 0,1% Kalhon GC as

B. INTRODUCTION

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

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

6. Antibody Calibrator: CALAB ...ml nº 1 vial "Lyophilized calibrator for anti-ReAg antibody. To be dissolved with EIA grade water as reported in the label, It contains felal bovine serum, positive plasma et 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The label is red colored.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume

reported on the label.

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

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

C. PRINCIPLE OF THE TEST HBeAg:

HBAQ, if present in the sample, is captured by a specific monodonal antibody, in the "I" incubation, after washing, a tracer, composed of a mix of two in the 2" incubation, after washing, a tracer, composed of a mix of two specific anti-HBAQ monodonal antibodies, labeled with peroxidese (HRP), is added to the microplate and binds to the captured HBAQ. The concentration of the bound enzyme on the solid phase is proportional to the amount of HBAQ in the sample and its activity is deleted by adding the chromogen/substrate in the 3" incubation.

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

8. Enzyme conjugate: [CONJ]
1/16m(Wal, Ready to use conjugate, it contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/0.7, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as pressurvaitives.

1x60ml/bottle, 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7,0+/-0,2, 0,05% Tween 20 and 0,1% Kalhon GC,

7. Wash buffer concentrate: WASHBUF 20X

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

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

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

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

11. Sulphuric Acid (HsSQL 0.3 M) X15ml/Val. It contains 0.3 M H2SQL solution. Attention: Inflant (H315, H319, P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Chromogen/Substrate: SUBS_TMB
11. Xf6mi/vial, Ready-to-use component. It contains a 50 mM cliratephosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03%, tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or HxOz,
Mote: To be stored protected from light as sensitive to strong

1x10ml/visit, Ready to use reagent, it contains recombinant HBaAg, bovine serum, buffered solution pH 8,0+/-0.1, 0.1% Kathon GC 0.09% sodium azide as presenvalives.

The reagent is blue color coded,

fetal

9. HBe Antigen: Ag-HBe The reagent is red color coded.

COMPONENTS

The kit contains reagents for total 96 tests,

1: Microplate: MICROPLATE

n° 1 coated microplate

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

 Negative Control: CONTROL |
 1x2,0ml/vial. Ready to use control. It contains bovine serum, 0,09% sodium acide and 0,1% Kathon GC as preservatives. The negative control is colorless

3. Antigen Positive Control: CONTROL + Ag

 1x1:0ml/vial:-Ready to use control: It contains 2% bovine serum albumin, non infectious recombinant +BeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The positive control is green color coded.

Package insert 12. Plate sealing foils n°2

ء 1°

E. MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable
- plastic figs.

 EA grade water (double distilled or detonised, charcoal treated to remove oxidizing chemicals used as disinfectants).

 Impriving to minute lange or higher.

 Absorbent paper insues.

- Calibrated ELISA microwell reader with 450nm (reading) and with
- 620-630nm (blanking) filters.
 Calibrated ELISA microplate washer.
- Vartex or similar mixing tools

F. WARNINGS AND PRECAUTIONS

- The kit has to be used by skilled and properly trained technical onnel only, under the supervision of a medical doctor responsible of
- protective laboratory clothes, tall-free gloves and glassies. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in blosfately procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication; Toosafety in Microbiological and Biomedical Laboratories*, ed. 1984.

 3. All the personnel involved in sample handling should be vaccinated for LBM and HAM for the Microbiological and Stormetical Laboratories*. personnel involved in performing the assay have to wear
- a. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective,

 The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
- Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room. 6 Do not interchange components between different lots of the kills, It is recommended that components between two kills of the same lot
- Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate should not be interchanged.
- Avoid cross-contamination between serum/plasma samples by using
- higs and changing them between the use of each one.

 10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) jabeja.

 11. Treat all specimens as potentially infective. All human serum disposable tips and changing them after each sample 9. Avoid cross-contamination between kit reagents cross-contamination between kit reagents by using disposable
- specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compilance with what reported in the Institutes of Health's publication. "Biosafety in Microbiological and Biomedical Laboratories," ed. 1984, 12. The use of disposable plastic-wate is recommended in the preparation of the washing southon or in transferring components into other containers of automated workstations, in order to avoid other containers of automated workstations, in order to avoid
- 13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generaled from the washing procedure, from residuals of controls and from samples has to be freated as potentially infective malerial and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 his or heal inactivation by autoclave at 12°C for 20 min.
 14. Accidental splits have to be adsorbed with paper issues soaked with household bleach for the processing the procedures of the process of t contamination 13. Waste pr
- discarded in proper containers designated for laboratory/hospital waste.

 5. The Stop Solution is an initiant, in case of spills, wash the surface with being of water

 15. Other waste materials generated from the use of the kit (example: 16. Other waste materials generated from the use of the kit (example: 16. used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and samples are concerning taboratory wastes.

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; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating
- 3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
- 4. Haemolysed and visibly hyperlipemic (mlky) samples have to be discarded as they could generate late results. Samples containing residues of thinn or heavy particles or microbial flaments and botter should be discarded as they could give rise to false results.

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

6. If particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0,2-0,80 filters to clean up the sample for testing.

H, PREPARATION OF COMPONENTS AND WARNINGS

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

Micropiate

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

Unused strips have to be placed back into the aluminum pouch, with the desicnant supplied, firmly zipped and stored at 2°.4°C, When opened the first time, unused strips are stable until the humidity indicator inside the desicnant bag turns from yellow to green,

Ready to use. Mix well on vortex before use Negative Control

. Antigen Positive Control

Ready to use. Mix well on vortex before use.

Ready to use, Mix well on vortex before use

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex; Note: The dissolved calibrator is not stable. Store it frozen in

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder, let fully dissolve and then gently mix on vortex, Note: The dissolved calibrator is not stable. Store it frozen in

7. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use, buring preparation avoid foreign 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°

Enzyme conjugate:
 Ready to use. Mix well on vortex before use.
 Avoid contamination of the liquid with oxidizing chemicals, air-driven dust
 Avoid contamination of the liquid with oxidizing chemicals, air-driven dust
 Avoid contamination of the liquid with oxidizing chemicals, air-driven dust
 Avoid contamination or air-driven dust.

တ

Ready to use. Mix well on vortex before use

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

10. Chromogen/Substrate:

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic Ready to use. Mix well on vortex before use

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

Ready to use. Mix well on vortex before use. Attention: Irritant (H315, H319; P280, P305+P351+P338, P337+P313, P362+P363) P302+P352 P332#P313

regenda

Warning Histatements: H315 – Causes skin irrilation H319 – Causes serious eye irritation

Precautionary P statements: P280 — Wear protective gloves/protective clothing/eye protection/face

Doe: | INS HBE CE/eng | Page |

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P302 + P332 – IF ON SKN. Wash with plenty of soap and water, P302 + P315 – If skin imation occurs: Get medical advicedationion, P305 + P351 + P336 – IF IN EPES: Rhose coulously with water for several minutes, Remove contact lenses, if present and easy to do,

P362 + P363 - Take off contaminated clothing and wash it before reuse. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention

I, INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE $\ensuremath{\mathrm{KIT}}$

- 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 comtact with the sample. Deconfarmination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a
- The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C). The guild incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both day incubators and water baths are suitable for the instrument is validated for the
- incubation of EUSA tests.

 The EUSA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panets, before using the kit or routine taboration years, 4-5 washing cycles (aspiration + dispensation of 500 degrees) and so the control of the city of the control of the and well characterized negative and positive retirence samples, and check to maint the values reported below in the section O "internat Californion". Regular califoration of the volumes delivered by, and Californion (decomanination and cleaning of needles) of the washer has to be carried out according to the instructions of the 30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kil controls manufacturer,

- manufacturer's instructions.
 When using an EUSA automated work station, all critical steps (dispensation, norbubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader, in addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set, Particular attention must be paid to
- Dia Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kil, in order to assure cumpliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kil.

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, containhated by visible Check that the liquid components are not containhated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H-2O2) is colouriess or pale blue by aspirating a small volume

- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.

 Set the ELISA incubator at +37°C and prepare the ELISA washer by

- 3 °9

M, ASSAY PROCEDURE

- incubation lines have a tolerance of ±5%.

 The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes Blanking is carried out on the well identified in the section. Assay Procedure, The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the measured. It should be regularly maintained according to the
- avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.

of it with a sterile plastic phetic. Check that no breakage occurred in transportation and no spillage of liquid is piezent listide the box (primary container). Check that the aluminium pouch, containing the minoroplate, is not punctured or damaged. Butter all the content of the 20x contentrated Wash Solution as

- Dissolve the Calibrator as described above and gently mix
- printing with the cllured washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for the common for the commo
- found in the validation of the instrument for its use with the kit.

 Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading,

 If using an automated work station, turn on, check settings and be sure to use the right assay protocot.

 Check that the micropitetics are set to the required volume,

 Check that all the other equipment is available and ready to use.
- In case of problems, do not proceed further with the test and advise the supervisor.

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

A) HBe Antigen:

- Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples, leave the A1 well empty for blanking purposes, leave the A1 well empty for blanking purposes, the ripeter 100 µl of the Negative Control in triplicate 100 µl of the Antigen Positive Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive
- marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0,100), incubate the microplate for 60 min at +37°C. Then dispense 100 μ I of samples in the proper wells. Check for the presence of samples in wells by naked eye (there is a
- ELISA automatic instruments Important note: Strips have to be sealed with the adhesive sealing foil, only when the lest is performed manually. Do not cover strips when using
- When the first incubation is finished, wash T_e microwells
- previously described (section t.3)

 Dispense 100 μl Enzyme Conjugate in all wells, except for A1, used for blanking operations

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

- Check that the reagent has dispensed properly and then
- incubate the microplate for 60 min at +37°C.

 10. When the second incubation is finished, wash the microwells as
- previously described (section I.3)
 11. Pipette 100 µl Chromoger/Substrale into all the wells, A1 included.
- Do not expose to strong direct light as a high
- Incubate the microplate protected from light at room temperature (18-24°C) for 20 minutes. Wells dispensed with positive control and
- positive samples will turn from clear to blue.

 13. Pipetite 100 µi Sujahuric Acid into all the wells using the same pipeting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.

 14. Measure the color intensity of the solution in each well, as described in section 1.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

B) HBe Antibody:

- Place the required number of strips in the plastic holder and carefully
- identify the wells for controls, calibrator and samples Leave the A1 well empty for blanking purposes.

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- 3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody
- Positive Control in single.

 Then dispense 50 µ of samples in the proper wells.

 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 450/620mm (samples show OD values higher than 0.100).

 Dispense then 50 µ of HBa Antiger in all the wells, except for A1. Incubate the micropiate 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 EUSA automatic instruments.

When the first incubation is finished, wash the microwells as previously described (section 1,3). Finally proceed as described for the HBeAg assay from point 8 to the

- Important notes:

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

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

 Declipator (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

N, ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples 1" incubation Temperature	100 ul 100 ul 60 min +37°C
Wash step	4-5 cycles
Enzyme Conjugate	100 ul
2 nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2 mix	100 u
3" incubation	20 min
Temperature	5.5
Sulphuric Acid	In 001
Reading OD	450nm

HBe antibody test

The ferringen

Controls and calibrator	50 ut
Samples	50 u
Neutralising antigen	50 ul
1 st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzymatic conjugate	100 u
2" incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2 mixture	100 u
3 ^{rrr} incubation	20 min
Temperature	17.3
Sulphuric Acid	100 Ld
Reading OD	450-1

An example of dispensation scheme is reported below

enda: B	ts.	PC	CAL	CAL	NC	NO.	NC	BLK		
LK = Blank	Sg	SB	S7	CAL S6	SS	S4	S3	523	2	
// N						j			لية	
I Ne		Г	Ī			ij		r.	4	
wiled									C71	
Con									6	
MA									7	
									8	
I									9	
									0.	
									1	
									12	

Z B O C M T O I O, INTERNAL QUALITY CONTROL

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

Control that the following data are matched:

ribe Amigen	
Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2,0
Positive Control (PC)	> 1,500 OD450nm

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

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

if they do not, don't proceed any further and perform the following

Problem	Check
Blank well > 0.100 OD450nm	 that the Chromogen/Substrate solution has not become contaminated during the assay.
Negative Control (NC) > 0,150 OD450nm	 that the washing procedure and the washer settings are as validated in the pre qualification study;
after blanking	2 that the proper washing solution has been used and the washer has been primed with it
variation > 36%	3, that no mistake has been done in the assay
	procedure (dispensation of positive control instead of negative control);
	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.
	that micropipettes have not become contaminated with positive samples or with the
	enzyme conjugate

Calibrator 1 that the procedure has been correctly	S/Co < 2 performed,	2, that no mistake has occurred during its	distribution (ex.: dispensation of negative	control instead);	Ihat the washing procedure and the washer	settings are as validated in the pre qualification	sludy;	4, that no external confamination	calibrator has occurred.	Positive Control 1, that the procedure has been correctly	< 1.500 OD450nm performed;	2, that no mistake has occurred during the	distribution of the control (dispensation	negative control instead of positive control);	 that the washing procedure and the washer 	settings are as validated in the pre qualification	study;	4 that no external conformation of the positive	The state of the s
has been		occurred a	sation of		dure and th	the pre qu		niammation		has been		occurred d	ol (dispen:	positive cor	dure and th	the pre qu		10000	IN IO LICIDE
correctly		during its	negalive		e washer	alification		of the		correctly		uring the	sation of	lrol);	e washer	allfication		o positivo	COUNTY OF

That the Chromogen/Substrate solution has not become contaminated during the sasily particular to the washing procedure and the washing self-up are as validated in the pre qualification sluty. The the proper washing solution has been sluty, The the proper washing solution has been given by the proper washing solution to the proper washing solution to the proper washing of procedure (Exc. dispersacion of one in the assay the procedure (Exc. dispersacion of positive control to the property of the procedure (Exc. dispersacion of positive control to the procedure (Exc. dis	lem	Check
3 6	450nm	 that the Chromogen/Substrate solution has not become contaminated during the assay
ğ	ontrol	1, that the washing procedure and the washer
)450nm	sludy:
	ď	that the proper washing solution has been used and the washer has been primed with it
	0,	before use:
procedure (Ex.: dispensation of positive cont	10%	3, that no mistake has been done in the assay
		procedure (Ex.: dispensation of positive control

Problem I that the Chemograph/Subtrale solution has 20 100 OD450mm and become contaminated during the assays Megative Control I had the washing procedure and the washing the sessing are as validated in the per qualification to 4 1000 OD450mm 2. That the washing procedure and the washing solution has bean strings are as validated in the per qualification to 400 or 100	HBe antibody	
Sonm WC/1.5	Problem	Check
obna strod Som	Blank well	
ttrat Dinm 94	> 0,100 OD450nm	
VC/1,5	Negative Control	1, that the washing procedure and the washer
VC/1.5	NC)	settings are as validated in the pre qualification
VC/1:5	< 1.000 OD450nm	sludy:
Q %	after blanking	2, that the proper washing solution has been
NO.7.5		used and the washer has been primed with it
35	coefficient of	before use;
7.5	variation > 10%	3, that no mistake has been done in the assay
v NO.		procedure (Ex.: dispensation of positive control
v NC/1.5		inslead of negalive control; no dispensation of
V CO		the Neutralizing Antigen; no dispensation of the
NC/1,5		Enzyme Conjugale);
v NC/1.5		4 that no contamination of the negative control
v NC/1,5		or of the wells where the control was dispensed
> NC/1,5		
> NC/1,5		nave not
V NC/1.5		contaminated with positive samples;
> NC/1.5		 that the washer needles are not blocked or
NO.17	Calibrator	artina haz hazin
	OD450nm > NC/1.5	a processor room security
distribution (ex.: dispensation of regalive control instead: no dispensation of the veturalizing Anigen; no dispensation of the Enzyme Conjugale); no dispensation of the Salme and the washer settings are as validated in the pre-dualification study;		mistake has occurred during
control instead: no dispensation of the Neutralizing Antigers; no dispensation of the Enzyme Conjugately. 3. That the washing procedure and the washer settings are as validated in the pre-qualification study;		(ex.: dispensation of
Neutralizing Anigen; no dispensation of the Enzyme Conjugate); 3. Ital the washing procedure and the washer settings are as validated in the pre-qualification study;		instead; no dispensation
Enzyme Conjugale); 3. that the washing procedure and the washer settings are as validated in the pre qualification study;		
 Inal the washing procedure and the washer settings are as validated in the pre qualification study; 		Enzyme Conjugate);
settings are as validated in the pre qualification study;		3, that the washing procedure and the washer
		settings are as validated in the pre qualification

Positive Control CD45pnm > NC/10	Calibrator CD450nm > NC/1,5
that the procedure has been correctly performed: that no misiate has occurred during the distribution of the control: that the washing procedure and the weather settings are as validated in the pre-qualification study.	i. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (exc. dispensation of regality control instead; no dispensation of the Neutralizing Anigsen; no dispensation of the Neutralizing Anigsen; 3. has the washing procedure and hie washer settings are as validated in the pre-qualification sudy; 4. that no external contamination of the self-index has occurred.

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

P. CALCULATION OF THE CUT-OFF

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

HBeAg:

NC + 0.100 = Cut-Off (Co)

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

HBeAb:

O INTERPRETATION OF RESULTS

Results are interpreted as follows:

HBeAg:

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

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HBeAb:

>1	0.9	< 0.0	Coff
Positive	1.1 Equivocal	Negative	Interpretation

Co = cut-off value S = OD450nm of the sample

An example of calculation for HBeAg assay is reported below.

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

Negative Control: Mean Value: 0.020 - 0.030 - 0.025 OD450nm 0.025 OD450nm

Positive Control: 2.489 OD4: Higher than 1.500 – Accepted Cut-Off = 0.025+0.100 = 0.125 Lower than 0_150 - Accepted 2,489 OD450nm

Calibrator: 0.520 - 0.540 OD450nm Mean value: 0.530 OD450nm S/Co higher than 2.0 - Accepted

Sample 1 S/Co < 0.9 = negativeSample 2 S/Co > 1.1 = positiveSample 1: 0.030 OD450nm Sample 2: 1,800 OD450nm

An example of calculation for HBeAb is reported below.

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

Negative Control: 2,100 – 2,200 – 2,000 OD450nm Mean Value: 2,100 OD450nm

Higher than 1,000 - Accepted

Positive Control: 0,100 OD450nm Lower than NC/10 – Accepted

Mean value: Cui-Off = (2,100 + 0,100) / 3 = 0,733 Calibrator: 0,720-0,760 OD450nm Mean value: 0,740 OD450nm

Sample 1: 0.020 OD450nm Sample 2: 1.900 OD450nm Sample 1 Co/S > 1.1 Sample 2 Co/S < 0.9 OD450nm < NC/1.5 - Accepted negalive

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important notes:

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

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Eriich Institute (PEI). The data obtained by examining the limit of detection on three lots is reported in the table below.

0303	0103/2	0103	HBE.CE F
0.25	0.25	0.25	EJ U/mJ HBeAg

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

BBI's Accurun 51 (S/Co)

HBE,CE	0103	0103/2	0303
×	4.1	4	4.0
2 x	1.6	1.7	1.6
4	0.9	0.9	0.9
CE X	0.6	0.6	0.5
16x	0,4	0.4	0.4

2. Diagnostic Sensitivity:

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

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

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

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

Data are reported below and compared with those reported by BBI for

two other commercial products.

27 27 22 23 23 23 23 23 23 23 23 23 23 23 23		E Abbon EIA S/Co 4.5 4.3	
	1.1	2.4	
25	1.0	21	
25	0.6	1.7	
77	0.2	0.8	1
28	0.2	0.5	
29	0.2	0.4	
30	0.2	0.3	
31	0.1	0.3	
32	0.1	0.3	

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

25	24	23	22	21	20	ŝ	160	17	ð.	15	Ä	13	12	11	10	9	8	7	a	CP1	4	3	2		Member
		ě,										×.1	^.	24			38	35	2		31	26	ch	Co)	PEI U/ml
0.3	0.2	0.4	0.3	0.3	0.2	0.2	0.2	0.3	0.5	0.4	5.0	0.9	0.2	3.4	0.3	16.6	29.2	30.1	23	1.1	29.4	30.1	17.5	33	HBE CE
0.2	0.2	0.1	0.1	1.0	0.1	0.1	0.2	0.2	0.1	0.1	0.2	1.4	1.2	3.6	0.2	10.8	31.9	24.6	6.9	2.2	23.5	37,1	21.9	7.0	Sorin EIA

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

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

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

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

No cross reaction were observed.

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

The values found were as follows: HBE.CE: lo1 # 0103 It has been calculated on two samples examined in 16 replicate in three different runs on three lots. 4. Precision

Negative Control (N = 16)

Mean values	151 run	2nd run	3 th run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
5V-V2	7.4	8.2	7,9	7.8
PE 4 10 - 100 - 400				

0.575

Average value 0.568 0.028

HBE,CE: jot # 0103/2

			400	
0.003	0.000	0.003	0 003	Std Deviation
0.032	0.030	0.031	0.033	OD 450nm
Average value	a run	200 700	157,520	Weath values

PEI 1 U/ml (N = 16)				
Mean values	20	2nd run	3 rd Euro	Average
OD 450nm		0.573	0.568	0.5
Std Deviation	0.026	0.025	0.024	0.025
6V W		4,3	4.2	14
SiCo	D.	4.4	4.4	4

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Mean values	181 run	2nd tun	J. run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0,003
CV %	9.7	9.8	9.2	9

B) HBe Antibody

Limit of detection
 The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erich Institute (PEI).
 The data obtained by examining the limit of detection on three lots is reported in the table below.

