P 2 Qualifative method in the mean DD450nm values in the squalitative method; calculate the mean DD450nm values for the Calibrators 0 and 10 U/ml and then check that the assay is valid.

Example of calculation

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

| Higher than 1_000 - Accepted | Calibrator 100 U/mi: | 200 - | Mean Value: | U/mt: | Lower than 0_150 - Accepted | Mean Value: | Callorator O O/mi: |
|------------------------------|----------------------|----------|---------------|-----------------------|-----------------------------|---------------|-----------------------|
| d. | 2,845 OD450nm | Accepted | 0.340 OD450nm | 0.350 ~ 0.330 OD450nm | 1 | 0.022 OD450nm | 0.020 - 0.024 OD450nm |

Q. INTERPRETATION OF RESULTS

Q.1 Qualitative results For qualitative interpret

For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of HBc igM ≥ 10 PEI U/ml.

2 to PEI U/ml. The control of the control of the sample objects results are therefore interpreted as a ratio of the sample objects may be the OD450mm of the OD450mm of the CO1450mm of the CO

| >1.1 Pc | 0.9 - 1.1 Eq | < 0.9 Ne | S/Co Interp |
|---------|--------------|----------|-------------|
| ositive | uivocal | gative | pretation |

O.2 Quantitative results
The calibration curve is used to determine the concentration of igM antibodies to HScAg in samples.

Samples with a concentration were than 5 PEI U/ml are considered negative for HSGAy.

considered in a gray-zone.

In the follow up of chronic heparitis, however, values higher of 5
PEL Umit may be considered positive for HBdgM, when in
presence of other clinical signs,
Samples with a concentration higher than 10 PEI Uml are considered positive for HBclgM.

- Important general notes:

 Important general notes:

 Important general notes:

 Important general not a 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 judgement errors and mainterpretations.

 A positive result is inflicative of HBV infection and therefore the patient should be treated accordingly.

 When rest results are transmitted from the aboratory to another facility, attention must be paid to avoid errorseous. data transfer
- Diagnosis of viral hepatilis infection has to be taken by and released to the patient by a suitably qualified medical

means of 1. Limit of detection
The limit of detection of the assay has been calculated by

| avidebau | 1 | 2.2 | 3 0 | n e | 5 6 | 300 | 5 5 | CO COURT OF | BCM.CE |
|----------|-------|-------|-------|-------|-------|-------|-------|-------------|--------|
| 0.040 | 58070 | 0,155 | 0.310 | 0.044 | 0.500 | 1,017 | 2000 | MUDGE | Lot # |
| -275.00 | 0.3 | 0,5 | 1,0 | | 100 | 7.0 | 0.5 | 3/00 | 0103 |
| 0.035 | 0.084 | 0.149 | 0.296 | 0.513 | 0.914 | 278.1 | 2,583 | 00450nm | E 10-1 |
| 1 | 0.3 | 0.5 | 3.0 | 1.7 | 3.1 | 0.7 | 9,7 | S/Co | 0103/2 |
| 0.044 | 0.093 | 0,161 | 0.321 | 0.582 | 1.095 | 2.053 | 2911 | 00450nm | Lot# |
| | 0.3 | 0.5 | 1.0 | 1.8 | 3,4 | 6,4 | 9.1 | S/Co | 0303 |

BBI Accurun # 113 lot # 48-9999-0621

| 128 x 0.086 | * | | 02 X 0 234 | 0.43U | 0 x | 784,1 × 4 | 2167 | 1 x 3.336 | DDI 113 UU450AR | | |
|-------------|-------|-------|------------|-------|-------|-----------|-------|-----------|-----------------|--------|----------------------|
| | L | - | - | H | 2.8 | 1 | 1 | 1 | m S/Co | П | The state of the |
| 0.000 | 0.082 | 0.133 | 0.234 | 0.427 | 0.807 | 1.413 | 2,385 | 3,195 | OD450nm | Lot 8 | 4 110 101 44 |
| | 0.3 | 0.4 | 0.8 | 1.4 | 2.6 | 4.6 | 7,8 | 10,4 | S/Co | 25:010 | 790-5566-04-# 101 51 |
| 0.000 | 0.089 | 0.122 | 0.248 | 0.410 | 0.856 | 1,429 | 2.385 | 3.259 | 00450nm | Lot # | 136 |
| | 0.3 | 0.4 | 0.8 | 1.3 | 2.7 | 4.5 | 7.5 | 10.3 | S/Co | 0303 | |

BBI - Panel code PHE 102

| Lot # 0103 | er | | 02 11.3 | | | | | | | | | | | | | | 05 11.3 06 12.1 07 0.1 07 0.1 08 9.2 09 11.2 10 11.7 11 5.9 12 12.7 13 11.6 14 7.0 |
|--------------|------|-----|---------|-----|-----|-----|------|---|-------|------|------------|--------------------|----------------------------|---------------------------------|-------------------------------------|---|--|
| Lot # 0103/2 | S/Co | 6,3 | 10.0 | 7.7 | | 3.4 | 11,4 | | 11.6 | 11.E | 0.1 8.5 | 11.6 0.1 8.5 | 11.6 0.1 6.5 11.7 | 0.1 6.5 11.7 10.2 | 11.6 0.1 11.7 10.2 11.4 | 11.6 0.1 6.5 11.7 10.2 5.8 | 0.1 0.1 10.2 10.2 11.4 11.4 11.0 |
| Lot# 0303 | 5/00 | 5.5 | 10.7 | 0 | 0,4 | 4.1 | 11,2 | - Constitution of the last of | 11.00 | 0.1 | 0.1 | 11.8 | 0.1 0.1 11.9 | 0.1 8.8 10.8 | 11.8 0.1 3.8 11.9 10.8 | 11.8 10.8 11.7 | 5.5 11.5 6 15 8 1. |
| Sorin FIA | S/Co | 20 | 2 | | 2,0 | 21 | 3.1 | 41 | | 0.2 | 0.2 | 23 | 223 | 0.2 2.3 2.4 2.4 2.1 | 2.0.2 2.1 2.1 5.2 | 3 5 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 | 242 242 242 242 242 |

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 faculte and chronic hepathts).

| M.CE | Lot # | 0103 | # 10.1 | 0103/2 | lot# | 5050 |
|---------|---------|-------|---------|---------|---------|-------|
| 1 U/mil | 0D450nm | S/Co | 00450nm | S/Co | 00450nm | S/Co |
| 90 | 2.752 | 8.9 | 2.883 | 9.7 | 2915 | 9 |
| 50 | 1047 | 0.3 | 4070 | | | 0.1 |
| 00 | 11811 | 7.0 | 1.8/2 | 6.7 | 2053 | 6,4 |
| 0.20 | 0.980 | 3.2 | 0.914 | ω. 1 | 1.095 | 3.4 |
| 10 | 0.544 | 1.8 | 0.513 | 1.7 | 0.592 | 8 |
| c | 0.310 | 1.0 | 0.296 | 5.0 | 0.321 | 10 |
| 2.5 | 0.155 | 0.5 | 0.149 | 0.5 | 0.151 | 3.5 |
| 25 | 0.084 | 0.3 | 0.084 | 0.3 | 2000 | 0 0 |
| ative | 0.040 | 10000 | 0.035 | | 0.044 | 20,00 |

| CE | Lot# | 0103 | Lot g | Lot 0 0103/2 | 0.00 | 0.55 |
|----|---------|------|---------|--------------|-------------|------|
| 13 | OD450nm | S/Co | 00450nm | S/Co | 0D450nm | SIC |
| 2 | 3,336 | 10.8 | 3,195 | 10.4 | 3 269 | 103 |
| | 2000 | | | | - challenge | 1000 |
| ľ | 2412 | 8,0 | 2,385 | 7.8 | 2/385 | 7.5 |
| 1 | 1,487 | 4.7 | 1.413 | 4.6 | 1,429 | 45 |
| 1 | 0.865 | 2.8 | 0.807 | 2.6 | 0.856 | 2.7 |
| × | 0.430 | 1.4 | 0.427 | 1,4 | 0.470 | 2 |
| × | 0.234 | 6,8 | 0.234 | 8.0 | 0.248 | 0 |
| ľ | 0.129 | 0.4 | 0.133 | 5,4 | 0.122 | 0 |
| × | 0.086 | 0.3 | 0.082 | 0.3 | 0.089 | 0.3 |
| We | 0.040 | | 0.040 | | 0.052 | |

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

| mber | Lot # 0103 | Lot# 0103/2 S/Co | Lo | Lot# 0303 S/Co |
|------|------------|---------------------|------|-------------------|
| 4 | 6.7 | 6,3 | | 5 |
| 12 | 11.3 | 10.0 | 35 | 17 |
| 3 | 9.5 | 7.2 | 50 | 4 |
| 4 | 5.0 | 3.4 | | |
| 5 | 11.3 | 11,4 | 72 | 3 |
| ch | 12.1 | 11.5 | 4 | 00 |
| 7 | 0.1 | 0.1 | 0 | - |
| 8 | 9.2 | 5.5 | 900 | 00 |
| 9 | 12.2 | 11.7 | 110 | 0 |
| 0 | 11.7 | 10.2 | 10 | CO |
| - | 5,9 | 5.8 | 5 | Co |
| 23 | 12,7 | 11,4 | 4 | .7 |
| G | 11,6 | 0.13 | - | ė. |
| * | 7.0 | 6,3 | ,on | 6 |
| 0 | 12.4 | 11.5 | 11.8 | tn |

R. PERFORMANCES

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

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

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

| 0103 Lot# 0103/2 Lot# SICO 0D450nm SICO 0D450nm 8.9 2.863 9.7 2.91 6.2 1.972 6.7 2.053 3.1 0.914 3.1 1.095 |
|--|
| Inn SiCo OD 3.7 3.3 9.7 3.3 9.7 3.4 3.1 4 3.1 4 3.1 6 9.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0 |
| 00000 |
| Lot# OD450nm 2 911 2 053 1,095 0,592 0,321 0,161 0,093 |
| |

| Lot # | 2010 | 1018 | CIECEG | | |
|--------|------|---|--------|-------------|------|
| 0450nm | S/Co | 0D450nm | S/Co | 00450nm | Sico |
| 3.336 | 10.8 | 3,195 | 10.4 | 3 269 | 103 |
| - | | 100000000000000000000000000000000000000 | | - option to | 0.00 |
| 2412 | 8.0 | 2,385 | 7.8 | 2.385 | 4 |
| 1,487 | 4.7 | 1.413 | 4.6 | 1 420 | 2.5 |
| 0.865 | 2.8 | 0.807 | 2.6 | 0.856 | 27 |
| 0.430 | 1.4 | 0.427 | 1.4 | 0.410 | 1 |
| 0.234 | 8.0 | 0.234 | 8.0 | 0.249 | 0 |
| 0.129 | 0.4 | 0.133 | 5.4 | 0.122 | 0 0 |
| 0.086 | 0.3 | 0.082 | 0.3 | 0.089 | 03 |
| 0.040 | | 0.040 | | 0.052 | |

| | Lot # 0103 | Lot # 0103/2 | FOX # 10.1 | |
|-----|------------|--------------|------------|-----|
| ber | S/Co | S/Co | SA | 0 |
| 5 | 6.7 | 6,3 | 50 | |
| 2 | 11.3 | 10.0 | 10. | 1 |
| | 9.5 | 7.2 | 8.4 | |
| ľ | 5.0 | 3.4 | | |
| 5 | 11.3 | 11,4 | 7 | 100 |
| | 12.1 | 11.5 | - | 00 |
| 1 | 0,1 | 0,1 | 1.0 | |
| | 9.2 | 5.5 | 00 | |
| | 12.2 | 11.7 | 11.0 | |
| | 11.7 | 10.2 | 10,8 | |
| | 5,9 | 5.8 | 5,8 | |
| 1 | 12.7 | 11.4 | 1 | 1 |
| | 31.6 | 0.17 | = | |
| | 7.0 | 6,3 | 6.6 | |
| | 12.4 | 11.5 | 17.E | |

| | Member # | 10 | 02 | 03 | 04 | 05 | 90 | 07 | 08 | 99 | 10 | 11 | 12 | 13 | 14 | cis. | 16 | 17 | co | 19 | 20 |
|---------------|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|-------|-------|
| Lot # 6103 | SiCo | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0,1 | 0.7 | 0.2 | 0.2 | 2.8 | 5,0 | > 12 | > 12 | > 12 | > 12 | > 12 | > 12 |
| Lot Abbott | S/Co | 0.1 | 0.1 | 0.1 | 0,1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0,1 | 0.1 | 0.1 | 3.7 | 6.4 | 6.2 | 55 | 5.5 | 4.00 | > 6.6 | > 6.6 |
| DiaSorin | S/Co | 0.1 | 0,1 | 0.1 | 0.1 | 1.0 | 0.1 | 0.1 | 0.1 | 0.5 | 0.1 | 1.0 | 0.1 | 0.7 | 0.9 | 4.51 | 4.5 | 4.3 | 4.3 | 4.4 | 5.2 |

3. Diagnostic Specificity:

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 pensie for negative stemally in a qualified Clinical Laboratory on pensie of negative semples from normal individuals and blood donors, classified resamples from normal individuals and blood donors, classified a pagative with a US FDA approved kit.

A local number of more than 400 negative specimens were tested. A diagnostic specificity > 59% has been found.

Moreover, the diagnostic specificity > 95% has been found, diagnostic specificity in the properties of the control of the properties of the prope

No interference was observed in the study.

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

Frozen specimens have also heen 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 radicals in three different runs, carried out on three different replicate in three different runs, carried lots. The values found were as follows:

BCM.CE: lot # 0103

| 6.01 | 10.7 | 12.3 | 99 | CV % |
|----------|----------------------|---------|-------|---------------|
| 0.006 | 0:005 | 0,006 | 0.005 | Std.Deviation |
| 0,053 | 0.051 | 0.053 | 0.055 | UD 4500M |
| value | | | | 25.454 |
| Sections | · G ^m eun | Sad sun | Strun | Same Agines |

TM No.

2nd nun

Tru out

"An overall value > 98% has been found in the study conducted on a total number of more than 200 samples, and a data of the A. Seroconversion panel produced by BBI, USA, code # PHM 936A, have also been studied; results are reported below with reference to two commercial kits (BBI's results).

Cal 50 U/m) (N = 16) Mean values

1st run

Zna run

nun b

2,109 0,101 4,8

2,048 0,088

Average value 2,070 0,109 5.2

2nd run

3" run

0.004 7.3

0.005

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| Lot Abbott DiaSorin | BBI Panel PHM 935A | | commercial kits (BBI's results) | been studied; results are reported below with |
|---------------------|--------------------|------|---------------------------------|---|
| BCM.CE: lot # 01 | | CV % | Std. Deviation | d below with OD 450nm |

| 55 | 5.6 4.5 | 6.2 4.5 | 6.4 0.9 | 3.7 0.7 | 0.1 0. | 0.1 0. | 0.1 0. | 0.1 0.1 | 0.1 0.1 | 0.1 0 | 0.1 0. | 0.1 0.1 | 0.1 | 0.1 | 0.1 0.1 | 0.1 0.1 | S/Co S/Co | EIA EIA | Abbott DiaSorin | |
|-------------|------------|----------|-----------------|-------------------|---------------|--------|-------------|------------------------|---------|--------------|---------------------|---------|------|---------------|----------|---------|-------------|---------------------|----------------------|-----|
| | | | | | | | | | | | | | | | | | 9 | | ŝ | |
| COLOGNICOCH | OH Doubles | On Aspen | Capito A single | Carlotte Contract | Cat 50 Himles | 24 AC | TOURINGTIES | Control of the Control | 20 4500 | Medin values | Cal a Citta (N = 16 | 2000 | CV % | Std.Deviation | OD 450nm | | Mean values | Cal 0 U/ml (N = 16) | BCM.CE: 101 # 0103/2 | 201 |
| l. | | J | V. | 9 | 3 | t. | L | L | ı | | Γ | | L | L | | | | Γ | 3/2 | |

unu psg 0.332

2rd run

3" 000

Average value 0.328 0.017

2nd run

Jan nan

| The second second second | Menn values | Cal S Ulm! (N = 16) | CV % | DOMENTAL DISC. | OD 430nm | Mean values | BCM.CE: lot # 0303 Cal 0 U/ml (N = 16) | CV% | Std. Deviation | OD 450mm | - |
|---|-------------|---------------------|------|----------------|----------|-------------|---|-----|----------------|----------|---|
| 100000000000000000000000000000000000000 | fast run | | 10,3 | 0,004 | 0.043 | Tet run | ш | 4.7 | 0.110 | 2,311 | |
| | 2nd run | | 121 | 0.005 | 0.042 | 2nd run | | 41 | 0.090 | 2,208 | |
| | 3 nm | | 6.01 | 0.004 | 0.040 | 3" nin | | 4,3 | 0.095 | 2.212 | |
| - Source | Salany | | 10.8 | 0,004 | 0.042 | Ayung | | 4.4 | 960'0 | 2.244 | |

| Mean values | Tel run | 2110 11/11 | 3"05 | мунади |
|--------------------|----------|------------|-------|---------|
| | | | | anjav |
| 09 458nm | 6.043 | 0.042 | 0.040 | 0.042 |
| Std. Deviation | 0,004 | 0.005 | 0.004 | 0.004 |
| CV % | 10,3 | 121 | 6.03 | 10 8 |
| Cal 5 U/ml (N = 16 | - | | | - |
| Mean values | Tast run | 2nd run | 3"run | Average |
| OD 450nm | 0.320 | 0.326 | 0.314 | 0.320 |
| Std Deviation | 0.023 | 0.024 | 0.026 | 0.024 |
| CV% | 7.1 | 7.4 | 8.2 | 7.5 |

| CV% | Sto. Deviati | UD 45000 | CHILD VIEW |
|-----|--------------|----------|------------------|
| 2.6 | gn 0.057 | 7 2,750 | es us ron |
| 3.1 | 0.067 | 2.163 | 2nd run |
| 3.6 | 0.076 | 2.092 | 3" 000 |
| 3,1 | 0.067 | 2 135 | Average value |

S. LIMITATIONS Frozen samples containing fibrin particles or aggregates may

Sacterial contamination of heat mactivation of the specimen may affect the absorbance values of the samples with consequent alteriation of the level of the analyte.

This test is suitable only for testing single samples and not generate false positive results

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

- REFERENCES

 1. Engyall E. and Perlmann P. J. Immunochemistry, 8, 871.
 874, 1971
- 5
- Engvall E. and Perlmann P., Jilmmunol. 109, 129-135, 1971
 Remington J.S. and Kiein J.O., In "Infectious diseases of the felus and newborn infant." Sanders, Philadelphia, London, Toronto.
 Volk W.A., In "Essential of Medical Microbiology." 2" ed. pp 729, G.B. Lippincott Company, Philadelphia, New York, Subset, Toronto. ω

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

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

(Milano) - Italy Phone +39 02 27007161 Fax +39 02 26007726

REF SAB.CE 96 Tests

HBs Ab

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A. INTENDED USE

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

B. INTRODUCTION

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

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

HBV is the most serious type of viral hepatitis and the only type causting chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or help fluits of an infected preson 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) perinatial from mother to halby at the hitch; (b) child: to child transmission: (c) unsafe injections and transfusions: (d) child transmission: (c) unsafe injections and transfusions sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in bouschold settings, and from reuses of unsteading neededs and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mather to infant programmes were implemented However, the majority of infections in these countries are acquired during young soulthood 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 and child to child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination workers have received hepatitis B vaccine and child-to-child transmission

Hepatitis B virus is not spread by contaminated food or water, and cannot be agreed custually 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 subrountment, about 5% are chronically infected infection is less common in Western Europe and North imerica, where less than 1% are chronically infected.

