Dr. Fooke-Achterrath Laboratorien GmbH, Habichtweg 16, D-41468 Neuss

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17.11.2023

Annex to the Declaration of Conformity

To whom it may concern:

Digitally signed by Ceaicovschi Tudor
Date: 2025.09.12 16:56:12 EEST
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

Dr. Fooke-Achterrath Laboratorien GmbH, Habichtweg 16, 41468 Neuss, Germany declares that the following list of allergens is the detailed information of the Product Name "Allergen-coupled Discs (Single Allergen, Allergen Mixes)" with the Product Number "Allergen Code", mentioned in the Declaration of Conformity.

Item code	Description
c 00068	Articaine
c 00082	Lidocaine
c 00088	Mepivacaine
c 00196	Epinephrine
c 00108	Ciprofloxacine
c 00125	Dexametazone
c 00056	Amoxiciline
c 00055	Cephalosporin
c 00153	Metronidazol
c 00089	Bupivacain
c 00172	Ketoprofen
c 00194	Azithromycin
c 00170	Clarithromycin
c 00083	Procain
c 00210	Tetracain

Sincerely,

DR FOOKE LABORATORIEN GMBH Habichtweg 16 41468 Neuss Tel (0 21 31) 29 84-0 · Fax (0 21 31) 29 84-184

Dr. Margrit Fooke-Achterrath
General Manager

Dr. Fooke-Achterrath Laboratorien GmbH, Habichtweg 16, D-41468 Neuss

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EC-Declaration of Conformity

For the purpose of 98/79/EC Directive on *in vitro* diagnostic medical devices

Product Name: **Allergene-coupled Discs (Sinlge Allergens, Allergen Mixes)**

Product Number: **Allergene Code**

The product is designed and manufactured for the purpose of 98/79/EC Directive on *in vitro* diagnostic medical devices in the sole responsibility of:

DR. FOOKE-ACHTERRATH Laboratorien GmbH
Habichtweg 16
D-41468 Neuss
Germany

The product meets all applicable requirements of Directive 98/79/EC.
The conformity assessment procedure followed Directive 98/79/EC Annex III.

This declaration is valid until 25th May 2027.

Neuss, 18th May 2022

DR. FOOKE-ACHTERRATH Laboratorien GmbH

Dr. Margrit Fooke-Achterrath
- General Manager -

CE IVD 2°C 8°C

Please read instructions for use before starting the assay

Specific IgE EAST

Enzym-Allergo-Sorbent-Test for the quantitative determination of allergen-specific IgE in human serum or plasma

REF 0560200PKL Σ 200 Determinations

REF 0561000PKL Σ 1000 Determinations

BACKGROUND

The worldwide frequency of allergies has increased significantly over the past decades. The term allergy is often used for Type I hypersensitivity reactions (immediate type reactions), whose symptoms generally occur within 30-60 minutes after contact with the allergen. The most frequent symptoms are: hay fever (rhinitis), conjunctivitis, hives (urticaria), allergic asthma and as the most dangerous manifestation anaphylaxis (the anaphylactic shock).

The allergens causing Type I hypersensitivity reactions are mostly proteins derived from the natural environment e.g. plant pollen, animal hair, food, mites, and insect venoms.

The characteristics of Type I allergies is the involvement of allergen specific immunoglobulins (antibodies) of class E (sIgE). Hence, the detection of sIgE is an important tool of modern allergy diagnostics.

INTENDED USE

The Specific IgE EAST is intended for the quantitative determination of sIgE in human serum or plasma. The results add to the diagnosis of type I allergies.

PRINCIPLE

The Specific IgE EAST for the quantitative measurement of specific IgE is carried out in mircotiterplates. During the first incubation step patient specimens are incubated on allergen coupled discs. Surplus serum components are removed from the well by washing whereas allergen specific IgE remains bound. Subsequently, alkaline phosphatase (AP)-labelled antibody is added forming allergen/-sIgE/anti-IgE conjugate complexes.

The wells are washed again, and the substrate solution p-nitrophenyl-phosphat (pNPP) is added and incubated, resulting in the development of a yellow colour if conjugate is present.

After stopping the enzymatic reaction with Sodium hydroxide (NaOH) the optical density (OD) of the coloured reaction product is measured spectrophotometrically at 405 nm (reference wave length 620 nm). The sIgE concentration of the patient sample is proportional to the OD. Calibrators with defined concentrations of IgE (calibrated against WHO) are assayed simultaneously with the patient samples to generate a calibration curve. Unknown IgE concentrations of the test samples are calculated from this curve.

KIT COMPONENTS

Enzyme kit	REF	**0560200PKL 0561000PKL**
Anti IgE Enzyme-Conjugate	CONJ AP E	1 x 10.4 mL 1 x 52 mL
Concentrated Washing Buffer (50x)	WASHBUF C 50x	1 x 30 mL 1 x 160 mL
Substrate Buffer	SUBBUF	1 x 50 mL 1 x 250 mL
Substrate Tablets	SUB PNPP	10 x 5 mg 50 x 5 mg
Stop Solution (1 N NaOH)	STOP NAOH	1 x 10 mL 1 x 52 mL

MATERIAL NEEDED, BUT NOT INCLUDED IN THE KIT

1. Reference unit	REF	**076000PQ**
Anti-IgE Reference discs	CALDISC	75 pieces
Calibrators (0.35, 0.7, 3.5, 17.5, 50, 100 IU/mL)	CAL (1-6)	6 x 0.8 mL

2. Allergen discs	REF	**Allergen-code**

3. Controls	REF	**07001/ 07002**
Positive Control	CONTROL +	1 x 0.5 mL
Negative Control	CONTROL −	1 x 0.5 mL

LABORATORY EQIUPMENT:

pipettes 10-100 µL, 200-1000 µL, Multipette, pipette tips, tubes for dilution of the specimens, graduated glass cylinder, ELISA-reader, covering foil, microplate-washer, incubator (optional), lab watch, distilled water.