0303	0103/2	0103	Lot ID	HBE CE
0.25	0,25	0.25	HBeAb	PEI U/ml

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

					i
Let 10	ž	2 ×	4 ×	60 ×	5
0103	10	8.0	0.6	0.4	O
0103/2	1.0	0.8	0.6	0.5	0
0303	0.5	8.0		0.4	0,

2. Diagnostic sensitivity: The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit. Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity. An overall value > 95% has been found in the study conducted on a total number of more than 200 samples. Moreover the Panel of Seroconversion code PHM 935B, produced by

8	21										
BECE	0,4	0,4	0.4	0	0.4	0.5	0.6	0.7	0.6	0.8	
Appon ElA	0.4	0.5	0.6	0.5	0.6	0.6	8.0	6.0	0.9	1.0	
Sorin EIA	co Un	0.6	0.5	0.6	0.5	0.6	0.7	0.7	0.7	6.0	

Finally the Performance Panet code PHU tested. Data are reported below and com-BBI for an other commercial product.

OE S	J 201. npared
OWN EIA	J 201, produced by BBI, was spared with those reported by

PEI U/M HBE

 Diagnostic specificity:
 The clinical specificity has been determined as described before for HBeAg.
The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98%

4. Precision: It has been calculated on two samples examined in 16 replicate in three different runs on three jobs. The values found were as follows:

HBE.CE: lot # 0103

Mean values	1strun	2110 1710	3º run	Average value
OD 450mm	2.484	2.420	2.471	2.458
Std. Deviation	0,129	0.160	0.142	0.144
CV %	5.2	6,6	5.7	5.9

8 0.848 0 0.051 6.1	0.878 5.7	0.800 0.060 7.5	0.867 0.043 5.0	OD 450nm Std Deviation CV % Co/S
- AVE	5.00	F2 517		Mean values

HBE_CE: lot # 0103/2

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

66I, was examined

Negative Control (N	16 16			
Mean values		2nd run	3" run	Average value
OD 450nm	2.316	2.361	2413	2,363
Std Deviation	0.127	0,144	0,146	0.139
CV %	5.5	6.1	6.0	5,9
PEI 0.25 U/ml (N = 16)	[8]			
Mean values		2nd run	3 rd rum	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046

HBE,CE: lot #0303

1.0

70 450	722	3415	3437	2 395
Std. Deviation	0.146	0.155	0.158	0.153
% A3	6.3	6.4	25.00	6.4

				The second name of the last of
Mean values	Strun	2nd (Un	3º run	Avisage valu
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.046	0.050
SA #2	en i	6.0	8.8	5,8
ColS	6.0	0.1	10	.0

BV IgN

for the determination of IgM antibodies Enzyme Immunoassay (ELISA) Ħ human serum and plasma to Hepatitis E Virus

for "in vitro" diagnostic use only -



DIA.PRO

Via G. Carducci nº 27 (Milano) - Italy 20099-Sesto-San Giovanni Diagnostic Bioprobes Srl

Phone +39 02 27007161 Fax +39 02 26007726 v-mail: info@diapro.it

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HEV IgM

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A INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Hepatitis C furus in human plasma and sera. The kit may be used for the determination of the acute phase of infection where IgM antibodies are generated before the other immunoglobulins and for the follow-up of HEV-infected patients. For "in vitro" diagnostic use only...

single-strand RNA virus, after being provisionally assigned to the Caliciviridae family, HEV was re-classified as the sole member of the genus Hepevirus, family Hepeviridae, in 2004. HEV is found in the stool of infected patients and present in 4 strains (1, 2, 3 and 4) differently spread geographically and strains (1, 2, 3 and 4) differently spread geographically and F Virus or HEV is a recently discovered agent of ty transmitted viral hepatitis, HEV is an un-enveloped

HEV is a serious problem in many developing countries since its first outbreak was reported in 1955 in New Delhi, India. women and chronic hepatitis carriers. high case-fatality rate has been found among pregnant

The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV antibodies based on recombinant immunodominant antigens derived from conservative regions of the four virus strains.

encoding for conservative and immunodominant determinants of all the 4 subtypes. C.PRINCIPLE OF THE TEST
Microplates are coated with HEV-specific recombinant antigens

The solid phase is first treated with the dittled sample and anti HEV IgM are captured, if present, by the antigens, Her washing out all the other components of the sample, in the 2rd incubation bound, anti-HEV IgM are detected by the addition of polyclonal specific anti hlgM antibodies, labelled with oxidase (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 HEV lgM present in the sample. A cut-off value let optical densities be interpreted into

ant-HEV IgM negative and positive results.

Neutralization of igG anti-HEV and Rheumatoid Factor, carried out directly in the well, is performed in the assay in order to block such kind of interferences.

D. COMPONENTS

Code EVM.CE contains reagents for 95 tests.

1. Microplate MICROPLATE

n° 1 microplate. 12 strips of 8 microwells coated with HEV specific recombinant antigens. Plates are sealed into a-bag-withdesiccant

2. Negative Control CONTROL

154.0m/kidal. Ready to use control. It contains 1% goat serum proteins, 10 mM Naccitate buffer, pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kalthon GC as preservatives. The negative control is yellow colour coded

3. Positive Control CONTROL +

1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, human anti HEV IgM, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC

as preservatives.
The Positive Control is dark green colour coded.

4. Wash buffer concentrate WASHBUF 20X 1x60m/bottle, 20x concentrated solution, Once diluted, the wash solution contains 10 mM phosphate buffer pH 7_0+/_0,2,0,05% Tween 20 and 0,1% Kathon GC,

5. Enzyme Conjugate CONJ

"Atfont/vial. Ready to use and red colour coded reagent, it contains Hoiserautish Peroxidase conjugated goat polycional antibodies to human igM, 5% 86A, 10 mM Tris buffer pH 6.84-f 0.11, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives

1x16ml/visit. Ready-to-use component, It contains 50 mM citate-phosphate buffer pH 3.5-3.8, 4% dimethylsuiphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.

Note: To be stored protected from light as sensitive to 6. Chromogen/Substrate SUBS TMB

strong illumination.

7. Sulphuric Acid H2SO4 0.3 M 1x15ml/vial. It contains 0.3 M H2SO4 solution

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

8. Specimen Diluent: DILSPE

9. Neutralizing Reagent: SOLN NTR 1x9mWal., Ready-Guse Reagent. It contains goat anti higG, 1x9m seain, 10 mM Na-citrate buffer pH 6.0 +/-0,1,0,1% Tween 20,0,09% Na-azide and 0.1% Kalhon GC as preservatives. 2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kalhon GC as preservatives. To be used to dilute the sample,

10. Plate sealing foils ก° 2

11. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 10ul) and disposable plastic tips.
EIA grade water (bidistilled or deionised, charcoal treated to

Ν remove oxidizing chemicals used as disinfectants).

Timer with 60 minute range or higher.

Absorbent paper tissues.

Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C, Calibrated ELISA microwell reader with 450nm (reading)

and with 620-630nm (blanking) filters Calibrated ELISA microplate washer.

Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

 The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical All the personnel involved in performing the assay have to responsible of the laboratory.

Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1994.

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

sale and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the

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ě hogen/Substrate bench surface from from strong li g light and he test is

6. Do not interchange components between different lots the kits. It is recommended that components between two k of the same lot should not be interchanged controlled refrigerator or cold room Upon receipt, store the kit at 2_8°C into a temperature

 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 between serum/piasma

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

samples by using disposable tips and changing them after each

Do not use the kit after the expiration date stated on

external container and internal (vias) justice.

11. Treat all specimens as potentially infective. All human serum specimens should be handed at Biosafely Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the institutes of Health's an whiteration: "Biosafely in Microbiological and Biomedical

Laborationes, ed. 1984.

12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

cross contamination.

13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chamical and biological substances. In particular, flouid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially interitive material and nactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 has on the stanctivation by authorate at 121°C for 20 min.

14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

15. The Sulphuric Acid is an irritant, in case of spills, wash the surface with plenty of water to the standard spotentially infective and disposed should be handled as potentially infective and disposed should be handled as potentially infective and disposed special spills.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1,Blood is drawn asoptically 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 Samples have to be clearly identified with codes or names in avoid misinterpretation of results. When the kit is

3. Haemolysed (red) and visibly hyperipemic ('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 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. Seria and plasma can be stored at 42°, 8°C for up to five days after collection. For imper storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozenthawed more than once as this may generate particles that could affect the test result. could give rise to false results.

5 if pacifies are present, centifupe at 2.000 rpm for 20 mio rittler using 0.20.20 will filest to clean up the sample for testing. H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not ponned out any relevant tass of anothly up to 6 resuse of the george angle up to 6

months

Allow the microplate to reach norm temperature (about 1 h), before opening the container. Check that the desiccent is not urned to dark green, indicating a defect of conservation, in this case call Dia Pro's customer service.

in presence of desiccant supplied, firmly zipped and stored Unused strips have to be placed back into the aluminium pouch +2° ...8°C.

to green indicator of humidity inside the desiccant bag turns from yellow When opened the first time, residual strips are stable till the

Positive Control

Ready to use. Mix well on vortex before use, Be careful not to contaminate the liquid with oxidizing chemicals Chromogen/Substrate:

Neutralizing Reagent

Ready to use

Sample Diluent

Ready to use. Mix well on vortex before use

Warning H statements:

H315 – Causes skin irritation. H319 – Causes serious eye irritation

Precautionary P statements P280 – Wear protective

Negative Control

Ready to use. Mix well on vortex before use.

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.

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 Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently Once diluted, the wash solution is stable for 1 week at +2,8° C

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.

Do not expose to strong illumination, oxidizing agents and If this component has to be air-driven dust or microbes, Iransferred

use only plastic, possible

Mix carefully on vortex before use

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

protective gloves/protective clothing/eye

> + P352 - IF ON + P313 = SKIN Wash With occurs: plenty Get of soap medical and

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advice/lettention.

2015 + p131 + p138 - IF IN EYES; Ringe cautiously with water for several minutes, Remove contact lenses, if present and easy to do, Continue rinsing,

237 + p313 - If eye irritation persists: Get medical

P362 + P363 - Take off contaminated clothing and wash if

L INSTRUMENTS AND TOOLS USED IN COMBINATION

WITH THE KIT

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 4-2%, Deconfamination of spills or residues of kit components should also be carried 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

out regularly,
out regularly,
the ELISA incubator has to be set at +37°C (tolerance of
+40.5°C) and regularly checked to ensure the correct
+40.5°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.

The ELISA washer is extremely important to the overall
performances of the assay. The washer must be carefully
validated and correctly optimised using the kil for outline bloodarby
tests, Usually 4.5 washing cycles (aspiration - dispensation
of 350ul/well of washing solution = 1 cycle) are sufficient to
ensure that the assay performs as expected. A soaking time
of 30.01 seep the testing solution = 1 cycle) are sufficient
of set correctly their number, it is recommended to true an
assay with the kit controls and well characterized negative
and positive reference samples, and check to march the
values reported below in the sections "Validation of Test"
and "Assay Fedformances". Regular calibration of the
values calculation of the control of

according to the instructions of the manufacturer.

Incubation times have a bleenze of ±2%.

The ELISA reader has to be equipped with a reading filter of 450mm and with a second filter (520-550m, storgly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 mm; (b) absorbance range from 0 to ≥ 20; (c) linearly to ≥ 20; repeatability ≥ 1%. Blanking is carried out on the well dentified in the section *4ssay 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 is

regularly maintained according to the manufacturer's instructions.

6 When using an ELISA automated work station, all critical sleps (dispensation, incubation, washing, reading, data handing) have to be carefully set, calibrated, controlled and regularly serviced in order to march the values reported in the sections "Validation of lest" 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 of dispensation and washing) has to be validated and correctly set Particular attention must be paid to avoid carry This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run. over by the needles used for dispensing and for This must be studied and controlled to mini

Dia, Pros customer service offers support to the user in the selfing and checking of instituments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instituments to be used with the kit.

- PRE ASSAY CONTROLS AND OPERATIONS
 Check the expiration date of the kit printed on the external label (primary container), Do not use if expired,
 Check that the liquid components are not contaminated by
- visible particles or aggregates,

 Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic
- pipette.
 Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch containing the microplate, is no purchased to dranaged.
 Dissolve the content of the Control Serum as reported.
- Ditute all the content of the 20x concentrated Wash Solution
- as described above.

 Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid
- reagents.
 Set the ELISA incubator at +37°C and prepare the ELISA. washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the
- instrument for its use with the kit.

 Check that the ELISA reader is furned on or ensure it will be turned on a least 20 minutes before reading.

 In It using an automated work station, turn on, check settings and be sure to use the right assay protocol.

 Check that the incropplettes are set to the required volume.

 Check that all the other equipment is available and ready
- to use. In case of problems, do not proceed further with the test and
- advise the supervisor,

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, laking care to maintain the same incubation time for all

- Dilute samples 1:101 into a property defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the negative Control as the Postive Control as they are ready to use, Mix carefully all the liquid components on
- votices and then proceed so described below.

 Place the required number of Microwells in the microwell holds; Leave & I well enruly for the operation of blanking.

 Disponse 50 µi of the Neutralizing Reagent (SCLN NTR) in all the wells of the samples. Do not add it in the wells used for Controls and in A 1!

 4. Disponse 50 µi of Negative Control in duplicate and 100 µl of Positive control in single. Then dispense 100 µl of diluted samples in each properly identified well.

 5. Incubate the microplate for 60 min at 437°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.

- Wash the microplate with an automatic washer by delivering and aspirating 300 pl/well of diluted washing solution as
- reported previously (section I.3).

 Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells.

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Important note: Be careful not to touch the psurface of the well with the tip filled with the Enzyme the plastic inner

- Incubate the microplate for 60 min at +37°C
- wells as in step 6

Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

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

- 11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive calibrators, the control serum and the
- positive samples from blue to yellow.

 12. Measure the colour intensity of the solution in each well, as described in section L5, at 450nm filter (reading) and at 620-630nm (background subfraction, strongly recommended), blanking the instrument on A1.

General Important notes:

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

 Reading has to earned out just after the addition of the Stop Solution and anyway not any larger than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background

N. ASSAY SCHEME

Method	Operations
Neutralizing Reagent	50 µl
(in sample wells only !)	
Negative and Positive Controls	100 μ
Samples diluted 1:101	100 µ
1" incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µ
2 nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µ
3 rd incubation	20 min
Temperature	1.1
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported below.

	Þ	œ	O	U	m	T	G	I
-	BLK	NC	N	PC	81	S2	S3	\$2
2	SS	S6	57	S	88	\$10	811	\$12
to								
4								
£								
d)								
7								
8				Ī				
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6							Ĭ	
=								
12								

BLK = Blank NC = PC = Positive Control NC = Negative Control control S = Sample

Legenda:

Q. INTERNAL QUALITY CONTROL A check is carried out on the controls 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	
nk well <	0.100 OD450nm value	
gative Control <	0.100 mean OD450nm value after lanking	fter
sitive Control >	> 0.500 OD450nm value	

PZZE

Problem	Check
> 0.100 OD450nm	1, that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC)	that the washing procedure and the washer settings are as validated in the pre qualification.
after blanking	Study. I that the proper washing solution has been used and the washer has been primed with ill before use; I had no mistake has been done in the assay procedure (dispression of positive control instead of registive control (1, that no containmation of the negative control (1, that no containmation of the negative control or of their wells has posurred due to positive amplies, to spills or to the enzymen confugilar. I that incompleties haven't got containmated with positive camples or with the enzymen of that the washer meedles are not blocked or in that the washer meedles are not blocked or
e 0.500 OD450nm	i. that the procedure has been correctly executed: 2 that no mistake has been done in the distribution of control fished of positive control fished of positive control in this case, the negative control will have an OD450m value > 0.160 ton. 3 that the washing procedure and the washer settings are as validated in the pre-qualification study.

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

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = NC mean OD450nm + 0.250

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

S/Co

Negative
1.2 Equivocal
Positive

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1eck	Kequirements		
ell.	< 0.100 OD450nm value		
Control	< 0.100 mean OD450nm value after blanking	value	after
Control	> 0.500 OD450nm value		

If the results of the lest 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	t, that the washing procedure and the washer settings are as validated in the pre-qualification.
> 0,100 OD450nm	sludy;
after blanking	2, that the proper washing solution has been used and the washer has been primed with it
	3, that no mistake has been done in the assay
	procedure (dispensation of positive control
	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;
	of the company of with the common of the com
	conjugate
	that the washer needles are not blocked or partially obstructed.
< 0.500 OD450nm	1. Ihat the procedure has been correctly executed;
	2. that no mistake has been done in the
	control instead of positive control in this case.
	the negative control will have an OD450nm
	value > 0.150, 100.
	3. that the washing procedure and the washer
	study:
	4. that no external contamination of the positive
	control has occurred

value found for the test is used for the interpretation ₫,

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

à.	
⋽.	
-	

*	Requirements		
	< 0.100 OD450nm value		
ontrol	< 0.100 mean OD450nm blanking	t value afte	after
ontrol	> 0.500 OD450nm value		

Problem	Check
ink well 100 OD450nm	that the Chromogen/Sustrate solution has not got contaminated during the assay
gative Control	that the washing procedure and the washer settings are as validated in the pre-qualification.
100 OD450nm	study:
er blanking	2 that the proper washing solution has been
	before use:
	3, that no mistake has been done in the assay
	procedure (dispensation of positive control
	inslead of negative control;
	4, that no contamination of the negative control
	or or men wells has occurred one to positive
	5. that micropipettes haven't got contaminated
	with positive samples or with the enzyme
	conjugale
	6. that the washer needles are not blocked or
	partially obstructed.
sitive Control	1. that the procedure has been correctly
0.500 OD450nm	
	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
	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
	and feel from the second

Repeatable false positive results were assessed for high liter RF positive samples, escaping the effect of the Neutralizing S. LIMITATIONS

obtained by the user. The following data must not be used instead or real figures An example of calculation is reported below:

Diagnosis of viral hepatitis E infection has to be done and released to the patient only by a qualified medical doctor.

erroneous data transfer.

Lower than 0,100 - Accepted Positive Control: 1,589-00450nm Higher than 0,500 – Accepted Cut-Off = 0,070+0,250 = 0,320 Negative Control: 0.060 - 0.080 OD450nm 0.070 OD450nm

Sample 1: 0.070 OD450nm Sample 2: 1.690 OD450nm Sample 1 S/Co < 1.0 = negative Sample 2 S/Co > 1.2 = positive

R. PERFORMANCES
Evaluation of Performances has been conducted on negative
and positive samples in an external clinical center with reference
to a FDA approved kit.

1. LIMIT OF DETECTION

The limit of detection of the product has been checked on the international reference reagent for HEV antibody supplied by NIBSC/WHO with code n° 95/584. This material was assessed to be positive also for anti HEV IgM, low titer. The observed value is about 1 IU/ml.

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY
They were checked on about 700 sample derived from acute infections. HEV Ab positive patients, random individuals under diagnostic examination and healthy individuals with a sensitivity set at about 5 Ulymi in order to assure the highest sensitivity and be able to detect primary infection at the earliest phase.

2.1 Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
A lotal of more 500 unselected donors and HEV negative hospitalized patients, including 1st time donors, were examined. The diagnostic specificity was assessed against a kit US FDA approved. A diagnostic specificity was assessed by sesting-more than 100 potentially interfering specimens (other infectious

diseases, patients affected by non viral hepatic diseas dialysis patients, pregnant women, haemolized, lipemic, etc.). A value of specificity of 100% was assessed. hepatic diseases

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

No interference was observed,
High reactive RF positive samples were observed to give origin to false positive results in not more than 5% of HEV Ab negative Neuronalia

Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and A negative result indicates that the patient has no detectable anti HEV IgM reactivity.

Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.

Any positive result should be confirmed by an alternative method before a diagnosis of wiral hepatitis is farmulated. When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid

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 and
internally on a total number of 100 positive specimens coming
from Germany, Mexico and from Burma,
A diagnostic sensitivity of 100% was found.

N

Important notes:

patient should be treated accordingly.

A positive result is indicative of HEV infection and therefore the

 PRECISION:
 PRECISION:
 If has been calculated on two samples, one negative and one positive, examined in 16 replicates in three separate runs, or values ranging between 5-15%, depending on OD450nm values, were found. The variability seen did not result in sample misclassification.

Frozen samples containing fibrin particles or aggregales after thawing have been observed to generate some false results.

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All the I/O Products manufactured by the company are under the control of a certified Cuality, Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and refessed into the market only if conforming with the EC technical Specifications and acceptance criteria.

Dia,Pro. Diagnostic Bioprobes Srt.
Via G. Carducci nº 27 – Sesto San Giovanni (MI) – Italy

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HV 10

Third generation Enzyme Immunoassay for the determination of IgG antibodies in human serum and plasma to Hepatitis E Virus

for "in vitro" diagnostic use only -



DIA.PRO

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A. INTENDED USE
Third generation Enzyme ImmunoAssay (ELISA) for the qualifative determination of IgG antibodies to Hepatitis E Virus in human plasma and sera.
The kit is intended for the follow-up of HEV-infected patients.

For "in vitro" diagnostic use only.

Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted wiral hepatitis. HEV is an un-enveloped single-strand RAIA virus after being provisionally assigned to the Caliciavindae lamily. HEV was re-classified as the sole member of the genus Hepevirus, lamily Hepeviridae, in 2004, HEV is tound in the stool of infected patients and present in 4 strains. (1, 2, 3 and 4) differently spread geographically and discounts.

HEV is a serious problem in many developing countries since its first outbreak was reported in 1955 in New Delhi, India.