Young children who become infected with HBV are the must likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between I to 4 years of size develop chronic infection. The risk of don'th from HBV related liver cancer or

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

Chronic hepatitis B in some patients is treated with drugs culled interferon or luminuting, which can help some patients. Pathents with currhosis are sometimes given liver transplants, with varying success, it is preferable to prevent this disease with varying than to try and care it.

to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991. WHO has colled 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 immunization programmes. effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a vaccine thave been used worldwide. Studies have shown that the resolution intranscular doses. Studies have shown that the resolution in the same of the contract of the same of the s Hepatitis B vaccine has an outstanding record of safety and

repatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV). This antigen is composed mainly of the type common determinant if and the type specific determinants of and vy. present only on the specific sensorbyzes. Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time egains the if determinant. Anil "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 convalencemen. The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitory of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified Microplates are coated with a preparation with sample specifically captures and H8Aq antibodies to the solid phase.

After washing, readured antibodies are decered by an H8Aq, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies, are scendingly bound to wells. by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of H8Ach in the sample and can be defected by an ELISA reader.

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

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

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests

Microplate: MICROPLATE

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

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

2. Calibration Curve: SAL N.

5.2.0 milval. Ready to use and striput coded standard curve, derived from RBAD positive pleans strated on WHO standard for anti-RBAD, positive pleans strated on WHO standard for anti-RBAD, [1" reference preparation 1977, lot 17-2-77), millim II CAL4 = 10 millim II CAL5 = 250 millim II CAL4 = 100 millim II CAL5 = 250 millim II CAL4 = 100 millim II CAL5 = 250 millim II CAL4 = 100 millim II CAL5 = 300 millim II CAL5 = 3

3. Wash buffer concentrate: MASHBUF 20X 1x50mlbottle. 20x concentrated solution.
Once diuted, the wash solution contains 10 mM phosphate buffer pH 7.0+40.2-0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate: CONJ

X15.0 milytal, Ready-foruse solution and rad color coded,
it contains inactivated purified HBs/4y of both subtypes aid and
ay labelled with HRP, 5% BBA, 10 mM firs buffer pH 68-4/-0,1,
0.3 mg/ml gentamizine sulphate and 0.1% Kathon GC as

5. Chromogen/Substrate: SUBS TMB
X16m/Vibl. Contains a 50 mM citrate-phosphate
solution at pH 35-38, 4% dimethylsulphoxide, 0,03
methyl-benzidine (TMB) and 0,02% hydrogen peroxide;
Note: To be stored protected from light as sen

phate buffered c. 0.03% tetra-coxide (H2O2).

6. Sulphuric Acid: [H2SQ4_0.3 M xt.5m/kval, Contains 0.3 M HsQL, solution Attention: Irritan (1435, H319, P280, P202+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363). sensitive ö

7. Specimen Diluent: DILSPE 1x8mi. 10 mM fris Buffered solution ph 7.4 +/-0,1,suggested to be used in the follow up of vaccination. It contains 0.09% sodium adde as preservatives.

8. Control Serum: 1 vial. Lyophilized.

9. Plate sealing foil n° 2 Contains fetal bovine serum proteins, nantibodies calibrated at 50 ± 10% WHO gentamicine sulphate and 0.1% Kathon GC.

10. Package insert n° 1

 Calibrated Micropipettes (100ul T NOT PROVIDED Out and 50ul) and di

plastic tips. EIA grade treated to disposable

õ water (double distilled or delonised, chargoal or remove oxidizing chemicals used as

Timer with 60 minute range or higher.

UI A W (dry or

Absorbent paper tissues,
Calibrated ELISA, micropiate thermostatic incubator
well), set at 47°C (+1°C tolerance).
Calibrated ELISA, microwell reader with 450nm (re
and with 620-630nm (blanking, strongly recomm (blanking, 450nm (reading) lly recommended)

00 14 filters.
Calibrated ELISA microplate
Vortex or similar mixing tools washer

similar mixing tools.

 WARNINGS AND PRECAUTIONS
 The kit has to be used by skilled and on echmical personnel only, under the supervision doctor responsible of the laboratory. properly trained ion of a medical

> weat protective laboratory cichties; talc-free gloves and glasses. The use of any sharp (needles) or cutting (thates) devices should be avoided. All the jestsonnel involved should be reinted in lobsafety procedures, as recommended by the Center for Disease Contiol, Alfaina U.S. and reported in the National Institute of Health's publication: Blossfey in Microbiological and Blomedical Laboratories; ed. 1994.
>
> 3. All the presonnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, each and faccines. l involved in performing the assay afory clothes, talc-free gloves and

disposable tips and changing them between between kit reagents by using them between the use of each

11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Allanta, U.S. nublication. "Biosafety in Microbiological and Biomedical control of the property of the p

preparation components a use of disposable plastic-ware is recommended in the ion of the liquid components or in transferring ents into automated workstations, in order to avoid

human a human anti HBs. 0 mIU/ml. 0.3 mg/ 3 as preservatives.

3 mg/ml

Cross containments 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 florn me washing procedure, from residuals of controls and from are less to be treated as potentially infective makeral and inactivated before waste. Suggested procedures of inactivation are freatment with a 10% final concentration of household bleach for the 18-his or heart mactivation by autoclave at 121°C for 20 min.

14. Accidental spills from samples and operations have to be adsorbed with paper tissues coached with household bleach and then with water. Tissues stould then be discarded in groper then with water. Tissues stould of then be discarded in groper then with water. Tissues stould then be discarded in groper than the control of the contro

G. SPECIMEN: PREPARATION AND WARNINGS Blood is drawn aseptically by venipuncture an

. Blood is drawn aseptically by enipuncture 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 clinic, EDTA

sale and effective.

4. The laboratory environment should be controlled so as to avoid containment such as clust or eir-born microbial agents, when opening kit vials and microbiates and when performing the last. Protect the Chromogen (TMB) from strong light and avoid whealon of the banch surface where the test is undertaken.

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

controlled refrigerator or cold room.

5. Do not infarchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

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

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each cannot be supervisor.

Do not use the kit after the expiration date stated on the external container and internal (visits) 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.

publication: "Biosafety Laboratories", ed. 1984 12. The use of disposal

surface with plenty of water

16. Other waste materials generated from the use of the ket example; the used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

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

3. Haemolysed ("ref.") and visibly hyperipemic ("milly") samples have to be discarded as they could generate late 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.

A Seria and plates make no textures, at Seria and plates make no texture at +2". B"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 freezed/hawed more than conce as this may 5. If particles are present, contribige at 2.000 pm for 20 min or filter using 0.2-0.5 til filters to clean up the sample for testing. Samples whose anti-HBBAG antibody concentration is expected to be higher than 250 millum should be distret before use either 1.10 or 11.00 in the Call D(1.10). Man 50 ul of he 1.10 distribut are distret by the Call of (1.10), in the Call of (1.10), in the call of the Cal

PREPARATION OF COMPONENTS AND WARNINGS

before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation. In this case, call Dia. Por's customer service. Unused strips have to be plaused back into the aluminum pouch, with the desiccant supplied, firmly zapped and stored at +2"-8"C.

After first opening, remaining strips are sable until the humarity, molicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Mix Well 9 vortex before use

Stare frozen in 98

dust only

vortex before use

| INS SAB CE/Eng | Page | 4 of 9 | Rev.: 5 | Date:

H. Pn...

1. Microplate

1. Microplate to re
Allow the microplate to re
Allow the microplate to re check that the desiccant has

Add the volume of ELISA grade water, reported on the label, the lyophised powder, let fully dissolve and then gently mix ordex.

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

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

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 plastic, and if possible, sterile disposable containers.

Ready to use Mix well on

7. Chromogen/Substrate: Ready to use. Mix well on writex before use. Ready to use. Mix well on writex before use. Avoid contamination of the liquid with oxidising chemicals, afforms dust or microbes. Do not expose to strong light, oxidising ogenis and metallic surfaces.
If this component has to be transferred use only plastic, and if

Sulphuric Acid: Ready to use. Mix well on

Attention: Imtant (F P305+P351+P338, (H315, H319; P280, P302 P 38, P337+P313, P362+P363) P352, P332+P313

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

serious eye irritation.

P332 + advice/att Precautionary P statements: P280 - Wear protective P305 + P351 + P338 - IF IN EYES; Rinse cautiously with water P280 – Wear protective gloves/gratective clothing/eye protection/face protection, P302 + P352 – IF ON SKIN: Wash with plenty of soap and water: P313 - If skin irritation occurs: Get medical

for several minutes. Remove contact tenses, if present and easy to do. Continue rinsing.

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

I INSTRUMENTS AND TOOLS USED IN COMBINATION
WITH THE KIT

Microphetes have to be calibrated to deliner the correct
volume required by the assay and must be submitted to
regular focuntamination (70% ethanol. 10% solution of
theach, hospital grade districtants) of those parts that
could accidentally come in contact with the sample or the
components of the kit. They should also be regularly
minimating in order to show a precision of 1% and a

The EUSA incubator has to be set at 437°C (becance of ±1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water temperature is maintained. Both dry incubators and water boths are suitable for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubations 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 kill for routine satisfication of a south of the satisfication of the stream of the satisfication of the satisfication of a south of the satisfication of th ço

positiva reference sample, and check for match the values reported below in the sections "Validation of Test" and Assay Perdomanoes." Reglater adiabation of the utimes delivered and maintenance (decontamination of the utimes delivered and maintenance (decontamination and cleaning in needles) of the washer has to be ceried out according to the instructions of the manufacturer.

In the instructions of the manufacturer.

In the contamination of the manufacturer.

In the contamination of the manufacturer.

In the contamination of the manufacturer (220-930mm and with a second filter (220-930mm and with a second filter (220-930mm strongly recommended) for banking purposes. Its standard performances should be (a) bandwidth total mailto: total the second filter (220-930mm strongly recommended) for banking purposes. Its standard performances should be (a) bandwidth total mm. (b) repositability total measured to the well demention of the section "Assay Procedure". The optical demention of the manufacturer is system of the reader has to be pallurated regularly to ansure regularly maintained according to the manufacturer is well of the manufacturer in the correct optical density is measured. It should be regularly maintained according to the manufacturer is well as the contamination of the manufacturer is severe.

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

values reported in the sections "Validation of Test" and "Asses protocol has to be installed interpretation of the seasy protocol has to be installed interpretation of the seasy protocol has to be in the washer and the reader. In addition, the liquid handling part of the stabiling hand season of the stabiling handling hand of the stabiling handling handled and controlled on minimize the possibility of confarmation of adjacent wells due to strongly reaches samples, leading to adjacent wells due to strongly reaches samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run. Sub Pro's customer service offers support to the user in the with the kit, in order to assume full compliance with the kit, in order to assume full compliance with the fauthern of new instruments used in combination.

undergoing vaccination for

present in people

Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100µl 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

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

Wash the microplate as reported in section I.3.

4. In all the wells except A1 and B1, pipelle 100 µl Enzyme Conjugate. Check that the reagent has been correctly added, incubate the micropiate at 437°C for 60 minutes.

Check the expiration date of the kit printed of

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

- pipette tip when dispensing the Enzymo Conjugate. Contamination might occur.

 Mix thoroughly the Enzyme Conjugate on vortex before use. Be careful not to touch the inner surface of the pipette tip when dispensing the Enzymo

Check that the liquid components are not contaminated by maked-eye visible particles or aggregates. Check that the Chromogen/Sustrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. See 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

5. Wash the microplate as described.
6. Pipette 100µl TMB/No. mixture in each well, the blank wells included. Check that the regigent has been correctly added.
Then incubate the microplate at room temperature for 20.

background might be ger Do not expose to strong direct light as a high

4 10 ţ,a

as described above.

Dissolve the Control Serum as described above.

Allow all the other components to reach foom temperature or damaged.

Dilute all the content of the 20x concentrated Wash Solution

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

(about 1 hr) and then mix as described.

Set the ELISA incubator at *37°C and prepare the ELISA washer by priming with the ditude washing solution, according to the manufacturers instructions. Set the right number of washing cycles as bound 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 fight assay protocol.

Check that the micropipettes are set to the required volume, 0. Check that all the other equipments are available and ready. M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder, Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccan at 2.8°C, sealed.

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 In case of problems, do not proceed further with the test and advise the supervisor. 2. Dispersis 50 u. Specimen Diluent in all the wells, except for the blank At. Then pigette 100µ of the Calibrator 0 mtl/mt in outplicate, 100µ of the Calibrator 10 mtl/mt in duplicate, 100µ.

If the Calibrator 250 mtl/mt in single, and then 100µ of samples have been correctly added. Then incubate the microplate at +37°C for 60 mt.

Wash the microplate as reported in section I.3.

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

Two procedures can be carried oul with the device according to

the request of the clinician

 Place the required number of strips in the micropiate holder. Leave A1 and B1 wells emply for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2.0°C, sealed. M.1 Quantitative analysis 4 ω

Then Dispense in all the wells to be used for the test, except for A1 and B1. 50µl of the Specimen Diluent.

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

important note:

De careful not to touch the inner surface of the well with the pipeths tip when dispensing the Enzyme Conjugate, Contamination inflat occur.

Mix thoroughly the Enzyme Conjugate on vortex before use.

5. Wash the microplate as described;
6. Pipette 100µt TMB/H2D; 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.

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

reading at 450nm. Finger prints could generate false positive results on reading.

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

N. ASSAY SCHEME (standard procedure)

| 100 ul 100 ul 100 ul 60 min +37°C 4-5 cycles 100 ul 60 min +37°C 4-5 cycles 100 ul 20 min r.1. 100 ul | Reading OD 450nm & 620nm | id | r.t. | 3" incubation 20 min | TMB/H2O2 mix 100 u | | remperature +37°C | 2" incubation 60 mil | Enzyme Conjugate 100 u | Wash step 4-5 cyc | Temperature +37°C | 1" incubation 60 mi | | Control Serum 100 u | | 00 4 |
|--|--------------------------|----|------|----------------------|--------------------|--|-------------------|----------------------|------------------------|-------------------|-------------------|---------------------|--|---------------------|--|------|
|--|--------------------------|----|------|----------------------|--------------------|--|-------------------|----------------------|------------------------|-------------------|-------------------|---------------------|--|---------------------|--|------|

| | | | rep. | | | Þ | G7 | C | O | m | 71 | တ | Œ |
|----------------------------|---------------|--------|---|------------|-----|------|------|------|------|------|------|------|--------|
| D SO 코 엑= | 20 | F | An example of reported below: | | | BLK | BLX | CALT | CAL1 | CAL2 | CAL2 | CAL3 | CALS |
| Temperature Sulphuric Acid | Reading OD | | le of d | | 2 | CAL4 | CAL4 | CALS | CAL5 | CS | CS | S1 | 822 |
| ation ura Acid | OC | 1 | ispensa | Mic | J | 53 | 54 | S | 88 | 57 | SB | 98 | n n |
| Ш | | | tion sc | Microplate | 4 | | | | | - | - | | - |
| | | , | heme | | e, | | | | 1 | | | | |
| 20 10 | 450nm & 620nm | 001111 | in qua | | 7 8 | 1 | - | | | | 1 | | 1 |
| 20 min r.1. 100 ul | 8. 620r | 0.0201 | ntitaliv | | ū | | 1 | | 1 | | | 1 | ŀ |
| | 3 | - | e assa | | 10 | - | + | + | + | + | + | + | 1 |
| | | | ays is | | 2 | - | 1 | t | 1 | 1 | 1 | | 1 |
| | 7 | | An example of dispensation scheme in quantitalive assays is reported below: | | | | | | 1 | | | | |

ote: Do not expose to strong direct light as a tigh might be generated. -O_INIERNAL-QUALITY-CONTROL

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 Stop the enzymatic reaction by pipette 100µl Sulphuric Acid
into sach well and using the same pipeting sequence as in step
6. Then measure the colour intensity with a microplate reader at
450mm (reading) and at 620-630mm (blanking, strongly
recommended), blanking the instrument on A1 and B1 wells. are as qualified. Contro! that the following data are matched: A validation check is carried out on the controls any lime the kit is used in order to verify whether the performances of the assay

| Coefficient of | Control Serum | 250 WHO mit/mi | 10 WHO mill/mi | Celibrator 0 WHO mill/mi | Blank well | raidmeters |
|-----------------------------------|---------------------------------------|-----------------|--|--------------------------------|-----------------|--------------|
| < 30% for the Calibrator 0 milumi | OD450nm = OD450nm CAL 50 mtU/ml ± 10% | > 1,500 OD450nm | OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0, 100 | < 0.200 OD450nm after blanking | < 0.100 QD450nm | Requirements |

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:

| | | coefficient of variation > 30% | Calibrator 0 mIU/ml > 0,200 | > 0.100 OD450nm | Problem |
|---|--|---|---|--|---------|
| the enzyme conjugate; 5. that micropipeties have not become contaminated with positive samples or with the enzyme conjugate enzyme conjugate enzyme conjugate of, that the washer needles are not blocked or partially obstructed. | 3. that no mistake has been done in the assay propodure when the dispersation of standards is certed out. 4. that no contamination of the Cal 0 mit/hit or of the wells where it was dispensed has no contamination. | that the proper washing solution has been used and the washer has been primed with it before use; | that the washing procedure and the washer settings are as validated in the pre-qualification study. | that the Chromogen/Substrate solution has not become conteminated during the assay | Check |

| reported below. | MI example of |
|-----------------|---------------|
| | dispensation |
| and the second | scheme |
| | 5 |
| | qualitative |
| | assays |
| | ß. |

| | L | > | m | O | ø | m | T | O | |
|------|----|-----|------|------------|----------|------|------|------|-----|
| | | BLK | CALT | CALT | CAL2 | CAL2 | CALS | Si | 522 |
| | 2 | 53 | | (S) (S) | ts di | 87 | 8.8 | 151 | 510 |
| | GI | | S 12 | | S T | | (S) | S 17 | S |
| Micr | 4 | | | | | | | | |
| opla | cn | | | Ц | | | | | 4 |
| 8 | œ | | | Ц | | | | | |
| | × | | 1 | Ц | | Ц | 4 | 1 | 1 |
| | 0) | | | | 1 | Ц | | 1 | 1 |
| | to | 1 | 1 | 1 | 1 | | | 1 | 1 |
| | ö | | 1 | | | 1 | | | 1 |
| | ** | | | | | | | | |
| | 13 | ı | | ı | | | 1 | | 1 |

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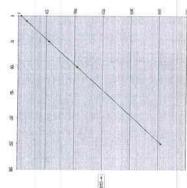
| Complete to | in ine procedure has been correctly |
|-----------------|--|
| OD460nm | |
| < Cat 0 + 0,100 | distribution (ex.) dispensation of a wrong |
| | |
| | 3. that the washing procedure and the washer |
| | pre qua |
| | 4. that no external contamination of the |
| | inderd has occurred |
| Calibrator 250 | 1. that the procedure has been correctly |
| < 1.500 OD450nm | 200 |
| 91 | stribution |
| | 3. Ihal the washing procedure and the washer |
| | sellings are as validated in the pre qualification |
| | study: |
| | 4 that no external contamination of the |
| | |
| Control Serum | First verify that |
| Different from | / performe |
| expected value | stribution (ex.: dispensation of a |
| | |
| | 3, the washing procedure and the washer |
| | |
| | 4, no external contamination of the standard |
| | has occurred |
| | 5, the Control Serum has been dissolved with |
| | the right volume reported on the label. |
| | If a mistake has been pointed out, the assay |
| | of this error |
| | If no mistake has been found, proceed as |
| | |
| | a) a value up to +/-20% is obtained: the overall |
| | Precision of the laboratory might not enable the |
| | test to match the expected value +/-10% |
| | Report the problem to the Supervisor for |
| | acceptance or refusal of this result. |
| | b) a value higher than +/-20% is obtained; in |
| | this case the test is invalid and the DiaPro's |
| | customer service has to be called |

P. RESULTS

P.1 Quantitative method
If the lest turns out to evalid, use for the quantitative method
an approved curve fitting program to draw the calibration curve
from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

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

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



Example of calculation

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

Mean Value; Lower than 0,200 – Accepted

Calibrator 250 mlU/ml: 2.845 OD450nm Higher than 1.500 — Accepted

Q. INTERPRETATION OF RESULTS

considered positive for anti HBsAg antibody.