SPECIMEN COLLECTION & PREPARATION

Either serum or plasma can be used in this test. No additives or preservatives are necessary to maintain the integrity of the specimen.
Specimens should be stored at 2-8°C and assayed within 48 hours after collection. If the assay cannot be performed within 48 hours or if the specimen has to be shipped, cap the specimen and keep it frozen. Repeated freezing and thawing should be avoided. Frozen specimens should be thawed at room temperature (RT, 20-25°C) and mixed thoroughly by gentle inversion before assaying. Samples should be tested undiluted. The use of haemolysed or lipemic specimens is not recommended.

PREPARATION OF REAGENTS

Allow all reagents to come to RT before use.
Enzyme conjugate: ready to use
Substrate Solution: to be prepared freshly
Stop Solution: ready to use
Calibrators and Controls: ready to use
Concentrated Washing Buffer:
The concentrated Washing Buffer has to be diluted 1:50 in distilled water. (Example: For 2 strips 10 mL of Washing Buffer is required. Therefore 200 µL concentrated Washing Buffer have to be diluted to a final volume of 10 mL with distilled water). The resulting Washing Buffer is stable for one week at RT.

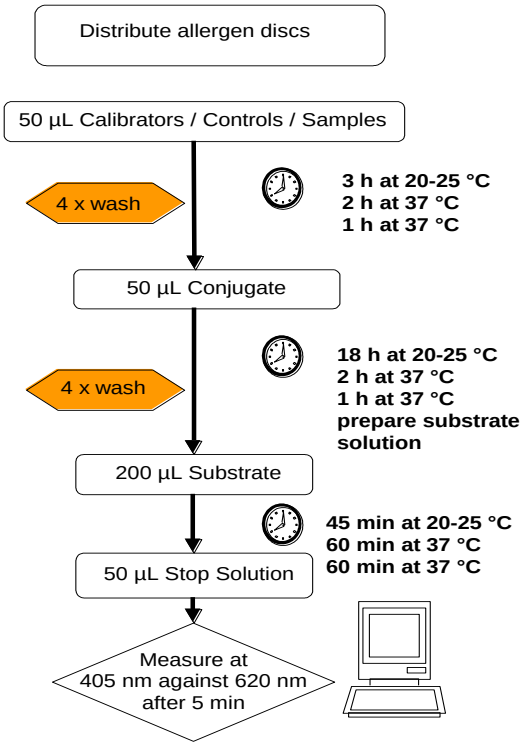
ASSAY PROCEDURE

1. Prepare a protocol for the assay run. It is recommended to test the calibrators and controls in duplicate determination.
2. Using plastic forceps, put reference- and allergen discs into test wells on the plate according to your protocol.
3. Pipette exactly 50 µL calibrator-, control- and patient samples directly onto the respective disc. Cover plate and incubate according to Table 1.
4. Following completion of the incubation time wash each well of the plate with an appropriate ELISA Plate Washer 4 x 1000 µL in “overflow”-modus with diluted Washing Buffer.
5. Pipette exactly 50 µL Anti-IgE-Conjugate onto each disc. Cover plate and incubate according to Table 1.
6. Prepare substrate solution approximately 1 h before use and store in the dark until use. Use one tablet for 5 mL Substrate Buffer.
7. Repeat washing as described in step 4.
8. Pipette 200 µL Substrate Solution into each well and incubate according to Table 1.
9. Add 50 µL Stop Solution to each well in the same order and interval as used for the substrate solution. It is recommended to mix the colour solution in the wells by knocking on the frame. Incubate plate for 5 min at RT. Read OD at 405 nm in a microplate reader (reference wavelength 620 nm) and calculate the results of the samples and controls as described on page 3.

Table 1: Incubation scheme

	Assay description		
	Long-time	**Short-time**	**Abbreviated**
Serum-incubation	3 h RT	2 h 37 °C	1 h 37 °C
Conjugate-incubation	18 h RT	2 h 37 °C	1 h 37 °C
Substrate-incubation	45 min RT	1 h 37 °C	1 h 37 °C

TEST SCHEME
Specific IgE EAST



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CALCULATION OF RESULTS

It is recommended to use validated software for the calculation of the results. For manual calculation, the mean OD [Δ 405 nm – 620 nm] values are calculated from the calibrators and controls. Generate a graph from the mean OD values of the six calibrators on half logarithmic paper (Abscissa: log IU IgE/mL; Ordinate: linear OD Δ 405 nm - 620 nm) to create a standard curve. The sIgE concentration and class of the patient sample is determined on the basis of this standard curve. The OD is mapped on the Ordinate and the result can be read out on the Abscissa. The standard curve and the controls should be in the acceptance range given in the Quality-Control-Certificates delivered with the kit. Otherwise, the test conditions should be verified and the test should probably be repeated.
The results are interpreted as follows:

Class	IU/mL sIgE	Interpretation
6	> 100	extremely high
5	50 -100	strongly high
4	17.50 - 50	very high
3	3.50 - 17.50	high
2	0.70 - 3.50	moderate
1	0.35 - 0.70	low
0	< 0.35	non detectable

EXPECTED VALUES

The clinical relevance of a positive test result varies significantly among the different allergens. Therefore, it is highly recommended for each laboratory to determine the normal range for each allergen individually. The above listed values can be used as a guideline for the interpretation.