A high case-fatality rate has been found among pregnant

women and chronic hepatitis carriers

The cloning and sequencing of HEV genome have led to the development of serological lests for the detection of anti HEV antibodies based on recombinant immunodominant antigens derived from conservative regions of the four virus strains.

C. PRINCIPLE OF THE TEST

Microplates are coated with HEV-specific recombinant antigens encoding for conservative and immunodominant determinants of all the 4 subtypes.

After washing out all the other components or the addition 2^M incubation bound ant-HEV igG are detected by the addition of polyclonal specific anti higG antibodies, labelled with HEV IgG are captured, if present, by the antigens.

After washing out all the other components of the sample, in the The solid phase is first treated with the diluted sample and anti

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

Code EVG.CE contains reagents for 96 tests

1. Microplate MICROPLATE

n° 1 microplate. 12 strips of 8 microwells coated with HEV specific recombinant antigens. Plates are sealed into a bag with

2. Negative Control CONTROL 3

1x4.0mWval. Ready to use control, it contains 1% goat serum proteins, 10 mM na-citrale buffer pH 6.0 +-0.1, 0.5% Tween 20, 0.09% Na-azide, and 0.1% Kalhon GC as preservatives. The negative control is plive green colour coded.

3. Positive Control CONTROL +

1x4.0ml/vial. Ready to use control, it contains 1% goat serum proteins, human antibodies positive to HEV, 10 mM Na-citrate burier pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Cathon GC as preservatives

The Positive Control is blue colour coded

4. Calibrator CAL Lyophilized calibrator. To be dissolved with volume of EIA grade water reported on the label. - the

It contains foetal bovine serum proteins, human antibodies to HEV whose content is calibrated on 1st WHO reference reagent for HEV antibody, NIBSC code \$5/564 at 1 flufin +/20%, 10 mM Na-citrate buffer pH 6.0 +/0.1, 0.3 mg/ml gentamicine sulphate and 0,1% Kafforn GC sas 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.

Wash buffer concentrate MASHBUF 20X 1x60m/bodtle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer ρH 7.0+/-0.2. 0.05% Tween 20 and 0.1% Kathon GC.

6. Enzyme Conjugate CONJ
X16mi/Wal, Ready to use and red colour coded reagent.
It contains Horseradish Peroxidase conjugated goal polycional
antibodies to human IgG. 5% 85A, 10 mM Tris buffer pH 6.84+
0.1, 0.1% Kathon GC and 0.02% gentamicine sulphale as

Inflimitial. Ready-to-use component, it contains 50 mM citate-prosphate buffer pH 3.5-8.8, 4% dimethy/suphoxide, 0.83%, letra-methy-benzidine or TMB and 0.02%, hydrogen peroxide or H2O2, hydrogen proxide or H2O2. 7. Chromogen/Substrate SUBS TMB

strong illumination.

8. Assay Diluent DILAS
1x6ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.1% Kalhon GC for the pre-treatment of samples and controls in the plate, blocking interference.

9. Sulphuric Acid H2SO4 0.3 M

1x15ml/vial. It contains 0.3 M HsSO₄ solution. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P382+P363).

10. Sample Diluent: DILSPE

1. Sample Diluent: DILSPE

1. Sonlains: 10 mM Na-citrate buffer pH 5.0

1.40.1, 0.5% Tween 20, 0.05% Naezride and 0.1% Kathon GC
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 Plate sealing foils n° 2

n° 1

Package insert

MATERIALS REQUIRED BUT NOT PROVIDED
 Calibrated Micropipettes (200ul and 10ul) and disposable

plastic tips.

- Timer with 60 minute range or higher. EIA grade water (bidistilled or deionised, charcoal treated to

- Absorbent paper tissues.
 Calibrated ELISA microwell reader with 450nm (reading).
 Calibrated ELISA microwell reader with 450nm (reading).
- and with 620-630nm (blanking) filters. Calibrated EUSA microplate washer. 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

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qualified by the national authority in that field (kinnistry of Health or similar entity) to carry out this type of analysis.

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 snould be avoided. All the personnel involved should be trained in biosalety procedures, as recommended by the Center for Disease Controt, Atlanta, U.S., and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Ripmorters in Americans."

Blomedical 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 laboralory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kti vials and micropiales and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is

controlled refrigerator or cold room. Upon receipt, store the kit at +2_8°C into a temperature

of the same lot should not be interchanged.

Check that the reagents are clear and do not contain visible heavy paticles or agoregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit 7. Do not interchange components between different lots of the kits. It is recommended that components between two kits

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

11. Do not use the kit after the expiration date stated on the sample.

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

12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosalety Level 2, as recommended by the Center for Disease Control, Atlanta U.S. in compliance with what reported in the Institutes of Health's container and internal (vials) *Biosafety in Microbiological Biomedical

Laborationes," ed. 1984.

13. The use of disposal plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

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 hardwated before waste. Suggested procedures of inactivation are treatment with a 10% final concernitation of household bleach for 16-18 his or heat inactivation by autoclave at 12.1°C for 20 min., 15. Accidental spills from samples and operations have to be adsorbed with paper listures soaked with household bleach and then with water, itssues should then be discarded in-proper-containers designated for laboratory/hospital-waste. 16. The Sulphuric Acid is an initiant, in case of spills, wash the surface with plenty of water 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 sould be handled as potentially infective and disposed wastes. cross contamination,
14. Waste produced during the
discarded in compliance with na use of the kit has to

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, EDT/ 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 take negative results. at 3. Samples have to the 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 used for the screening of blood units, bar code labeling and

Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they 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 electronic reading is strongly recommended.

could give rise to false results.

5. Sera and plasma can be stored at +2°..8°C for up to seven days after collection. For lunger storage periods, samples can be stored frozen at +20°C for several months. Any foozen 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 pm for 20 min or filter using 0,2-0.8b eithers to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A sludy 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

 Microplates:
 Allow the microplate to reach room temperature (about 1 horizon amening the container. Check that the desiccent is: turned to dark green, indicating a defect of storing. In this case call Dia Pro's customer service. 33

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at

to green opened the first time, residual strips are slable till the ħe bag turns from yellow

2. Negative Control

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.

L Calibrator

Dissolve carefully the content of the lyophilized vial with volume of EIA grade water reported on its label. ŧ

Mix well on vortex before use:
Handle this component as potentially infective, even if HEV eventually present in the control, has been chemically chemically

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

Wash buffer concentrate

Once diluted, the wash solution is sibble for 1 week at +2,8° C. 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 The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use

Enzyme conjugate: Ready to use. Mix well on vortex pefore use.
 Be careful not to contaminate the floud with oxidizing chemicals air-driven dust or microbes.

II this component has to be transferred use only plastic, possibly

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Ready to use. Mix well on vortex before Be careful not to contaminate the liquid

If this component has to be transferred use only plastic, possible

sterile disposable container

Sulphuric Acid

Legenda

Warning H statements:
H315 – Causes skin irritation
H319 – Causes serious eye irritation

to do Continue rinsing P337 + P313 - If eye protection/face protection.

P302 + P352 - IF ON SKIN: Wash with plenty of soap and P305 + P351 + P338 - IF IN EYES: Rinse cauliously with water for several minutes. Remove contact lenses, if present and easy P280 -Precautionary P statements: P362 + P363 — Take off contaminated clothing and wash P332 + P313 -Wear protective lf skin irritation persists: irritation occurs: gloves/protective Get Gel medical clothing/eye medical

Sample Diluent

- 1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular docunfamination (nousehold actional). The 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 +22%. Deconfamination of spills or residues of kit compronents should also be carried of spills or residues of kit compronents should also be carried.
- validated and correctly optimized using the kit controls and reference panels, potice using the kit for returne behavioury tests. Usually 4-5 wasning cycles (aspiration + dispensation of 350chwaler of washing solution = Troycle) are sufficient to ensure that the essay performs as expected. A soaking lime of 20-20 seconds between cycles is suggested, in order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test"

and "Assay Performances" Negular calibration of volumes delivered by, and maintenance (decontaminal and cleaning of needles) of the washer has to be carried and cleaning of needles) of the washer has to be carried.

air-driven dust or microbes.

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

Ready to use, Mix well on vortex before use

P305+P351+P338, P337+P313, P362+P363) Ready to use, Mix well on vortex before use, Attention: Irrilant (H315, H319; P280, P302+P352, P332+P313,

Ready to use, Mix well on vortex before use

I, INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

- The ELISA incubator has to be set at +37°C (tolerance of +7.0,5°C) and regularly checked to ensure the correct temperature is maintained. Both dy incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully

according to the instructions of the manufacturer, 20

- 4. Incubation times nave a tolerance of ±5%.

 The ELISA reader has to be pulpped with a reading filler of 450nm and with a sector filler (620.650nm, strongly recommended) for blanking purposes. Its slandard performances should be (a) bandwith ≤ 10 nm. (b) absorbance range from 0 to ≥ 2.0; (c) linearly 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, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections. Visitation 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 fund 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 rumber of samples to be tested exceed 20-30 units per run.
- Dia,Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit,

L. PRE ASSAY CONTROLS AND OPERATIONS

- Check the expiration date of the kit printed on the external
- label of the kit box. Do not use if expired.
 Check that the fliquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogarisobstrale is coloriess or pale blue by aspirating a small volume of it with a steffic transparent plastic project. 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 Check that no breakage occurred in transportation and spillage of liquid is present inside the box. Check that
- or damaged.

 Dilute all the content of the 20x concentrated Wash Solution
- 4, 10
- as described above.

 Dissolve the Calibrator as described above.

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

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

 Check that the ELISA reader has been turned on at least 20 minutes before reading.
- If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol. Check that the micropipettes are set to the required volume, theoret that it is other equipment is available and ready.
- 5
- 11. In case of problems, do not proceed further with the test and advise the supervisor.

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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.

In case the test is carried out automatically with an ELISA workstation, we suggest to make the instrument aspirate 200 uit Sample Diluent and then 10 uit sample. When the appropriate sample well of the microplate, Before the next sample is aspirated, needles have to be duly washed to avoid any processor of the propriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any processor of the propriate sample with the propriat

any cross-contamination among samples.

Do not dilute controls/calibrator as they are ready to use. Dispense 200 ul controls/calibrator in the appropriate

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

- -Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of
- ω blanking.

 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!

 Add 200 ul of Sample Dilutent (DILSPE) to all the sample wells, then dispense 10 ul sample in each properly identified well. Mits gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully dispense the sample môt is divent.

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

- Dispense of samples has turned to dark blue Dispense 50 ul Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color
- Incubate the microplate for 45 min at +37°C.

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

- Wash the microplate with an automatic Wash the micropiate with an automatic washer by delivering and aspirating 300ul/well of diluted washing
- solution as reported previously (section 1.3).
 Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer, Check that this red colored component has been dispensed in all the wells,

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

- Incubate the microplate for 45 min at +37°C
- Wash microwells as in step 6.

S = Sample

10 Pipette 100µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the micropiate at room temperature (18-24°C) for 15 minutes.

- 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.

 12. Measure the color intensity of the solution in each well, as described in section 1.5, al 450nm filler (reading) and at 620-630nm (background subtraction, strongly recommended).
- blanking the instrument on A1.
- If the second filter is not available ensure that no finger pmts are present on the bottom of the microwel before reading at 450mm. Finger prints could generate false positive results on reading. Freading has to be carried out just after the addition of the feading 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.

Method Controls & Calibrator(*)	Operations 200 ui
Samples	200ul dil.+10ul
Assay Diluent (DILAS)	50 ul
1 st incubation	45 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	In 001
2 nd incubation	45 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/HZOZ	100 ul
3 ⁷⁰ incubation	15 min
Temperature	111
Sulphuric Acid	100 uf
Reading OD	450nm

The Calibrator (

- The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation. The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.

An example of dispensation scheme is reported below

×	BLK -	22	ca	4		Ċ/s	cn co		6 7	6 7 8	6 7 8 9
00	K	\$3									
O	NC	54									
0	NC	SS									
m	CAL(*)	38									
71	CAL(*)	S7									
G	DG.	88			П						
	ņ	50									

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

N. ASSAY SCHEME

Problem	Check
Blank well > 0 100 OD450nm	that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control	1, that the washing procedure and the washer
(NC)	settings are as validated in the pre qualification
> 0,050 OD450nm	sludy;
after blanking	that the proper washing solution has been
	used and the washer has been primed with it
	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;
	with positive samples or with the enzyme
	conjugale
	6, that the washer needles are not blocked or
	partially obstructed.
Positive Control	1, that the procedure has been corractly
< 1,000 OD450nm	executed;
	2. Ihat 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
	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 prolifered

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

Check Requireme

If Calibrator has used, verify the following data

above operate as follows:	If the results of the	
fallows:	If the results of the test doesn't match the requirements sta	
	E C	

Problem	Check
Calibrator	1 that the procedure has been correctly executed;
S/Co < 1.1	 that no mistake has been done in its distribution (e.g.: 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.

O, INTERNAL QUALITY CONTROL A check is carried out on the controls any time the kit is used in order to verify whether their ODA50nm values are as expected and reported in the lable below.

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Negative Control (NC) Check < 0.100 OD450nm value < 0.050 mean 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

A negative result indicates that the patient has not been infected by HEV 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 HEV infection and therefore the patient should be treated accordingly or the blood unit should be

Important notes:

- interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.

 By definition, any positive result should be confirmed by an attemptive method before a diagnosis of viral hepatitis is
- When lest results are transmitted from the laboratory to an informatics center, attention has to be done to avoid
- erroneous data transfer.

 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

tated Sample 1: 0.070 OD450nm Sample 2: 1.590 OD450nm Sample 1.5/Co < 0.9 = negative Sample 2.5/Co > 1.1 = positive Lower than 0.050 – Accepted
Positive Control: 2,189 OD450nm Negative Control: Mean Value: S/Co higher than 1_1 - Accepted Mean value: Cut-Off = 0.020+0.350 = 0.370 Higher than 1,000 - Accepted 0,620 OD450nm 0,620 OD450nm 0.020 OD450nm 0.019 - 0.020 - 0.021 OD450nm

S/Co = 1.7

Anyway, if all other parameters (Blank, Negative Positive Control), match the established requirements, may be considered valid, Control, s, the test

P: CALCULATION OF THE CUT-OFF The tests results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = NC mean OD450nm + 0.350

important note: When the calculation of results is done by the operative system of an ELISA automated work station be sure and generate the right interpretations of results
Q.INTERPRETATION OF RESULTS werenive system or an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and paper is the cate-off. The value found for the lest is used for the interpretation results as described in the next paragraph

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

>1.1	0.9 - 1.1	< 0.9	2/40
Positive	Equivocal	Negative	interpretation

R. PERFORMANCES

R. PERFORMANCES

Fivaluation of Performances has been conducted on negative and positive samples in an external clinical center with reference to a FDA approved kit.

1. LUNIT OF DETECTION

The limit of detection of the assay has been calculated by means of 1st WHO reference reagent for HEV antibody, NISSC code 95/554. The assay shows an analytical sensitivity of about

2 DIAGNOSTIC SPECIFICITY AND SENSITIVITY They were checked with a sensitivity set at 0.25 WHO IU/ml more than total 700 samples. 9

2.1 Diagnostic Specificity.

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

A total number of more than one hundred 1-5 years old children, by definition negative for HEV and policy sea steep rever chanced to eat uncooked swine meal and get therefore infected, were tested; a value of 100% specificity (negativity) was assured at a sensitivity set at a cut-off of 0.25 WHO (Jume).

of a commercial Confirmation kit, However, these samples were detected positive with a commercial CE-marked ELISA. From this study a diagnostic specificity of 10% was observed. Moreover, the Diagnostic Specificity was observed. Moreover, the Diagnostic Specificity was also assessed by testing more than 100, potentially interfering specimens (other infectious diseases, patients affected by mon viral hepath diseases, dialysis patients, pregnant women, heemolized, liperatic, etc.). A value of specificity of 10% was assessed. As the searchity, due-to-the-method-of-specimen-preparation has been observed. Both plasma, defined with different standard techniques of preparation (citrate, EDTA and hepathn), and sera have been used to determine the value of specificity. Frozen specimens have been used to determine the value of specificity. Frozen specimens have been used to other things the part of the plants. In addition, a total of more 500 unselected donors, including 1st time donors, and HEV negative hospitalized patients, coming from Italy, were examined maintaining the sensitivity of 0.25 WHO IUIm. About 5% of such population turned out to be repeatedly positive; confirmation was not carried out in absence repeatedly positive; confirmation was not carried out in absence

No interference was observed.

2.2 Diagnostic Sensitivity
It is defined as the probability of the assay of scoring positive in

The diagnostic sensitivity has been assessed externally (Institute of Virology, University of Milan) and internally on a total number of 200 positive specimens (maintaining a sensitivity set at a cut-off of 0.25 WHO IU/ml); a diagnostic sensitivity (or correlation with a commercial reference kit manufactured in Europe and CE marked) of 100% was found.

PRECISION.
 PRECISION.
 It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs.
 CV values ranging between 5-10%, depending on OD450nm values, were found. The variability seen did not result in sample misclassification.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

- REFERENCES

 9 1. Eliner PD.Neu HC. Viral agents of gastroenteritis. In Understanding infectious disease. St.Louis: Mosby-Year Book, 1982, pp 183-186.

 1900, pp 183-1

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC lechnical specifications and acceptance criteria.

Dia Pro Diagnostic Bioprobes Srl Carducci nº 27 – Sesto San Giovanni (MI) – Italy

Via G.



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

for "in vitro" diagnostic use only -



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HDV Ab

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A, INTENDED USE
Competitive Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis Delta Virus or HDV in human plasme and sera with a "two-steps"

For "in vitro" diagnostic use only, The kit is used for the follow-up of patients infected by HDV

B. INTRODUCTION

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

Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously

occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a palient with chronic HBV infection can support HDV repiticulion indefinitely, usually with a less severe illness appearing as a clinical exacerbation. The determination of HDV specific serological markers (HDV Ag, HDV Ab, HDV (gN and HDV (gG)) represents in these cases an important tool to the clinican for the classification of the etiological agent, for the follow up of infected patients and their treatment. The detection of HDV total antibodies allows the classification of the litness and the monitoring of the

C. PRINCIPLE OF THE TEST

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

D. COMPONENTS
 Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: [MICROPLATE]
8xi2 -microwell-strips coaled with recombinant HDV-specific antigen and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control: CONTROL - 1x2.0ml/vial, Ready to use. Contain *x2.0mil/val. Ready to use. Contains goat serum proteins, 100 mM. Tris-HCI buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.1% Kathon GC as preservatives. The negative control is

3. Positive Control: CONTROL 1
72.0 m/Wai: Ready to use. Contains goat serum proteins, high liter and HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 ++0.1, 0.09% Sodium Azide and 0.1% Kathon GC as preservatives.

4. Calibrator: CAL.....)

n' 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low liter human antibodies to HDV, 0.2 mg/mi gentamicine sulphate and

0.1% Kalhon GC as preservatives.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle 20x concentrated solution.

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

6. Enzyme conjugate: CONJ

Intelligible Really-druste solution, Contains 5% bowine serum albumine, 10 mM lits buffer pH 68 +/-01, Hovescaleb, peroxidase conjugated ambody to HDV in presence of 0.2 mg/ml gentiamicine sulphate and 0.1% Kathon GC as preservalives. The component is colour coded red.

7. Chromogen/Substrate: SUBS_TMB
1. Xf6ml/Wal; Contains a 50 mM critate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 0.03% teltra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H202; Note: To be stored protected from light as sensitive to strong illumination.

Plate sealers n° 2

8. Sulphuric Acid: [H2SO4_O.3.M]
7x15mWal. Contains 0.3 M H2SO4 Solution.
7x15mWal. Contains 0.3 M H2SO4 Solution.
Attention: Intrant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Instructions for Use nº 1

- E. MATERIALS REQUIRED BUT NOT PROVIDED

 1. Calibrated Micropipettes in the range 10-1000 ul and
- disposable plastic tips.

 EIA grade water (double distilled or deionized, charcoal treated to remove oxidizing chemicals used
- Timer with 60 minute range or higher.
- ō, Absorbent paper tissues.

 Calibrated ELISA microplate thermostatic incubator (dry or
- wet) set at +37°C, Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
- Calibrated EUSA microplate washer. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

- 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.
- All the personnel involved in performing the assay have to wear protective laboratory clothes, lab-free gloves and glasses. The use of any sharp (needes) 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.

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

The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H2O2) from storng light and avoid vibration of the bench surface where the test is undertaken, upon receipt, store the kit at +2_8°C into a temperature

Do not interchange components between different lots of controlled refrigerator or cold room It is recommended that components between two

8 kils of the same lot should not be interchanged.
Check that the reagents are clear and do not contain Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.

each sample.

Avoid cross-contamination between kit reagents by using them between the use of

ဖ ō

each one.

10. Do not use the kit after the expiration date stated on it external (primary container) and internal (vials) labels.

11. Treat all specimens as potentially infective. All human was serum specimens should be handled at Biosatert Level 2, as recommended by the Center for Disease Control. It Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication. "Brosately in Microbiological and Biomedical Laboratories", ed. 1984.

12. The use of disposable plastic laboratories", ed. 1984.

13. The use of disposable plastic laboratories of automated in the preparation of the washing solution or in transferring components into other containers of automated

12,

workstations, in order to avoid contamination.

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, fujuri waste generated from the wasting 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 has on heal inactivation by autoclave at 171°C for 70 min. 13

12.1°C for 20 min...

14. Accidental splits have to be adsorbed with paper issues scaled with household bleach and then with water. Tissues should then be discarded in proper containers designated for abboretopythospital waste.