In the follow up of vaccination recipients, however, the value of 20 WHO mill/mill is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection. international medical literature, Samples with a concentration higher than 10 WHO mIU/ml are Samples with a concentration lower than 10 WHO mILI/ml are considered negative for anti HBsAg antibody by most of the

Important notes:

- Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

Example of Calibration Curve :

| - 4 | ų | ā | 2 | 8 | <u>u</u> | ä |
|-------|---|----|-----|---|----------|---|
| | | | | | | |
| et: | | | | | | |
| 8 | | | | | | |
| el | | | | | | |
| 3 | | | | X | | |
| ta - | | | | | | |
| y III | | T. | | | | |
| | | | 100 | | | |

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 II and 10 mIU/mI and then check that the assay is valid.

Calibrator 0 mlU/ml: 0.020 - 0.024 OD450nm 0.022 OD450nm

Calibrator 10 mlU/ml: 0,250 – 0,270 OD450nm Mean Value: 0,260 OD450nm Higher frian Cal 0 + 0,100 – Accepted

- 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, afternton must be paid to avoid erroneous
- data transfer.

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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).

Mean values 1st run

2nd run 3" run

OD 450nm 2.998 3.000 3.259 Std Deviation 0.152 0.151 0.158 CV % 5.1 5.0 4.8

Average value 3.085 0.153

1. LIMIT OF DETECTION:

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

| millimi MHO | SAB.CE Lot # 1002 | SAB.CE Lot # 1001 | SAB.CE Lot # 1002 |
|----------------|----------------------|----------------------|----------------------|
| 50 | 0.933 | 0.812 | 0.846 |
| 10 | 0.219 | 0.192 | 0.194 |
| 51 | 0.110 | 960.0 | 0.104 |
| 2,5 | 0.057 | 0.058 | 0.067 |
| Std 0 | 0.021 | 0.015 | 0.023 |

2.1 Diagnostic Specificity

has been observed

Calibrator 10 mit/mi (N = 16)
Mean values 1st run

2nd run

3" run

Average value 0.234 0.016

0.226 0.238 0.015 0.017 6.5 7.0

OD 450rm 0.050 0.051 0.050 Std Deviation 0.005 0.006 0.006 CV % 10.0 10.9 11.9

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

| Cattor a con o migra | (a: - n) | No. of Contract of | 4 | |
|----------------------|-------------|--|--------|-------|
| SQUIDE LINE | unit del | 200 pun | も | , , |
| OD 450mm | 0.038 | 0.038 | 0.039 | 0 |
| Std Deviation | 0.003 | 0.004 | 0.005 | 0 |
| CV % | 8.6 | 9.5 | | 10.0 |
| Calibrator 10 mlU | ml (N = 16) | | | |
| Mean values | UNE 352 | 2nd run | 3" run | . ^ |
| OD 450mm | 0,250 | 0.243 | 0.244 | 0 |
| Std. Deviation | 0.020 | 0.023 | 0.017 | 0,020 |
| The Control | 0 | 0.0 | 7.6 | . 0 |

SAB.CE: lot # 1002

Mean Values 1st run

2nd nun

3" 1011

0.048

0,048 0,050 0,004 0,006 6.4 11.5

velue 0.049 0.005 9.8

| 1 9 | SAB.CE Lot # 1002 | SAB.CE Lot # 1001 | SAB.CE Lot # 1002/2 |
|-----|----------------------|----------------------|------------------------|
| 0 | 0.933 | 0.812 | 0.846 |
| 0 | 0,219 | 0.192 | 0.194 |
| | 0.110 | 0.096 | 0.104 |
| èn. | 0.057 | 0.058 | 0.067 |
| 0.0 | 0.021 | 0.015 | 0.023 |

Calibrator 10 mIU/ml (N = 16)
Mean values 1st run

0.249 0.021 8.3

0.242 0.023 9.6

3" nun

Mean values 1st cun

2nd nun

3" пл

Average value 3.603 0.156

OD 450nm 3.544 3.653 3.812 Std.Daviation 0.153 0.176 0.138 CV 4, 4,3 4,8 3,8

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

It is defined as the probability of the assay of scoring negative in

SAB,CE: lot # 1002/2

Calibrator 6 miU/mi (N = 16) Mean values (strun

2nd nun

3" run

Average value 0.050 0.005

the absence of specific analyse.

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

A diagnostic specificity of 98,6% was assessed.

Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients diffected by non-viral hepatic diseases, patients diffected by non-viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipening, etc.) against the European company. A value of specificity of 100% was assessed.
Finally, both human plasma, derived with different standard techniques of preparation (cirtate, EDTA and heparin), and human seria have been used to determine the specificity.

No laise 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 preserve of specific analyte.

10s vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.

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

Calibrator 250 mlU/mi (N = 16) Mean values 1st run 3.526 0.137 3.457 0.143 2nd run 3,499 0,162 4.6 3" run agenow

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

4. ACCURACY
The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was rufed out up to 10,000 mill/limit.

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.

- Englatif et al., Jimmunobenisty, 8, 871-874, 1971.
 Englatif et al., Jimmunobenisty, 8, 871-874, 1971.
 Englatif et al., Jimmunol, 109, 129-135, 1971.
 Remington J.S. and Klein, J.O. in "Infectious diseases of the fets and newborn infant". Sanders, Philadelphia, London, Toonson, 100 rose, 100 rose,

- 5. Sindhand JR, et al., Ann.Int.Med., 83 : 838, 1975.
 5. Barker LE., Dodd R.J., Sandler S.G., in "viral Heparitis: babratory and Clinical Science F. Epoinhardt, J. Deinhardt et s. M. Deker Inc., New York, 215-230, 1983.
 7. Cossart Y., Britt Med Bull., 28: 158, 1972.
 8. Mashawar I.K. et al., Jurmunol., 105: 1055, 1971.
 9. Mushawar I.K. et al., Jurmunol., 105: 1055, 1974.
 9. Mushawar I.K. et al., Ann.J.Clin-Pathol., 76; 773, 1991.
 10. Howard C.R., Immunol. Inday, 5: 155, 1984.
 11. Auch R.D., Lamoet 7874, 190-193, 1974.
 12. Jilg W. et al., J. Hepatol 9: 201-207, 1988.
 13. P. Crovari et et, Boll Ist. Sieroter, Milan., 63: 14-16, 1984.
 14. M.Davidson et al., J.Natl.Cancer Inst., 59: 1451-1467, 1977.
 15. F. Soyorkey et al., J.Natl.Cancer Inst., 59: 1451-1467, 1977.
 16. S. Hadler et al., N.E.J.Med., 315: 209-274, 1986.
 7. J.H. Hochraglie et al., Hepatology, 7: 758-763, 1987.
 19. W.Lig et al., J. Hapatol., 6: 201-207, 1988.
 20. P. Michel et al., Nephrologie, 7: 114-117, 1986.
 21. W. Szmuness et al., N.E.J.Med., 301: 383-385, 1980.
 22. M. Szmuness et al., N.E.J.Med., 301: 383-385, 1980.
 23. A.J.Cuskerman et al., in "Hepatitis Viruses of Man" Academic Press, London, 1979.

All the IVB 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 nº 27 – Scito San Giovanni (Mf) - Italy

0318

CV IQN

the quantitative/qualitative determination Enzyme ImmunoAssay (ELISA) for human serum and plasma of IgM antibodies to **Hepatitis C Virus**

for "in vitro" diagnostic use only -



DIA.PRO

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(Milano) - Italy Phone +39 02 27007161

REF CVM.CE

HCV igM

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A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM antibodies to Hepatitis C Virus in human plasma and sera. The kit is mainly intended for the follow-up of HCV chonic patients submitted to anti-viral pharmaceutical treatment. For 'in vitro' diagnostic use only.

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

of patients. Interferon combined with Ribavirin is effective in about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone. Treatment with interferon alone is effective in about 10% to 20%

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 sufference and cellular damage of the liver. During the pharmaceutical treatment HCV IgM may represent a marker for the illow-up of the efficiency of the drug itself, monitoring the balance between its effectiveness and the side effects, that often may be heavy for the passent.

C. PRINCIPLE OF THE TEST

Microplates are coated with HCV immunodominant synthetic antigens (core peptide, recombinant NSS, NS4 and NS5 peptides). In the 1st incubation, the solid phase is treated with dulled samples and anti-HCV lgM are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2st incubation bound anti-HCV lgM are detected by the addition of anti-HgM antibody, labeled with peroxidase (HRP). The entryme captured on the solid phase, acting on the sufferdichromogen mixture, generates an optical signal that is proportional to the amount of anti-HCV lgM antibodies 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

Neutralization of tgG antLHCV, carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

D. COMPONENTS

Each kit contains sufficient reagents to perform 95 tests.

Microplate: MICROPLATE
 Stips x 8 microwells coated with HCV-specific synthetic antigens (core, NS4 and NS5 peptides and recombinant NS3). Plates are sealed into a bag with desiccent.

Calibration Curve: CAL N° ...
 6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on an internal Gald Standard (in absence of a defined.)

It contains chemical inactivated HCV IgM positive human plasma, 100 mM Tris buffer pH 7,44-9.1, 0.2% tween 20, 0.09% sodium acide and 0.1% kathon GCs as preservatives. The Calibration Curve is coded with blue allmentary dye.

> Important Note: Even inactivated, handle If plasms has been this component as

potentially

3. Wash buffer concentrate: WASHBUF 20X1
1x80mi/hotite 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.

Rinzyme conjugate: [CON.]
 At Stankhal. Ready, to use and red colour coded, it contains that stankhal. Ready to use and red colour coded, it contains thorseradish peroxidase conjugated polyclonal antibodies to human light, 5% BSA, 10 mM Tits buffer pH 6,844.01, 1, 0.1% Kalhon GC and 0.02% gentamicine sulphate as preservatives.

5. Chromogen/Substrate: SUBS TMB
1.416m/Wals, it condains 50 mM citrate-phosphate buffer pH 3.51.416m/Wals, it condains 50 mM citrate-phosphate buffer pH 3.53.8. 4% dimethylsuphoxide, 0.03% tetra-methyl-benzidine (or
1.02% hydrogen proxide (or H2Oz).
1.02% hydrogen proxide

strong illumination.

6. Sulphuric Acid: [<u>H2SO4 0.3 M</u>]

'kt/Sm/vallt contains 0.3 M H:SOx solution,
'kt/Sm/vallt contains 0.3 M H:SOx solution,
Attention: Iritiant (4315, H319; p-280, P-902+P-382, P-332+P-313,
P-305+P-351+P-338, P-337+P-313, P-362+P-363).

7. Specimen Diluent: DILSPE

2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.2% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample,

B. Neutralizing Reagent: SOLN NEUT TX6mWhat. It contains goal anti-fligG; 2% casein, 10 mM Nativabilities buffer pH 6.0 +/-0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

9, Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
 Calinated Microphetts (1000, 100 and 10ul) and disposable plastic lips.
 ElA grade water (bidistilled or deionised, charcoel treated to 2. ElA grade water (bidistilled or deionised, charcoel treated to 3.

remove oxidizing chemicus used as disinfectants).

3. Timer with 60 minute range or higher.

4. Assorbent paper tissues.

5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at *37°C(+4).67°C belance).

6. Calibrated ELISA microwall reader with 450nm (reading) and with £20.950nm (blanking) filters.

7. Calibrated ELISA microwall washer.

8. 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 technical personnel only.

doctor responsible of the laboratory,

2. All the personnel involved in performing the assay have to
wear protective laboratory clothes, tale-free gloves and glasses.
The user of any strator (needles) or cutting (blades) devices
should be avoided. All the personnel involved should be trained
in bloaslety procedures, as recommended by the Center for
Disease Control, Atlanta, U.S. and reported in the National
Institute of Health's publication: "Bloaslety in Microbiological and
Biomedical Laboratories", ed. 1984.

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

4. The laboratory environment should be controlled so as to swell contaminants such as dust or six-born -microbins agreets, when opening it value and microplates and when performing the last. Protect the Chromogen/Substrate (or TMB) from strong light and solid vibration of the bench surface where the test is undertaken.

Upon receipt, slore the kit at 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 controlled refrigerator or cold room.

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

crass-confamination between

samples by using disposable tips and changing them after each

 Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted disposable tips Avoid cross-contamination between kit reagents by using yosable tips and changing them between the use of each

on an opened kit did not pointed out any relevant loss of octivity up to six 6 uses of the device and up to 6 months.

11. Treat all specimens as optentially inhective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S., in compliance with what reported in the Institutes of Health's publications. "Biosafety in Microbiological and Biomedical shortwines."

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 Laboratories", ed. 1984. 12. The use of disposa

cross containmation.

13. Waster 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 paticular, liquid waste generated from the wasting procedure, from residuats of controls/cabinations and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of household bleach for 15-18 has or heat inactivation by autoclave

at 121°C for 20 min.

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

15. The Subhuric Acid is an intilant. In case of spills, wash the surface with planty of water.

16. Other waste materials generated from the use of the kit example: tips 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 dechanques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with cirate. EDTA and beparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and

electronic reading is strongly recommended.

3. Haernolysed (Ted') and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results.

Samples containing residues of fibrin or heavy particles or microbial filiaments and bodies should be discarded as they could give rise to false results.

4. Sera and plasma can be stored at *2", 8°C for up to five days after-collection—Furningur-storage-patieds—Samples Gain-Bu stored fouzer at "20"C for several monities. Any foozer assumples should not be inczent/hawed more than once as this may generate particles that could affect the last result.

If bendices are present, centrifuge at 2.000 tpm for 20 min or 5. If particles are present, centrifuge at 2,000 rpm for 20 min or filter using 0,2-0,8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desicrant is not urned 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 desicant supplied, firmly atpeed and stored at +2...8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desicant bag turns from

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 bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles. Note: Once diluted, the wash solution is stable for 1 week of 12.8°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.

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, possibly sterile disposable containers, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and

sterile disposable container

If this compan

tent has to be transferred use only plastic, possibly

metallic surfaces.

Ready to use component. Mix carefully on vortex before use

Ready to use component. Mix carefully on vortex before use Neutraling Reagent

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

H319 - Causes serious eye inflation Warning H statements: Causes skin irritation

P280 — Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 — IF ON SKIN: Wash with plenty of soap and Precautionary P statements

- If skin

to do. Continue rinsing. P337 + P313 - II advice/attention P362 + P363 — Take before reuse P351 + P338 - IF IN EYES, Rinse causiously with water weral minutes. Remove contact lenses, if present and casy off contaminated clothing and wash eye

irritation persists: Get

medica

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I, INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamnation (household alcoho), 10% solution of bleach, hospital grade distributions) of those parts that of spills or residues of kit components should also be carried 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

out regularly.

Out regularly.

Out 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 carefully validated and correctly optimised using the kit controls/calibrators and reference pensits, before using the kit for routine laboratory tests, Lisually 4.5 washing cycles (aspiration + dispensation of 350u/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 supposted to refer to set orrectly their number, it is recommended to tun an assay with the kit controls/calibrators and well characterized negative and positive reference samples, and check to match the values exported below in the section finamal Quality Control. Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer have to be carried out according to the instructions of the manufacturer.

4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a seading filter of 450m and with a second filter (620-630m, strongly recommended) for blanking purposes. Its standard performances should be (a) barnwidth ≤ 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.

regularly serviced in order to match the values reported in the section "internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader, in addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensation and washing, has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensation of the station with the station of the station and the station of the stati When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, data steps (dispensation, incubation, washing, reading, data steps (dispensation) have to be carefully set, calibrated, controlled and handling) have to be carefully set, calibrated, controlled and

requirements_described_Support_is_also_provideri_fer_fer_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 of er). Do not use if expired on the externa

label (primary container). Do not use if expl Check that the liquid components are not

visible particles or aggregates,
Check that the Chromogen/Substrate is colourless or pale
blue by aspirating a small volume of it with a sterile plastic

Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.

Dilute all the content of the 20x concentrated Wash Solution

as described above,

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

reagents.

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

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

unred on at least 20 minutes before reading.

9. If using an automated work sation, turn on, check satings and be sure to use the right assay producol.

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

QUANTITATIVE ASSAY

Place the required number of strips in the plastic holder and

carefully identify the wells for calibrators and samples.

Dilute samples 1:101 dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before

use. Do not difful the Calibrators as they are ready-to-use. Leave the A1+91 wells emply for blanking purposes. Discense 50 µI houtlaining Reagent 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 Calibrators in duplicate followed by 100 µl 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

In all the wells, except AT+BT, Conjugate. Incubate the microplate pipette 100 µi Enzyme e for 60 min at +37°C

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

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- microwells as previously described (section 1.3)

 Pipette 100 µl Chromogen/Substrate into all the wells the second incubation <u>6</u>; finished, wash the

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

- 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.

 12. Pipette 100 µl Sulphuric Acid into all the wells using the same pipeting 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 both.

- M.2 QUALITATIVE ASSAY
 Place the required number of strips in the plastic holder and carefully dentify the wells for califorators and samples.
 Diffue samples 1:101 dispensing 1 ml Sample Diffuent into a disposable tube and then 10 ut sample; mix on vortex before use, Do not diffue the Calibrators as they are ready-to-use,
 Leave the A1 well empty for blanking purposes.
 Dispense 50 µl Neutraizing Reagent in all the wells, except the Calibrators.
- the Calibrators.
 Then pipette 100 µl of Calibrator 0 arbU/ml in duplicate,
 —100-pl of Calibrator 10 arbU/ml in duplicate and finally 100
 µl 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 1.3)
 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 1.3)

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.