HSA coupled allergens

Low molecular substances (Haptens) e.g. Penicillin and Isocyanates are coupled to the discs by a protein (Human Serum Albumin / HSA). In rare cases patient samples can contain HSA specific IgE. Therefore reaction against HSA itself has to be tested for each patient sample by running the HSA-Control Disc test and comparing the results to the Allergen-HSA-Conjugate.

Recommended interpretation:

The sIgE concentration against the HSA Conjugate is measured in parallel to sIgE to HSA. The concentration obtained from the HSA disc has to be subtracted from the concentration obtained from the respective HSA conjugate.

Alternative interpretation:

The result for the Allergen-HSA-Conjugate is calculated by multiplying the OD-Value of the HSA Control Disc by the factor 2.

Cut off = OD (HSA control disc) X 2

OD Allergen-HSA-Conjugate > Cut off: positive result.

MEASURING RANGE

This ELISA detects IgE concentrations in the range between 0.35 and 100 IU/mL. Samples with IgE concentrations above 100 IU/mL should be diluted and retested to obtain the exact concentration.

PRECISION

Variability and Reproducibility

1. ***Intra***-Assay-Variability

Specimen	Mean [IU/mL]	CV (%)
1 (n=10)	22,57	7,45
2 (n=10)	10,48	7,14
3 (n=10)	11,57	9,54

2. ***Inter***-Assay-Variability

Specimen	Mean [IU/mL]	CV (%)
1 (n=17)	23,41	7,91
2 (n=20)	10,49	7,54
3 (n=20)	10,93	10,79

LINEARITY

Five randomly selected sera show a linear behaviour (≤ ±20%) in three consecutive dilution steps. Based on the heterogeneity of human serum or plasma samples varying results can not be excluded.

SPECIFICITY

In physiological concentrations no cross-reactivity to other Ig-classes could be observed using this sIgE test.

LIMITATIONS OF THE METHOD

This sIgE test shows the following limitations:

- A negative test result does not exclude a Type I allergy
- The test result has to be considered in the context of the patient's history and the clinical findings

LITERATURE

1. Ishizaka K, Ishizaka T, und Hornbrook MM: **Physicochemical Properties of Human Reaginic Antibody IV. Presence of a Unique Immunoglobulin as a Carrier of Reaginic Activity** *J Immunol* 1966, **97**:75-85.
2. Hamilton R: **Radioimmunoassay in the Assessment of Allergic Disease***, Ligand Quarterly* 1979, **2**:13-19.
3. Johansson S, Bennich H, Berg T: **The Clinical Significance of IgE**, *Progress in Clin. Immunol* 1972, **1**.
4. Kjellman M: **Immunoglobulin IgE and Atopic Allergy in Childhood.** *Linkpoing University Medical Dissertations* No 36 1976.
5. Wittig H, Bellot J, Fillippi I, Royal G: **Age-related Serum IgE Levels in Healthy Subjects and in Patients with Allergic Disease.** *J Allergy Clin Immunol* 1980, **66**:305-313.
6. Gleich G, Averbeck A and Swedlund H: **Measurement of IgE in Normal and Allergic Serum by Radioimmunoassay**. *J Lab and Clin Med* 77 (1971) 690-698.
7. Arbeitsgruppe der Deutschen Diagnostika Gruppe e.V. (DDG). **Gute Labordiagnostische Praxis GLDP, Konzept einer „Guten Labordiganostischen Praxis".** *Clin Lab* 1999, **45**: 569-80.