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

16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used to sample should be seen to the kit (example: tips used for samples and controls, used to sample should be seen to the kit (example: tips used for samples and controls, used to sample should be seen to the kit (example: tips used for samples and controls, used to sample should be seen to the kit (example: tips used for samples and controls, used to the sample should be seen to the sample should be sample should be sample should be sample should be seen to the sample should be
5

16 microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

SPECIMEN: PREPARATION AND RECOMMANDATIONS Blood is drawn aseptically by venepuncture and plasma

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 citrate, EDTA and heparin. is for clinical laboratory analysis. No influence observed in the preparation of the sample with

Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic

activity of the conjugate.

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.

Haemolysed fred) and visibly hyperipermic ("miley") samples have to be discarded as they could generate label results. Samples containing residues of florin or heavy particles or microbal flaments and bodies should be discarded as they

Sera and plasma can be stored at +2*_8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozen/thaved more than once as this may generate particles that could affect the test

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.

PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

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:

dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C. When thewed do not freeze again; discard it. Low positive control, Add precisely the water, reported on its label, to the lyoph y the volume of EIA grade fyophilized powder; let fully

The whole content of the 20x concentrated solution has to be diluted with EIA grade water up to 1200 mi and mixed gently end-over-end before use. During preparation avoid floaming as the presence of bubbles could impact on the efficiency of the 5. Wash buffer concentrate washing cycles.

Note: Once diluted, the wash solution is stable for 1 week at

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 and if possible, sterile disposable containers

7. Chromogen/Substrate

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 Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals.

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

Warning H statements

H315 – Causes skin imitation H319 – Causes serious eye imitation

protection/face protection, P302 + P352 - IF ON SKIN: Wash with plenty of soap and

Precautionary P statements P280 – Wear protectiv

protective

gloves/protective

clathing/eye

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I. INSTRUMENTS AND TOOLS USED IN COMBINATION

Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontaination (70%, ethanol, 10%, southor, 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%.

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 incubations provided that the instrument is validated for the incubations provided that the instrument is validated for the incubation of ELISA tests.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be capefully validated and correctly optimized using the kill controls/cabitablor and reference panels, before using the kill controls/cabitablor and reference, panels, before using the kill controls/cabitablor and reference, panels, before using the kill for routine laboratory tests, Usually 4.5 washing solidion = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20.30 seconds between cycles is suggested, in order to set correctly their number, it is recommended to run an assay with the kill controls/calibrator and well characterized negative and positive reference samples, and check to match the values reported below in the sections: "Validation of the volumes delivered and maintenance (decontamination and cleaning of condition) of the volumes. of needles) of the washer has to be carried out according to the instructions of the manufacture

4. Incubation times have a loterance of ±5%,
The ELISA micropiate reader has to be equipped with a feating filter of 450mm and with a second filter (620-630mm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to ≥ 20; (c) linearity to ≥ 20; repeatability ≥ 1%. Blanking is carried out on the well dentified 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 restaurance of the correct optical density is measured. It should be regularly maintained according to the manufacturer's restaurance. regularly maintained instructions.

6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data hearting) 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 the validated and correctly set. Particular differior 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 Dia Pro's customer service offers support to the user in the stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

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.

for several minutes, Remove contact tenses, it present to do, Continue mising,

P337 + P313 - If eye irritation persists: Get advice/attention. P362 + P363 - Take off contaminated clothing and wash if

P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water

Remove contact lenses, if present and easy

medica

P332 + P313 - If skin irritation occurs:

Get medical

L. PRE ASSAY CONTROLS AND OPERATIONS

Check the expirition date of the kit printed on the external label (primary container). Do not use if expired.

Check that the fluidud components are not containmated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic piette, Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.

Dilute all the content of the 20x concentrated Wash Solution

as described above, as described above and gently mix. Dissolve the Calibrator as described above and gently mix.

Allow all the other components to reach room (about 1 hr) and then mix gently on vortex mix gently on vortex all liquic

reagents.

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

Check that the ELISA reader is turned on or ensure it will be

turned on at least 20 minutes before reading.

If using an automated work station, turn on, check settings and be sure to use the fight assay protocol,

Check that the micropipelies are set to the required volume, to Check that all the other equipment is available and ready

 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.

Leave A1 well empty for the operation of blanking.

Store the other strips into the bag in presence of the desiccant at +2_8°C, sealed. 1. Place the required number of strips in the microplate holder

2. Pipette 100 µl of Negative Control in triplicate, 100 µl Positive Control in single and then 100 µl of samples. Check Inal controls and samples have been correctly added.

Then incubate the microplate at +37°C for 60 min.

Wash the microplate as reported in section 1.3.

4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Then incubate the microplate at +37°C for 60 min.

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

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6. Pipatte 100 µl TMB/HzO2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at room temperature for 20 min.

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

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

8. Measure the colour intensity of the solution in each well, as described in section 1.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly bzu-530nm filter (background subtraction, recommended), blanking the instrument on A1.

- If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450mm. Finger prints could generate false positive results on reading. Reading has should ideally be performed immediately after the addition of the Stop Soulian but definitely no longer than the finite state of the chromogen can occur leading to a higher background. The use of the Calibrator, a low positive control, is not mandatory for the assay as the CAL obes not enter into the cut-off calculation. The CAL may be used as a low titer positive control when a laboratory internal quality verification is required by the management. When used for such purpose, dispense 100 ut of it, possibly in duplicate.

N. ASSAT SCHEME

Controls/Calibrator Samples	100 ш
1 st incubation	60 min
Temperature	+37°C
Washing step	4-5 cycles
Enzyme Conjugate	100 u
2 nd incubation	60 min
Temperature	+37°C
Washing step	4-5 cycles
TMB/H2O2 mix	100 ut
3 ^{re} incubation	20 min
Temperature	1,1
Sulphuric Acid	100 ul
Reading OD	450nm & 620nm

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

E C	I	G	Ŧ	m	b	c	œ	Þ		
enda:	H S1	PC	CAL	CAL	NO.	NC	NO.	무듯	1	
	89	88	S7	SS	SS	22	S3	S2	2	
m		ij						ſï	ω	١
BLK = Blank		Ī	Ī		ii				44	
Blan								Ī	cn	1
*									6	Action to the same
z									7	910
NC = Negalive									8	
ieoal									9	
									10	
Contro									11	
									12	

CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL A check is performed on the negative and positive controls any time, and on the Calibrator in addition when the kit is used for the first lime, in order to verify whether the expected OD450mm or Co/S values have been matchade in the analysis.
Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 DD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking if lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation < 30%
Positive Control (PC)	OD450 nm < NC/10
Calibrator (CAL)	PC < 0D450nm < (NC+PC)/5

proceed to the next section.
If they don't, do not proceed any further and perform the If the results of the test match the requirements stated above

Dealdren	At make
Problem	Check
> 0.100 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control	1. that the washing procedure and the washer
Ñ.	settings are as validated in the pre qualification
< 1,000 OD450nm	study;
after blanking	that the proper washing solution has been used and the washer has been primed with it
coefficient of	before use:
variation > 30%	3. Ihat 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
	partially obstructed.
Calibrator	1. that the procedure has been correctly
00450nm	formed;
Outside the range	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
	4 mat no external contamination of
Positive Control	1 that the procedure has been correctly
OD450nm	
> NC/10	2, that no mistake has occurred during
	distribution of the control (dispensation
	negative control instead of positive control).
	3. that the washing procedure and the washer
	settings are as validated in the pre qualification
	study;
	4. that no external contamination of the positive
	control has occurred

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

P. RESULTS

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

Cut-Off = (NC + PC) / 5

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station.

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Q. INTERPRETATION OF RESULTS

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

>1.1	0.9 - 1.1	< 0.9	ColS
Positive	Equivocal	Negative	interpretation
			113

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

Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample. A positive result is indicative of HDV infection and therefore the patient should be treated accordingly.

Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.

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

An example of calculation is reported below.

The following data must not be used instead or real figures

Mean Value: 2.100 GD4 Higher than 1.000 – Accepted Negative Control: 2.100 - 2.200 - 2.000 OD450nm 2.100 OD450nm

Lower than NC/10 - Accepted

0.100 OD450nm

Cut-Off = (2.100 + 0.100) / 5 = 0.440

Within the range PC ≤ OD450nm < (NC+PC)/5 - Accepted 0.300-0.260 OD450nm

Sample 1: 0.020 OD450nm Sample 2: 1.900 OD450nm Sample 1: Co/S > 1.1 Sample 2: Co/S < 0.9

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 58/79/EC)_

LIMIT OF DETECTION:

In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA.

ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results. The table below reports the OD450nm shown by th preparation when diluted in Fetal Calf Serum to prepare limiting dilution curve, in three different lots.

this

Co/S values

Accurun	DAB.CE OD450	1102 Ca/S	DAB.CE	 0103 Co/S	
# 127	OD450	Co/S	OD450	Co/S value	Co/S OD450 value nm
1×	0.171	3.0	0.163	2.9	Н
2x	0.187	27	0.176	2.6	Н
88 1	0.296	1.7	0.285	1.6	1.6 0.271
16x	0.417	1.2	0.405	1.1	-
32x	0.514	1.0	0,490	0.9	
54x	0.717	0.7	0.700	0.7	
128x	1.063	0.5	1.006	0.5	0.5 1.015
CTRL (-)	2.484	MANNA	2.261	MIMILIA	JIIJIIII 2.114

DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The diagnostic performances were evaluated in a clinical rial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Govanni Bartista hospital, Torino, Italy, on more than 400 samples against a reference kit. Negative, positive and potentially interfering samples were examined in the trial.

Soft plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed. Results are briefly reported in the tables below:

Spe	ŭ
chicity	SHIVILY
V	1
100	40
8	Ü

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

DAB.CE: lot #1102

Negative Control	(N = 16)			
Mean values 1500	tst run	2nd run	37700	Average
OD 450nm	2.342	2,428	2.433	2.401
Std Deviation	0.113	0.106	0.122	0,114
CV%	4	4.4	5.0	4.7

Meson values	1strum	2nd run	3 nun	Average
OD 450nm		0.289	0.286	
Std.Deviation	0.023	0.027	0.026	
EV %		9.3	9.1	111
SACO	1.6	7.7	17.7	

DAB_CE: lot #0103

Mean values	1st run	2nd tun	3" 140	Aver
	The state of			valu
OD 450nm	2.208	2.237	2,246	2,230
Std.Deviation	0.105	0.108	0.108	0,10
EV %	4.7	4	4.8	4.8

0.00	CV% 9.8 8.5	Std.Deviation 0.026 0.024	OD 450nm 0.269 0.277	Mean values 1st run 2nd run
1.7	SE CA	0.025	0.266	3"11111
1.7	9.3	0.025	0.271	Average

DAB,CE: lot # 0403

Mean values:	Unit 385	2nd run	3" run	Average
OD 450nm	2.246	2.221	2.182	2.216
Std.Deviation	0.097	0.103	0.118	9.106
% A3	4.3	4.0	N.	4.8

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 # conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia, Pro Diagnostic Bioprobes Sri
Via G, Carducci n° 27 – Sesto San Giovanni (M1) – Italy

Mean values	1st run	2nd run	3" run	Avorage
OD 450nm	0.286	0.273	0.280	0.280
Std.Deviation	0.027	0.023	0.026	0.025
CV %	8.3	8.5	1.6	9.0
Co/S	1.6	1.7	1.6	1.6

0318

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

S. LIMITATIONS

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

This test is suitable only for testing single samples and not pooled ones. Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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the quantitative/qualitative determination Enzyme ImmunoAssay (ELISA) for in human serum and plasma of IgM antibodies to Hepatitis C Virus

for "in vitro" diagnostic use only -



DIA.PRO

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REF CVM.CE 96 Tests

HCV IgM

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A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantiative/qualitative determination of IgM antibodies to Hepatitic C Vins in human plasma and sera. The kit is mainly intended for the follow-up of HCV chronic patients submitted to anti-vinal pharmacoutical treatment. For fin vitro diagnostic use only.

B. INTRODUCTION

chronic viral hepatitis C. Antiviral drugs, such as Interferon taken alone or in combination with Ribavirin, can be used for the treatment of persons with

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

Active production of HCV antigens in the liver of chronic patients generates spikes of IgM antibodies production and release of liver specific enzymes, similar to what happen in HBV chronic patients, The presence of anti viral IgM is usually correlated to a

phase of sufferance and cellular damage of the liver.

During the pharmaceutical freatment HCV light may represent a
marker for the follow-up of the efficiency of the drug itself,
monitoring the balance between its effectiveness and the side
efficis, that often may be heavy for the patient.

C. PRINCIPLE OF THE TEST

Microplates are coated with HCV immunodominant synthetic antigens (core peptide, recombinant NS3, NS4 and NS5

samples and anti HCV IgM are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2th riccubation bound anti-HCV IgM are descreted by the addition of anti higM antibody, labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substratel/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HCV IgM antibodies peptides), incubation, the solid phase is treated with diluted in the 1st incubation, the solid phase is treated with diluted

present in the sample.

The presence of IgM in the sample may therefore be quantitated by means of a calibration curve able to determine the content of

the antibody in arbUml. Neutralization of IgG anti-HCV, carried out directly in the well, is performed in the assay in order to block midriferences due to this class of antibodies in the determination of IgM.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

 Microplate: MICROPLATE
 strips x 8 microwells coated with HCV-specific synthetic antigens (core, NS4 and NS5 peptides and recombinant NS3). Plates are sealed into a bag with desiccant.

international one) or IGS, ranging: CAL 2 = 10 arbU/ml

6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on an Internal Gold Standard (in absence of a defined

2. Calibration Curve: CAL N° ...

CAL 5 = 100 arbU/ml CAL 4 = 50 arbU/ml CAL 6 = 250 arbU/ml

It contains chemical inactivated HCV IgM positive human It contains chemical inactivated HCV IgM positive human plasma. 100 mM Tris buffer pH 7.44-0.1, 0.2% Tween 20, 0.09% sodium azide and 0.1% kalton GCs as preservalives. The Calibration Curve is coded with blue alimentary dye.

Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

3. Wash buffer concentrate: MASHBUF 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 0.05% Kathon GC.

5. Chromogen/Substrate: SUBS_TMB 1x16ml/vial. It contains 50 mM dibtle-phosphate buffer pH 3.53.8., 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% typidogen persione (or H.20-a. Note: To be stored protected from light as sensitive to

4, Enzyme conjugate: CONJ 1xf6ml/vial. Ready to use and red colour coded, it contains 1xf6ml/vial. Ready to use and red colour coded, it contains thorseradish peroxidase conjugated polyclonal antibodies to human IgM, 5% BSA, 10 mM Tids buffer pH 6.8+40.1, 0.1% Kathon GC and 0.02% genlamicine sulphate as preservatives.

6. Sulphuric Acid: [H2SO4 0.3 M] 1x15ml/viallt contains 0.3 M H2SO4 solution. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, Attention: Irritant (H315, H319; P380, P302+P352, P332+P313, Attention: Irritant (H315, H319; P380, P302+P352, P332+P313, Attention: Irritant (H315, H319; P380, P302+P352, P332+P313, P380, P302+P352, P332+P313, P380, P302+P352, P332+P313, P380, P302+P313, P380, strong illumination

7. Specimen Diluent: <u>DILSPE</u>
2x60m/Wal, It contains 7% casein, 10 mM Na-citrate buffer pH
6.0 +/-0,1 0.2% resen 20, 0.09% Na-azide and 0.1% Karinon
GC as preservatives. To be used to dilute the sample. P305+P351+P338, P337+P313, P362+P363).

8. Neutralizing Reagent: SOLN NEU1

1x8mtlvial, It contains goal anti htgG, 2% casein, 10 mM Nactrate buffer pH 6.0 +/-0_1, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

Plate sealing foils n°2

10. Package insert n°1

- E. MATERIALS REQUIRED BUT NOT PROVIDED
 I. Calibrated Micropipetes (1000, 100 and 10ul) and disposable platic figs.
 EIA grade water (bidistilled or deionised, charcoal treated to
- remove oxidizing chemicals used as disinfectants).
 Timer with 60 minute range or higher.

- Absorbent paper tissues.

 Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance). Calibrated ELISA microwell reader with 450nm (reading)
- and with 620-630nm (blanking) filters. Calibrated ELISA microplate washer.

Vortex or similar mixing tools.

- WARNINGS AND PRECAUTIONS
 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.

 All the personnel involved in performing the assay have to
- in biosafety procedures, as recommended by the Center for Disease Control, Atlanta; U.S. and reported in the National 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 Biomedical Laboratories", ed. 1984. Institute of Health's publication: "Biosafety in Microbiological and
- All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available

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4 The laboratory environment should be controlled so as to avoid conteminants such as duelt or air-born microbial agents, when opening kit vials and micropilares and when performing the test. Protect the Chromrogen/Substates (or TMB) from strong light and avoid vibration of the bench surface where the test is

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

of the same lot should not also be interchanged.

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 6. Do not interchange components between different lots of the kits. It is recommended that components between two kits.

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

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

Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant toss of activity up to six 6 uses of the device and up to 6 months.
 Test all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Conter for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication. "Biosafety in Microbiological and Biomedical processing the content of

Laboratories", ed. 1984. Laboratories", ed. 1985. 12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

cross contamination.

3. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concarning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls/calibrators and from samples has to be treated as potentially infective material and inactivation are treatment with a 10% final concentration of household bleach for 15-18 hrs or heal inactivation are treatment with a 10% final concentration of household bleach for 15-18 hrs or heal inactivation are

14. Accidental spills from samples and operations have to be adsorted with pager tissues socked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an initiant in case of spills, wash the

surface with pienty of water.

6. Other waste materials generated from the use of the kit (example; lips used for samples and controls/calibrators, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laborationy analysis. No influence has been observed in the preparation of the sample with citrate, EDTA

Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and

have to be discarded as they could generate faise results. Samples containing residues of fibrin or heavy particles or microbial fillaments and bodies should be discarded as they could give rise to faise results. electronic reading is strongly recommended
3. Haemolysed ("red") and visibly hyperlipernic ("milky") samples

4. Sera and plasma can be stored at +2", 9"C for up to five days after collection. For longer storage periods, samples can be stored fozen at -20"C for severeal months. Any fozen samples should not be trozen/thawed more than once as this may

generate particles that could affect the test result.

5. If particles are present, centifuge at 2.000 rpm for 20 min or filter using 0.2-0.80 filters to clean up the sample for testing.

H, PREPARATION OF COMPONENTS AND WARNINGS

Allow the micropiale 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 storing.

In this case call Dia Pro's customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desoccant supplied, firmly zipped and stored at +2_3°C. When opened the first time, residual strips are stable till the indication of humidity inside the desicant bag tumer from yellow to green,

Calibration Curve

Ready to use components. Mix carefully on vortex before use.

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidsitiled 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 42.18°C.

Enzyme conjugate:

Enzyme conjugate:

Ready to use. Mix well on vortex before use.

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

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

Do not expose to strong illumination, metallic surfaces. air-driven dust or microbes Ready to use. Mix well on vortex before Be careful not to contaminate the liquid with oxidizing chemicals oxidizing agents and

4 10

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

Ready to use component. Mix carefully on vortex before use

Neutraling Reagent

Ready to use component. Mix carefully on vortex before use

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attendion: Initian. (H315, H319; P280, P302-P352, P332-P313, Attendion: Initian.) (H315, H319; P280, P302-P363).

Legenda:

H315-Warning H statements Causes skin irritation

H319 - Causes serious eye irritation

protection/face protection.

P302 + P352 - IF ON SKIN: Wash with plenty of soap and P.280 protective gloves/protective clothing/eye

- If skin irritation occurs: Get medical

off contaminated diathing

WITH THE KIT I, INSTRUMENTS AND TOOLS USED IN COMBINATION

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 regulatly 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 Micropipettes have to be calibrated to deliver the correct

out regularly.

Out regularly.

The ELISA incubator has to be set at +37°C (tolerance of +10.5°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 of or the incubations, provided that the instrument is validated and correctly optimised using the kill performances of the assay. The washer must be carefully validated and correctly optimised using the kill for routine laborationy tests. Usually 4-5 washing cycles (aspiration + dispensation of 350.0/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20.30 seconds between cycles is suggested in order to set correctly their number, it is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill is recommended to run an assay with the kill controls/califorations and check to market and positive reference samples, and check to market and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and maintenance (decommended and decommended and de instructions of the manufacturer,

4. Incubation times have a locarance of ±5%.

The ELISA microplate reader has to be equipped with a reading filter of 450m and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 20; (c) linearity to ≥ 20; repeatability ≥ 1%. Blanking is carried out on the well destified 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 reference of the section of the section of the manufacturer is referred to the section of the section of the manufacturer is referred to the section of the manufacturer.

instructions.

6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the waster and the reader, in addition, the tight which is the protocol of the station (dispensation and trained handling part of the station (dispensation and adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested 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 or mended when the number of samples to be tested

P305 + P351 + P338 – IF IN EYES; Rinse cauliously with water for several minutes, Remove contact lenses, if present and easy to do. Continue mising, 10 do. Continue mising, 11 eye irritation persists: Get medical advice/attention.

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exceed 20-30 units per run.

Dia Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the

requirements described. Support is also provided for the installation of new instruments to be used with the kit.

500 43cm

L PRE ASSAY CONTROLS AND OPERATIONS
Check the expiration date of the kit printed of on the external

label (primary container). Do not use if expired:
Check that the liquid components are not contaminated by
visible particles or aggregates.
Check that the Chromogen/Substrate is colourless or pale

blue by aspirating a small volume of it with a sterile

pipette.
Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the

က microplate, is not punctured or damaged.