- 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.

 12. Pipette 100 µl Sulphuric Acid into all the walls using the ——same pipeting 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 I.5 using a 450nm titler (reading) and a

620-630nm filter (background subtraction recommended);-blanking-the-instrument or A1;

General important notes:

- If the second filter is not available onsure that no finger prints are present on the bottom of the microwell before reading at 450mm. Finger prints could general lake positive results on reading prints from the addition of the Reading has to be carried out just after the addition of the TMB after its addition. Some self-oxidation of the TMB after its addition. Some self-oxidation of the TMB chromogen can occur leading to high background

N. ASSAY SCHEME

| Reading OD | Sulphuric Acid | Temperature | 3" incubation | TMB/H2O2 | Wash step | Temperature | 2 incubation | Enzyme conjugate | Wash step | Temperature | 1 th Incubation | Samples diluted 1:101 | Calibrators (no SOLN NEUT I) | Neutralizing Reagent | Wethod |
|------------|----------------|-------------|---------------|----------|------------|-------------|--------------------------|------------------|------------|-------------|----------------------------|-----------------------|------------------------------|----------------------|------------|
| 450nm | 100 ul | r.t. | 20 min | 100 µ | 4-5 cycles | +37°C | 60 min | IU 001 | 4-5 cycles | +37°C | 60 min | 100 ш | 100 μ | - | Operations |

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

| b., | × | Ø | O | O | m | m | 0 | Ξ | ď |
|-----|------|-----|------|-------|------|------|------|------|----------------------------|
| | BLK | BLK | CALT | CAL1 | CAL2 | CAL2 | CALS | CALS | enda. |
| 2 | CAL4 | | 777 | 172.5 | 1 | CALS | | | Legenda: BIK = Blank // CA |
| 3 | S3 | S4 | 25 | 88 | S7 | S8 | SB | \$10 | 306 |
| 4 | | | | | | | | | 2 |
| Óì | | | | | | | | | - |
| G) | | | | | | | | | dispersion of |
| 7 | | | | | | | | | |
| 00 | | | | | | Ü | | | 2 2 |
| £ | | | | | | | | ij | |
| 10 | | | | | | | | | 2 |
| | | | | | | | | | I |
| 53 | 1 | T | 1 | 1 | | 1 | 1 | T | I |

An example of dispensation scheme in qualitative assays

| | A | œ | C | 0 | E (| m | 0 | Ξ |
|-----|-----|------|------|------|------|----|-----|-----|
| | BLK | CALT | CALT | CAL2 | CAL2 | S | SZ | \$3 |
| 2 | 84 | SS | S6 | S7 | SS. | 88 | 510 | SI |
| 3 | | | | | l | | | |
| 4 | | | | | I | | | |
| cn | | | | | | | | |
| m | | | | | Ц | | | |
| 7 | | | | | | | | |
| œ | | | | | | | | 1 |
| t,O | | | 1 | 1 | 1 | | | |
| 3 | | | | | | | | |
| | | | | | | | | |
| 12 | | | | 1 | | | | |

Strongly vignories

| Neutralizing Reagent | 50 µl |
|-----------------------|------------|
| samples diluted 1:101 | 100 1 |
| " incubation | 60 min |
| emperature | +37°C |
| Vash step | 4-5 cycles |
| nzyme conjugate | 100 Jul |
| incubation | 60 min |
| emperature | +37°C |
| Vash step | 4-5 cycles |
| MB/H2O2 | 100 M |
| incubation | 20 min |
| emperature | 1.1 |
| Sulphuric Acid | 100 H |
| Reading OD | 450nm |

Calibrator 10 arbU/ml

t. that the procedure has been correctly enzyme conjugate 5, that the waster needles are not blocked or the enzyme conjugate:

5. that micropipottos have not become contaminated with positive samples or with the

Microplate

| | | | arc. Diamonifi | 250 arbU/ml > 3.500 OD450nm | | | 250 arbU/ml < 2,000 OD450nm | | | < CAL 0 + 0,100 | 10 arbU/ml |
|--|---|---|--|---|--|--|--|--|---|-----------------|------------|
| enzyme conjugate 6. that the washer needles are not blocked or partially obstructed. | the enzyme conjugate; 5. that micropipeties have not become contaminated with positive samples or with the | procedure: 4. that no contamination of the Cal 250 arbU/ml, or of the wells where this was dispensed, has occurred due to positive samples, to spits or to | intel the proper washing solution has been used and the washer has been primed with it before use; that no mistake has been done in the assay | 1 that the washing procedure and the washer settings are as validated in the pre qualification study: | that no external contamination of the calibrator has occurred. | distribution of the califoration. 3. that he wasting procedure and the waster settings are as validated in the pre qualification study. | that the procedure has been correctly performed; | that no external contamination of the calibrator has occurred. | inat the washing procedure and the washer settings are as validated in the pre-qualification study: | distribution; | ietaka |

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

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

| Calibrator 3,500 : 250 arbU/ml | Calibrator OD450nm : 10 arbU/ml 0.100 | Calibrator < 0.20 | Blank well < 0.10 | Parameter |
|-----------------------------------|--|--------------------------------|-------------------|--------------|
| 1,500 > CD450nm > 2,00 | | < 0.200 OD450nm after blanking | QD450nm | Requirements |
| 10 | > OD450nm CAL 0 arbU/m | olanking | | nents |

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)

If the test turns out to be valid, interpretation of results is carried out in the quantitative assay from the mean OD450nm value of the Calibration Curve elaborated with an appropriate curve

P. RESHLTS

litting system (suggested : 4 parameters).

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

correct interpretation of results.

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

following checks

Q. INTERPRETATION OF RESULTS

Q.1 QUANTITATIVE ASSAY

Problem

Blank well

> 0.100 OD450nm

Calibrator
0 arbUml
> 0.200 OD450nm

that the Chromogen/Substrate solution has not become contaminated during the assay
 that the washing procedure and the washer settings are as validated in the pre qualification.

Subjy.

2. that the proper washing solution has been used and the washer has been primed with it before use:

3. that no michae' has been done in the assay procedure (dispersation of positive calibrators Concentrations in ad-Ulmi are obtained elaborating OD450nm of samples on the fitted calibration curve.

The concentration of IgM is from Literature correlated proportionally with the liver damage produced by antibodies to HCV upon virus replication in hepatocities.

A decrease in IgM concentration upon pharmacological treatment is usually officially acknowledged as a sign of recovery and therapeutic efficacy.

Q.2 QUALITATIVE ASSAY

procedure (dispensation of positive calibrators instead of Call O arbUm);
4. that no contamination of the Call 0 arbUm, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to

following lable: rest results are interpreted as a ratio of the sample OD450nm ratio (S) and the cut-off value (Co), or S/Co, according to the

| · · · | ^1. | S/Co |
|------------|------------|------------------|
| 0 Positive | 0 Negative | o Interpretation |

A negative result indicates that the patient has not developed lgM antibodies to HCV. positive result is indicative of an ongoing HCV active infection

Important notes:

- 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 to another facility, attention must be paid to avoid erroneous data transfer.
- Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

 The results of this ELISA assay should be implemented with other diagnostic and clinical tests, anyway
- An example of calculation is reported below.

The following data must not be used instead or real figures

Lower than 0:200 - Accepted Mean Value: 0.060 - 0.080 OD450nm 0.070 OD450nm

CAL 2: Mean Value 0.210 OD450nm Higher than CAL1+0.100 = accepted 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.

1. Limit of detection

No international standard for HCV IgM Antibody detection has been defined so far by the European Community. In its absence, an internal Gald 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

Soft- plearna, derived with different standard techniques of preparation (citrate, EDTA and hepatri), and sera have been used to determine the specificity. No dise reactivity due to the method of specimen preparation has been observed. Frozen specimens have also been tested to check whether samples freezing interfers with the performance of the test. No interference was observed on clean and particle free

| Speci | Sensi |
|--------|--------|
| ficity | tivity |
| | - |
| v | V |
| > 98 | > 98 |

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Reproducibility:
 Thas been calculated on two samples examined in replicates in different runs. Results are reported below summarized in a different runs.

| CV % | Std. Deviation | OD450nm | Average values N = 48 |
|------|----------------|---------|--|
| 11.3 | 0.027 | 0.241 | Calibrator 2 10 arbU/ml |
| 6.9 | 0.113 | 1.632 | Calibrator 2 Calibrator5 10 arbU/ml 100 arbU/ml |

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, per-tistled positive with an analytical system developed in house by the clinical laboratory where the study was conducted. Positive samples were collected from patients with a clinical history of HCV Infection (acute and chronic).

In addition some Senoconversion Panels, purchased from Bostion 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 anti-HCV antibodies with the reference kit in use in the laboratory, including potentially

Interfering specimens.
A panet of potentially interfering samples (RF+, bemolised, lipemic, etc.) was also examined. No interference was observed

on the samples examined. Both plasma, derived wi

samples.
The Performance Evaluation provided the following values:

1

0318

S. LIMITATIONS

False positivity has been assessed on less than 2% of the normal population, mostly due to high titers of RF.

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

BIBLIOGRAPHY

1. Krasavisev FL Zhavoronck SV Milsura VM Demohito AP.
Zh Mikrobiol Epidemiol Immunobiol, 2006 Mar-Apr.(2):57-51

Papatheodoridis GV, Delladetsima JK, Katsoulidou A, Sypsa V, Albrecht M, Michel G, Hatzakis A, Tassopoulos NC. J Hepatol. 1997 Jul;27(1):36-41;

3. Martinelli AL, Brown D, Braun HB, Michel G, Dusheiko GM, J Hepatol. 1996 Jan;24(1):21-6.

Pawlotsky JM, Darthuy F, Ramine J, Pellet C, Udin L, Stuyver L, Roudol-Thoravel F, Duvoux C, Douvin C, Mallat A, et al. J Med Virol, 1995 Nov;47(3):285-91.

Stransky J, Honzakova E, Vandasova J, Horejsova M, Kynol J, Nemecek V, Horak J. Acta Virol. 1996 Apr;40(2):61-5.

6. Nikolawa LI, Blokhina NP, Tsurikova NN, Voronkova NV, Mirrinoshvili MI, Braginsky DM, Yastrebova ON, Booynitskaya OB, Isaeva OV, Michailov MI, Archakov AI, Gut. 2000 Nov;47(5):598-702.

7. Bizollon T. Ahmed SN, Guichard S, Chevallier P, Adham M, Ducert C. Baulleux J, Trepo C. J Med Virol. 1998 Nov;56(3):224-9,

Tran A, Yang G, Dreyfus G, Rouquie P, Durant J, Rampal A, Rampal P, Benzaken S, Am J Gastroenterol. 1997 Oct;92(10):1835-8.

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 if conforming with the EC technical specifications and acceptance criteria.

ICV 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

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(Milano) - Italy

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

HCV Ab

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A. INTENDED

Version 4.0 Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatilis 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.

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

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

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

relative mutability of its genome, which in turn is probably related to the high properatly (60%) of inducing chronic infection. HCV is clustered into several distinct, grouppes 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 E), which together account for the vast majority of cases of viral hepatitis. It is an enveloped RNA virus in the fluviridae family which appears to have a narrow host disease and the response to treatment. Hepatitis C virus (HCV) is one of the viruses (A, B, C, D, and

infection leads to liver cancer are still unclear. Hepatitis C also exacerbates the sevenity of underlying liver disease when it coexists with other hepatic conditions. In particular, liver disease progresses more rapidly among persons with develops in 1% to 5% of persons with chonic infaction over a period of 25 to 30 years. Most patients suffering from liver cancer who do not have hepatitis B virus infection have evidence of HCV infaction. The mechanisms by which HCV symptomatic, About 80% of newly infected patients progress to develop chronic infection. Chribosis develops in about 10% to 20% of persons with chronic infection, and liver cancer clinical symptoms ranges from 15 to 150 days. In acute infections, the most common symptoms are faigue and pandice: however, the majority of cases between 60% and 70%),—even those that develop chronic infection, are a The incubation period of HCV infection before the onset of

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

blood products. In many developing countries, where unscreened blood and blood products are still being used, the major means of transmission are unsterilized injection 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 chomot CLV infection are current and farmer injecting drug users and those with a history of transfusion of unscreened blood or In hoth developed and developing countries, high risk groups practices are at risk if they use or re-use unsterdized tools. equipment and unscreened blood transfusions; In addition, people who use traditional scarification and circumcision include injecting drug users, recipients of unscreened blood

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

the confirmation of a positive EIA result. Testing for HCV circulating by amplification tests 18th (e.g. polymerase chain reaction or PCR, branched DNA assay) is also being utilized to confirmation of seedulgical results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the presence of active infection and a potential for spread of the infection and or/the development of chronic fiver disease. Inggnostic tests to the clinical diagnosts and to make better decisions regarding medical management of a patient. Diagnostic tests medical management of a patient. Diagnostic tests commercially available today are based on Enzyme immunosachent assays (BIA) for the detection of HCV specific antibodies. RIAs can detect more than 95% of chronically infected patients but can detect noted by 50% to 70% of scutte infections. A recombinant immunobable assay (RIBA) that identifies antibodies which react with individual HCV Diagnostic tests for HCV are used to prevent infection

about 10% to 20% of patients, Interferon combined with ribavirin is effective in about 30% to 50% of patients. Antiviral drugs such as interferon taken alone or in combination with ribavirin, can be used for the treatment of persons with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in Antiviral drugs such as interferon Ribaxarin_does_not-appหมา-ฉ-bc-offective_when-used-alone

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

control practices in health care settings, including appropriate servilization of medical and dental equipment:
(d) promotion of behaviour change among the general public and health care workers to reduce overnee of injections and to use safe injection practices and do Risk reduction counselling for persons with high-risk days and sexual Some studies however, have shown the presence of virus montralizing antibodies in patients with HCV infection. In the bisection of virus models to prevent infection the bisection of virus including of blood and organ denotes (b) Virus inactivation of plasma derived products (c) implementation and maintonance of infection control practices in health one settings, including practices

processing.
The nucleocapsid-encoding region and encodes for two o envelape dived in the r structural components, a nucleocapsid elope glycoproteins, and functional in the virus replication and protein

onservative among the isolates obtained all over the world.

C. PRINCIPLE OF THE TEST

Micropiates are constant with HCV-specific antigens derived from force and in the regions encoding for conservative and immunodominant antigents determinants (Core peptide, recombinant NS3, NS4 and NS5 peptides).

The solid phase is first treated with the dutated sample and HCV Ab are captured, if present, by the antigens, and the sample, in the 2"incubation bound HCV antibodies, ligG and lgM as well, are detected by the addition of polyclonia specific anti-higG&M antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/principle in the control device should be amount of anti-HCV antibodies gresent in the substrate/principle in the control devices an optical signal that is proportional to the amount of anti-HCV antibodies present in the sample. A nut-off value for optical derivative should be supported into HCV antibody negative and positive results.

D. COMPONENTS

Code CVAB CE contains reagents for 192 to

Microplate MICROPLATE
 ** 2 microplates
 ** 2 microplates
 ** 2 microplates
 ** 2 microplates
 ** 3 microwells coated with Core peptide, recombinant NS3, NS4 and NS5 peptides. Plates are sealed into a bag with NS3, NS4 and NS5 peptides.

2. Negative Control CONTROL 1 contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 5.0 +4.0.1, 0.5% Tween 20. 15.9% Nacadde and 11.1% Kathon GC as preservatives. The negative control is olive green colour coded.

3. Positive Control CONTROL +
154.0mt/vial. Ready to use control. It contains 1% goat serum
proteins, human antibodies positive to HCV, 10 mM Na-citate
buffer pH 6.0 +4-0.1, 0.5% Tween 20, 0.09% Na-acide and 0.1%
Kathori GC as preservatives. The Positive Control is blue colour

4. Calibrator n° 2 viais. Ly n*2 viais. Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label. In contains betal bowine serum proteins, human antibodies to HCV whose content is calibrated on the NIBSC Working Standard code 99/588-003-WII, 10 mM Nacitate buffer pH 60 +/-0,1,0,3 mg/ml gentamicine sulphate and 0.1% Kathon GC as ator CAL

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

wash solution 0.05% Tween nl/bottle. 20x concentrated solution. Once diluted, the solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 6 Tween 20 and 0,05% Kathon GC.

6. Enzyme Conjugate CONJ Zx16mi/vial. Ready to use and pink/red colour coded reagen. It contains Horseradish Peroxidase ponjugated goat polydoral antibodies to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1. 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate SUBS TMB|
2x16nl/vial. Ready-to-use component. It contains 50
mM chrate-phosphate buffer pt 3.5-3.6, 4% dimethylsulphoxide,
0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen
perovide or H2O2.