PRECAUTIONS FOR USERS

1. In compliance with annex I of European directive 98/79/EC the use of *in-vitro* diagnostic medical devices is intended to secure suitability, performance and safety of the product by the manufacturer. Therefore the test procedure, information, precautions and warnings stated in the instructions for use have to be followed strictly. The kit has only to be used as described on page 1 (intended use).
2. The test must be performed according to this instruction, which contains all necessary information, precautions and warnings. The use of the test kit with analyzers and similar equipment has to be validated. Any change in design, composition of the test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes resulting in false results and other incidents. The manufacturer is not liable for any results obtained by visual analysis of patient samples.
3. The kit is intended for use by trained and qualified professionals carrying out research or diagnostic activities only. Pregnant women should not perform the test.
4. Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
5. For *in-vitro* diagnostic use only. Use only once. Do not use components exceeding the expiry date. Do not combine reagents of other suppliers or kit components of different lots (unless specified on page 1) with this kit.
6. Do not use kit components when the package of the component is damaged. Please check all solutions prior to use for microbiological contamination. Cap vials tightly immediately after use to avoid evaporation and microbiological contamination. Do not interchange screw caps of the reagent vials.
7. The kit was evaluated for use at the temperatures specified in the Testing scheme (see page 2). Higher or lower temperatures may result in values not meeting the quality control ranges.
8. The washing procedure is absolutely important. Improper washing will cause erroneous results. It is recommended to use a multichannel pipette and an automated washer.
9. To avoid cross-contamination and false-positive results it is recommended to perform all pipetting steps properly. Use only clean pipette tips, dispensers and lab ware.
10. Test components based on human serum were tested using a CE marked method for the presence of antibodies against HIV 1 / HIV 2, Anti-HBc, and Anti-HCV as well as for hepatitis antigen HBsAg and were found to be negative. Nevertheless, material based on human serum should be handled as potentially infectious (BIOHAZARD).
11. Some kit components may contain bovine serum albumin, of which according to the manufacturer no infectious potential is known. Due to the eventual occurrence of undetectable infectious agents we recommend to handle any product of animal origin as potentially infectious.
12. The following safety rules should be followed with all reagents:
    - Do not get in eyes, on skin, or on clothing (P262). Do not breathe spray (P260). Pipetting should never be done by mouth, but with suitable pipetting devices.
    - IF SWALLOWED: rinse mouth. Do NOT induce vomiting (P301/330/331)
    - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303/361/353).
    - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing (P303/340).
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305/351/338)
    - Don't eat, drink or smoke while performing the test. Keep away from food, feed and beverage.
    - Wear protective gloves/protective clothing/eye protection (P280). Wash hands thoroughly after handling (P264) and care for your skin.
    - Material safety data sheet is available on request.
13. Stop Solution and SubBuf cause severe skin burns and eye damage (H314).
14. TMB in high concentrations may be potentially mutagenic. Due to the low concentration of TMB in this substrate solution a mutagenic effect can be ruled out, if it is properly used.
15. p-NPP is harmful if swallowed (H302). Diethanolamin (SubBuf) may cause damage to organs through prolonged or repeated exposure (H373). Get medical advice/attention if you feel unwell (P314).
16. The preservatives (Bronidox) are toxic to aquatic life, but their concentration is not hazardous to environment anymore. On disposal, flush large volumes of reagents with plenty of water.
17. Waste containing serum must be collected in separate containers containing an appropriate disinfectant in sufficient concentration. This material has to be treated according to national biohazard and safety guidelines or regulations.
18. We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.

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LOT	CE	IVD	2 °C 8 °C		REF					Σ		
Lot- Number	European conformity	For *in-vitro* diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manufactured by	Biohazard

TITLE: Product Group Assays for the quantitative Determination of specific IgE in human Serum or Plasma with allergen coupled discs and affiliated quantitative Reference Systems

1 Table of Contents

2 Product group

REF Enzyme kit	REF Reference unit
0540200PKL 0541000PKL	074000PQ
0560200PKL 0561000PKL	076000PQ
0541001PKL	074010PQ
0561001PKL	076010PQ

3 Background

The worldwide frequency of allergies has increased significantly over the past decades. The term allergy is often used for Type I hypersensitivity reactions (immediate type reactions), whose symptoms generally occur within 30-60 minutes after contact with the allergen. The most frequent symptoms are: hay fever (rhinitis), conjunctivitis, hives (urticaria), allergic asthma and as the most dangerous manifestation anaphylaxis (the anaphylactic shock).
The allergens causing Type I hypersensitivity reactions are mostly proteins derived from the natural environment e.g. plant pollen, animal hair, food, mites, and insect venoms.

The characteristics of Type I allergies is the involvement of allergen specific immunoglobulins (antibodies) of class E (sIgE). Hence, the detection of sIgE is an important tool of modern allergy diagnostics.

4 Intended Use

The specific IgE EAST is intended for the quantitative determination of sIgE in human serum or plasma. The results add to the diagnosis of type I allergies.

5 Principle of Test

This sIgE test for the quantitative measurement of specific IgE is carried out in mircotiterplates. During the first incubation step patient specimens are incubated on allergen coupled discs. Surplus serum components are removed from the well by washing whereas allergen specific IgE remains bound. Subsequently, alkaline phosphatase (AP)-labelled antibody is added forming allergen/-sIgE/anti-IgE conjugate complexes. The wells are washed again, and the substrate solution p-nitrophenyl-phosphat (pNPP) is added and incubated, resulting in the development of a yellow colour if conjugate is present.
After stopping the enzymatic reaction with Sodium hydroxide (NaOH) the optical density (OD) of the coloured reaction product is measured spectrophotometrically at 405 nm (reference wave length 620 nm). The sIgE concentration of the patient sample is proportional to the OD. Calibrators with defined concentrations of IgE (calibrated against WHO 75/502) are assayed simultaneously with the patient samples to generate a calibration curve. Unknown IgE concentrations of the test samples are calculated from this curve.

6 Material

6.1 Enzyme Kit 0540200PKL, 0541000PKL with quantitative Reference system 074000PQ

Enzyme kit	REF	**0540200PKL** **0541000PKL**
Anti IgE Enzyme-Conjugate	CONJ AP E	1 x 10.4 mL 1 x 52 mL
Concentrated Washing Buffer (50x)	WASHBUF C 50x	1 x 30 mL 1 x 160 mL
Substrate Buffer	SUBBUF	1 x 50 mL 1 x 250 mL
SubstrateTablets	SUB PNPP	10 x 5 mg 50 x 5 mg
Stop Solution (1 N NaOH)	STOP NAOH	1 x 10 mL 1 x 52 mL

Reference unit	REF	**074000PQ**
Anti-IgE Reference discs	CALDISC	2 x 25 piec.
Calibrators (0.35, 0.7, 3.5, 17.5 IU/mL)	CAL (1-4)	4 x 0.8 mL