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

1 hr) and then components to reach room temperature then mix gently on vortex all liquid

set the ELISA incubator at 33°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions, Set the right number of washing cycles as found in the validation of the instrument for its use with the kild.

Check that the ELISA reader is furned on or ensure it will be

turned on at least 20 minutes before reading.

9. If using an automated work station, turn on, check settings and be sure to use the right assay profitocing the control of the properties are set to the required volume.

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
below, taking care to maintain the
the samples in testing. according to what reported same incubation time for all

Two methods of analysis are possible, as described below:

M.1 QUANTITATIVE ASSAY

Place the required number of strips in the plastic holder and carefully identify the wells for calibrations and samples.
 Diute samples 1:101 dispensing 1 mt Sample Diluent into a disposable tube and then 10 ut sample; mix on vortex before use. Do not diffuld the Collinators as they are ready-buse.
 Leave the A1+81 wells empty for blanking purposes.
 Dispense 50 µt Neutralizing Reagrent in all the wells, except A1+81 wells, used for blanking operations and the wells.

used for the Calibration Curve.
In the identified positions pipette 100 µl of the Calibrations in duplicate followed by 100 µl of diluted samples. Check that calibrators and samples have been correctly added. Incubate the microplate for **go min at +37°C**, death

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

When the first incubation is finished, wash the microwells as

previously described (section 1.3) In all the wells, except A.1+B.1, Conjugate incubate the microplate e 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

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wwent the second incubation is finished, wash the microwells as previously described (section 13) 10. Pipette 100 µl Chromogen/Substrate into all the wells, A1+B1 included,

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

- 11. Incubate the microplate protected from light at countries, wells dispensed with positive samples and with positive calibrators will turn from clear to blue.

 12. Pipette 100 JL Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from blue to
- yellow.

 13. Measure the color intensity of the solution in each well, as described in section 1.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or

- M.2 QUALITATIVE ASSAY

 I. Place the required number of strips in the plastic holder and carefully deafully the wells for calibrators and samples.

 2. Dilute samples 1:101 dispensing 1 in Sample Diluent into a disposable ubus and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators as they are ready-to-use.
- Leave the A1 well empty for blanking purposes,
 Dispense 50 µl Neutralizing Reagent in all the wells, except
 A1 well used for blanking operations and the wells used for
- Then pipelle 100 µi of Calibrator 0 arbU/ml in duplicate, 100 µl of Calibrator 10 arbU/ml in duplicate and finally 100 μί of diluted samples. Check that Calibrators and samples have been correctly added
- Incubate the microplate for 60 min at +37°C.

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

- When the first incubation is finished, wash the microwells as
- previously described (section I.3)
 In all the wells, except A1, pipette 100 µl Enzyme
 Conjugate, 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.

9. When the second incubation is finished, wash the

microwells as previously described (section 13) 10. Pipette then 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.

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

 2. Pipette 100 µi Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from blue to
- 3 , Measure the color intensity of the solution in each well, as described in section L5 using a 450nm filter (reading) and a

620-630nm filter (background subtraction, recommended), blanking the instrument on A1. strongly

- General Important notes:

 1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading out just after the addition of the Stop Solution and anyway not any longer than 20 trinutes after its addition. Some self oxidation of the TMB otherwayen can occur leading to high background.

N. ASSAY SCHEME

Neutralizing Reagent Neutralizing Reagent Calibrators (no SOLN NEUT I) Samples diluted 1:101 1st incubation Temperature Mask sea	50 µl 100 µl 100 µl 60 min +37°C
Wash step	4-5 cycles
Enzyme conjugate	100 H
2 nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	In 001
3 rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ut
Reading OD	450nm

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

Microplate

	Þ	œ	C	U	m	T	0)	I
_	BLX	BLK	CAL1	CAL1	CAL2	CAL2	CAL3	CAL3
2	CAL4	CAL4	CAL5	CAL5	CALE	CAL6	S	\$2
3	S3	\$4	SS	SS	S7	SS.	SS	S10
4								
Oi								
a								
7					T			
00						П		
ø	7	i	П		Ī	٦	П	П
10								
14								
12				7		П	T	

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

Microplate

	Þ	œ	O	O	m	m	0	I,	Leg
1	BLX	CAL1	CALT	CAL2	CAL2	St	S2:	S3	Legenda BL
2	S4	SS	SS	57	88	S9	810	211	25
3									= Blank // CAL = Callbrators //
4									AL=
tri									Calle
6									noteni
7									11 2
8									S
10									= 00
5	Ţ								CS = Control Serum II
::	ď								Serur
12									17.31

S = Sample

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o, INTERNAL QUALITY CONTROL A validation check is carried out any time the kit is used in order to verify whether the performances of the assay are as qualified, Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.100 OD450nm
Calibrator	< 0.200 OD450nm after blanking
0 arbU/ml	
Calibrator	OD450nm > OD450nm CAL 0 arbU/ml -
10 arbU/ml	0.100
Calibrator 250 arbU/ml	3,500 > OD450nm > 2,000

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:

blem	Check	
11 D450nm	that the Chromogen/Substrate solution has not become contaminated during the assay	C 0
7	1. that the washing procedure and the washer	of sa
_	settings are as validated in the pre qualification	∏
D450nm	sludy;	200
ring	2, that the proper washing solution has been	
	used and the washer has been primed with it	
	before use;	2
	3, that no mistake has been done in the assay	treal
	procedure (dispensation of positive calibrators	reco
	instead of Cat 0 arbU/ml);	
	4. that no contamination of the Cal 0 arbU/ml,	
	or of the wells where this was dispensed, has	00
	occurred due to positive samples, to spills or to	1
	the enzyme conjugate.	1001
	5 that micropipettes have not become	VIII
	contominated-with-positive-samples-or with-the-	Olici
	enzyme conjugate	
	6, that the washer needles are not blocked or	
	partially obstructed	
4	1. that the procedure has been correctly	
=		
	2, that no mistake has occurred during its	
100	distribution:	

Problem	Check
> 0,100 OD450nm	 that the ChromogervSubstrate solution has not become contaminated during the assay
Calibrator	1. that the washing procedure and the washer
0 arbU/ml	settings are as validated in the pre qualification
> 0,200 OD450nm	sludy;
after blanking	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 calibrators
	instead of Cat 0 arbU/ml);
	4. that no contamination of the Cal 0 arbU/ml,
	or of the wells where this was dispensed, has
	occurred due to positive samples, to spills or to
	the enzyme conjugate;
	5 that micropipettes have not become
	ve-samples-c
	enzyme conjugate
	6, that the washer needles are not blocked or
	partially obstructed
Calibrator	 that the procedure has been correctly.
10 arbU/mi	
	2, that no mistake has occurred during its
< CAL 0 + 0,100	
	ihat the washing procedure and the washer
	settings are as validated in the pre qualification
	study;
	4 that no external contamination of the

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

P. RESULTS

If he lest turns cut to be valid, interpretation of results is carried
out in the quantitative assay from the mean OD450 m value of
the Calibration Curve elaborated with an appropriate curve
fitting system (suggested: 4 parameters).

In the qualitative assay interpretation of results is done on the mean OD450nm value of the Calibrator 10 arbU/ml (or CAL 2) by means of the following formulation:

Mean OD450nm CAL 2 = cut-off (Co)

correct interpretation of results. 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 generate the

Q INTERPRETATION OF RESULTS

QUANTITATIVE ASSAY

amples on the fitted calibration curve,
concentration of IgM is from Literature correlated
portionally with the liver damage produced by antibodies to centrations in arbU/ml are obtained elaborating OD450nm

very and therapeulic efficacy / upon virus replication in hepatocites, decrease in IgM concentration upon pharmacological innert is usually clinically acknowledged as a sign of

QUALITATIVE ASSAY

st results are interpreted as a ratio of the sample OD450nm ue (S) and the cut-off value (Co), or S/Co, according to the puring table:

Positive	> 1.0
Negative	< 1.0
Interpretatio	S/Co

A negative result indicates that the patient has not developed IgM antibodies to HCV. A positive result is indicative of an ongoing HCV active infection

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.

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

 The results of this ELISA assay should be anyway implemented with other diagnostic and clinical tests:

An example of calculation is reported below:

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

Lower than 0,200 - Accepted Mean Value: 0.060 - 0.080 OD450nm 0.070 OD450nm

Cut-Off or $Co = 0.210$	Higher than CAL	Mean Value:	CAL 2:
0.210	Higher than CAL1+0,100 = accepted	0.210 OD450nm	0.200 - 0.220 OD450nm

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Sample 1: Sample 2: Sample 1 Sample 2

0.080 OD450nm 1.800 OD450nm S/Co < 1.0 = negative S/Co > 1.0 = positive

R. PERFORMANCE CHARACTERISTICS Evaluation of Performances has been conducted on selected panels carried out in a clinical external center and internally.

No international standard for HCV IgM Antibody detection has been defined so far by the European Community. In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of chronic HCV infection, has been defined in order to provide the device with a constant and Limit of detection

excellent sensitivity.

2. Diagnostic Sensitivity and Specificity.
The diagnostic performances were evaluated in a study conducted in an external clinical center, with excellent experience in the diagnosis of infectious diseases and HCV.
The Diagnostic Sensitivity was studied on about 200 samples, pre-lested positive with an analytical system developed in house by the clinical laborationy where the study was conducted. Positive samples were collected from patients with a clinical history of HCV infection (eaute and chornic), in addition some Servoconversion Panels, purchased from Boston Biomedical Inc., USA, were examined.
The diagnostic specificity was determined on panels of more than 300 negative samples from normal individuals and blood donors, classified negative for ant HCV antibodies with the reference kit in use in the laboratory, including potentially interfering specimens.

A panel of potentially interfering samples (RF*, herrolised, lipemic, etc.) was also examined. No interference was observed on the samples examined.

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 freazing indefferes with the performance of the lest. No interference was observed on clean and particle free

The Performance Evaluation provided the following values:



Reproducibility:
 It has been calculated on two samples examined in replicates in different runs. Results are reported below summarized in a table:

6.9	13	CV %
0.113	0.027	Std.Deviation
1.632	0.241	OD450nm
Calibrator5 100 arbU/ml	Calibrator 2 10 arbU/ml	Average values N = 48

S. LIMITATIONS
False positivity has been assessed on less than 2% of the normal population, mostly due to high titens of RF.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Managament 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 (Ml) – Italy

0318

TCV A

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

- for "in vitro" diagnostic use only -



DIA.PRO

Diagnostic Bioprobes Srl 20099 Sesto San Giovanni Via G. Carducci nº 27

disease and the response to treatment,

(Milano) - Italy Fax +39 02 26007726 e-mail: into(@diapro.it Phone +39 02 27007161

96.192.480,960 Tests REF-CVAB.CE--

HCV Ab

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A. INTENDED USE Version 4.0 Enzyme ImmunoAssay (ELISA) for the Version 4.0 Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis C Virus in human plasma and sera. The kil is intended for the screening of blood units and the follow-up of HCV-infected patients.

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

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-"Hepatitis C is a viral infection of the liver which had been transfusion hepatitis and its tendency to induce persistent

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 noscomial exposures (e.g., blood transfusions, unsafe injection practices) and high-risk vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chunically 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 HCV is a major cause of acute hepatitis and chronic liver unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No hehaviours (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 bepatitis. It is an enveloped RNA virus in the relative mutability of its genome, which in turn is probably related to the high propensity (80%) of inducing chronic infection. HOV is clustered into several distinct grouplyses which may be important in determining the severity of the 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 fluviviridue family which appears to have a narrow host

clinical symptoms ranges from 15 to 150 days. In acute nifections, the most common symptoms are faigue and jundice: however, the majority of cases thetween 50% and 70%), even those that develop chonic infection, are a symptomatic About 50% of newly infected patients progress to develop chronic infection. Cirrbosis develops in about 10% 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 hapatitis B wins infection have evidence of HCV infection. The mechanisms by which HCV infection leads to liver concer are still unclear. Hepatitis C also exocerbates the severity of underlying liver disease when it coexists with other bepatic conditions. In particular, to 20% of persons with chronic infection, and liver cancer The incubation period of HCV infection before the onset of

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

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 inflection are current and former injecting drug users and those with a history of transfusion of unscrewed blood or major means of transmission are unsterilized injection equipment and unscreened blood transfusions, In addition, blood products, practices are at risk if they use or re-use unsterilized tools. people who use traditional scarification and circumcision blood products. In many developing countries, where unscreened blood and blood products are still being used, the

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

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) through screening of donor blond 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 for confirmation of serological results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the pressure of active infection and a potential for spread of the infection and or/the development of chronic 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 Diagnostic tests for HCV are used to prevent infection reaction or PCR-branched DNA-assay)-is also being utilized liver disease.

Antiviral drugs such as interferon taken alone or in combination with ribovirin, con be used for the treatment of persons with chomic 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 about 10% to 20%, of patients. Ribavirin does not appear to be effective when used alone.

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

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Some studies, however, have shown the presence of virus neutralizing antiholics in patients, with HCV infection. In the absence of a vaccine, all precautions to powent infection must be taken including (a) screening and testing of blood and organ decroses (b) Virus inservation 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 counseiling for persons with high-risk drug and sexual practices.

and two envelope glycoprateins, involved in the virus replication encodes for structural components, a nucleocapsid replication and functional and protein

The nucleocapsid-encoding region seems to be the conservative among the isolates obtained all over the world. nucleocapsid-encoding

C. PRINCIPLE OF

Micropiates are coaled with HCV-specific antigens derived from core and "ns" regions encoding for conservative and immunodominant enlighent determinants (Core peptide, recombinant MS3, 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 vasishing out all the other components of the sample, in the 2" incubation bound HCV antibodies, 19G and 19M as well, are At antibodies, tabelled with percudiate (HRP).

The enzyme captured on the solid phase, acting on the substrate/bromogen mixture, generators on optical signal final proportional to the amount of anti HCV antibodies present in the sample. A cut-off value let optical densities be interpreted into an encoder and the sample of

D. COMPONENTS Code CVAB,CE co

contains reagents for 192 lests

MICROPLATE

n° 2 microplates
12 strips of 8 microwells coated with
NS3, NS4 and NS5 peptides. Plates Core peptide, recombinant are sealed into a bag with

2. Negative Control CONTROL.

134.0ml/wal. Ready to use control. It contains 1% goat serum prateins, 10 ml Na-citrate buffer pH 5.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azitle and 0.1% Kathon CC as preservatives. The negative control is olive green colour coded.

3. Positive Control CONTROL + 1x4.0mt/Not. 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% these 120, 0.05% Na-acide and 0.1% Kathon GC as preservatives. The Positive Control is blue colour

4. Calibrator CAL

7. Velss. Lyophilized calibrator. To be dissolved with the volume of EA, grade water reported on the label, it contains feetal bovine serum proteins, human antibodies to HCV whose content is calibrated on the NIBSC Working Standard code 99/SBR-003-WI, 10 mM Na-citrate buffer pH 6.0 +/-0,1, 0.3

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 <u>WASHBUF 20X</u> 2x50m/bortle. 20x concentrated solution. On wash solution contains 10 mM phosphate buffer 0.05% Tween 20 and 0.05% Kathon GC. Once diluted, the pH 7_0+/-0,2

Zx16mi/viai. Ready to use and pink/red colour coded reagent. It contains Horisaradish Peroxidase conjugated goal polyclonal antibodes to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6,84/-0,1, 0,1% Kathon GC and 0,02% 6. Enzyme Conjugate 2x16ml/vial, Re gentamicine sulphate as

7. Chromogen/Substrate SUBS_TMB 2x16m/Wal. Ready-to-use component, It contains 50 mM citrate-phosphate buffer pt 35-38, 4% dimethy/sulphoxide, 0.03% letta-methyl-benzidine or TMB and 0.02% hydrogen

peroxide or H2O2.

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

8. Assay Diluent DILAS

1x15m/Val. 10 mM. Inis buffered solution pH 8,0 +/-0.1 containing 0.1% & suthon GC for the pre-treatment of samples and controls in the plate, blocking interference.

Sulphuric Acid H₂SO₄

Attention: Irritant (H315; H319; P280; P302+P352; P332+P313 P305+P351+P338; P337+P313; P362+P363) 1x32mi/bottle 0.3 M ins 0.3 M H₂SO₄ solution

10. Sample Diluent:

2x50mi/bottle. It contains 1% gual serum proteins, 10 mi/ Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kalhon GC as preservatives. To be used to diute the

Sample.

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

11. Plate sealing foils

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

Code	Number of tests	12 Pack insert	11,Plate seal foils	10,SampleDituent	9 Sulphuric Acid	8 Assay Diluent	/ Chromog/Subs	6 Enz. Conjugate	5 Wash buff conc	4 Calibrator	3_PositiveControl	2 NegativeControl	1 Microplate	
CVAS CE 95	96	n, 1	n° 2	1x50ml/vial	1x15mi/vial	1x8ml/vial	1x16ml/vial	1x16ml/viai	1x60ml/bottle	nº 1 vial	1x2_0ml/vial	1x2 0ml/vial	n°1	
TVAR CE 480	480	٦	n° 10	5x50ml/bottles	2x40ml/bottle	1x40ml/bottle	2x40ml/battles	2x40ml/battles	5x60ml/bottles	n° 5 vials	1x10ml/vial	1x10ml/vial	ວ ທ້	
CVAR PE GEO	960	ח" ו	n° 20	4x125ml/bottles	2x80ml/bottles	1x80ml/bottle	4x40ml/bottles	4x40ml/bottles	4x150ml/bottles	n° 10 vials	1x20_mt/vial	1x20_ml/vial	n°10	

MATERIALS Calibrated N

plastic tips.

EIA grade water (bidistilled or deionised, charcoat treated to

Micropipettes REQUIRED

8UT :

ul and

PROVIDED 10ul) and disposable

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remove oxidizing chemicals used as disinfectants)
Timer with 60 minute range or higher.

Absorbent paper tissues:
Calibrated ELISA microplate thermostatic incubator

to provide a temperature of +37°C, calibrated ELISA microwell reader with and with 620-630nm (blanking) filters. Calibrated ELISA microplate washer, Vortex or similar mixing tools, 450nm (reading) capable.

F. WARNINGS AND PRECAUTIONS

 The kit has to be used by skilled and pre-lechnical personnel only, under the supervision. used by skilled and properly trained , under the supervision of a medical

doctor responsible of the laboratory.

When the kil is used for the screening of blood units and blood components, it has to be used in alaboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.

All the personnel involved in performing the assay have to wear protective laboratory dothes, telic-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 bicsafety procedures, as recommended by the Center for Disease Control, Allanta, 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 contembrants such as dust or air-born microbial agents, when opening it values and micropiates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid whorloon of the bench surface where the test is

undertaken.
6. Upon receipt, store the kit at 2_8°C into a temperature

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.

2. Check that the reagents are clear and do not contain visible heavy pericles of aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit

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

Do not use the kit after the Avoid cross-contamination between kit reagents by using sosable tips and changing them between the use of each

12. Treat all specimens as potentially infective. All human serum specimens should be handled at Blosafety 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 liner and internal (vials) fabels expiration date stated on the

Laboratories", ed. 1984.

13. The use of disposable plastic-ware is recommended in the figure components or in transferring components into automated workstations, in order to avoid 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 12 fr C or 20 min., 15. Accidental spills from samples and operations have to be assorbed with paper lissures scaked with household bleach and then with water, Tissures should from be discarded in proper containers designated for laboratory/hostpila waste, 16. The Sulphuric Acid is an initiant. In case of spills, wash the 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 surface with plenty of water

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1.Blood is drawn aseptically by ventipuncture 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 order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and 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 electronic reading is strongly recommended

4. Heemolysed (red) and visibly hyperipperiic ("milky") samples have to be discarded as they could generate talso results.

Samples containing residues of fichin or heavy particles or microbial illaments and bodies should be discarded as they could give rise to false results.

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

5. Il particles are present, centrifuge at 2.000 pm for 20 min or filler using 0.2-0.8v filters to dean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A sludy 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

 Microplates:
 Allow the microplate to reach room temperature (about before opening the container. Check that the desiccant iturned to dark green, indicating a delect of manufacturing, in this case call Dia Pro's oustomer service. Check that the desiccant is not

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 esn

3. Positive Control
Ready to use Mix
component as poten Ready to use Mix well on vorlex before use Handle component as potentially infective, even if HCV, eventupresent in the control, has been chemically inactivated. Handle this

4. Calibrator

volume of EIA grade water reported on its label.

Mix well on vortex before use. With ine

present in t as control, has been chemically

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

The 20x concentrated solution has to be difuted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some sait 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 beat washing efficiency.

 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

air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and Ready to use. Mix well on vortex before use. Be careful not to contaminate the liquid with oxidizing chemicals

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

Assay Diluent

Ready to use, Mix well on vartex before use

P305+P351+P338; P337+P313; P362+P363) Ready to use, Mix well on vortex before use.

Attention: Irrilant (H315; H319; P280; P302+P352; P332+P313;

gloves/protective dothing/eye protection/face

P332+P313 – If skin irritation occurs: Get medical advice/eltention.
P345 + P351 + P336 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact tenses, if present and easy to do. P302 + P352 - IF ON SKIN: Wash with plenty of soap and water

P337 + P313 - If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated dollring and wash it before reuse.

 Sample Diluent:
 Ready to use. Mix well on vortex before use

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

- Micropipiettes 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 hose 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.

 The ELISA incubator has to be set at +37°C (tolerance of +1-0.5°C) and regularly checked to ensure the correct emperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that he instrument is validated for the incubation of ELISA tests. The ELISA waster is extinently important to the overall particurances of the assay. The waster-must be carefully

validated and correctly optimized using the kit controls and reference panels, before using the kit for routine abcoratory tests. Usually 4-54 washing sopties (aspiration + dispensation of 350 ulwell of washing solution = 1 cycle) are sufficient to ensure that the essay performs as expected. A soaking sine of 26-30 securids between cycles is suggested, in order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "visitation of 1 fest" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out

according to the instructions of the manufacturer.