Note: To be stored protected from light as sensitive to

 Assay Diluent DILAS
 1x15ml/vial. 10 mM tris buffered solution containing 0.1% Kalhon GC for the pre-trea and controls in the plate, blocking interference, e pre-treatment 1 8.0 +/-0.1 1 of samples

9. Sulphuric Acid <u>FESOr. D.3 M</u> 1x32m/botte. It contains 0.3 M H43O4 solution. Attention: Inflant (H315; H319; P280; P202+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Sample Diluent:

2x30m/bottle. It contains 1% goat serum proteins, 10 mM.
Na-citate buffer pH 6,0 4-4.1, 0.5% Tween 20, 0.09% Na-azide
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

11. Plate sealing foils n° 4

Package insert ٦° ۲

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

| 2 | | _ | _ | 9 | 00 | 7 | 0 | ch | 4 | 44 | N | 144 |
|----------------|---------------|--------------------|--|--|--|----------------|----------------|-----------------|---|---|--|--|
| umber of tests | 2 Pack insert | 1 Plate seal foils | 0.SampleDiluent | Sulphuric Acid | Assay Diluent | Chromog/Subs | Enz, Conjugate | Wash buff conc | Calibrator | PosttveControl | .NegativeControl | Micropiate |
| 96 | ກໍ | n° 2 | 1x50ml/vial | 1x15ml/vial | 1x8ml/vial | 1x16ml/vial | 1x16ml/vial | 1x60ml/bottle | nº 1 vial | NZ.Qmirvai | 1x2,0ml/vial | n°1 |
| 480 | n° 1 | n° 10 | 5x50ml/bottles | 2x40ml/bottle | 1x40ml/bottle | 2x40ml/bollles | 2x40ml/bollles | 5x60ml/boilles | n° 5 vials | Tx10ml/vial | 1x10ml/vial | ສິຕິ |
| 980 | 2,1 | n° 20 | 4x125ml/boliles | 2x80ml/battles | 1x80ml/boite | 4x40ml/boldes | 4x40ml/boldes | 4×150mi/bottles | n° 10 vials | 1x20.ml/vial | 1x20_ml/vial | n°10 |
| | 480 | n° 1 n° 1 n° 1 | n° 1 0 0° 10 | 1 1x50ml/hall 5x50ml/bottles n° 1 n° 1 n° 1 n° 1 480 | 1x15milvial 2x40milbottle 1x15milvial 5x50milbottles 5x50milvial 5x50milbottles n° 2 n° 1 n° 1 n° 1 n° 1 480 | XiSm/Wai | Xi.familyoid | X46m/kval | X86m/bodie Sessembonies X16m/vai Zw46m/bodies X46m/vai Zw46m/bodies Xx6m/vai Zw46m/bodies Xx6m/vai Zw46m/bodies Xx6m/vai Zw46m/bodies Xx5m/vai Zw46m/bodies | n*1 Vails 1x50m/bode 1x50m/bode 1x5m/vail 2x40m/bodies 1x5m/vail 2x40m/bodies 1x5m/vail 1x40m/bodies 1x5m/vail 1x5m/vail 1x50m/bodies 1x5m/vail 1x50m/bodies 3x5m/vail 1x50m/bodies 3x5m/vail 3x50m/bodies 3x7 0 n' 1 1x50m/bodies 4x00m/bodies 4x00m/bodies | n 1 val n 75 vals n 1 val n 75 vals n 1 val n 75 vals t 1 x6nmbottle 5x6nmbottles 2x46nmbottle 5x40nmbottles 1x6nmbottle 2x40nmbottles 1x6nmbottle 2x40nmbottles 1x6nmbottle 2x40nmbottles 1x6nmbottle 2x40nmbottles 1x15nmbottle 2x40nmbottles 1x15nmbottle 2x40nmbottles 1x15nmbottle 2x40nmbottles 1x15nmbottles 1x | de l'Az-Domivial (X. Inmivial (|

REQUIRED BUT NOT

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| BUT NOT PROVIDED | (200ch and 10ut) and disposable

remove oxidizing chemicals used as disinfectants)
Timer with 60 minute range or higher, EIA grade water (bidistilled or deionised, charcoal treated to

before waste. Suggested procedures of inactivation are ireatment with 70% mild procedures of inactivation are ireatment with 70% mild procedure at 121°C for 20 min., 15. Accidental spits from samples and operations have to be adsorted with paper tissues soaked with nousehold bleach and then with water, Tissues should then be discarded in proper containers designated for lapsoratorythospital waste, 16. The Sulphurc Acid is an irritant, in case of spits, wash the enteres with instructions and the surface with instructions and the surface with instructions and the surface with instructions are supported to the surface with instructions and the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instructions are sufficiently as the surface with instruction of the surface with instru

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Absorbent paper tissues.

Calibrated ELISA microplate thermostatic incubator

capable

<u>ب</u> With 450nm (reading)

to provide a temperature of +37°C.

6. Calibrated ELISA microwell reader will and with 620-630nm (blanking) filters, Calibrated ELISA microplate washer.

7. Calibrated ELISA microplate washer.

8. 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 acctor 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 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 his type of analysis.

3. All the presonnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or culting (taldes) devices should be avoided. All the personnel involved should be trained in blosslety procedures, as recommended by the Center for Disease Control, Alaina, U.S. and reported in the National Institute of Health's publication: "Blossfety in Microbiological and Brinnelfred!"

safe and effective 5. The laborate Biomedical Laboratories", ed. 1984.

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

5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vals and micropiates and when performing the test. Protect the Chromogen/Substrate 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 trolled refrigerator or cold room.

 Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged

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

 Avoir cross-conformination between serum/plasma samples by using disposable tips and changing them after each sample. Avoid tips and changing them between between reagents by using in the use of each

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

12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosately Level 2, as recommended by the Center for Disease Control, Maharla U.S. in compliance with what reported in the Institutes of Health's publication. Biosately in Microbiological and Biomedical Laboratories, ed. 1984.

use of disposable plastic-ware is recommended in the on of the liquid components or in transferring ants 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 wasting procedure, from residuals of confuss and from samples has to be treated as potentially infective material and inactivated

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

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

17. Other waste materials generated from the use of the

surface with plenty of water

1. Slood is drawn aseptically by verilpuricture and plasma or sarum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA

and heparin.

2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation or results. When the kit is used for the screening of blood units, bar code labeling and best of the screening of blood units.

electronic reading is strongly recommended.

4. Haemolysed (red) and visibly hyperiplemic ("mility") 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. Seria and plasma can be storied at +2*-8*°C for up to seven days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozen/thewed more than once as this may generate particles that could affect the test result.

6. If particles are present, controlling at 2.000 rgm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

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

1. Microplates:
Allow the microplate to reach room lemperature (about 1 hr) before opening the container. Check that the desiccent is not turned to dark green_indicating a defect of manufacturing.

In this case call Dia Pro's customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow

2. 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.

Dissolve carefully the content of volume of EIA grade water reporte Mix well on vortex before use: reported on its label. the

as potentially y infective e even if HCV

Note: When dissolved the Calibrator is not stable. Store

5. Wash buffer concentrate:

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

 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

Ready to use. Mix well on vortex before use. Be careful not to contaminate the liquid with oxidizing chemicals 7. Chromogen/Substrate: Ready to use, Mix well on vortex before Do not expose to strong illumination, oxidizing agents and metallic surfaces. If this comparient has to be transferred use only plastic, possible driven dust or microbes

8. Assay Diluent: Ready to use. Mix well on vortex before use.

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

Precautionary P statements:

P280 — Wear protective gloves/protective dorbing/eye protection/face protection.

P281 — P382 — FP ON SKNN: Weak with plenty of scap and water.

P382 + P313 — If also initiation occurs (ad medical shriocalisation.

P385 + P381 + P383 — IF NR PYES: Rive occurred with water for several minutes. Remove contact lesses, it present and dary to do.

Continue rinsing.

P337 + P313 - If eye initiation persists: Gel medical advice/attention.

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

Sample Diluent

Ready to use, Mix well on vortex before use

WITH THE KIT I. INSTRUMENTS AND TOOLS USED IN COMBINATION

- Micropipeties have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular deconfarmation (household alcoha), 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
- The ELIXA inclubator has to be set at +37°C (tolerance of +/0.5°C) and regularly checked to ensure the correct
 temperature is markaned. Both dy incubators and water
 baths are suitable for the incubations, provided that the
 markument is validated for the incubation of ELIXA tests.
 The ELIXA washer is extremely important to the overall
 performances of the assay. The washer-must be carefully

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 soeking time validated and correctly optimized using the kit controls and e using the kit

- of 20-30 seconds between cycles is suggested in order to set contractly their number, it is recommended to run an assay with the lat cuntrols and well characterized hegative and positive reference samples, and check to match the values reported below in the sections. Validation of the values reported below in the sections. Validation of the values reported below in the sections. Validation of the values reported below in the sections. Validation of the values reported below in the section section of the values reported below in the section and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

 4. Including the reader has to be equipped with a reading filter of 450m and with a second filter (620-630m, sistingly) recommended) for blanking purposes, its standard performances should be (a) bendulath. < 10 mm. (b) absorbance range from 0 to 2.00 (c) linearly to 2.00 (d) repeatability 2 1%. Blanking is carried out on the well destribed 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 mainteined according to the manufacturer 's inverteriories." When using
- B. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data harding) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O Thernal 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, he input handling part of the station (dispensation and washing) has to be validated and controller he needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of consmittation of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.

 7. When using automatic devices in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label mem with the same label peaked of from the original vial. This operation is important in order to avoid mismatching contents of visits, when transferring them. When the test is over, return the secondary labeled containers to 2.8°C, firmly remortal.
- firmly capsed.

 Dia Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit. In order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

- L. PRE ASSAY CONTROLS AND OPERATIONS

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

 2. Check that the liquid components are not contaminated by naked-eye visitie particles or aggregates. Check that the Chromogen/Substrate is colories or pale blue by septrating a small volume of it with a sterile transparent plastic pipette. Check that no breakage cocurred in transportation are no spillage of highes the sterile transparent plastic pipette. Office that no breakage cocurred in transportation are no spillage of highes the sterile transportation are no spillage of highes the sterile transportation are not spillage of highes the sterile transportation are not spillage of highes the sterile transportation are not spillage of highes the spillage of high spillage of highes the spillage of highes the spillage of high spillage of highes the spillage of high spillage of highes the spillage of high spillage of hig
- or damaged.

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

 as described above.

 Dissolve the Calibrator as described above.

 Allow all the other components to reach room temperature (about 4 th) and then mix as described.

- for routine laboratory

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. It check that all the other equipment is available and ready. 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

M. ASSAY PROCEDURE

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

All the mixture is then carefully dispensed directly into the appropriate sample well of the mixturale. Before the next sample is applicate, needles have to be duly washed to avoid

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

- 12
- Disperse 200 ul of Negative Control in triplicate, 200 ul Calibrator in duplicate and 200 ul Positive Control in single in proper wells. Do not diffule Controls and Calibrator as they are pre-diuted, ready to use;
 Add 200 ul of Sample Diuent (DILSPE) to all the sample wells; then disperse 10 ul sample in each properly identified well. Mx gently the plate, avoiding overflowing and confaminating adjacent wells, in order to fully disperse the sample into its diluent.

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

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

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

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

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

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

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 colour of dispensed samples has turned to dark bluish-green while the colour of the negative control has remained ofive green.

It is strongly recommended to check that the time tap 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.

Ļ,

- Manual assay:

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

An example of dispensation scheme is reported below

solution as reported previously (section 1.3).

Pipatte 100µi Enzyme Conjugate into each walf, except the 1 bearing well, and cover with the sealer. Check that this pink/red coloured component has been dispensed in all the wash the microplate with an automatic washer delivering and aspirating 350ul/well of diluted was

asher by

- 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
- 10.0
- Pipette 100µl Chromogen/Substrate mixture into each we the blank well included. Then incubate the microplate room temperature (18-24°C) for 15 minutes.

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

- 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 furn the positive control and positive samples from blue to yellowibrown.
 Measure the colour intensity of the suition in each well, as described in section 1.5 at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on Ari.

- Important notes:

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

 Positive results or reading, and the second of the Stip Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can

- occur leading to high background.

 Shaking at 350 ±150 ppn during incubation has been proved to increase the sensitivity of the assay of about 20%. The Calibrator (CAL) does not affect the cut-off calculation and therefore the leaf results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.

N. ASSAY SCHEME

| earling OD | siphuric Acid | emperature | incubation | MB/H202 | Wash slep | emperature | " Incubation | nzyme conjugate | Vash step | emperature | " incubation | Assay Diluent (DILAS) | ontrols & Calibrator | tethod |
|------------|---------------|------------|------------|---------|------------|------------|--------------|-----------------|------------|------------|--------------|--------------------------|----------------------|------------|
| 450nm | In 601 | r.t. | 15 min | 100 ut | 4-5 cycles | +37°C | 45 min | In 00 t | 4-5 cycles | +37°C | 45 min | 200ul dil.+10ul 50 ul | 200 ut | Operations |

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| 587 | I | 9 | 71 | m | O | C | B | Þ | | |
|-----------------------|----|-----|-----|-----|----|-----|----|-----|-----|------------|
| Legendo: | Si | PC | CAL | CAL | NC | NC | NC | BLK | 1 | |
| | 89 | SS. | S7 | S6 | SS | \$2 | S3 | 52 | 2 | |
| BLK = Blank | | | | | | | | | 3 | |
| Blank | | | | | | Ī | | | 23. | |
| Z, | 15 | | | | | | | | ы | S |
| NC # Negative Control | ŀ | | | | | | | | £. | Micropiate |
| galive: | | | | | | | | | 7 | plate |
| Control | | | | Ì | | | | | 02 | |
| | | | | | | | | | 60 | |
| | | | | | | | | | 10 | |
| | | | | | | | | | | |
| | | | | | | | | | 12 | |

CAL = Calibrator PC = Positive Control S = Sample

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

| Check | Requirements |
|--------------------------|--|
| Blank well | < 0,100 OD450nm value |
| Negative Control (NC) | < 0.050 mean OD450nm value after blanking |
| Calibrator | S/Co > 1.1 |
| Positive Control | > 1.000 OD450nm value |

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

| Problem | Check |
|------------------|--|
| Blank well | 1. that the Chromogen/Sustrate solution has not |
| > 0.100 OD450mm | gol conteminated during the assay |
| Negative Control | 1. That the washing procedure and the washer |
| (NC) | sellings are as validated in the pre qualification |
| > 0,050 OD450nm | study; |
| 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 control |
| | instead of negative control; |
| | 4, that no contamination of the negative control |
| | or of their wells has occurred due to positive |
| | samples, to spills or to the enzyme conjugate; |
| | 5 that micropipettes haven't got contaminated |
| | with positive samples or with the enzyme |
| | conjugate |
| | 5, that the washer needles are not blocked or |
| | partially obstructed. |
| Calibrator | 1, that the procedure has been correctly |
| | execuled; |

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

CALCULATION OF THE CUT-OFF

he tests results are calculated by means of a cut-off value elermined with the following formula on the mean CD450nm alue of the Negative Control (NC):

NC + 0.350 = Cut-Off (Co)

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

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

Q, INTERPRETATION OF RESULTS

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

| > 1.1 Positive | 0.9 - 1.1 Equivoc | < 0.9 Negative | S/Co Interpretation |
|----------------|-------------------|----------------|---------------------|
| Ve | cal | ive | ation |

ny patient showing an equivocal result should be tested again na second sample taken 1-2 weeks later from the patient and negative result indicates that the patient has not been infected HCV or that the blood unit may be transfused.

xamined. The blood unit should not be transfused, positive result is indicative of HCV infection and therefore the attent should be treated accordingly or the blood unit should be

- interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations. Itematics and solution is a supervision to the confirmed by an attituding method capable to detect 19G and 19M antitlodies (confirmation test) before a diagnosis of viral hepatitis is

S/Co < 1,1

As proved in the Performance Evaluation of the product, the assay is able to delect seroconnersion to and HCV core antibodes earlier than some other commercial Ks. Therefore a positive result, not confirmed with these commercial Kis, does not have to be nuted out as a false positive result. The sample has to be anyway submitted to a confirmation test (supplied upon request by DiaPra srt. code CCONF)

< 1.000 OD450nm

1, that the procedure has been correctly

that no external contamination of the

2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead of control security.) 3. that he washing procedure and he washer settings are as validated in the pre qualification study.

As long as the assay is able to detect also IgM antibodies some discrepant results with other commercial products for the detection of anti-HCV antibodies - lacking anti-high conjugate in the formulation of the enzyme tracer and titlerefore missing jold reactivity - may be present. The extension positivity of the sample for antibodies to HCV should be then positivity of the sample for antibodies to HCV should be then confirmed by examining also IgM reactivity, important for the diagnosis of HCV infection.

2. that no mistaker has been done in the distribution of controls (dispersation of negative control riselead of positive control, in this case, the negative control will have an OD450nm value > 0.150, too.

When lest results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid

4 that no external contamination of the positive control has occurred: Juitate the washing-procedure and die-washer settings are as validated in the pre qualification

erroneous data transfer.

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

Mean Value Negative Control: The following data must not be used instead or real ower than 0,050 - A 0.019 - 0.020 - 0.021 OD450nm

Mean value: 0.540 OD450nm S/Co higher than 1,1 – Accepted Sample 1: 0,070 OD450nm Sample 2: 1,690 CD450nm Sample 1: VCo < 0,9 = negative Sample 2: S/Co > 1,1 = positive Lower than 0.050 – Accepted
Positive Control: 2,189 OD450nm
Higher than 1,000 – Accepted
Cut-Off = 0.020+0.350 = 0.370 Calibrator: 0.550 - 0.530 OD450nm 0.540 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).

I, LIMIT OF DETECTION

The limit of detection of the assay has been calculated by means of the British Working Standard for anti-HCV, NIBSC code 999588-003-WII. The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined

| | Nonetice placema 0.3 | 20 78 | 4× 0,7 0 | 2× 1.1 1 | 1X 2.0 2 | Factor S/Co S | Dilution Lot#1 Lo |
|--|----------------------|-------|----------|--------------|----------|---------------|-------------------|
|--|----------------------|-------|----------|--------------|----------|---------------|-------------------|

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

| Lot ID | Accurun 1 Series | · · |
|--------|---------------------|-----|
| 1201 | 3000 | |
| 0602 | 3000 | |
| 1202 | 3000 | _, |

In addition, n° 7 samples, tested positive for HCV Ab with Ortho HCV 3.0 SAVe, code 930820, lot, # EXEMS.1, were diluted HCV. Ab negative plasma in order to generate limiting dilutions and then tested again on CVAB.CE. lot, # 1202, and Ortho. The following lable reports the data obtained:

| 7 | on | cn | 46. | w | 2 | | Sample n" |
|-------|-------|------|-------|-------|-------|-------|-------------------|
| 135 X | 128 X | 85 X | 128 X | 256 X | 256 X | 256 X | Limit |
| 3.2 | 2.2 | 3.3 | 2.5 | 2.4 | 1.9 | 1.9 | CVAB.CE S/Co |
| 22 | 0,8 | 1.4 | 3.2 | 1.0 | 0.7 | 1.3 | Ortho 3.0 S/Co |

figures

An example of calculation is reported below

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2.1 Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the distance of specific enalyte, in addition to the first study, the absence of specific enalyte, in addition to the first study, where a total of 5043 unselected blood donors, [210 hospitalized galennis and 162 potentially interfering specimens (other infectious diseases, Ecoli antotally positive, patients affected by non-viral hepatic diseases, dialysis

patients, prognant women, hemplized, licemic, etc.) were examined, the diagnositic 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.

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 hepparin), and sera have been used to determine the value of specificity. Frozen speciments have been tested, as well, to check for interferences due to collection and storage. No interference was observed.

Diagnostic Sensitivity

2,2

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

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

genotypes of HCV were lested as well, Furthermore, most of seroconversion panels available from Boston Biomedica Inc., USA, (PHV) and Zeptometrix, USA.