6.2 Enzyme Kit 0560200PKL, 0561000PKL with quantitative Reference system 076000PQ

Enzyme kit	REF	**0560200PKL** **0561000PKL**
Anti IgE Enzyme-Conjugate	CONJ AP E	1 x 10.4 mL 1 x 52 mL
Concentrated Washing Buffer (50x)	WASHBUF C 50x	1 x 30 mL 1 x 160 mL
Substrate Buffer	SUBBUF	1 x 50 mL 1 x 250 mL
SubstrateTablets	SUB PNPP	10 x 5 mg 50 x 5 mg
Stop Solution (1 N NaOH)	STOP NAOH	1 x 10 mL 1 x 52 mL

Reference unit	REF	**76000PQ**
Anti-IgE Reference discs	CALDISC	3 x 25 piec.
Calibrators (0.35, 0.7, 3.5, 17.5. 50, 100 IU/mL)	CAL (1-6)	6 x 0.8 mL

6.3 Enzyme Kit 0541001PKL with quantitative Reference system 074010PQ

Enzyme kit	REF	**0541001PKL**
Anti IgE Enzyme-Conjugate	CONJ AP E	1 x 75 mL
Concentrated Washing Buffer (50x)	WASHBUF C 50x	1 x 500 mL
Substrate Buffer	SUBBUF	1 x 350 mL
SubstrateTablets	SUB PNPP	70 x 5 mg
Stop Solution (1 N NaOH)	STOP NAOH	2 x 125 mL

Reference unit	REF	**74010PQ**
Anti-IgE Reference discs	CALDISC	6 x 8 piec.
Calibrators (0.35, 0.7, 3.5, 17.5. IU/mL)	CAL (1-4)	4 x 2.5 mL

6.4 Enzyme Kit 0561001PKL with quantitative Reference system 076010PQ

Enzyme kit	REF	**0561001PKL**
Anti IgE Enzyme-Conjugate	CONJ AP E	1 x 75 mL
Concentrated Washing Buffer (50x)	WASHBUF C 50x	1 x 500 mL
Substrate Buffer	SUBBUF	1 x 350 mL
SubstrateTablets	SUB PNPP	70 x 5 mg
Stop Solution (1 N NaOH)	STOP NAOH	2 x 125 mL

Reference unit	REF	**76000PQ**
Anti-IgE Reference discs	CALDISC	9 x 8 piec.
Calibrators (0.35, 0.7, 3.5, 17.5. 50, 100 IU/mL)	CAL (1-6)	6 x 2.5 mL

7 Performance evaluation according to EN 13612

Considering the broad range of IVD MDs covered by Directive 98/79/EC many items described in EN 13612 will depend on the level of complexity of the IVD MD and their applicability have to be proven individually.

7.1 The variability and reproducibility was tested: REF 0540200PKL, 0541000PKL

7.1.1 Intra-Assay

Specimen	Mean [IU/mL]	CV (%)
1 (n=10)	17.45	1.02
2 (n=10)	9.95	8.53
3 (n=12)	13.56	6.05

7.1.2 Inter-Assay

Specimen	Mean [IU/mL]	CV (%)
1 (n=16)	17.09	3.84
2 (n=22)	13.22	7.00
3 (n=22)	3.84	8.28

7.1.3 Linearity

Five randomly selected sera show a linear behaviour (≤ ± 20%) in three consecutive dilution steps. Based on the heterogeneity of human serum- or plasma samples varying results can not be excluded.

7.1.4 Specificity

In physiological concentrations no cross-reactivity to other Ig-classes could be observed using this sIgE test.

7.2 The variability and reproducibility was tested: REF 0560200PKL, 0561000PKL

7.2.1 Intra-Assay

Specimen	Mean [IU/mL]	CV (%)
1 (n=10)	22.57	7.45
2 (n=10)	10.48	7.14
3 (n=10)	11.57	9.54

7.2.2 Inter-Assay

Specimen	Mean [IU/mL]	CV (%)
1 (n=17)	23.41	7.91
2 (n=20)	10.49	7.54
3 (n=20)	10.93	10.79

7.2.3 Linearity

Five randomly selected sera show a linear behaviour (≤ ± 20%) in three consecutive dilution steps. Based on the heterogeneity of human serum- or plasma samples varying results can not be excluded.

7.2.4 Specificity

In physiological concentrations no cross-reactivity to other Ig-classes could be observed using this sIgE test.

8 Literature

1. Ishizaka K, Ishizaka T, und Hornbrook MM: **Physicochemical Properties of Human Reaginic Antibody IV. Presence of a Unique Immunoglobulin as a Carrier of Reaginic Activity.** *J Immunol* 1966, **97**:75-85.
2. Hamilton R: **Radioimmunoassay in the Assessment of Allergic Disease.** *Ligand Quarterly* 1979, **2**:13-19.

3. Johansson S, Bennich H, Berg T: **The Clinical Significance of IgE**. *Progress in Clin Immunol* 1972, 1.
4. Kjellman M: **Immunoglobulin IgE and Atopic Allergy in Childhood.** *Linkpoing University Medical Dissertations* No 36 1976.
5. Wittig H, Bellot J, Fillippi I, Royal G: **Age-related Serum IgE Levels in Healthy Subjects and in Patients with Allergic Disease.** *J Allergy Clin Immunol* 1980, **66**:305-313.
6. Gleich, G, Averbeck, A, and Swedlund, H: **Measurement of IgE in Normal and Allergic Serum by Radioimmunoassay**. *J Lab and Clin Med* 1971, **77**: 690-698.
7. Arbeitsgruppe der Deutschen Diagnostika Gruppe e.V. (DDG). **Gute Labordiagnostische Praxis GLDP, Konzept einer „Guten Labordiganostischen Praxis".** *Clin Lab* 1999, **45**:569-580.