Incubation times thave a bletance of ±5%.

The ELSA, microplate reader has to be equipped with a reading filter of 450mm and with a second filter (250-630mm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm. (b) assorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 1%. Banking 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 required to expense the section of the results of the control of the section of the results of the control of the section of the section of the results of the section of the regularly maintained according to the manufacturer s be

instructions.

6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handing) 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 poerating 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 and controlled to minimize the passibility of contamination of sections. is recommended for blood screening when the number of adjacent wells. The use of ELISA automated work stations samples to be tested exceed 20-30 units per run.

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 containines 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 over, return the secondary labeled containers to 2.8°C, certain contains.

œ Dia Pros' customer service offers support to the user in the setting and checking of instruments used in combination with, the still, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kft.

- Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
 Check that the liquid components are not contaminated by native-laye 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.

 Dilute all the content of the 20x concentrated Wash Solution
- 4 0
- Dissolve the Calibrator as described above.

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

M_ASSAY PROCEDURE

The assay has to be carried out below, taking care to maintain the the samples in testing.

11, Pipette 100, Il Sulphurio Adol 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 yellowitowin.

12. Measure the colour intensity of the solution in each well, as

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

Incubate the microplate for 45 min at +37°C.

Wesh microsells as in step 6.

Pipette 100µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature [183-4°C) for 15 minutes.

200 ut 1 Wells controls/calibrator :5 ite appropriate

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 plive green

below for the Manual Assay

occur leading to high background.
Shaking at 350 ±500 pm during incubation has been powed to increase the sensitivity of the assay of about 20%.
The Calibrator (CAL) does not affect the cut-off calculation and therefore the least results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.

delaying the first washing operation accordingly

- Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of well empty
- Dispense 200 ul of Negative Calibrator in duplicate and 200 in proper wells. Do not dilute Controls and Calibrator 200 ul Positive Control in single ilute Controls and Calibrator as Control in triplicate, 200

'n

they are pre-diluted, ready to use i.

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.

ω

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.

- Dispense 50 ut Assay Diluent (DILAS) into all the control/calibrator and sample wells. Check that the color of samples has timed to dark blue. Incubate the microplate for 45 min at +37°C.

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

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

Check that the ELISA reader has been turned on at least 20°C check that the ELISA reader has been turned on at least 20°C. Wash the microplate with an automatic washer by delivering and aspirating 350 Julywell of dictor washing solution as reported previously (section 1.3). Fuperiar (160) forzyme Confugate into each well except the "blanking well, and cover with the sealer, Check that this Doc.:

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wells, except A1.

pink/red coloured component has been dispensed in all

- If using an automated and be sure to use the right assay protocol workstalion, turn it on, check settings
- Check that the micropipettes are set to the required volume Check that all the other equipment is available and read

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate,

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

according to what reported same incubation time for all

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

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

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

positive results on reading, positive results on reading has to be carried out just after the addition of the Reading has to be carried out just after than 20 minutes Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can

If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm, Finger prints could generale false

blanking the instrument on A1,

described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended)

For the next operations follow the operative instructions reported

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

N. ASSAY SCHEME

Assay Diluent (DILAS) Controls & Calibrator 200ul dă.+10ul 50 ul 45 min +37°C 4-5 cycles 100 ul 45 min +37°C Operations 200 ul 4-5 cycles 100 ul 15 min

An example of dispensation scheme is reported below

	A	u	o	0	ш	F	G	I
1	BLK	NC	NO	NC	CAL	CAL	PC	S
2						57		
u								
44								
cn								
0								
7								
CO.								
60								
ö								
1								
12								

BLK = Blank NC = Negative Control S = Semple CAL = Calibrator PC = Positive Control S = Semple

Legenda

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 lable below. O, INTERNAL QUALITY CONTROL

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

study. 4. that no external contamination calibration has occurred. 1. that the sprocedure has been exculted. 2. that no extended has been done distribution of controls (dispersation of control instead of positive control, in it the negative control will have an O value 3 has the washing procedure and the	ti no external contamination (for reas occurred.) (for the occurred.) (et al. the procedure has been done to one the controls (dispensation of the reason of the control in the control
2. that no mistake has been doub distribution (ex. disponsation of control firstead of control serum) 3. that the washing procedure and the settings are as validated in the pre-quality. 4. that no external contamination (all present the procedure has been executed) 1. that the procedure has been done executed: 2. that no mistake has been done distribution of control instead of positive control. In the regative control with they are 0. 150, ico. 3. that the washing procedure and the	2 that no mistake has been don distribution (e.g. dispensation of control featural) 3, that the weating procedure and the settings are as validated in the pre-quastropy. 4, that no external contamination of the procedure has been danged that the procedure has been danged that no mistake has been danged stribution of control instead of positive control in the negative control will have an Occurred as the procedure and the negative control will have an Occurred as the procedure and the negative control will have an Occurred as the procedure and the negative control will have an Occurred that the washing procedure and in the negative control set of the pre-quastropy are as evaluated to the pre-quastropy are as evaluated to the pre-quastropy are as evaluated to the pre-quastropy are as evaluated to the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to the pre-quastropy and the pre-quastropy are as evaluated to
oxionized. Intel no missiste has been don distribution (ex.: disponsation of control ferral of control ferral of control ferral of control ferral of control ferral of the procedure and the settings are as railfasted in the pre-quite auditor has control for the secoured. Intel the procedure has been one control instead of positive control in the procedure has been of the procedure of the proce	a visible of missive has been don distribution (ex.: dispensation of control instead of control serum). That the westing procedure and the softings are as validated in the pre-question of the pre-question of the pre-question of the procedure has been done executed. 1. that he procedure has been done executed. 2. that no missive has been done of the pre-question of control instead of positive control, in the regarding series are suitable on the pre-question of the serious and the washing procedure and the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious and the serious are suitable of the pre-question of the serious are suitable of the pre-question of the serious are suitable of the pre-question of the serious are suitable of the
control freezed of control serum) 3. that the weaking procedure and the settings are as validated in the pre quasury. 4. that no external contamination contamination has been executed: 1. that the procedure has been executed: 2. that no mistake has been done distribution of controls (dispensation of control integral of positive control, in it the negative control with have an O value 5 0,150,1co.	control freetaal of control serum) 3. that the washing procedure and the settings are as validated in the pre-que study: 4. that no external contamination challenging are as validated in the procedure has been done executed. 2. that no mistake has been done distribution of controls (dispersation of control instead of positive control. In the negative control will have an Other than the washing procedure and in the settings are as readistated in the pre-que study. 4. that no external contamination of the
1. that the procedure has been executed: 2. that no mistake has been done distribution of control (dispersation of control instead of positive control in it in the regalitive control with have an O value > 0.150, loo. 3. that the washing procedure and the	1 that the procedure has been executed: 2 that no missive has been dane distribution of controls (dispersation of control instead of positive control. In the negative control will have an Otal value > 0,180,100 procedure and in elements are as readistated in the pire questions. 3 that the washing procedure and study. 4 that no external contamination of the
distribution of controls (dispensation of regional of surfusion of controls (dispensation of regional of regional dispensation of regional matter of 150 (co.) 3. that the washing procedure and the we	2, that no nistate has been done in distribution of controls (discrepation of neg control instead of positive control in this the negative control will have an OD44 value? 0,150, top conclure and the wastings are are satisfacted in the pire qualification? 4 that no external contamination of the both
entillerin etc un heishilev es ere ennites	sludy: 4 that no external contamination of the posi-

should these problems happen, after checking, report any

. CALCULATION OF THE CUT-OFF

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

NC + 0.350 = Cut-Off (Co)

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

Important nate: 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:

>1,1	0.9 - 1.1	< 0.9	S/Co
Positive	Equivocal	Negative	Interpretation

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

Important notes

- 2 Integratation of results should be done under the supervision of the responsible of the laboratory to reduce the nisk of judgment errors and misinterpretations. Any positive result should be confirmed by an alternative method capable to detect JGS and JGM antibodies (confirmation test) before a diagnosis of viral hepatitis is
- 3. As proved in the Performance Evaluation of the product, the assay is able to detect seroconversion to anti HCV core entitlodies seatler than some other commercial kits. Therefore a positive result, not confirmed with these commercial kits, obes 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 srt. code CCONF)
- 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: Jesking anti-light conjugate in the formulation of the enzyme tracer and therefore missing IgM resolutily: nay be present. The result in the formulation of the shape tracer and therefore missing IgM resolutily: nay be present. The stap of the properties of the PCV should be then positivity of the sample for antibodies to HCV should be then confirmed by examining also igM reactivity, important for the
- Ç'n diagnosis of HCV infection.

 When test results are transmitted from informatics centre, attention has to re transmitted from the laboratory to an attention has to be done to avoid
- ø erroneous data transfer.

 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

Negative Control: 0.019 – 0 Mean Value 0.020 OD Lower than 0.050 – Accepted The following data must not be used instead or real figures 2 189 OD450nm 0,020 OD450nm 0,019 - 0,020 - 0,021 OD450nm

Sample 2: 1,690 OD450nm Sample 1 S/Co < 0,9 = negative Sample 2 S/Co > 1,1 = positive Sample 1: S/Co higher than 1.1 - Accepted Calibrator Cut-Off = 0.020+0.350 = 0.370Higher than 1,000 - Accepted 0.550 - 0.530 OD450nm 0.540 OD450nm 0,070 OD450nm S/Co = 1.4

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 delection of the assay has been calculated by means of the British Waxing Standard for anti-HCV, MBSC code 99/588-003-WI. The table below reports the mean Ob450nm values of this standard when diluted in negative plasma and then examined.

Negative plasma	8 X	4 X	×	1×	Factor	Dilution
0.3	0.5	0.7	1.1	2.0	S/Co	Lot#1
03	0.5	0.8	1.2	2.0	S/Co	Lot # 2

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

SOC	0602	1201	CVAB.CE Lot ID
OUUE	3000	3000	Accurun 1 Series
6	15	1.5	S/Cc

In addition, n°.7 samples, tested positive_for HCV Ab with Ortho HCV 3.0 SAVe, code 930820, lot, # EXE085-1, 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	Limit	CVAB.CE S/Co	S/Co
-	256 X	1.9	1.3
2	256 X	1.9	0.7
ω	256 X	2.4	1.0
4	128 X	2.5	3.2
S	85 ×	F.E	1.4
.s	128 X	2.2	0.8
-7	135 X	3.2	2.2

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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 4 where a total of 5043 unselected blood donors of 152 potentially interfering specimens (other infectious diseases, Ecoil antibody interfering specimens (other infectious diseases). examined, the diagnostic specificity was recently assessed by testing a total of 2876 negative blood donors on six different tots, A value of specificity of 100% was found. patients, pregnant women, hemolized, lipemic, etc.) were positive, patients affected by non viral hepatic diseases, dialysis

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 sear have been used to determine the value of specificity, prozen specimens have been tested, as well, to check for No interference was observed. interferences due to collection and storage.

2.2 Diagnostic Sensitivity

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, themally more than other 50 positive samples were was found, themally more than other 50 positive samples were sted, providing a value of diagnostic sensitivity of again 100%. Positive samples from infections carried out by different genotypes of HCV were tested as well. It defined as the probability of the assay of scoring positive in

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,

HCV 10165	HCV 6212	HCV 10039	PHV 920	PHV 919	PHV 918	PHV 917	PHV 916	PHV 915	PHV 914	PHV 913	PHV 912	PHV 911	PHV 910	PHV 909	PHV 908	PHV 907	PHV 906	PHV 905	\$106 AHd	PHV 901	Panel
o	9	5	10	7	œ	10	co	4	9	4	3	c/s	5	3	13	7	7	9	7	-1	Nº samples
C)	an	2	ø,	u	N	on	4	ယ	cn	2	L	ω	ta)	2	10	w	7	ω	2	00	DiaPro*
4	7	0	a)	3	0	6	w	0	55	2		u	3	2	00	2	7	4	4	Ф	Ortho"

Note: "Positive samples detected -- HCV v 3.0

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

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

EFS Panel Ac HCV

e	S/Co	SICo	SICo	-
HCV 1	2.2	74 74	Ç0	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	S, S	positive
HCV 5	1.6	100	1.6	positive
HCV 6	0.4	0.4	0.4	negative

PRECISION:
 PRECISION:
 If 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)

0.000 0.007	OD 450nm 0,396 0,403 0,418 1	trun 2ndrun 3"run A	CV% 8.7 5.6 7.9 Cal #2 – 7K (N = 16)	0.008		s istrum 2ndrum 3 num
0.026	0.406	Average	7.7	0.007	0.096	Average value

Lot # 0602

S/Co

1.1 1.1 1.2

-

Negative Sample	e (N = 18)			
Mean values	istrun	Znd run	39 000	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	fst run	2nd run	3" run	Average
OD 450nm	0.400	0.395	0.393	0,396
Std.Deviation	0.021	0.025	0.026	0.024

Lot # 0602/2

S/Co

5.4 6.2 1.2 1.2

10.

Mean values	1st run	2nd run	3" run	Average
OD 450nm	0.067	0.091	0.066	0.089
Std.Deviation	0.009	0.007	0.008	0.008
CV%	10.0	3	0	
THE PARTY OF THE P	1	8.2	8.0	or to
中 サイーンフレー	16)	8.2	8.0	or to
Mean values	16) 1strun	8.2 2nd run	3 rd run	Average
Dia 1	16) 1strun 0.386	2nd run 0.390	3 st run 0.391	Average 0.389
Mean values OD 450nm Std.Deviation	16) 1strun 0.386 0.023	2nd run 0.390 0.021	3" run 0.391 0.023	Average 0.389 0.022
Mean values OD 450nm Std.Deviation CV %	16) 1strun 0.386 0.023	2nd run 0.390 0.021 5.3	3 rd run 0.391 0.023 5.8	Average 0.389 0.022

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

Repeatable false positive results, not confirmed by RTBA 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.

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Dia.Pro. Diagnostic Bioprobes Srt. Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy

HAV AK

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera

for "in vitro" diagnostic use only -



DIA.PRO

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(Milano) - Italy

HAV Ab

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A. INTENDED USE

Competitive Enzyme immunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera. The kit is used for the follow-up of patients infected by HAV. For "in vitro" diagnostic use only.

B. INTRODUCTION

Hepatitis A Virus as follows Disease Control or CDC, Atlanta, USA, defines

reported vaccine preventable diseases in the world, despite the licensure of hepatitis A vaccine in 1995. Widespread vaccination of appropriate susceptible populations would substantially lower disease incidence and potentially climinate indigenous transmission of hepatitis A virus (HAV) Hepatitis A continues to be one of the most frequently

15-50 days). The illness caused by HAV infection typically has an abrupt onest of symptoms that can include fever, malaiss, sacrexia, nausea, abdominal dissonatort, days urine, and jaundies. The likelihood of having symptoms with HAV infection is related to the person's age. In children less that payears of age, most (70%) infections are asymptomatic: if years of age, most (70%) infections are asymptomatic: if joundice or elevation of liver enzymes, when the concentration of virus in stool is highest. The concentration of virus in stool electhrees after joundice appears: Children and infants can shed HAV for longer periods than adults, up to several months after the onset of climbal lifness. Chronic shedding of HAV in faces does not occur however, shedding transport of the contract of produce either asymptomatic or symptomatic infection in humans after an average incubation period of 28 days (range HAV. a 27-nm RNA agent classified as a picornavirus, can liver enzyme elevation patients. Signs and symptoms usually last less than 2 months, although 10%-15% of symptomatic persons have prolonged or relapsing disease lasting up to 6 months. In interted persons, HAV replicates in the liver, is excreted in hin letted signs, and is shed in the stool. Peak infectivity of infected bill, and is shed in the stool. Peak infectivity of infected symptometic, with jaundice occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 Illness does occur it is not usually accompanied by jaundice.

Among older children and adults, infection is usually persons occurs during the 2-week period before onset of occurs soon after infection and persists through the period of

which appears early in the course of infection, remains detectable for the person's lifetime and confers lifeting protection seating the disease. Commercial diagnosis test are available for the detection of IgM and total (IgM and Igf) hepatitis on the basis of clinical or epidemiologic features alone. Sembogic testing to detect immunoglobilin M (igM) anti-HAV serabid proteins of HAV (igM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most persons, IgM anti-HAV becomes detectable 5-10 days before the onese of symptoms and ean persist for up to 6 months after infection, Immunoglobilin G (igG) anti-HAV. anti-HAV in serum Hepatitis A cannot be differentiated from other types of viral

persons during the acute phase of infection by using nucleic acid amplification methods, and nucleic acid sequencing bas been used to determine the relatedness of HAV isolates. HAV RNA can be detected in the blood and stool of most

HAV infection is acquired primarily by the fecal-oral route by either person copresson confact or ingestion of contaminated food or water. On rare occasions, HAV infection has been transmitted by transfusion of blood or blood products collected from denores during the viremic phase of their infection. In experimentally infected nonhuman primates, HAV has been desected in saliva during the incubation period however, transmission by saliva has not been demonstrated

environment for months, fleating foods at temperatures greater than 185 F (85 C) for 1 minute or disinfecting surfaces with a 1:100 dilution of sodium hypothelatic fundamental bleach) in tap water is necessary to inactivate HAV. Depending on conditions, HAV can be stable in the

Because most children have asymptomatic or unrecognized infections, they play an important role in HAV transmission and serve as a source of infection for others. In one study adults without an identified source of infection, 52% of their bouseholds included a child less than 6 years old, and the presence of a young child was associated with HAV presence of a young child was associated with transmission within the household. In studies an identified source of infection was performed, 25%-40% the contacts less than 6 years old had serologic evidence acute HAV infection (Ig/M anti-HAV). serologic testing of the household contacts of adults without

Hepatitis A typical sequence of events following infection Journalies ME lgG

Weeks after exposure

10 11 12

C. PRINCIPLE OF THE TEST The assay is based on the principle of competition where the antibodies in the sample compete with an anti-HAV specific antibody, labeled with HRP, for a fixed amount of antigen on the

A purified and inactivated HAV is coated to the microwells. The patient's serum/plasma is added to the microwell and antibodies to HAV are captured by the solid phase.

The plate is washed to remove unbound conjugate and then the After washing, the enzyme conjugate is added and binds to the free HAV antigen, if still present.

chromogen/substrate is added.

In the presence of peroxidase the coloriess substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is inversely proportional to the amount of antibodies to HAV present in the sample. An additive is added to the sample directly into the well to block interferences able to mask the presence of antibodies, mostly appearing in the follow up of vaccination.

REF AVAB.CE 96 Tests

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Each kit contains sufficient reagents to perform 96 tests

8x12 microwell strips coated with purified and inactivated HAV, sealed into a bag with desiceant, Allow the microplate to reach room temperature before opening. Reseal unused strips in the bag with desiceant and store at 2.8°C°.

2. Negative Controt: CONTROL 1 174, Dmitval. Ready to use, Contains bovine serum proteins, 10 mM phosphate buffer ph 7.44-0.1, 0.02% gentamicine submate and 0.1% Kathon GC as preservatives. The negative control is

1'44 (Innt/ust, Ready to Use. Contains bovine serum proteins, anti HAV antibodies at a concentration higher than 100 WHO mU/mt, 10 mM phosphate buffer pH 7,4+/-0,1, 0,02% gentamicine sulphate and 0,1% Kathon GC as preservatives, The positive control is colour coded green,

4. Calibrator: CAL.....
nº 1 vial. Lyophilized. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, anti HAV antibodies at a concentration of about 10 WHO mlU/ml, 10 mM phosphate buffer pH 7.44/4-1, 0.02% gentamicine suphate and

0.1% Kathon GC as preservatives.

Note: The volume necessary to dissolve the content of the valume vial may vary from lot to lot. Please use the right volume

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

1x60ml/bottle, 20x concentrated solution.

n. to be diluted

ģ

1200ml with distilled water before use.

peroxidase conjugated antibody, specific to HAV, in presence of 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives, 6. Enzyme conjugate: CONJ The reagent is colored with a red dye. solution, Contains Horseradish

7. Chromogen/Substrate: SUBS TMB vs. fight/will. Contains a 50 mM citrate-phosphale solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine and 0.02% hydrogen peroxide of HoDs. Note: To be stored protected from light as sens sensitive buffered e or TMB

 Specimen Diluent: DILSPE
 1x8ml. Buffered solution suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide and 0.1% Kathon k green.

9. Sulphuric Acid: H2SO4 GC as preservatives. The reagent is color coded dark M H₂SO₄ solution.

1x15mil/vial. Contains 0.3 M HzSO₄ solution. Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P352+P363)

10. Plate sealing foils

Package insert

Calibration Curve: CAL N°... 5x2.0 mi/Vial. Ready to use and col ranging: 0-5-10-50-100 WHO milUml. and colour coded standard curve

> (CAL1=0mIU/ml, CAL4=50mIU/ml CAL2=5mIU/ML

Contains serum proteins, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

Standards are blue colored. CAL5=100mIU/ml) CAL3=10miU/ml

MATERIALS REQUIRED BUT NOT PROVIDED

Micropipettes (150ut, and 50ul) and

- disposable plastic tips.

 EIA grade water (double distilled or delonised, charcoal treated to remove oxidizing chemicals used as Timer with 60 minute range or higher.
- o Absorbent paper tissues.

 Calibrated EUSA micropide thermostatic incubator (dry or wet) sec at 4.37°C (-t/4.0.1°C loterance).