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 | 506 AHd | PHV 908 | PHV 907 | PHV 906 | PHV 905 | PHV 904 | PHV 901 | Panel |
|-----------|----------|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------|
| 9 | 9 | 5 | 10 | 7 | OI | 10 | 8 | 4 | 9 | A | ω | 5 | 5 | 3 | 13 | 7 | 7 | 9 | 7 | 11 | N° samples |
| on | 5 | 2 | on | (2 | 2 | on | 4 | ω | O1 | N | - | 3 | Ç. | 2 | 10 | 3 | 7 | 3 | М | 9 | DiaPro* |
| 4 | 7 | 0 | 6 | 3 | 0 | en. | ω | 0 | 5 | 2 | _ | 3 | u | 2 | 8 | 2 | 7 | 4 | 4 | 9 | Ortho" ** |

Note: * Positive samples detected -- HCV v.3.0

Finally the Product has been tested on the panel EFS Ac HCV. iot in 01/08.03.22C/01/A, supplied by the Elablissement 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 Papel Ac HCV

| negative | 0.4 | 0.4 | 0.4 | HCV 6 |
|-------------------------|--------|----------|---------|--------|
| positive | 1.6 | 1.8 | 1.6 | HCV 5 |
| positive | 5.5 | 6.5 | 5,2 | HCV 4 |
| positive | 1.6 | 1.7 | 1.5 | HCV 3 |
| positive | 2.1 | 2.0 | 1.6 | HCV 2 |
| positive | 2.6 | 2.4 | 2.2 | HCV 1 |
| expected | SiCo | SICo | S/Co | Sample |
| TiLot# 2 Lot# 2 Results | Lot# 2 | T-01 # 2 | LOI # 1 | |

PRECISION:
 Thas 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)

| OD 450nm Std Deviation CV % | 0.094 0.008 8.7 | 0.099 0.007 6.6 | 0.096 0.008 7.9 | Average value 0.096 0.007 7.7 |
|-----------------------------------|-----------------------|-----------------------|-----------------------|---|
| Cal # 2 - 7K (N = 16) | 16) | | | |
| Mean values | 1st run | 2nd run | 3" run | Average |
| OD 450nm | 0.396 | 0,403 | 0.418 | 0.406 |
| Std.Deviation | 0.023 | 0.029 | 0.027 | 0.026 |
| CV% | 5.9 | 7.1 | 6.4 | 6.5 |
| | | | | |

Lot # 0602

SICo

1.1 1.1 1.2

Negative Sample (N = 16) 2nd run 3"run Average OD 450nm 0.097 0.096 0.094 0.096

| Std.Deviation CV % | 0.009 | 0.010 | 0.008 | 0.009 |
|-----------------------|---------|---------|---------------------|------------------|
| Cal # 2 - 7K (N = 16) | = 16) | | | |
| Mean values | 1st run | 2nd run | 3 rd run | Average value |
| OD 450nm | 0.400 | 0.395 | 0.393 | 0.396 |
| Std.Deviation | 0.021 | 0,025 | 0.026 | 0.024 |
| CV % | 5.4 | 6.2 | 8.8 | 6.1 |
| S/Co | 1.2 | 1.2 | = | 1.2 |

Lot # 0602/2

| Negative Sample (N = 15) | Mean values | 1st run | 0.087 | Std.Deviation | 0.009 | CV % | 10.0

| Mean values | ist run | 2nd run | 244 145 | Aug |
|---------------|---------|---------|---------|-------|
| OD 450nm | 0.386 | 0.390 | 0.391 | 0.3 |
| std.Deviation | 0.023 | 0.021 | 0.023 | 0.022 |
| CV % | 6.0 | 53 | CT: 00 | th. |
| S/Co | 10 | 12 | 12 | |

The variability shown in the tables above did not result in

S_LIMITATIONS

Sc_LIMITATIONS

Repealable false positive results, not confirmed by RiBA or similar confirmation techniques, were assessed as less than 0.1% of the normal population,

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

REFERENCES

- CDC. Public Health Service inter-agency guidelines for screening donors of blood, plasma, organs, tissues, and semen for evidence of hepatitis B and hepatitis C MMWR 1991;40(No. RR-4):1-17.
- McCuillan GM, Aller MJ, Moyer LA, Lambert SB, Margolis HS, A population based serologic study of hepatitis C virus infection in the United States. In RZZEITO M, PLORAL RH, Gent) L., Vermer G, sea Vral Hepatitis and Liver Disease. Edizion Minerva Medira; Turin, 1997, 267-70.
- Dufour MC, Chronic liver disease and cirrhosis, in Evenhart JE, ed. Digashive diseases in the United States epidemiotogy and impact, US Oppartment of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. "Washington, DC. US. Government Printing Office, 1994; NIH publication no. 94-1447, 615-
- B. N Engl J Med 1975;292;767-70 Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV, Transfusion-associated hepatitis not due to viral hepatitis type A or
- Choo CL, Kup G, Weiner AJ, Overby LR, Bradley DW, Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome Science 1989;244,359-62.
- Kuo G, Choo OL, Aller HJ, et al., An assay for circulating artibodies to a major etiologic virus of human non-A, non-B hepatitis, Science 1989;244:362-4.
- Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatilis C vrus in prospectively followed fransitision recipients with acute and chord non-A, non-B hepatilis. N Engl. J Med 1989;321:1494-1500.

2nd run 3^m run Averrage 0.091 0.088 0.089 0.007 0.008 0.008 8.2 8.6 8.9

- Aach RD, Stavens CE, Hollinger FB, et al. Hepatits C virus intechar in post-transfusion hepatitis. An analysis with first- and second generation assays. N Engl J Med, 1991;325:1325-9.
- 4. Alter MJ, Margolis HS, Krawczynski K, Judson, FN, Mares A, Alexander WJ, et al. The natural history of community-acquired hepatitis C in the United States, N Engl J Med 1992;327:1899-1905.

ŝ

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- Alter MJ, Epidemiology of hepatitis C, Hepatology 1997;26:62S-5S
- Alter MJ, Hadler SC, Judson FN, et al. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection, JAMA 1990;264:2231-35,
- 6 Aller HJ, Holland PV, Purcell RH, et al., Posttransfusion hepatitis after exclusion of commercial and hepatilis-B antigen-positive donors, Ann Intern Med 1972;77:691-9.
- Alter HJ, Purcell RH, Holland PV, Feinstone SM, Morrow AG, Moritsugu Y, Clinical and serological analysis of transfusion-associated hepalitis, Lancet 1975;2:838-41.
- Seeff LB, Wright EC, Zimmerman HJ, McCollum RW, VA Cooperative Studies, Group, VA cooperative study of post-transfusion hepatitis and responsible risk factors, Am J Med Sci 1975;270:355-62.

- 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

After_MJ_Epidemiology of hepatitis C in the west, Semin Liver Dis 1995;15:5-14

Donahue JG, Nelson KE, Muáoz A, el al, Antibody to hepatitis C virus among cardiac surgery patients, homosexual men, and intravenous drug usors in Baltimore, Mayland, Am J Epidemiol 1991;134:1206-11.

Zeldis JB, Jain S, Kuramolo IK, et al. Seroepidemiology of viral infections among intravenous drug users in northern California, West J Med 1992;156:30-5.

Fingerhood Mi, Jasinski DR, Sullivan JT, Provalence of hepatitis C in a chemically dependent population, Arch Intern Med 1993;153:2025-

18

Garlein RS, Vishov D, Galai N, Doherty, MC, Nelson, KE, Viral inhetitons in short-term injection drug ssors; the prevalence of the hepatils C, hepatils B, human inmunopelicency, and human 1-lymphotropic viruses, Am J Pub Health 1996;86:555-61;

19

20 Brettler DB, Alter HJ, Deinstag JL, Forsberg AD, Levins PH, Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients, Blood 1990;76:254-6

Troisi CL, Hollinger FB, Hools WK, et al. A multicenter study of viral hepatitis in a United States hemophilic population, Blood 1993;81:412-8.

Kumar A, Kulkarni R, Murray DL, et al. Serologic markers of viral hepatitis A, B, C, and D in patients with hemophilia, J Med Virology 1993;41:205-9.

23 Tokars JI, Miller ER, Alter MJ, Arduino MJ, National surveillance of dialysis associated diseases in the United States, 1995, ASAIO Journal 1998;44,98-107.

 Weinstock HS, Bolan G, Reingold AL, Polish LB: Hepatilis C virus infection among patients attending a clinic for sexually transmitted diseases, JAMA 1993;269,392-4. Osmond DH, Charlebois E, Sheppard HW, et al. Comparison of risk factors for hepatitis C and hepatitis B virus infection in homosexual men. J Infect Dis 1993;157:55-71.

26 Thomas DL, Camoon RO, Shapiro CN, Hook EW III, Alter MJ, Hepatilis C, hepatilis B, and huntan immunodeficiency virus infections among non-intravenous drug-using patients altending clinics for sexually transmitted diseases, J Infect Dis 1994;169:989-5.

27_ Buchbinder SP, Kaiz MH, Hessot NA, Liu J, O'Malley PM, Alter, MJ. Hepatilis C virus infection in sexually active homosexual men, J Infect 1994;29:263-9

Thomas DL, Zenliman JM, Atter HJ, et al. Sexual transmission of hepatilis C virus among palients altending sexually transmitted diseases officis in Ballimore-an analysis of 309 sex partnerships. J Infect Dis 1996;171:768-75

29 Thomas DL, Factor SH, Kelen GD, Washington AS, Taylor E Jr, Quinn TC, Viral hepatitis in health care personnel at The Johns Hopkins Hospital, Arch Intern Med 1993;153:1705-12.

Cooper BW, Krusell A, Titon RC, Goodwin R, Levitz RE, Seroprevalence of antibodies to hepatitis C virus in high-risk hospital personnel, Infect Control Hosp Epidemiol 1992;13:82-5.

Dia Pro, Diagnostic Bioprobes Srl. Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy

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0318

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM class antibodies to Hepatitis A Virus in human plasma and sera

- for "in vitro" diagnostic use only -



DIA.PRO

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REF AVM.CE S1521 98

HAV IgM

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A. INTENDED USE

Enzyme immuno/assay (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.

Hepatilis A Virus as follows The Center for Disease Control or CDC, Atlanta, USA, defines

Hepatitis A continues to be one of the most frequently 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 preschally eliminate indigenous transmission of hepatitis A virus (HAV) infection.

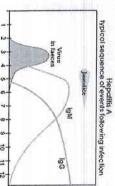
usually symptomatic, with jaundice occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 months, although 10%-13% of symptomatic presents have prolonged or rulipsing disease lasting up to 6 months. In infected pursons, HAV replicates in the liver, is excreted in bile, and is shoul in the stool. Feek infectivity of infected persons occurs during the 2-week periad before onest of jaundice or elevation of liver enzymes, when the concentration of virus in stool is highest. The concentration of virus in stool declines after jaundice appears. Children of virus in stool declines after jaundice appears. Children shedding of HAV in faces does not occur; however, shedding can occur in persons who have relapsing illness. Virenia occurs soon after infection and persists through the period of and infants can shod HAV for longer periods than adults, up to several munths after the onset of clinical illness, Chronic urine, and jaundice. The likelihood of having symptoms with 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 jaundice. Among older children and adults, infection is typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark produce either asymptomatic or symptomatic infection in humans after an average incubation period of 28 days (range, 13-50 days). The illness caused by HAV infection HAV, a 27-nm RNA agent classified as a picornavirus, can

Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of chircle or epidemologic features alone. Seenlagic testing to detect immonoglobulin M (IgM) antibody to the capsid protests of thAV (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 onset of symptoms and can persist for up to 6 months after infection, namonoglobulin G (IgG) anti-HAV, which appears early in the course of infection, remains discretable for the person's lifetime and comiers lifeting protection against the disease. Commercial diagnostic tests are available for the detection of IgM and stool of most persons during the section of IgM and stool of most persons during the section place of infection by using meletic acid amplification metalizing and aucher and sequenoming has been used to determine the relatedness of HAV isolates.

HAV infection is acquired primarily by the feedborni route by either person-to-person contact or ingestion of contaminated foot or water. On one occasions, HAV infection has been transmitted by transfusion of blood or phase of their infection. In experimentally infected menchunan primates, HAV has been detected in saliva during the incubation period: however, transmission by saliva has not been demonstrated. blood products collected from donors during the virenie

environment for months, Heating foods at temperatures groater than 185 F 685 C) for 1 minute or disinfecting surfaces with a 1100 dilution of soidium hypothoriste fact, household bleach) in tap water is necessary to inactivate Depending on conditions, HAV can be stable in

Escatas most children have asymptomotic or unrecognized infections, they play an important role in HAV transmission and serve as a source of infection for others. In one study of adults without an identified source of infection, 32% of their households included a child less than 6 years old, and the presence of a young child was associated with HAV transmission within the household. In studies where an identified source of infection was performed, 25%-40% of the contacts less than 6 years old had sevologic evidence of acute HAV infection (IgM anti-HAV). serologic testing of the household contacts of adults without most children have asymptomatic



Weeks after exposure

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "golf capture" where IgM. The assay is based on the principle or first captured by the solid phase coaled with ant higM antibody.

Alter washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are discused by the addition of a purified preparation of inactivated HAV, labelled with an antibody conjugater with peroxidase (HAP). In abelied with an antibody conjugate with peroxidase (HAP).

After incuration, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.

In the presence of peroxidase the confortes substrate is hydrolysed to a colored end-product, whose optical identity may be detected and its proportional to the amount of antibodies to HAV present in the sample.

'12 strips of 8 breakable wells coated with anti-human IgM antibody, affinity purified, and sealed into a bag with desicoant. Bring the microplete to room temperature before opening the bag. Unused strips have to be returned into the bag and the bag thats to be sealed and stored back to 2.8°C, in presence of the desicoant.

2

2. Negative Control: CONTROL_1
1x4.0 ml/vial, Resay to use control. It contains goat serum
proleins, 10 mM tris buffer pH 6,0+/-0,1, 0,1% Tween 20,
0,09% sodium azide and 0,1% Kathon GC as preservatives,
The regative control is colourless,

1x4.0 mil/tet. Ready to use control, it contains anti FAV IgN, goat serum proteins, 10 mM iris buffer pH 6,0x4.0,1, 0,1%. Tween 20, 0,03% sodium azide and 0,1%. Kathon GC as preservalives. The positive control is green colour coded. CONTROL +

3. Positive Control:

4. Calibrator: [AL...]

N* 1 typphilizad vial. To dissolved with EIA grade water as reported in the label. It contains and HAV IgM, 2% BSA, 10 mM list buffer pH 6,0+0,1,0,09% sodium azide and 0,1% Kalhon GC as preservalives.

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

5. Wash buffer concentrate: [WASHBUF 20X]
1x50mllottle, 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.

Enzyme conjugate 20X: CONJ 1x0.8 ml/vial. 20X concentrated

1x0.8 milvisi. 20X concentrated solution. It contains thorsectish peroxidase conjugated antibody specific to HAV in presence of 10 mM Tris buffer pd 1,84-7,1, 2% BSA, 0,1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

7. HAV Antigen: [Ag HAM]

X16 mWall. Ready-fouse solution, it contains machivated and stabilised HAV in presence of 10 mM Tris buffer pH 5,8+0.1,

2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as

The reagent is red colour coded

 Specimen Dituent: DILSPE 2x60.0 m/vial, Project buffered solut samples, It contains goal secum protein 6.0+-0.1, 0.1% Tween 20, 0.09% so Kalthon GC as preservatives.

The reagent is blue colour coded. solution for the dilution of proteins, 10-mM-tris buffer pH 9% sodium azide and 0.1%

strong Discrete description of the control of the control

It contains 0.3 M H2SO4 solution

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

11. Plate sealing toils

insert n. 1

- MATERIALS REQUIRED of 10ul, NOT 100ui PROVIDED and 1000ы
- disposable plastic tips. EIA grade water (double distilled to remove Bulzipixo chemicals or deignised used charcoal used as
- incubator (dry or
- disinfectants)

 Timer with 60 minute range or higher.

 A Absorbent paper lissues.
 Calibrated ELISA micropiate thermostatic included the paper of the 450nm (reading)
 - o

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.

2. All the personnel involved in performing the assay have to wear protective laboratory clothes, a lab-free glooves and glassess. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in blossiety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Blossiety in Microbiological and Biomedical Laboratories", ed. 1984,
3. All the presonnel involved in sample handing should be vaccinated for HBV and HAV, for which vaccines are available, safe and effortives.

sale and effective.

4. The laboratory environment should be controlled so as to avoid containments such as dust or air-born microbial agents, when opening kit valis and microbiates and when performing the test, Protect the Chromoger/Subtrate (TMB & HO20) from strong light and avoid vibration of the bench surface where the test is undertaken.

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

5. Upon receipt, store the kit at 2.4°C into a temperature controlled refrigeration 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 tot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the liaboratory supervisor to initial the necessary procedures.

8. Avoid cross-contamination between serum/plasme

samples by using disposable tips and changing them after each

sample.

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

für. Do not use nie kit after the expiration date stated on external (primary confainer) and mismal (vials) labels. Treat all specimens as potennally infective. All humans seum specimens should be handled at Biosafely Level 2, as recommended by the Center für Disease Control. Allanta. Lis. in compliance with what reported in the Institutes of Health's publication: Biosafely in Microbiological and Biomedical Laboratories', ed. 1984.

11. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring promponents into other, contoiners of automated-workshalions. In one. fu. Do external

order to avoid contamination.

12. Waste produced during discarded in compliance with the produced to the prod iste produced during the use of the kit has to be qu'in compliance with indicinal directives and laws ing laboratory waste of chemical and biological cess. In periodier, and the process are periodier and from produce, from esiduals was control and from that to be reasted as potentially infective material and

inactivated. Suggested procedures of inactivation are irearment with a 10% ling concentration of household beach for 15-18 hrs or heat inactivetion by autoclave at 121°C for 20 min.

13. Accidental spills have to the adsorbed with paper issues soaked with household bleach and hen with water. If ssues should then be discarded in proper containers designated for laboratory/hospital waste.

14. The Stop Solution is an initiant. In case of spills, wash the

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS
1. Blood is drawn asspitically 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

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 reading is strongly recommended.

3. Samples confaining residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they

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. any

Antibody coated microwells:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiscoant has not turned dark green, indicating a defect in conservation, 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°2°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from

Ready to use. Mix well on **Negative Control**

vortex before use.

3. Positive Control: Ready to use. Mix w component as potential present in the control. well stially has t on vortex before use. Handle this infectious, even if HAV, eventually s been chemically inactivated.

Add-the volume of EUSA 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

Note: When the is not stable Store

The whole content of the or diluted 20x with bidistilled water The whole content of the

oncentrated solution has to be and mixed gently end-over-end

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Once diluted, the wash solution is stable for 1 week at 2-8° C. During preparation avoid framing 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.

surface with plenty of water

5. Ofter waste maternals generated from the use of the kit (example: tips used for samples and controls, used micropiales) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes. 6. Enzyme conjugate:
20% prepartion, Mix well on vortex.
20% prepartion, Mix well on vortex.
Avoid contamination of the liquid with oxidizing chemicals, or microbes when the reagent is aspirated to be used.

, dus

Handle this component as potentially has been chemically inactivated.

Ready to use, Mix well on vortex before use.
Handle this component as potentially infectious, even if HAV

6+7, HAV Antigen/Antibody complex:
About 5-10 min before its use, diule the 20X concentra
About 5-10 min before its use, diule the 20X concentra
Enzyme Conjugate in the proper volume of HAV Antig
Enzyme Conjugate in the proper volume carefully,
Example: To run 2 strips, diffue 100 ul Enzyme Conjugate
into 2 ml of HAV Antigen.
Note: This immunocomplex is not stable; discard the exceen

X concentrated f HAV Antigen,

20X

mocomplex is not stable; discard the exceeding

could give rise to laise 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 fozen at +20"C for several months. Any fozen samples should not be freezelfhawed more than once as low antibodies may get damaged and as this procedure may generate particles that could affect the test result.

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

Chromogen/Substrate:

Ready to use. Mix well on vortex

Sample Diluent:

Ready for use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemi driven dust or microbes.

Do not expose to strong light, oxidizing agents and surfaces. If this component has to be transferred plastic, and if pagishle, sterile disposable container. vortex before use. ne liquid with oxidizing chemicals and

asu d metallic use only

10, 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).