9 History

Revision	Released	
01	**24.09.2003**	
02	**05.03.2004**	
03	**06.06.2007**	Editorial changes, insert of "Performance evaluation according to EN 13612"

Name	Date:	Signature:
Issued by: **Jens Sperveslage**	**06.06.2007**	
Reviewed by: **Dr. M. Mahler**	**06.06.2007**	
Released by: **Dr. M. Fooke**	**06.06.2007**	

LISTE DER ALLERGENE

LIST OF ALLERGENS

DR FOOKE

Alle Produkte sind CE gekennzeichnet
All products are CE marked

Pollen Pollens

Bäume Trees

Code	Deutsch	English	Latein/Latin
t1	Ahorn	Maple	Acer negundo
t2	Erle	Alder	Alnus glutinosa
t3	Birke	Birch	Betula pendula
t4	Hasel	Hazelnut	Corylus avellana
t5	Buche	Beech	Fagus silvatica
t6	Sadebaum	Sade Tree	Juniperus sabina
t7	Eiche	Oak	Quercus alba
t8	Ulme	Elm	Ulmus spp.
t9	Olive	Olive	Olea europea
t10	Walnuss	Walnut	Juglans regia
t11	Platane	Plane	Platanus acerifolia
t12	Salweide	Willow	Salix alba
t13	Jasmin	Jasmin	Jasminum spp.
t14	Pappel	Poplar	Populus spp.
t15	Esche	Ash	Fraxinus excelsior
t16	Kiefer	White Pine	Pinus silvestris
t17	Kastanie	Chestnut	Aesculus hippocastanum
t18	Eukalyptus	Eucalyptus	Eucalyptus spp.
t19	Mimose	Mimosa	Mimosa spp.
t20	Liguster	Privet	Ligustrum vulgare
t21	Flieder	Lilac	Syringa vulgaris
t22	Weißdorn	Hawthorn	Crataegus spp.
t23	Zypresse	Cypress	Cupressus sempervirens
t24	Zeder	Cedar	Juniperus spp.
t26	Holunder	Elder	Sambucus nigra
t27	Linde	Lime Tree	Tilia cordata
t28	Robinie	Robinia	Robinia pseudoacacia
t29	Kirsche	Cherry	Prunus avium
t30	Mesquite	Mesquite	Prosopis velutina
t31	Melaleuca	Tea Tree	Melaleuca leucadendron (alternifolia)
t32	Orange	Orange	Citrus sinensis
t33	Lombard. Pappel	Lombardy Poplar	Populus nigra italica
t34	Mandel	Almond	Prunus amydalus
t35	Fichte	Fir	Picea abies
t36	Akazie	Acacia	Acacia spp.
t37	Eibe	Yew	Taxus bacchata
t38	Tanne	Fir tree	Abies concolor
t39	Pecan	Pecan	Carya pecan
t40	Pinie	Pine	Pinus pinea
t41	Dattelpalme	Datepalm	Phoenix dactylifera
t43	Thuja	Thuja	Thuja spp.
t50	Magnolie	Magnolia	Magnoliaceae
t70	Maulbeerbaum	Mulberry	Morus alba / rubra
t71	Japanische Zeder	Japanese Cedar	Cryptomeria japonica

Pollen Pollens

Bäume Multi-Allergene Trees Multi-Allergens

Code	Deutsch			Code	English		
Tx1	Bäume frühblühend	t2	Erle	Tx1	Trees early	t2	Alder
		t4	Hasel			t4	Hazelnut
		t8	Ulme			t8	Elm
		t12	Salweide			t12	Willow
		t14	Pappel			t14	Poplar
Tx2	Bäume spätblühend	t1	Ahorn	Tx2	Trees late	t1	Maple
		t3	Birke			t3	Birch
		t5	Buche			t5	Beech
		t7	Eiche			t7	Oak
		t10	Walnuss			t10	Walnut
Tx3	Bäume Mischung 3	t3	Birke	Tx3	Trees Mix 3	t3	Birch
		t7	Eiche			t7	Oak
		t8	Ulme			t8	Elm
		t24	Zeder			t24	Cedar
		t30	Mesquite			t30	Mesquite
Tx4	Bäume Mischung 4	t1	Ahorn	Tx4	Trees Mix 4	t1	Maple
		t3	Birke			t3	Birch
		t5	Buche			t5	Beech
		t7	Eiche			t7	Oak
		t11	Platane			t11	Plane
		t14	Pappel			t14	Poplar
Tx5	Bäume Mischung 5	t1	Ahorn	Tx5	Trees Mix 5	t1	Maple
		t3	Birke			t3	Birch
		t7	Eiche			t7	Oak
		t8	Ulme			t8	Elm
		t28	Robinie			t28	Robinia
		t31	Melaleuca			t31	Tea Tree
Tx6	Bäume Mischung 6	t9	Olive	Tx6	Trees Mix 6	t9	Olive
		t11	Platane			t11	Plane
		t23	Zypresse			t23	Cypress
Tx7	Bäume Mischung 7	t2	Erle	Tx7	Trees Mix 7	t2	Alder
		t3	Birke			t3	Birch
		t9	Olive			t9	Olive
Tx8	Bäume Mischung 8	t2	Erle	Tx8	Trees Mix 8	t2	Alder
		t3	Birke			t3	Birch
		t4	Hasel			t4	Hazelnut
		t7	Eiche			t7	Oak
		t12	Salweide			t12	Willow
Tx9	Bäume Mischung 9	t2	Erle	Tx9	Trees Mix 9	t2	Alder
		t3	Birke			t3	Birch
		t4	Hasel			t4	Hazelnut
		t5	Buche			t5	Beech
		t7	Eiche			t7	Oak
		t8	Ulme			t8	Elm
		t11	Platane			t11	Plane
		t12	Salweide			t12	Willow
Tx10	Bäume Mischung 10	t3	Birke	Tx10	Trees Mix 10	t3	Birch
		t7	Eiche			t7	Oak
		t8	Ulme			t8	Elm
		t9	Olive			t9	Olive
		t11	Platane			t11	Plane
		t12	Salweide			t12	Willow
		t14	Pappel			t14	Poplar