 Calibrated EUSA microwell reader with 450mm (reading) and with 520-50mm (blanking) filters.

 Calibrated EUSA micropide washer.

 Vortex or stmilar mixing loots.
- 8 7

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.

wear protective laboratory clothes, latic-free glooves and glasses. The use of any sharp (needes) or cutting (blass) devices should be avided. All the personnel involved should be trained in blossfely procedures, as recommended by the Center for Disease Control, Allana, U.S., and reported in the valional institute of Health's publication. "Biossafely in Microbiological and Biomedical Laboratories", od. 1844.

3. All the personnel involved in sample handling should be vaporimated for HBV and HAV, for which vaccines are available. All the personnel involved in performing the assay have to

avoid contaminants such as dust or sir-born microbial agents, when opening kit vials and microplates and when performing the test. Protect her Chromogen (TMB) from storing light and avoid vibration of the bench surface where the test is undertaken.

5. Upon reseipt, store the kit at 2.4°C, into a temperature controllet refrigerator or cold room.

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits. safe and effective.

4. The laboratory environment should be controlled so as to

of the same lot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.

8. Avoid cross-contamination between serum/plasma.

sample.

9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each a. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each

what reported in the Institutes of Health's publication. "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984. should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with 10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels. Treat all specimens as potentially infective. All human serum specimens

order to avoid contamination.

12. 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 in Microbiological and Biomedical Laboratories", ed. 1884.

11. 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

> 16-18 his or heat inactivation by autoclave at 12.1°C for 20 min.
>
> 3. Accidental spills have to be adsorbed with paper tissues scaled with hosper tissues scaled with hosper beach and then with water. Tissues should then be discarded in proper containers designated for substances. In particular, liquid waste generated from the washing procedure, from residuads of frontios and from samelar has to be treated as potentially infective material and machination. Supposed procedures of inachination are treatment with a 10% first concentration of household bleach for

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

5. Other waste materials generated from the use of the kit (exampler this used for samples and controls, used microplates) should be hardled as potentially infective and disposed according to national directives and laws concerning laboratory

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical aboratory analysis. No influence has been observed in the preparation of the sample with citate. EDTA

Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the

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).

conjugate, generating false negative results.

3. Samples have to be clearly identified with codes or names in order to avoid mishing-pretation of results. When the kit is used for the screening of blood units, bar code labeling and reading is strongly recommended.

4. Hearnolysed and visibly hypertipentic ("miky") samples have to be discarded as they could generate false results. Semples containing medicuse of finitin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results. Samples can be stored forcer and plasma can be stored at +2..8°C for up to five days after collection. For longer storage periods, samples can be stored forcer at -20°C for several months. Any forcer samples should not be freeze-thwaved more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifice at 2.000 pm for 20 min or filter using 0.2-0.8 utilities to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on opened kit has pointed out no relevant loss of performances up to 3 months from first opening.

1. Antigen coated microwells:

Allow the microplate to reach room temperature (about 1 hr) above opening the container. Check that the destocant has not turned dark green, microplang a deed in conservation. In this case, call Dia Pro's customer service. Unused strips have to be placed back into the aluminum pouch, with the descreant supplied firmly zipped and stored at *2" 8"C. When opened the first time, unused strips are stable until the humidity indication inside the descreant say turns from yellow to humidity indication inside the descreant bay turns from yellow to

Negative Control

Ready to use. Mix well on vortex before use

Ready to use. Mix well on vortex before use

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

vortex, adiquots The whole content of the 20x The dissolved calibrator is not stable; store it frozen in sat -20°C. INS AVAB, CE/Eng | Page | 4 of 9 | Rev.: 3 | Date: 2017/06

and-over-end before use.

Once diluted, the wash solution is stable for 1 week at 2-5° C.

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

diluted with bi-distilled water up to 1200ml and mixed

n has to be iixed gently

6. Enzyme conjugate:
Ready fu use, fix well on vortex before use.
Avoid contamination of the liquid with problems chemicals, or microbes. If this component has to be transferred, use plastic, and in possible, sterile disposable containers.

dust only

7. Chromogen/Substrate: Ready to use. Mix well on vortiex before use. Avoid contamination of the liquid with oxidizing chemicats, 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. Mix well on vortex before use.

Legenda:

Warning H statements: H315 – Causes skin irritation H319 – Causes serious eye irritation.

Procautionary 9 atterments:

P280 — Wear proluctive gloves/protective dichling/eye protection/face protection.

P301 - P352 — FON SKIN: Wash with plenty of scap and water. P302 - P353 — IF ON SKIN: Wash with plenty of scap and water. P303 - P353 — IF Skin initiation occurs: Get medical advise/attention. P303 - P353 + P353 — If N EYES. Ranse caudiously with water for several minitude. Remove contect lenses, if present and seasy to do. Continue rissing.

P303 + P313 — If sign irrialize resists: Get medical advise/attention. P303 + P313 — Take off contaminated clothing and wash it before reuse. 8 8

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

WITH THE KIT

Micropherises have to be calibrated to deliver the correct volume required by, the assay and must be submitted to regular decontamination (household abcohol, 10%, solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in conflact with the sample. They should accidentally come in conflact with the sample. They should accidentally come in conflact with the sample. They should accidentally come in conflact with the sample. They should accidentally come in conflact with the sample. They should accidentally conflact the conflact of the conflact should accidentally and the present of the conflact should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.

The ELISA incubator has to be set al ±37°C (tolerance of +1-0.5°C) and regularly checked to ensure the correct baffs are suitable for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. The ELISA, washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correstly optimized using the 4t controls and reference panels, before using the fit for routine laboratory tests. 4.5 washing toyles (aspitation + dependent on the assay performs as expected. A soaking time of 20.30 sepands between cycles is suggested. In order to a sea correctly their number. It is recommended to run an extraction of the correctly their number. It is recommended to run an

Control*. Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to assay with the kit controls and well characterized negative and positive reference samples, and check to makeh the values reported below in the section O Tinternal Quality

the instructions of the manufacturer.

Incubation times have a bit berance of £5%.

The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter (520-530nm, strongly recommended) for blanking purposes Blanking is carried out on the well identified in the section "Assay Procedure." The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

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 dispensating and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wills. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-20 units nor nu.

exceed 20-30 units per run.

Die Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.

2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+HCs) is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary containing). Check that the aluminum pouch, containing the microrotias is reaf nurstrand or advanced. microplate, is not punctured or damaged.

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

Dissolve the Calibrator as described above and gently mix.

Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid

reagents
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 sycles as found in the validation of the instrument for its use with the kt.
7. Check that the ELISA reader is turned on or ensure it will be

on at least 20 minutes before reading.

If using an automated work station, turn on, check settings and be sure to use the right assay protocol. Check that the micropipettes are set to the required volume.

÷ 9 Check that all the other equipment is available and ready

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

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

Leave A1 well empty for the operation of blanking.

Store the other strips into the bag in presence of the desiccant at +2,,8°C, sealed.

 Dispense 50 ul Specimen Diluent in all the wells identified for samples and controls/calibrator, except for A1.
 Then pipette 100 µi of Negative Control in triplicate, 100 µi of Calibrator in duplicate, 100 µi Positive Control in single and then 100 ul of samples.

Wash the microplate as reported in section I.3.

Check that the reagent has been or microplate at +37°C for 60 minutes

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

5. Wash the microplate as described

Pipette 100 µi TMB/HzOz mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the micropiate at room temperature for 20

background might be generated.

A1 well.

Important notes:

If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450mm. Finger prints could generate false positive results on reading to performed immediately a positive results on reading to performed immediately false should ideally be performed immediately false. Reading has should ideally be positive results on reading that the addition of the Stop Solution but definitely no longer than the addition of the Stop Solution but definitely motioned to the solution of the Stop Solution but definitely motioned to the solution of the Stop Solution but definitely motioned to the solution of the Stop Solution but definitely motioned to the solution of the solution

N. ASSAY SCHEME

Reading OD 4	Sulphunc Acid	emperature	3" incubation	MB/H2O2 mix	Washing step	emperature	" incubation	nzyme Conjugate	Washing step	emperature	"incubation	Samples	Controls/Calibrator(*)	Specimen Diluent
450nm & 620nm	100 ul	1.1.	20 min	100 ul	4-5 cycles	+37°C	60 min	100 ul	4-5 cycles	+37°C	60 min	100 LI	100 ui	50 ul

1. Place the required number of strips in the microplate holder

4. In all the wells except A1, pipelle 100 µl Enzyme Conjugate Check that the reagent has been correctly added. Incubate the

Pipette 100 µl Sulphuric Acid into each well to stop the enzymatic reaction using the same pipeting sequence as in step 6. Then measure the color intensity with a microplate reader at 450mm (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on

chromogen can occur leading to a higher background.

Check that controls/calibrator and samples have been correctly added. Incubate the microplate at +37°C for 60 min.

Important note: Do not expose to strong direct light as a high

(*) Important Notes:

The Calibrator (CAL) does calculation, therefore it doe results calculation. it does not affect the test's not affect the Cut Off

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The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.

An example of dispensation scheme is reported in the

	===	C	5	5	C	ō	Ö	×		
,	98	88	\$7	Se	85	22	S3	S2	2	
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			Ċ						6)	Microplate
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1									9	
									6	
									1	
İ									12	

O. INTERNAL QUALITY CONTROL

 Not mandatory NC = Negative Control
PC = Positive Control

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

Ensure that the following parameters are met:

Parameter	Requirements
ank well	< 0.100 OD450nm value
egative Control	> 0.750 mean OD450nm value
Ō	after blanking
	coefficient of variation < 30%
sitive Control	< 0.300 OD450nm value

220

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

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

Problem	Check
Blank well	1. that the Chromogen/Substrate solution
> 0.100	has not become contaminated during the
OD450nm	assay
Negative	1, that the washing procedure and the
Control (NC)	washer settings are as validated in the
< 0.750	pre qualification study;
OD450nm after	2, that the proper washing solution has
blanking	been used and the washer has been
	primed with it before use;
coefficient of	3, that no mistake has been done in the
variation > 30%	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

Positive Control > 0.300 OD450nm performed; 2. that no mistake has occurred during the distribution of the control (ex.: dispensation of negative control instead of the positive one); 4. that no external contamination of the 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 1. that the procedure has been correctly

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

Calibrator has used, verify the following data

alibrator 10 mIU/ml (WHO)	Parameter
) Co/S > 1.0	Requirements

If the results of the lest doesn't match the requirements stated above, operate as follows:

		Ca/S < 1,0	Calibrator	Problem
pre qualification study; 4. that no external contamination of the calibrator has occurred.	 that the washing procedure and the washer settings are as validated in the 	 that no mistake has occurred during its distribution; 	1, that the procedure has been correctly	Check

may be considered valid. Anyway, if all other parameters (Blank, Negative Posilive Control), match the established requirements, Control, the test

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = (NC+PC)/3

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

Important note: When the colculation of results is performed by the poperating system of an ELISA automated work station, make time operating system of an ELISA automated work station, make sure 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 Cut-Off value and the OD450nm of the sample (or Co/S) according to the following

>1.1	0.9 - 1.1	< 0.9	000
Positive	Equivocal	Negative	interpretation

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

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Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample, A positive result is indicative of a past or recent HAV infection and therefore the patient should be treated accordingly.

An example of calculation is reported below

The following data must not be used instead or real figures

Higher than 0,750 - Accepted Negative Control: Mean Value: 0.100 OD450nm 1.900 - 2.000 - 2.100 OD450nm 2,000 OD450nm

Cut-Off = (2.000 + 0.100) / 3 = 0.700

Lower than 0,300 - Accepted

Sample 1: 0,050 OD450nm Sample 2: 1,900 OD450nm Sample 1 Co/S > 1,1 Sample 2 Co/ < 0,9 Mean value: U
Co/S > 1 - Accepted 0.400-0.360 OD450nm 0.380 OD450nm positive negative

Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of
- Ν judgement errors and misinterpretations.

 When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous
- data transfer.

 Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical

R. PERFORMANCE CHARACTERISTICS

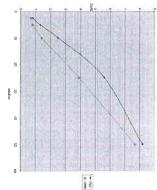
Limit of detection

The limit of delection of the assay has been calculated by means of the 2"d international Standard surphiled by WHO.
Two control samples, supplied by Boston Biomedica Inc., USA, with code Accurun 52 and 120, were also examined.
The sensitivity shown by the assay is < 10 WHO mIU/ml or < 5 pell mU/ml.

Results of Quality Control are given in the following table

6.6	0.115	Accurun 120	12.7	0.060	Accurun 52
	2.217	Neg. Control		2.217	Neg. Control
0.8	0.849	2.5		1,015	2.5
1.3	0.587	ı		0.943	5
2.5	0.304	10		0.543	10
5.6	0.137	25	1	0.197	25
8.2	0.093	50	7.7	0.099	50
Co/S	0D450	mU/mi	Co/S	OD450	mlU/ml

Curves are reported below



2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in a clinical trial on The diagnostic sensitivity has been tested in a clinical trial on panels of samples classified positive by a US FDA approved sit. An overall value of 100% has been found in the study conducted on a total number of more than 200 samples.
Sensonversion and performance panels have also been studied. Results obtained by examining two panels supplied by Boston Bromedeca Inc., USA, are responded below.

Seroconversion Panel: PHT 902

0,120	0,123	0.090	1,501	1,878	PHT902	librator 0,470	(+) 0,084	-(-) 1,968	sample Co-sonni colo piasorin
5,7	5,6	7,6	0,5	0,4	i	1,5	8,1	0,3	Cina

Performance Panel: PHT 201

Sample	0,169 0,169	CoiS 4,0	DiaSorin	Sample 14	Sample OD450nm ColS DisSozin/Sample OD450nm ColS DisSorin 1 0,169 4,0 pos 14 0,139 4,9 pos 2 0,137 5,2 pos 15 0,115 6,9 pos	5 4 G
N N	0,132	4 52	Dos Dos	5 5	0,115	5.9
	0,143	4.	pos	16	0,167	2
4	0,104	9,8	pos	17	0,086	8,0
ın	0,438	1.6	pos	18	0,160	4.3
on	0,121	5,7	pos	19	0,175	3,9
7	0,127	5,4	pos	20	1,772	0,4
00	0,150	4,6	pos	21	0,090	7,5
9	0,115	5,9	pos	22	0.201	4
10	0.094	7,3	pos	23	0.281	2,4
17	0,070	8,8	pos	24	0,134	ı
Ñ	1,814	0,4	gen	25	0,142	14
13	0.097	7.1	pos	Neg	1,780	0.4

3. Disgnostic specificity.

The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of speciment preparation has been observed, because the process specimens have also been tested to check whether this interferes with the performance of the test, No interference was observed on clean and particle free samples.

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

No cross reaction were observed.

The Performance Evaluation study conducted in the external reference center on more than 1000 samples has provided a

 Precision

A. Precision

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

Test #1

CV %	Std. Deviation	OD450nm	Sample
2.7	0.065	2,425	Negative
3.9	0.023	0.608	Low Pos.

Test#2

CV %	td. Deviation	OD450nm	Sample
4.5	0.107	2.373	Negative
6,0	0.034	0.573	Low Pos.

Test #3

Sample	Negative	Low
OD450nm	2.478	0.5
Std. Deviation	0,108	0.0
CV %	4.4	

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

S. LIMITATIONS OF THE PROCEDURE

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

This test is suitable only for testing single samples and not

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

8

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IAV IgN

"Capture" Enzyme Immuno Assay (ELISA) tor the determination of IgM class antibodies to Hepatitis A Virus in human plasma and sera

for "in vitro" diagnostic use only -



DIA.PRO

(Milano) - Italy 20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl

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HAV igM

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A. INTENDED JSU

Enzyme ImmunoAssay (ELISA) for the determination of IgM class antibodies to Hepatitis A Virus in human plasma and sera with the "capture" system. The kit may be used for the identification of the viral agent causing hepatitis in the patient and the follow up of the acute phase of the infection, For "in vitro" diagnostic use only.

B. INTRODUCTION

The Center for Disease Control or CDC, Atlanta, USA, defines Hepatitis A Virus as follows:

vaccination of appropriate susceptible populations would substantially lower disease incidence and potentially reported vaccine preventable diseases in the world, despite the licensure of hepatitis A vaccine in 1995. Widespread (HAV) infection Hepatitis A continues to be one of the most frequently eliminate indigenous transmission of hepatitis A virus

if illness does occur, it is not usually accompanied by jaundice. Anong older children and adults, infection is usually symptomatic, with jaundice occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 months, although 10%-15% of symptomatic operons have prolonged or rehipsing disease lasting up to 6 months. In infected persons, HAV raphiteds in the liver, is excreted in bile, and is shed in the sool. Feak infectivity of infected and infants can shed HAV for longer periods than adults, up to several months after the onset of clinical illness. Chronic shodding of HAV in faces does not occur however, shedding can occur in persons who have relapsing illness. Viremia occurs soon after infection and persists through the period of HAV infection is related to the person's age. In children less than 6 years of age, most (70%) infections are asymptomatic if illness does occur, it is not usually accompanied by humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection HAV, a 27-nm RNA agent classified as a picornavirus, can produce either asymptomatic or symptomatic infection in liver enzyme elevation. of virus in stool declines after jaundice appears. Children persons occurs during the 2-week period before onset of jaundice or elevation of liver enzymes, when the typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausca, abdominal discomfort, dark concentration of virus in stool is highest. The concentration urine, and jaundice. The likelihood of having symptoms with

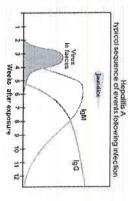
protection against the disease. Commercial diagnostic tests are available for the detection of IgM and total (IgM and most persons, IgM anti-HAV becomes detectable 5-10 days before the onset of symptoms and can persist for up to 6 months after infection. Immunoglobulin G (IgG) anti-HAV, which appears early in the course of infection, remains detectable for the person's lifetime and confers lifelong hepatitis on the basis of clinical or epidemiologic fleatures alone. Serologic testing to detect immunoglobulin M (IgM) attibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In (gG) anti-HAV in serum Hepatitis A cannot be differentiated from other types of viral

HAV RNA can be detected in the blood and stool of most persons during the acute phase of infection by using nucleic acid sequencing has acid amplification methods and nucleic acid sequencing has buch used to determine the relatedness of HAV isolates

> HAV infection is acquired primarily by the ficultural route by either person-torperson contact or ingestion of contaminated food or water. On rare orusining, HAV infection has been transmitted by transfusion of blood or during the incubation period; however, transmission saliva has not been demonstrated. blood products collected from donors during the viremic phase of their infection. In experimentally infected nonhuman primates, HAV has been detected in saliva

HAV environment for months. Heating foods at temperatures greater than 185 F (85 C) for 1 minute or disinfecting surfaces with a 1:100 dilution of sodium hypochlorite (i.e., Depending on conditions, household bleach) in tap water is necessary to inactivate HAV can be stable in the

an identified source of infection was performed, 25%-40% of the contacts less than 6 years old had serologic evidence of presence of a young child was associated with transmission within the household. In studies and serve as a source of infection for others. In one study of adults without an identified source of infection, 52% of their households included a child less than 6 years old, and the acute HAV infection (IgM anti-HAV). serologic testing of the household contacts of adults without infections, they play an important role in HAV transmission secause most children have asymptomatic



c. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid

phase coated with anti high antibody.

After washing out all the other components of the sample and in particular ligit antibodies, the specific light captured on the solid phase are detected by the addition of a purificed preparation of inactivated HAV, labelled with an antibody conjugated with percodases (HAP).

After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added. In the presence of percoldase the coloriess substrate is hydrolysed to a colored end-product, whose optical density may be detected and is proportional to the amount of antibodies to HAV present in the sample.

REF AVM.CE

reagents for 96

1. Microplate: MICROPLATE
12 strips of 8 breakable wells coated with anti-human IgM
antibody, affinity purified, and sealed into a bag with desiccat.
Bring the microplate to room temperature before opening the
bag. Unused strips have to be returned into the bag and the
bag thas to be sealed and stored back to 2.8°C, in presence of
the desiccant.

2. Negative Control: CONTROL 2. Negative Control: CONTROL 1. 1x4.0 milytal. Ready to use control. It contains goat serum proteins. 10 mM firs buffer p.H 6.0+4.0.1. 0.1% Tween 20. 0.09% sodium azide and 0.1% Kathon GC as preservatives. The negative control is colourless

3. Positive Control:

1.44.0 ml/vial. Ready to use control, it contains anti HAV IgM, goal serum proteins, 10 mM tris buffer pH 6,04-0,1, 0.1% Tween 20, 0.09% sodium azide and 0,1% Kathon GC as preservatives. The positive control is green colour coded.

reported in the label. It contains anti HAV IgM, 2% BSA, 10 mM Iris buffer pH 6,0+0.401, 0.09% sodium azide and 0.1% Kathon GC as preservatives.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

WASHBUF 20X

1x60mi/bottle, 20x concentrated solution.

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

6_Enzyme conjugate 20X: CONJ 1x0.8 ml/vial, 20X concentrated solution,

1x0.8 ml/vial, 20X concentrated solution, it contains Hoiseradish peroxidase conjugated antibody specific to HAV in presence of 10 mM Tris buffer pH 6.8-4, 1,1.2% BSA, 0,1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

The reagent is red colour coded 7. HAV Antigen: [Ag HAM]

Ix 16 mWill. Ready-to-use solution, it contains inactivated and stabilised HAV in presence of 10 mM. Tris buffer pH 6.8+0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as

8. Specimen Diluent: DILSPE 2x60.0 ml/vial. Proteic buffered solution to samples. It contains goat serum proteins, 10 (6.0+/0.1, 0.1% Tween 20, 0.09% sodium Kalhon GC as preservatives. The reagent is blue colour coded on for the dilution of s, 10 mM tris buffer pH dium azide and 0.1%

9, Chromogen/Substrate: SUBS TMB IX16m/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.6, 0.03% letra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of Hz0z. Note: To be stored protected from light as sensitive to

It contains 0.3 M H2SO4 solution.
Attention: Irritani (H315, H319; P280 P332+P313, P305+P351+P338, P337+P313, P280 P362+P363) P302+P352

> 11, Plate sealing foils 7° 2

12. Package insert л° 1

E. MATERIALS REQUIRED MATERIALS REQUIRED BUT NOT PROVIDED Calibrated Micropipettes of 10ul, 100ul and 1000ul and

N disposable plastic tips.