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

Precautionary P statements: P280 - Wear protective

P280 — Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 — IF ON SKIN: Wash with plenty of soap and

advice/attention, P362 + P363 - Take of before reuse. for several minutes. Remove contact easy to do. Continue rinsing. P337 + P313 - If eye irritation advice/attention. P305 + P351 + P338 – IF IN EYES: Rinse cautiously with + P313 = 8 skin irritation persists: lenses, occurs: if present Get water

clothing and

wash

3

LINSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipetus have to be calibrated to deliver the correct volume (tolerance +/5%) required by the assay and must be submitted to regular deconfamination (household be submitted to regular deconfaminati

allochiol. 10%, solution of bleach, hisspital grade disinfectables, of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Deconfamination of spits 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 truences of ±2%. To (tolerance of ±0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water that 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 carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. 4-5 washing cycles (aspiration + dispensation of 3500/Weil to 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 maich the values reported below in the socion. Regular calibration of the volumes delivered to wash and maintenance. 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

4. Incubation limes have a lobrance of ±5%.
5. The ELISA reader has to be epulpped with a reading filler of 450nm and with a second filter (520-630nm, 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

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 "interned Cuality Control." The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader, in addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of FLISA automated work stations is encommended when of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per

un. 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.

Check the expiration date of the kit printed on the external

- label (primary container). Do not use the device if expired.

 Check that the fluud components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate—is—colourless—or—pale—bue—by—aspirating a small volume of it with a sterile plastic ploate. 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 Soliution as described above. N

- 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
- රා 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 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 eutomated work station, turn on, check settings and be sure to use the right assay protocol. Check that the micropipettes are set to the required

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

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Sample Diluent into a dilution tube; mix gently on vonex
- Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of
- Dispense 100 µl Negative Control in triplicate, 100 µl Positive Control in single and 100 µl Calibrator in duplicate in proper wells. Do not dilute controls and the calibrator 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
- dispensed. Incubate the microplate for 60 min at +37°C

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

- About 5-10 minutes before use, prepare Antigen/Antibody immunocomplex as previously. า/Antibody the HAV described
- Wash the microplate with an automatic washer reported previously (section 1.3) as
- Pipette 100 µ HAV Antigen/Antibody complex into each well, except the 1st blanking well, and cover with the sealer. Check that all wells are red coloured, except A1.

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

- Incubate the microplate for 60 min at +37°C
- 10. Wash microwells as in step 7.
- 11 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.

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

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- 12. Pipette 100 µl Sulphurlo Acid into all the wells to stop the enzymatic reaction using the same pipetting sequence as in step 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 450nm 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 finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false
- reading at 450mm. Finger prints could generate false positive results on reading. 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 | Sulphuric Acid | emperature | " incubation | MB/H2O2 mix | Vashing | emperature | " incubation | IAV & Tracer | Vashing | emperature | "incubation | Controls&Calibrator (*) samples diluted 1:101 |
|------------|----------------|------------|--------------|-------------|------------|------------|--------------|--------------|------------|------------|-------------|---|
| 450nm | 100 ul | 11 | 20 min | 100 ut | 4-5 cycles | +37°C | 60 min | 100 ul | 4-5 cycles | +37°C | 60 min | 100 ul |

- 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
- An example of dispensation scheme is reported in the table

| | A | w | 0 | O | m | m | G | 1 | CAL |
|-----------|-----|----|----|----|--------|--------|-----|----|--|
| | BLX | NC | NO | NC | CAL(*) | CAL(*) | De. | S1 | Legenda: BLK = Blank CAL(*) = Calibrator - Not mendatory |
| 2 | S2 | S3 | S4 | SS | Se | S7 | SS. | 88 | alor - N |
| 3 | | | | | | | | | er - Noi mand |
| 24 | | | | | | | | | ndator |
| 5 6 7 | | | | | | ľ | | j | , š |
| 6 | | | | | | | | | = Nog |
| 7 | | | | | | | | | BULL |
| co | | | | | | | | | NC = Registre Control PC = Positive Control |
| 9 | | | | | | | | Ī | |
| 10 | | | | | | | | | S = Sample |
| 1 | j | | | | | | | 3 | ample. |
| 12 | | | Ü | | | | | ĺ | |

order to verify whether the expected OD450nm or S/Co values have been matched in the analysis. O, INTERNAL QUALITY CONTROL

| Blank well < 0.100 OD450nm value Negative Control < 0.150 OD450nm value after blanking mean value (NC) coefficient of variation < 30% | Parameter | Requirements |
|---|-------------------------------------|---|
| 0.0 | Blank well | < 0.100 OD450nm value |
| | Negative Control mean value (NC) | < 0.150 OD450nm value after blanking coefficient of variation < 30% |

If the results of the test match the requirements stated above, proceed to the next section, if they do not, do not proceed any further and perform the following checks:

| Problem | Check |
|------------------|---|
| Blank well | t, that the Chromogen/Substrate solution has |
| Negative Control | 1 that the washing procedure and the washer |
| (NC) | settings are as validated in the pre qualification |
| > 0,150 OD450nm | study: |
| after blanking | 2 that the proper washing solution has been |
| | used and the washer has been primed with it |
| coefficient of | before use; |
| variation > 30% | 3 that no mistake has been done in the assay |
| | procedure (dispensation of positive control |
| | instead of negative control; |
| | 4. that no contamination of the negative control |
| | or of the wells where the control was dispensed |
| | has occurred due to positive samples, to spilis |
| | or to the enzyme conjugate; |
| | 5. Ihat micropipettes have not become |
| | contaminated with positive samples or with the |
| | enzyme conjugate |
| | 6 that the washer needles are not blocked or |
| | partially obstructed |
| Positive Control | 1. that the procedure has been correctly |
| < 0.500 | performed, |
| OD450nm | 2 that no mistake has occurred during the |
| | distribution of the control (dispensation |
| | negative control instead of positive control). |
| | Ihat the washing procedure and the washer |
| | settings are as validated in the pre qualification |
| | sludy, |
| | 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.

If Calibrator has used, venify the following data

| Parameter | Requirements |
|------------|--------------|
| Calibrator | S/Co > 1 |

above, operate as follows If the results of the test doesn't match the requirements stated

| | | | | | | | S/Co < 1 | Calibrator | T. T. WILLY WILL THE |
|-------------------------|---|--|--|------------------|--|--|------------|--|----------------------|
| callbrator has occurred | a that an external contamination of the | settings are as validated in the pre qualification | 3 that the washing procedure and the washi | control instead) | distribution (e.g.: dispensation of negative | 2. that no mistake has occurred during its | performed; | 1. that the procedure has been correct | Templeton |

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 lest 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 out-off

Cut-Off = NC + 0,250

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

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

Q. INTERPRETATION OF RESULTS

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

| 200 |
|-----|
| 44 |
| m |
| 22 |
| m |
| 0 |
| 20 |
| 35 |
| Þ |
| z |
| Ω. |
| m |
| () |
| 30 |
| 20 |
| 잗 |
| 2 |
| 53 |
| 7 |
| 21 |
| 発 |
| 23 |
| 丑 |
| 82 |
| ų, |
| |

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 t the following preparations:

misdefers with the performance of the test. No intoference was observed on clean and particle free samples. Samples cenved from patients with different viral. (HCV, HDV, HEV, and non viral pathologies of the liver that may intofere with the test were examined.

Frozen specimens have also been tested to check whether this

No cross reaction were observed.

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

Densting J. Hepaths A Virus Identification, characterization and epidemiologic investigations. Progress in liver desease VII, Popper. E., Schaffter, F., (eds), pp. 323-370,—New York, Gruner and Stration, 1873,

2. Duerneyer W., Van der Veen J., Koster B., 'ELISA in Hepatitis A., Lancet, I.: 323-524, 1978

2. Parry J.V., (1981) 'Hepatitis A infection: guidelines for the development of satisfactory seasys for laboratory diagnoss's. The Institute of Medical Laboratory Sciences, 38, 303-311.

4. Lindberg J., Frostner G., Hansson B.G. et al. Speciogic markers of hepatitis A and B in chronic active nepatitis." Scandinavian Journal of Gastroenlerology, 13:525-

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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 (1100) and then further diluted in Sample Diluent to generate a limiting

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:

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 33-45, Orlando Academic Press, Inc. 1984 Barbara J.A., Howell D.R., Briggs M., Parry J.V., Post transfusion hepatitis A". Lancet (1982), 1-738.

| # 51 1:100 | 1:16 | 1:80 | 1:40 | # 121 1:200 | Accurun 1:10 | Preparation Oiluti |
|------------|------|------|------|-------------|--------------|--------------------|
| 0 4.2 | | -11 | | 0 4.1 | | ons S/Co |

Diagnostic Sensitivity:

Any patient showing an equivocal result, should be re-tested by examining a second sample after 1-2 weeks from first (esting. A positive result is indicative of an HAV infection event and therefore the patient should be lreated accordingly.

A negative result indicates that the patient is not undergoing an acute infection by HAV.

0.8 - 1.2S/Co < 0.8

Interpretation Equivocal Positive Negative

The following data must not be used instead or real figures

An example of calculation is reported below:

The 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

An overall value of 100% has been found in the study conducted on a lotal number of more than 100 samples. A seroconversion panel has also been studied

Results obtained by examining a preparation supplied by Boston Biomedica Inc., USA, are reported below,

Seroconversion Panel: PHT 902

| 5 0,669 | 4 1,988 | 3 1,956 | 2 0,042 | 1 0,037 | PHT902 | TRL (+) 1,736 | CTRL (-) 0,048 | | Sample Chaptum S/Co his Sonn Keler. |
|---------|---------|---------|---------|---------|--------|---------------|----------------|-------|-------------------------------------|
| 9 2,2 | 38 6,7 | 8,8 | 12 0,1 | 37 0,1 | | 5,8 | 18 0,2 | | unm S/Co |
| 1,5 | 6,7 | 6,8 | 0.3 | 0,3 | | | | S/Co | nasoni |
| pos | pos | pos | neg | neg | | | Ĵ | Score | Keler |

Interpretation

Important notes

Sample 1: 0,070 OD450nm
Sample 2: 1,590 OD450nm
Sample 1 S/Co < 0,8 = negative
Sample 2 S/Co > 1,2 = positive

S/Co higher than 1.0 - Accepted

0.550 - 0.530 OD450nm 0.540 OD450nm

S/Co = 1.7

Calibrator:

Cut-Off = 0.060+0.250 = 0.310

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

- When test-results are transmitted from the laboratory to hepatitis is confirmed
- dala transfer.
 Diagnosis of viral hepatits infection has to be taken by and released to the patient by a suitably qualified medical another facility, attention must be paid to avoid erroneous
- 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
- preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the
 - Diagnostic Specificity:
 The diagnostic specificity has been determined on panels of specimens-negative with the reference kit, derived from normal individuals and blood donors of European origin.
 Both plasma, derived with different standard techniques of

the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

Diagnosis of an infectious disease should not be established on

pooled ones

consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with False positivity has been assessed as less than 2% of the normal population, mostly due to high titers of RF, Frozen samples containing fibrin particles or aggregates may

generale false positive results

S. LIMITATIONS

Dia.Pro Diagnostic Bioprobes Srl Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

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 technical specifications and acceptance criteria

 Sample
 Negative
 Low Pos.

 OD450nm
 0.058
 0.719

 d. Deviation
 0.008
 0.052

 CV %
 14.3
 7.2

Test#1

Test#2

Sample Negative Low Pos. OD450nm 0.048 0.709 itd Deviation 0.007 0.063 CV % 13.9 8.9 Test#3

| 7.7 | 13.4 | % A3 |
|----------|----------|----------------|
| 0,055 | 0.007 | Std. Deviation |
| 0.713 | 0.050 | OD450nm |
| Low Pos. | Negative | Sample |

0

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

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

e-mail: info,a diapro.in Fax +39 02 26007726

Phone +39 02 27007161

HAV Ab

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Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera, The kill is used for the follow-up of patients infected by HAV. For 'in vitro" diagnostic use only. A. INTENDED USE

The Center for Disease Control or CDC, Atlanta, USA, defines Hepatitis A Virus as follows:

Hepatitis A continues to be one of the most frequently responded 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 igenous transmission of hepatitis A virus (HAV)

mulaise, autorexia, natusea, abdominal discomfort, durk urine, and joundoe. The likelihood of having symptoms with 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 joundine. Among older children and adults, infection is usually symptomatic, with joundine occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 months, although 10%-15% of symptomatic persons have persons occurs during the 2-week period before onset of jaundice or elevation of liver enzymes, when the concentration of virus in stool is highest. The concentration of virus in stool is highest. The concentration of virus in stool declines after jaundice appears. Children and infants can shed HAV fur longer periods than adults, up to several months after the onset of clinical illness. Chronic boddies of the virus in some content of the concentration of the content HAV, a 27-nm RNA agent classified as a picornavirus, can produce either asymptomatic or symptomatic infection in shedding of HAV in feces does not occur however, shedding can occur in persons who have relapsing illness. Vironia occurs soon after infection and persists through the period of prolonged or relapsing disease lasting up to 6 months. In infected persons, HAV replicates in the liver, is excreted in humans after an average incuhation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include lever, bile, and is shed in the stool, Peak infectivity of infected liver enzyme elevation. period of 28 days (range,

Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of chinical or epidemiologic features alone. Semiogic testing to detect immunoglabulin M (IgM) anti-HAV) is required to endirm a diagnossis of acute HAV infection. In most persons, IgM, anti-HAV becomes detectable 6-10 days for the persons. before the onset of symptoms and can persist for up to 6 months after infection. Immunoglobulin G (IgG) anti-HAV anti-HAV in serum protection against the which appears early in the course of infection, remains detectable for the person's lifetime and confers lifelong ire-awiibible for the detection of IgM-and-total (IgM and IgO)

persons during the acute phase of infection by using nucleic acid amplification methods, and nucleic acid sequencing has been used to determine the relutedness of HAV isolates. HAV RNA can be detected in the blood and stool of most

HAV infection is nequired primarily by the freal-oral route by either person to person contact or ingestion of contaminated for or water. On tere constaints, HAV infection has been transmitted by transitusion of blood or blood products collected from denors during the viremic phase of their infection. In experimentally infected nonhuman primates, HAV has been detected in saliva during the incubation period however, transmission by saliva has not been demonstrated.

HAV Depending on conditions, HAV can be stable in the 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 sedium hypochlorite (i.e., household bleach) in tap water is necessary to inactivate

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 of adults without an identified source of infection, 22% of their households included a child less than 6 years old and the presence of a young child was associated with HAV an identified source of infection was performed, 25%-40% of the contacts less than 6 years old had sevologic evidence of acute HAV infection (IgM anti-HAV). presence of a young child was associationsmission within the household. In serologic testing of the household contacts of adults without

Hepatitis A typical sequence of events following infection Virus In faeces Jaundice Mgl igG

Weeks after exposure

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 coaled to the microwells.

The patient's serum/plasma is added to the microwell and

antibodies to HAV are captured by the solid phase.

After washing, the enzyme conjugate is added and binds to the free HAV antigen, if still present.

The plate is washed to remove unbound conjugate and then the chromoger/substrate is added.

In the presence of pervolute, whose optical density in the presence of pervolute, whose optical density may be detected and is inversely proportional to the amount of antibodies to HAV present in the sample. One of the well-to block interferences able to mask the presence of antibodies, mostly appearing in the follow up of vaccination.

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D, COMPONENTS

Each kit pontains sufficient reagents to perform 96 tests

1. Microplate MICROPLATE

8x12 microwell strips coaled with purified and inactivated HAV, 8x12 microwell strips coaled with purified and inactivated HAV, sealed into a bag with desicrant, Allow the microplate to reach room lemperature before opening. Reseal unused strips in the bag with desicrant and store at 2,8°C°.

2. Negative Controt: <u>CONTROL</u> 1 1x4.0mt/val. Ready to use. Contains bovine serum proteins, 10 mM phosphate buffer pH 7.4++0.1, 0.02% gentamiche sulphate and 0.1% Kalmin GC as preservatives. The negative control is color coded pale yellow.

3. Positive Controt: CONTROL 1 1x4.0ml/val. Ready to use. Contains bowine serum proteins, anti HAV amtibodies at a concentration higher than 100 WHO mlU/ml, 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. 0.7 6

C_A

n° 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 mtll/mt, 10 mM phosphate buffer pH 7.44-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GCI 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 1x50m/bottle. 20x concentrated solution, to be diluted up to 1x50m/bottle. 20x concentrated solution, to be diluted up to 1x50m/with distilled water before use. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/0.2, 0.05% Tween 20 and 0.05% Kathon GC.

x inmivas. Ready-to-use solution. Contains Horseradish perovidase conjugated anibody, specific to HAV, in presence of 10 mM Tris buffer pH 6.8+/1.01, 7.2% BSA, 0.1% Kathon GC and 0.02% gentamicine subhale as preservatives. 1x16ml/vial. Ready-to-use speroxidase remains. The reagent is colored with a red dye.

7. Chromogen/Substrate: SUBS TMB
1. X16mi/val. Contains a 50 mM dirate-phosphate buffer solution at pri 3.5-3.8. 0.03% tetra-methyl-benzidine or TI and 0.02% hydrogen peroxide of H2O2.

Note: To be stored protected from light as sensitive buffered or TMB 6

8. Specimen Diluent: DILSPE

strong illumination

1x8mt. Buffered solution suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide and 0.1% Kathon GC as preservatives. The reagent is color coded dark green.

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

10. Plate sealing foils n° 2

7. 1

Calibration Curve: CAL N°.... 5x2.0 ml/vial. Ready lo use and colour coded standard ranging: 0-5-10-50-100 WHO mlU/ml. curve

> (CAL1=0mIU/ml, CAL4=50mIU/ml CAL3=10miU/ml

CAL4=50mil/imi CAL5=100mil/imi), CAL5=100mil/imi), Contains section proteins, 0.3 mg/mi gentamicine sulphate and 0.1% Kalhon GO as preservatives.
Standards are blue colored.

MATERIALS REQUIRED BUT NOT PROVIDED

(150ut 100ut and 50ul) and

disposable plastic tips.

EIA grade water (double distilled or delonised charcoal treated to remove oxidizing chemicals used as disinfectants).

3. Timer with 60 minute range or higher,
4. Absorbent paper tissues.
5. Calibrated ELISA, microplate Thermostatic incubator (dry or we't) set at 437°C (4-40,1°C (beleanne).
6. Calibrated ELISA microyelli reader with 450nm (reading) and with 520-650nm (blanking) fillers.
7. Calibrated ELISA microplate washer.
8. 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.

wear protective laboratory clothes, tale-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 biossifely procedures, as recommended by the Center for Disease Control. Allania, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1894.

3. All the presonnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available. 2. All the personnel involved in performing the assay have to

sale and effective.

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

Lipon receipt, store the kit at 2-8°C into a temperature controlled terfigerator or cold troom.

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

of the same lot should not be interchanged.

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

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

sample.

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

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 subcernals, should be handled a Biosafely Level 2. as recommended by the Center for. Dispesse Control, Allania, U.S. in compliance with what reported in the Institutes of Health's publication: Biosafely in Microbiological and Biomedical Laborationers, ed. 1984.