Pollen

Bäume

Multi-Allergene

Code	Deutsch		
Tx13	Bäume Mischung 13	t5	Buche
		t7	Eiche
		t9	Olive
		t12	Salweide
		t36	Akazie
		t40	Pinie
Tx14	Bäume Mischung 14	t2	Erle
		t4	Hasel
		t8	Ulme
		t9	Olive
		t12	Salweide
Tx15	Bäume Mischung 15	t3	Birke
		t5	Buche
		t7	Eiche
		t9	Olive
		t10	Walnuss
Tx16	Bäume Mischung 16	t7	Eiche
		t8	Ulme
		t11	Platane
		t12	Salweide
		t14	Pappel
Tx17	Bäume Mischung 17	t3	Birke
		t7	Eiche
		t8	Ulme
		t11	Platane
		t16	Kiefer
		t18	Eukalyptus
		t19	Mimose
Tx18	Bäume Mischung 18	t3	Birke
		t7	Eiche
		t8	Ulme
		t11	Platane
		t18	Eukalyptus
		t36	Akazie
		t40	Pinie
Tx19	Bäume Mischung 19	t3	Birke
		t6	Sadebaum
		t7	Eiche
		t8	Ulme
		t30	Mesquite
Tx20	Bäume Mischung 20	t2	Erle
		t4	Hasel
		t7	Eiche
		t8	Ulme
		t14	Pappel
Tx21	Bäume Mischung 21	t3	Birke
		t7	Eiche
		t9	Olive
		t12	Salweide
		t14	Pappel
		t16	Kiefer
Tx23	Bäume Mischung 23	t5	Buche
		t7	Eiche
		t11	Platane
		t14	Pappel
		t36	Akazie

Pollens

Trees

Multi-Allergens

Code	English		
Tx13	Trees Mix 13	t5	Beech
		t7	Oak
		t9	Olive
		t12	Willow
		t36	Acacia
		t40	Pine Tree
Tx14	Trees Mix 14	t2	Alder
		t4	Hazelnut
		t8	Elm
		t9	Olive
		t12	Willow
Tx15	Trees Mix 15	t3	Birch
		t5	Beech
		t7	Oak
		t9	Olive
		t10	Walnut
Tx16	Trees Mix 16	t7	Oak
		t8	Elm
		t11	Plane
		t12	Willow
		t14	Poplar
Tx17	Trees Mix 17	t3	Birch
		t7	Oak
		t8	Elm
		t11	Plane
		t16	White Pine
		t18	Eucalyptus
		t19	Mimosa
Tx18	Trees Mix 18	t3	Birch
		t7	Oak
		t8	Elm
		t11	Plane
		t18	Eucalyptus
		t36	Acacia
		t40	Pine Tree
Tx19	Trees Mix 19	t3	Birch
		t6	Sade Tree
		t7	Oak
		t8	Elm
		t30	Mesquite
Tx20	Trees Mix 20	t2	Alder
		t4	Hazelnut
		t7	Oak
		t8	Elm
		t14	Poplar
Tx21	Trees Mix 21	t3	Birch
		t7	Oak
		t9	Olive
		t12	Willow
		t14	Poplar
		t16	White Pine
Tx23	Trees Mix 23	t5	Beech
		t7	Oak
		t11	Plane
		t14	Poplar
		t36	Acacia

Pollen

Bäume

Multi-Allergene

Code	Deutsch		
Tx24	Bäume Mischung 24	t2	Erle
		t4	Hasel
		t7	Eiche
		t8	Ulme
		t11	Platane
		t12	Salweide
		t14	Pappel
Tx25	Bäume Mischung 25	t1	Ahorn
		t3	Birke
		t4	Hasel
		t7	Eiche
		t8	Ulme
		t14	Pappel
		t23	Zypresse
Tx26	Bäume Mischung 26	t1	Ahorn
		t2	Erle
		t3	Birke
		t7	Eiche
		t12	Salweide
		t14	Pappel
Tx27	Bäume Mischung 27	t2	Erle
		t4	Hasel
		t7	Eiche
		t8	Ulme
		t10	Walnuss
		t11	Platane
		t12	Salweide
		t14	Pappel
TTx7	Bäume Mischung T7 *	t9	Olive
		t12	Salweide
		t16	Kiefer
		t18	Eukalyptus
		t19	Mimose
		t31	Melaleuca
TTx8	Bäume Mischung T8 *	t9	Olive
		t12	Salweide
		t16	Kiefer
		t18	Eukalyptus
		t19	Mimose