EIA grade water (double distilled or defonised, charcoal lo remove oxidizing chemicals used

disinfectants). Timer with 60 minute range or higher,

r (dry or

4. Absorbent paper lissues,
5. Calibrated ELISA microplate thermostatic incubator (drivet) set at 43.77 (4.40.1° to loterance).
6. Calibrated ELISA microplate rader with 450nm (read and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools. 450nm (reading)

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 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. F. WARNINGS AND PRECAUTIONS

wear protective laboratory clothes, tato-free glovies and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosately procedures, as recommended by the Center for Disease Control, Marianta, U.S., and reported in the National Institute of Health's publication. "Biosafely in the Marianta U.S., and reported in the National Institute of Health's publication." Biosafely in All the personnel involved in performing the assay have to

Microbiological and Biomedical Laboratories", ed. 1984.

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

4. The laboratory environment should be controlled so as to

4. The laboratory environment should be controlled so as lo avoid confaminish such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromoger/Subrate (TMB & H2O2) from strong light and avoid vibration of the bench surface where the test is undertaken.

controlled refrigerator or cold room Upon receipt, store the kit at 2-8°C into a temperature

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits.

 Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laborationy supervisor to initiate the necessary procedures,
 Avoid cross-contamination between serum/plasma of the same lot should not be interchanged.

7. Check that the reagents are clear a

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

sample 9. Av

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

10. Do not use the kit after the expiration date stated on external (primary container) and internal (valas) labels. Treat all specimens as potentially infective. All human serum speciments should be handled at Bosefley Level 2, as recommended by the Center for Disease Control, Allanda, U.S., in compliance with what reported in the Institutes of Health's publication:

Blosafety in Microbiological and Biomedical Laboratories, ed. 1984.

11. The use of disposable plastic-were is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in

order to avoid contamination.

12. 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 fur the 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

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soaked with household bleach and then should then be discarded in proper contain Accidental spills have to be adsorbed with paper fissues ked with household bleach and then with water. Tissues

15. 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 the control of the contro surface with plenty of water 15. Other waste materials iaboratory wastes

SPECIMEN: PREPARATION AND RECOMMANDATIONS

been observed in the preparation of the sample with citrate, EDTA and heparin. Blood is drawn aseptically by venepuncture and plasma serum is prepared using standard techniques of preparation samples for clinical laboratory analysis. No influence h has

order to avoid misinterpretation of results. Wu used for the screening of blood units, bar code

could give rise to false results.

4. Sera and plasma can be stored at +2"...8"C for up to five 4. Sera and plasma can be stored at set and set and set at each collection. For longer storage periods, samples can be stored frozen at -20"C for several months. Any frozen as amples should not be freeza/thawed more liber once as light antibodies may get damaged and as this procedure may generate particles that could affect the test result.

5. It particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0,2-0,0b filters to chean up this sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS A study conducted on an opened kit has not pointed out relevant loss of activity up to 3 months. t any

1. Antibody coated microwells

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

Unused strips have to be placed back into the aluminium pouch, with the desiccent supplied, firmly zapped and stored at 12°-8°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green,

Ready to use. Mix well on vortex before use.

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

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

aliquots at -20° C.

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

5. Wash buffer concentrate: The whole content of the concentrated solution has to be diffused 20x with bidistilled water and mixed gently end-over-end before use. Once diffused, the wash solution is stable for 1 week at 2-6° C.

containers designated <u>g</u>

laboralory/hospital waste.

14. The Stop Solution is an irritant. In case of spills, wash the

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.

7. HAV Antigen:
Ready to use. Mix well on
Handle this component a Enzyme conjugate:
20X preparation, Mix up on vortex.
Avoid contamination of the liquid with oxidizing chemicals, or microbes when the reagent is aspirated to be used.

Mix well on vortex before use

as

potentially

infectious, even if HAV

, dust

6+7, HAV Antigen/Antibody complex:
About 5-10 min before its use, divite the 20X concentrated Enzyme Conjugate in the poper volume of HAV Antigen, necessary for the assay. Then mix on vortex carefully, Example: To run 2 strips, divide 100 ul Enzyme Conjugate 20X into 2 ml of HAV Antigen.

Note: This immunocomplex is not stable; discard the exceeding has been chemically inactivated

DTA and heparm.

DTA and heparm.

Samples have to be clearly identified with codes or names in sider to avoid misinferpretation of results. When the kit is seen from the screening of blood units, bar code labeling and

reading is strongly recommended.

3. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they

volume.

Ready to use. Mix well on Avoid contamination of the Sample Diluent

9. Chromogen/Substrate Ready to use, Mix well on vortex before use

driven dust or microbes,

Do not expose to strong light, oxidizing agents and metallic surfaces, if this component has to be transferred use only , and if possible, sterile disposable container,

contamination of the liquid

vortex before use,

with oxidizing chemicals, air-

10. Sulphuric Acid:

Ready to use. Mix well on vortex before use,
Attention: Infraint (H315, H319; P280, P302+P35
P332+P313, P305+P351+P338, P337+P313, P362+P363).

P302+P352

Legenda:

Warning H statements:
H315 – Causes skin irritation,
H319 – Causes serious eye irritation

advice/attention.

P305 + P351 + P338 = IF IN EYES: Rinse cauliously 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,

P322 + P353 = Toke off contaminated clothing and wash it. protection/lace protection.
P302 + P352 - IF ON SKIN: Wash with plenty of soap water.
P332 + P313 - If skin irritation occurs: Get med Precautionary P statements: P280 – Wear protective protective gloves/protective clothing/eye medical and

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

Micropipelles have to be calibrated to deliver the correct volume (tolerance +/-5%) 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. Decontemination of spills or residues of kill components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a frueness of ±2%. 32°C (Glerance of +4/0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water temperature is maintained. Both dry incubators and water haths are suitable for the incubations of EUSA lests. The EUSA washer is extremely improfant to the overall performances of the assay. The washer must be carefully incurred.

- validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboration of sests. 4-5 washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested, in order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the
- Incubation times have a tolerance of ±5%.
 The ELISA reader has to be equipped with a reading filter
 of 450nm and with a second filter (620-630nm, strongly
 recommended) for banking purposes Blanking is carried
 out on the well dentified in the section Assay Procedure;
 out on the well dentified in the section. The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the
- assay protocol has to be installed in the operating system of the unit and validated as for the waster 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 confamination of adjacent wells. The use of EUSA automated work stations is recommended when the number of samples to be tessed exceed 20-30 units per steps (dispensation incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to march the values reported in the section O "internal Quality Control". The manufacturer's instructions.

 When using an ELISA automated work station, all critical
- 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

- Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
 Check that the fluid comproments are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colourless or pade blue by aspirating a small volume of kwilth a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of Ingluid is present inside the box (primary container). Check that the aluminium pourth, containing the
- microplate, is not punctured or damaged.

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

- Δ D Dissolve the Calibrator as described above and gently mix, Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid
- 0 reagents.
 Set the ELISA incubator at +37°C +/-0,1°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kill.

 Check that the ELISA reader is turned on or ensure it will
- and be sure to use the right assay protocol.

 Check that the micropipettes are set to the required
- 10, Check that all the other equipment is available and ready

M. ASSAY PROCEDURE

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

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Sample Diluent into a dilution tube; mix gently on vortex
- Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of
- Dispense as they are ready to use
- Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue coloured and that controls and calibrator have been
- Incubate the microplate for 60 min at +37°C

instruments

- About 5-10 mir Antigen/Antibody minutes before use, ody immunocomplex y prepare described
- Wash the microplate with an automatic washer reported previously (section 1.3).
- Pipette 100 µl HAV Antigen/Antibody complex into each well, except the 1st blanking well, and cover with the seafer. Check that all wells are red coloured, except A1.

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

- Incubate the microplate for 60 min at +37°C
- =;

- be turned on at least 20 minutes before reading.
 If using an automated work station, turn on, check settings
- to use.
- 11_ In case of problems, do not proceed further with the test and advise the supervisor.

- Positive Control in single and 100 μl Calibrator in duplicate in proper wells. Do not dilute controls and the calibrator 100 µl Negative Control in triplicate, 100 µl

be regularly maintained according to the

- 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

- Wash microwells as in step 7.
- Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the micropiate at room temperature (18-24°C) for 20 minutes.

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

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- 12. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction using the same pipetting sequence as in slep 10. Addition of acid will turn the positive control and positive samples from blue to yellow
- Measure the colour intensity of the solution in each well, as described in section 1.5, at 450mm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

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

N. ASSAY SCHEME

Reading OD 4	Sulphuric Acid 1	Temperature	3" incubation 21	TMB/H2O2 mix 1	Washing 4-5	Temperature +	2" incubation 66	HAV & Tracer 1	Washing 4-5	Temperature +	1 st incubation 60	samples diluted 1:101	Controls&Calibrator (1)
450nm	100 ul	17.	20 min	100 Ld	4-5 cycles	37°C	nin 08	100 uf	4-5 cycles	+37°C	60 min		III OU

(*) Important Notes:

- The Calibrator (CAL) does not affect the Cut calculation, therefore it does not affect the to results calculation test's Off
- The Calibrator (CAL) used only if a laboratory Management,

An example of dispensation scheme is reported in the table

	Œ	G	T	m	0	C	Ø	P	1	
	S	PC	CAL(*)	CAL(*)	NC	NC	NO	BLX	_	
2 120	89	SB	S7	Se	SS	22	S3	S2	2	
200									3	
1				ſ					4	
5									un	1
									cn	and obtate
									7	0
									00	
									9	
									10	
					Г				12	
				Ŋ					12	

CAL(*) = Calibrator PC = Pasiuve Canirol S = Sample

O. INTERNAL QUALITY CONTROL

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

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control mean value (NC)	< 0.150 OD450nm value after blanking coefficient of variation < 30%
Positive Control	> 0.500 OD450nm

proceed to the next section: If they do not, do not proceed any further and perform the following checks: If the results of the test match the requirements stated above

If Calibrator has used, verify the following data

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

Calibrator	Parameter	
SiCo > 1	Requirements	

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator	that the procedure has been correctly
S/Co < 1	performed;
	2. that no mistake has occurred during its
	distribution (e.g.: dispensation of negative
	control instead)
	3, that the washing procedure and the washer
	settings are as validated in the pre qualification
	sludy;
	4, that no external contamination of the
	calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical-calculation, in order to define the following cut-off

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Cut-Off = NC + 0.250

The value found for the test is used for the interpretation esults as described in the next paragraph. 잌

Important nate: 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 and the Cut-Off value (or S/Co) according to the following table:

>1.2	0.8 - 1.2	< 0.8	S/Co in
Positive	Equivocal	Negative	iterpretation

A negative result indicates that the patient is not undergoing an acute infection by HAV.

Any patient showing an equivocal result, should be re-tested by examining a second sample after 1-2 weeks from first testing. A positive result is indicative of an HAV infection event and therefore the patient should be treated accordingly.

An example of calculation is reported below:

The following data must not be used instead or real figures

Lower than 0.150 – Accepted Positive Control: 2.189 OD450nm Higher than 0.500 - Accepted Mean Value: Negative Control: 0.050 - 0.060 - 0.070 OD450nm 0.060 OD450nm

 $Cut ext{-}Off = 0.060 + 0.250 = 0.310$

Mean value: 0.540 0U450 S/Co higher than 1.0 – Accepted 0.550 - 0.530 OD450nm 0.540 OD450nm S/Co = 1.7

Sample 1: 0.070 OD450nm Sample 2: 1.690 OD450nm Sample 1 S/Co < 0.8 ≈ negetive Sample 2 S/Co > 1.2 = positive

Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.

 Any positive result should be confirmed by an alternative method (confirmation test) before a diagnosis of viral
- hepatitis is confirmed.

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

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection
In absence of a defined international standard for HAV IgM, the limit of detection of the assay has been calculated by means of the following preparations:

- Accurun # 121 supplied by Boston Biomedica Inc. USA Accurun # 51 supplied by Boston Biomedica Inc., USA
- curve (accurun # 121).
 Results of Quality Control are given in the following table: These preparation were prepared according to the manufacturer's instructions, diluted in Sample Diluent (1:100) and then further diluted in Sample Diluent to generate a limiting

Accurun #51				#121	Accurun	Preparation
1:100	1:1600	1:800	1:400	1:200	1:100	Dilutions
4.2	1.0	.1.9	2.8	4.1	5,4	S/Ca

An overall value of 100% has been found in the study conducted on a total number of more than 100 samples. A seroconversion panel has also been studied.

Seroconversion Panel: PHT 902

Diagnostic Specimenty:The diagnostic specificity has been determined on panels of specimens, negative with the reference kit, derived from normal

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

Samples derived from patients with different viral (HCV, HDV, HBV, HEV) and no viral pathologies of the liver that may interfere with the test were examined.

Diagnostic Sensitivity:
 The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
 Positive samples were collected from patients carrying HAV acute Infection, confirmed by clinical symptoms and analysis.

Results obtained by examining a preparation supplied Boston Biomedica Inc., USA, are reported below. è

	4 1,988	3 1,956	2 0,042	1 0,037	PHT902	CTRL (+) 1,736	CTRL (-) 0,048		Sample OB450nm S/Co DiaSonn Keler
27	6,7	6,6	0,1	0.1		5,8	0,2		Sico
,	6,7	6,8	0,3	0.3				S/Co	DiaSoni
pos	pos	pos	neg	Sec				Score	Keler.

specimens, negative with the reference kit, derived from normal individuals and blood donors of European origin.

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

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Na cross reaction were observed.

The Performance Evaluation study conducted in a qualified external reference centre on more than 500 samples has provided a value > 88%.

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:

neferences 1. Dienstag

characterization and epidemiologic investigations". Progress in liver desease VI. Popper E., Schaffiner F. (eds), pp 343-370, New York, Gunner and Stratton, 1979.

2. Duermeyer W., Van der Vean J., Koster B. "ELISA in Hepatilis A. Inancel. I. 823-824, 1978

3. Parry J.V., (1981) "Hepatilis A infection: guidelines for the development of satisfactory assays for laboratory diagnosis". The Institute of Medical Laboratory Sciences, 38, 303-311.

3. Lindberg J., Frosrer G., Hansson B.G., et al.

"Serologic markers of hepatilis A and B in chronic active hepatilis". Scandinavian Journal of Gastroenterology, 13:525-627, 41379. Ë "Hepatitis A Virus identification,

hepatitis". 527, 1978. Zachoval R., Dienstag J.L., Purcell R.H. "Tests for hepatitis A virus antigen and antibody" in "Hepatitis A", Gerety R.J. (Ed), pp 33-46, Orlando, Academic Press, Inc. 1984 Barbara J.A., Howell D.R., Briggs M., Parry J.V.. "Post transfusion hepatitis A". Lancet (1982), 1-738.

specifications and acceptance criteria

Sample Negative Low Pos. OD450nm 0.048 0.709

0.048 0.007 13.9

Test # 2

Negative Low Pos. n. 0.058 0.719 on 0.008 0.052 14.3 7.2

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 1345s rule. Each lot is submitted to a qualify control and released into the market only if conforming with the EC technical

Test#1

Dia Pro Diagnostic Bioprobes Sri Vla G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

7

 sample
 Negative
 Low Pos.

 OD450nm
 0.050
 0.713

 Std. Deviation
 0.007
 0.055

 CV %
 13.4
 7.7

Test#3

False positivity has been assessed as less than 2% of the normal population, mostly due to high thers of RF. Frozen samples containing their particles or aggregates may generate false positive results.

Bacterial containination or head inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the tevel of the analyte.

This test is suitable only for testing single samples and not may. the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be S. LIMITATIONS Diagnosls of an infectious disease should not be established on poolea ones

Certificate

mdc medical device certification GmbH

certifies that



Research and Production area building 36, Office 211, Koltsovo 630559 Novosibirsk region Russian Federation

with the locations listed in the attachment

for the scope

Design and development, production and distribution of medical devices for in vitro diagnostics (PCR, ELISA, Biochemistry)

has introduced and applies a

Quality Management System

The mdc audit has proven that this quality management system meets all requirements of the following standard

EN 120 13482

Medical devices – Quality management systems – Requirements for regulatory purposes

EN 120 13486:2016 + AC:2016 - 150 13486:2016

2018-07-13 2020-07-03 2020-07-03 2013100017

2018-07-13

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Head of Certification Body

Head of C



O-SW-) 9005-06-00 Denteche Akkreditierungsztelle Akkreditierungsztelle

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1:C Declaration of conformity EIA-1-17	AO Vector-Best
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EC DECLARATION OF CONFORMITY

of Annex I Directive 98/79/EC of 27 October 1998 regarding in vitro diagnostic medical devices. on pages 2-3 are in conformity with applicable provisions and fulfill the essential requirements AO Vector-Best hereby ensures under own responsibility and declares that the products listed

Classification of products:

Other devices (all devices except Annex II and self-testing devices)

Harmonized standards applied:

supplied by the manufacturer (labelling). Terms, definitions and general requirements. In vitro diagnostic reagents for professional use); EN ISO 15223-1:2012 (Symbols to be used with medical devices. Application of risk management to medical devices). reduction of risk of infection related to in vitro diagnostic reagents); EN ISO 14971:2012 (Medical medical devices, Evaluation of stability of in vitro diagnostic reagents); EN 13641:2002 (Elimination or (Performance evaluation of in vitro diagnostic medical devices); EN 23640:2013 (In vitro diagnostic devices. Quality management systems. Requirements for regulatory purposes); EN 13612:2002 device labels, labelling and information to be supplied); EN ISO 13485:2012+AC:2012 (Medical EN ISO 18113-1:2011; EN ISO 18113-2:2011 (In vitro diagnostic medical devices, Information

Conformity assessment procedure

Annex III (not including section 6)

Manufacturer:

Address: 630559, Koltsovo, Novosibirsk Region, Research and Production area, building 36, office 211, Russian Federation, tel. +7 (383) 336-73-46, tel./fax +7 (383) 332-67-49 AO Vector-Best

European authorized representative

Bioron GmbH

621 5720 916 Address: Rheinhorststr. 18, D-67071 Ludwigshafen, Germany, tel.: +49 (0) 621 5720 915, fax: +49 (0)

Date: 2017/10/16



General Director AO Vector-Best Murat Khusainov

Valid until: 2022/07/03

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EIA-1-17	Declaration of conformity	AO Vector-Best
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Pepsinogen 2-EIA-BEST	Pepsinogen 1-EIA-BEST	IgG-Transglutaminase-EIA- BEST	IgA-Transglutaminase-EIA- BEST	Ascaris-IgG-EIA-BEST	Echinococcus-IgG-EIA-BEST	Yersinia-IgM-EIA-BEST	Yersinia-IgA-EIA-BEST	Yersinia-IgG-EIA-BEST	Trichinella-IgG-EIA-BEST	Toxocara-IgG-EIA-BEST	VectoMumps-IgM	VectoMumps-IgG	VectoEBV-VCA-IgM	VectoEBV-EA-IgG	VectoEBV-NA-IgG	Adenovirus-antigen-EIA-BEST	Rotavirus-antigen-EIA-BEST	VectoMeasles-IgM	VectoMeasles-IgG	Vectohep A-IgG	Product name
Enzyme immunoassay kit for the determination of nepsinogen? concentration in blood serum	Enzyme immunoassay kit for the determination of pepsinogen 1 concentration in blood serum	Enzyme immunoassay kit for the quantitative determination of IgG to tissue transglutaminase in blood serum (plasma)	Enzyme immunoassay kit for the quantitative determination of IgA to tissue transglutaminase in blood serum (plasma)	1 0	Enzyme immunoassay kit for the detection of IgG to Echinococcus granulosus antigens in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgM to causative agents of yersiniosis	Enzyme immunoassay kit for the detection of IgA to causative agents of yersiniosis	Enzyme immunoassay kit for the detection of IgG to causative agents of yersiniosis	Enzyme immunoassay kit for the detection of IgG to Trichinella antigens in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgG to Toxocara antigens in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgM to mumps virus in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgG to mumps virus in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgM to viral capsid antigen of Epstein-Barr virus in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgG to early antigens of Epstein-Barr virus in blood serum (plasma)	Enzyme immunoassay kit for the detection of IgG to nuclear antigen of Epstein-Barr virus in blood serum (plasma)	Enzyme immunoassay kit for the detection of human adenovirus antigen	Enzyme immunoassay kit for the detection of human rotavirus antigen	Enzyme immunoassay kit for the detection of IgM to measles virus in blood serum (plasma)	Enzyme immunoassay kit for the quantitative and qualitative determination of IgG to measles virus in blood serum (plasma)	Enzyme immunoassay kit for the qualitative and quantitative determination of IgG to hepatitis A virus	Identification data
D-3764	D-3762	D-3760	D-3758	D-3452	D-3356	D-3206	D-3204	D-3202	D-3152	D-2752	D-2604	D-2602	D-2176	D-2172	D-2170	D-1654	D-1652	D-1358	D-1356	D-0362	REF