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 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 hindigical

16-18 hts or heat inactivation by autoclave at 121°C for 20 min.

13. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water, Tissues should then be discarded in proper containers designated for substances in particular, liquid waste generated from the wasteing procedure, from residuos of controls and from samples, has to be treated as potentially infective material and machined.

Suggested procedures of machine

iaboratory/hospital waste.

14. The Stop Solution is an irritant. In case of spills, wash the

 Other waste materials generated from the use of the kit (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. surface with pienty of water 15. Other waste materials generated from

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by verigurature and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysts. No influence has been observed in the preparation of the sample with citatle, EDTA

and heparin.

2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the

conjugate, generating laise negative results,

3. Samples have to be clearly identified with codes or names in

order to avoid misinterpretation of results. When the kit is

used for the screening of blood units, bar code labeling and

reading is strongly recommended.

4. Haennolysed and visibly hyperlipenic ("mility") 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 freezer/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, certifying at 2,000 pm for 20 min or filter using 0.2-0.8 utilities to deen up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS A study canducted on opened kit has pointed out no rel loss of performances up to 3 months from first opening. relevant

. Antigen coated microwells:

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

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at *2*-8* °C. When opened the first time, unused strips are stable until the humidify indicator inside the desiccant hall form yellow to Allow the micropiale to reach noom temperature

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

Ready to use. Mix well on vortex before use

 Calibrator: Add the volume of ELISA grade water, the tyophilized powder, let fully dissolv dissolve and then reported on the label re and then gently mix 9 5

vortex. The dissolved calibrator is not stable; store it frazen in aliquots.at.—20°C.

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end-over-end before use.

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

During preparation avoid foaming as the presence of bubb diluted with bi-distilled water 20x up to 1200ml solution has I and mixed s to be

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

of bubbles

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

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use,
Ready to use. Mix well on vortex before use,
Avoid confamination of the flouid with oxidizing chemicals, airdriven 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 Dittent:
Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:

Ready to use. Mix well on vortex before use. Attention: Imitant (H315: H319: P280: P302+P352: P332+P313:

P305+P351+P338; P337+P313; P362+P363)

Warning H statements: H315 – Causes skin imitation. H319 – Causes senous eye imitation.

Precautionary Pataments:
P230 — Was protective glovesprotective definingleye protection/face
protection,
protection, 1935 — IF ON SOCIETY Wash with piems of snap and water.
P332 + P315 — IF SN SOCIETY Wash with premis of snap and water.
P332 + P315 — If SN SOCIETY SNAP called advisoration on.
P335 - P315 — P316 — IF NE EYES. Rines called with water for several minutes. Remote contact invests, if present and easy to discussed minutes.

several minutes. Remove contact tenses; if present and easy to or Continue rinsing. Part 1971 - If the initiation persists; Set medical advice/attention. P382 + P383 - Take off contaminated dothing and wash it before reuse

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct

volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hespital grade disnitretants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be regularly maintained in order to show a precision of 1% and a tureness of £2%.

The ELISA incubator has to be saf at 47°C (hierance of +1.

Charles are suitable for the incubations provided that the instrument is maintained. Both dry incubators and water boths are suitable for the incubations provided that the instrument is validated for the incubation of ELISA rests.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly primized using the kit controls and reference panels, before using the kit for outline thorothary tests. 4-S washing cycles (aspiration + dispensation of 350 ulwell of washing solution = 1 cycles) are sufficient to ensure that the assay performs as expected. A casking time of 20-30 seconds between orthes is suggested. In order to set of correctly their number it is recommended to run an

Control. Regular calibration of the volumes delivered by, and markenine (deconlamination and delaring of needles) of the washer has to be carried out according to the instructions of the manufacturer, assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section O "internal Quality

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 Internat 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 controlled necessarily set. Particular attention must be paid to avoid carry over by the needles used for dispensating and for washing. This must be suited and controlled to miximise the careful to the controlled of the controlled of the controlled of the careful to the controlled of the careful to the careful and controlled to minimize the possibility of contamination of adjacent wells. The use of EUSA automated work stations is recommended when the number of samples to be tested instructions, When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data

- 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+H502) is colorless or pale blue by aspirating a small volume of i with a sterile pastic pipette. Check that no breakege 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 purclured or damaged. 2
- Dissolve the Calibrator as described above and gently mix.

 Allow all the other components to reach room temperature
- က ceagents.

 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
- 7 instrument for its use with the kit.

 Check that the ELISA reader is turned on or ensure it will be
- ထ turned on at least 20 minutes before reading. If using an automated work station, turn on, check settings
- 10° and be sure to use the right assay protocol,
 Check that the micropipeties 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.

- 4. Incubation times have a tolerance of ±5%.
 5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter (520-630nm, 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 manufacturer is regularly maintained according to the manufacturer is
- 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 kil, in order to assure compliance 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
- Dilute all the content of the 20x concentrated Wash Solution
- 1 hr) and then mix gently on vortex all liquid

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.

- 1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.

 Store the other strips into the bag in presence of the desiccant
- Wash the micropiate as reported in section 1.3. Check that controls/calibrator and samples have been correctly added. Incubate the microplate at +37°C for 60 min.
- Check that the reagent has been correctly added, Incubate the microplate at +37°C for 60 minutes.

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

Important note: Do not expose to strong direct light as a high

enzymatic reaction using the same pipetting sequence as in step 6. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (hackground subtraction, strongly recommended), blanking the instrument on A1 well, Pipette 100 µl Sulphuric Acid into each well to stop

- 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 be performed immediately and positive results on reading be performed immediately there the addition of the Stop Solution but definitely no longer than an immediate afterwards. Some self-oxidation of the
- chromogen can occur leading to a higher background.

N. ASSAY SCHEME

| Reading OD 450nm | Sulphuric Acid 1 | Temperature | 3 ¹⁰ incubation 20 | 1B/H2O2 mix | Washing step 4-5 | Temperature + | 2 ^m incubation 60 | Enzyme Conjugate 1 | Washing step 4-5 | Temperature + | 1" incubation 60 | Samples 1 |
|------------------|------------------|-------------|-------------------------------|-------------|------------------|---------------|------------------------------|--------------------|------------------|---------------|------------------|-----------|
| 450nm & 620nm | 100 ut | 12 | 20 min | 100 ul | 4-5 cycles | -37°C | 60 min | 100 ut | 4-5 cycles | +37°C | 60 min | 100 ul |

- Dispense 50 ul Specimen Diluent in all the wells identified for samples and controls/calibration, except for A1.
 Then pipette 100 ul of Negative Control in triplicate, 100 ul of Calibrator in duplicate, 100 ul Positive Control in single and then 100 ul of samples
- In all the wells except A1, pipette 100 µl Enzyme Conjugate

inner surface of the

5: Wash the microplate as described

6. Pipette 100 µl TMB/HzOz 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

1 2 A BLK S2 B NC S3 C NC S4 D NC S5 E CAL(1) S6 F CAL(1) S7

If the results of the test doesn't match the requirements stated above, operate as follows:

C. 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 Co/S values have been matched in the analysis. Ensure that the following parameters a

| Positive Control < 0.300 OD450nm value | Positive Control |
|---|------------------|
| coefficient of variation < 30% | |
| after blanking | (NC) |
| Negative Control > 0,750 mean OD450nm value | Negative Control |
| < 0.100 OD450nm value | Blank well |
| Requirements | Parameter |

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:

| | variation > 30% | blanking coefficient of | Negative Control (NC) < 0,750 OD450nm after | 8lank well > 0.100 OD450nm | Problem |
|---|---|---|---|---|---------|
| positive samples, to spills or to the enzyme conjugate; 5, that micropipettes have not become contaminated with positive samples or with the enzyme contiguate to the enzyme of the think obstructed are not blocked or partially obstructed. | assay procedure (dispensation of positive control inslead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to | been used and the washer has been primed with it before use; 3. that no mistake has been done in the | Inat the washing procedure and the washer settings are as validated in the pre qualification study; Inat the proper washing solution has | 1. that the Chromogen/Substrate solution has not become contaminated during the assay | Check |

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(*) important Notes

results calculation calculation, therefore it does not The Calibrator (CAL) does not affect the Cut Off affect the test's

Positive Control > 0.360 OD450nm

1. That the procedure has been correctly

2. that no mistake has occurred during the distribution of the control (ex. dispensation of negative control instead of the positive one);

3. that the washing procedure and the washer settings are as validated in the

pre qualification study;
4. Ihat no external contamination of the

positive control has occurred

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 table below:

3 4 5 6 7 8 9 10 11 12 to the supervisor for further actions, if any of the above problems have occurred, report the problem Parameter Requirements Calibrator 10 mIU/ml (WHO) | Co/S > 1.0 If Calibrator has used, verify the following data

| | 100 | | The second secon | |
|------|--|-----------|--|--|
| | BLK = B | ank | NC = Negative Control | |
| =Cal | Calibrator - Not mandators | nandatory | PC = Positive Control | |
| 음 | | | | |

| OD450nm or Co/S values | Problem | Check |
|------------------------|------------|--|
| | Calibrator | 1. that the procedure has been correctly |
| are met: | | performed; |
| | Co/S < 1_0 | 2, that no mistake has occurred during its |
| | | distribution; |
| equirements | | 3, that the washing procedure and the |
| chamounta | | washer settings are as validated in the |
| D450nm value | | pre qualification study; |
| ean OD450nm value | | 4. That no external contamination of the |
| ing | | 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 lest results are calculated by means of a cut-off value determined with the following formula:

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

the operating system of an ELISA automated work station, make sure that the proper formulation is used to calculate the cut-off Important note: When the calculation of results is performed by 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 | Co/S |
|----------|-----------|----------|----------------|
| 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 intial 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

Negative Control: 1,900 – 2,000 OD4
Mean Value: 2,000 OD4
Higher than 0,750 – Accepted 1,900 - 2,000 - 2,100 OD450nm 2,000 OD450nm

0,100 OD450nm

Calibrator: 0.

Mean value: 0.

Co/S > 1 - Accepted Cut-Off = (2.000 + 0.100) / 3 = 0.700 Lower than 0.300 - Accepted 0.400-0.360 OD450nm 0.380 OD450nm

Sample 1: 0 050 OD450nm Sample 2: 1 900 OD450nm Sample 1 Co/S > 1 1 Sample 2 Co/ < 0.9 positive negative

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 lest results are transmitted from the laboratory to
- another facility, attention must be paid to avoid erroneous
- data transfer.

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

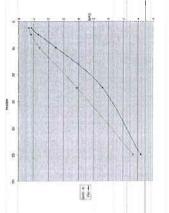
R. PERFORMANCE CHARACTERISTICS

1. Limit of detection
The limit of detection of the assay has been calculated by means of the 2rd International Standard supplied by WHO.
Two control samples, supplied by Boston Blomedica 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 mUml.

Results of Quality Control are given in the following table:

| Accurum 52 | Neg. Control | 2.5 | cn | 10 | 25 | 50 | mlU/ml OHW |
|-------------|--------------|-------|-------|-------|-------|-------|---------------|
| _ | | 1.015 | 0.943 | 0.543 | 0.197 | 0.099 | 0D450 nm |
| 12.7 | 8 | 0.7 | 0.8 | 1.4 | 3.9 | 7.7 | CoiS |
| Accurun 120 | Neg. Cor | 2.5 | 5 | 10 | 25 | 50 | mU/mf |
| 0.115 | 2.217 | 0.949 | 0.587 | 0.304 | 0.137 | 0.093 | OD450 |
| 0,0 | | 0.8 | 1.3 | 2.5 | 5.6 | 8.2 | Co/S |

Curves are reported below



on a total number of more than 200 samples, Seroconversion and performance panels have also been Diagnostic sensitivity:
 The diagnostic sensitivity has been lested in a clinical trial on
 panels of samples classified positive by a US FDA approved kit.
 An overall value of 100% has been found in the study conducted studied, Results obtained by examining two panels supplied by Boston Biomedica Inc., USA, are reported below.

Seroconversion Panel: PHT 902

| Sample CTRL (-) | 0D450nm Co/S BiaSorin 1,968 0,3 | 0,3 | Dias |
|--------------------|------------------------------------|-----|------|
| CTRL (+) | 0,084 | 8,1 | |
| Calibrator | 0,470 | 1,5 | |
| PHT902 | | | |
| -1 | 1,878 | 0,4 | neg |
| 22 | 1,501 | 0,5 | neg |
| t _s | 0,090 | 7,6 | pos |
| 4 | 0,123 | 5,6 | pos |
| C/A | 0 100 | 5.7 | nns |

Performance Panel: PHT 201

| Sample | 00450nm | C0/S | DiaSorin | Sample | Sample CD450nm Co/S DiaSorin Sample OD450nm Co/S DiaSorin | Co/S | Dia |
|--------|---------|------|----------|--------|---|----------|-----|
| - | 0,169 | 4,0 | pos | 4 | 0.139 | 49 | pos |
| 22 | 0,132 | 5,2 | pos | 55 | 0.115 | sh ip | pos |
| 64 | 0,143 | 4.0 | pos | 6 | 0.167 | 4.1 | pos |
| 4 | 0,104 | 6,6 | pos | 17 | 0,086 | 0,8 | pos |
| Č. | 0,438 | 1,6 | pos | 18 | 0,160 | 4.3 | pos |
| cn | 0,121 | 5,7 | pos | ú | 0,175 | 3,9 | pos |
| 7 | 0,127 | 5,4 | pos | 20 | 1,772 | 0,4 | gen |
| œ | 0,150 | 4,0 | pos | 21 | 0,090 | 7,6 | pos |
| 10 | 0,115 | 5,9 | Bod | 22 | 0,201 | 3,4 | pos |
| 16 | 0.094 | 7,3 | pos | 23 | 0.281 | 2,4 | pos |
| 11 | 0.070 | 9,8 | spd | 24 | 0.134 | Ch | pos |
| 12 | 1,814 | 0,4 | neg | 25 | 0.142 | 44 C0 | pos |
| 13 | 0,097 | 7.1 | pos | Neg | 1,780 | 0.4 | neg |

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classified negative with a US FDA approved in Capacitied negative with a US FDA approved in Capacitied techniques of People and seral have been used to determine the specificity. No laise reactivity due to the method of specimen preparation have also been observed. Fozors specimens have also been tested to check whether this interferes with the performance of the test. No interference was Diagnostic specificity:
 The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors,

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. observed on clean and particle free samples,

No cross reaction were observed,

The Performance Evaluation study conducted in the external eference center on more than 1000 samples has provided a

| Test # 1 | replicates in three separate runs is reported below: | samples of different anti-HAV reactivity, examined in | The mean values obtained from a study conducted on |
|----------|--|---|--|
| | | | |

Precision

| CV % | Std, Deviation | OD450nm | Sample |
|------|----------------|---------|----------|
| 27 | 0.065 | 2.425 | Negative |
| 3.9 | 0.023 | 0.608 | Low Pos. |

Test # 2

| CV % | Std. Deviation | OD450nm | Sample |
|------|----------------|---------|----------|
| 4.5 | 0.107 | 2.373 | Negative |
| 6.0 | 0.034 | 0,573 | Low Pos. |

Test #3

| Sample | Negative | Low Pos |
|----------------|----------|---------|
| 0D450nm | 2.478 | 0.554 |
| Std. Deviation | 0.108 | 0.023 |
| 200 | | |

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

3,4

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 lest 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

REFERENCES

- CDC, Summon 1998;46:1-87, 1997, MMWR
- CDC. Prevention of hepatitis A through active or passive immunization, Recommendations of the Advisory Committee on Immunization Practices (ACIP), MMWR 1996;45(RR-15),
- Krugman S, Giles JP, Viral hepatitis: new light on an old disease JAMA 1970;212:1019-29.
- Hadler SC, Websier HM, Erben JJ, Swanson JE, Maynard JE, Hepatitis A in day-care centers: a communitywide assessment, N Engl J Med 1980;302:1222-7
- Lednar WM, Lemon SM, Kirkpatrick JW, Redfield RR, Fields ML, Kelley PW, Frequency of illness associated with epidemic hepatilis A virus infection in adults. Am J Epidemiol 1985;122:226-33.
- Skinh | P. Mathiesen LR, Kryger P. M tler AM, Faecal excretion of hepatitis A virus in patients with symptomatic hepatitis A infection, Scand J Gastroenterol 1981;16:1057-9. Glikson M, Galun E, Oren R, Tur-Kaspa R, Shouval D, Relapsing hepatitis A, Review of 14 cases and literature survey, Medicine 1992;71:14-23,

16 W

- Tassopoulds NC, Papaevangelou GJ, Ticehursi JR, Purcell RH, Fesal excretion of Greek strains of hepatitis A virus in patients with hepatitis A and in experimentally infected chimpanzees, J Infect Dis 1986;154:231-7.
- Rosenblum LS, Villarino ME, Nainan OV, et al. Hepatitis A outbreak in a neonated infersive daire unit: risk factors for fransmission and evidence of prolonged viral excretion among preterm infants. J Infect Dis 1991;164:476-82.
- Sjogren MH, Tanno H, Fay O, et al. Hepatitis A virus in stool during clinical relapse. Ann Intern Med 1987;106 221-6.
- 11. Lemon SM. The natural history of hepatitis A: the potential for transmission by transfusion of blood or blood products. Vox Sang transmission by transfu 1994;67(suppl 4):19-23.
- Bower WA, Nainan OV, Margolis HS, Duralion of viremia in naturally-acquired hepatilis A viral infections, (Abstract 103) in: Abstracts of the Infectious Diseases Society of America, 35th Annual Meering, Alexandria, VA: Infectious Diseases Society of America, 1997.
- ζà Liaw VF, Yang CY, Chu CM, Huang MJ, Appearance and persistence of hepatitis A IgM antibody in acute clinical hepatitis A observed in an outbreak, Infection 1986;14:156-8.
- 14. Stapleton JT. Host immune response to hepatitis A virus. J Infect Dis 1995;171(suppl 1):S9-14.
- 5 Hulin YJF, Pool V, Cramer EH, et al. A multistate, outbreak of hepatitis A. N Engl J Med 1999;340:595-802. foodbame
- 6 Soucie JM, Robertson BH, Bell BP, McCaustland KA, Evalt BL, Hepatitis A virus infections associated with dotting factor concentrate in the United States, Transfusion 1998;38:573-9.
- Cohen JI, Feinstone S, Purcell RH, Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. J Infect Dis 1989;160:687-90.

17

- McCaustland KA, Bond WW, Bradley DW, Ebert JW, Maynard JE. Survival of hepatitis A virus in foces after drying and storage for 1 month. J Clin Microbiol 1932;16:957-5.
- Favero MS, Bond WW. Disinfection and sterifization. In: Zuckerman AJ, Thomas HC, eds. Viral hepatitis, scientific basis and clinical management New York, NY: Churchill Livingston, 1993;565-75.
- 20 Staes C, Schlenker T, Risk I, et. al. Source of infection among persons with acute hepautis A and no identified risk factors. Sall Lake County, Ulah. 1996 [Abstract 302]. Clin Infect Dis 1997-25-411.