Pollens

Trees

Multi-Allergens

Code	English		
Tx24	Trees Mix 24	t2	Alder
		t4	Hazelnut
		t7	Oak
		t8	Elm
		t11	Plane
		t12	Willow
		t14	Poplar
Tx25	Trees Mix 25	t1	Maple
		t3	Birch
		t4	Hazelnut
		t7	Oak
		t8	Elm
		t14	Poplar
		t23	Cypress
Tx26	Trees Mix 26	t1	Maple
		t2	Alder
		t3	Birch
		t7	Oak
		t12	Willow
		t14	Poplar
Tx27	Trees Mix 27	t2	Alder
		t4	Hazelnut
		t7	Oak
		t8	Elm
		t10	Walnut
		t11	Plane
		t12	Willow
		t14	Poplar
TTx7	Trees Mix T7 *	t9	Olive
		t12	Willow
		t16	White Pine
		t18	Eucalyptus
		t19	Mimosa
		t31	Tea Tree
TTx8	Trees Mix T8 *	t9	Olive
		t12	Willow
		t16	White Pine
		t18	Eucalyptus
		t19	Mimosa

* nur als biotinyliertes Reagenz verfügbar
* available only as biotinylated reagent

Pollen
Kräuter und Blumen

Pollens
Weeds and Flowers

Code	Deutsch	English	Latein/Latin
w1	beifußbl. Ambrosie	Common Ragweed	Ambrosia arthemisiifolia
w2	ausd. Ambrosie	Western Ragweed	Ambrosia psilotachya
w3	dreil. Ambrosie	Giant Ragweed	Ambrosia trifida
w4	falsche Ambrosie	False Ragweed	Ambrosia acanthicarpa
w5	Wermut	Wormwood	Artemisia absinthium
w6	Beifuß	Mugwort	Artemisia vulgaris
w7	Margerite	Ox Eye Daisy	Leucanthemum vulgare
w8	Löwenzahn	Dandelion	Taraxacum officinale
w9	Spitzwegerich	English plantain	Plantago lanceolata
w10	Weißer Gänsefuß	Lamb's Quaters	Chenopodium album
w11	Salzkraut	Saltwort	Salsola kali
w12	echte Goldrute	Goldenrod	Solidago spp.
w13	Spitzklette	Common Cucklebur	Xanthium strumarium
w14	Fuchsschwanz	Amaranth	Amaranthus retroflexus
w15	Melde	Scale	Artriplex spp.
w16	Weidenröschen	Willow herb	Epilobium spp.
w17	Aster	Aster	Callistephus chinensis
w18	Sauerampfer	Sorrel	Rumex acetosella
w19	Glaskraut 2	Wall pellitory 2	Parietaria judaica
w20	Brennnessel	Nettle	Urtica dioica
w21	Glaskraut 1	Wall pellitory 1	Parietaria officinalis
w22	Chrysantheme	Chrysanthemum	Chrysanthemum segetum
w23	Dahlie	Dahlia	Dahlia cultorum
w24	Besenradmelde	Firebush	Kochia scoparia
w25	Kamille (echte)	Camomile	Matricaria chamomilla
w26	Narzisse	Narcissus	Narcissus spp.
w27	Nelke	Carnation	Dianthus caryophyllus
w28	Rose	Rose	Rosa spp.
w29	Sonnenblume	Sunflower	Helianthus spp.
w30	Tulpe	Tulip	Tulipa spp.
w31	Heidekraut	Heather	Calluna vulgaris
w32	Raps	Rape	Brassica rapa
w33	Malve	Mallow	Malva spp.
w34	Klee	Clover, sweet	Meliotus spp.
w35	Geranie	Geranium	Geranium spp.
w36	Primel	Primerose	Primula spp.
w38	Rispenkraut	Marsh, elder rough	Iva annua
w39	Lupine	Lupine	Lupinus luteus
w40	Hyazinthe, blau	Hyacinth, blue	Hyacinthus spp.
w41	Luzerne	Alfalfa	Medicago sativa
w43	Oleander	Oleander	Nerium oleander
w44	Lilie	Lily	Lilium spp.
w45	Euphorbie	Euphorbia	Euphorbia spp.
w46	Azalee	Acalee	Acalea spp.
w47	Hibiscus	Hibiscus	Hibiscus spp.
w49	Begonie	Begonia	Begonia semperflorens
w50	Forsythie	Golden bell	Forsythia suspensa
w52	Arnika	Arnica	Arnica montana
w53	Johanniskraut	Rose of Sharon	Hypericum perforatum
w54	Lavendel	Lavender	Lavandula
w55	Maiglöckchen	Lily of the valley	Convallaria majalis
w58	Fresie	Fresia	Fresia spp.
w59	Gerbera	Gerbera	Gerbera spp.
w62	Yucca	Yucca	Yucca spp.
w64	Fuchsie	Fuchsia	Fuchsia spp.
w65	Aloevera	Aloevera	Aloe barbadensis
w66	Hartriegel	Cornel	Cornus
w67	Ginseng	Ginseng	Panax ginseng

Weitere Allergene auf Anfrage. Further allergens on request

Pollen
Kräuter und Blumen Multi-Allergene

Pollens
Weeds and Flowers Multi-Allergens

Code	Deutsch			Code	English		
Wx1	Kräuter Mischung 1	w1	beifußbl. Ambrosie	Wx1	Weed Mix 1	w1	Common Ragweed
		w6	Beifuß			w6	Mugwort
		w7	Margerite			w7	Ox Eye Daisy
		w8	Löwenzahn			w8	Dandelion
		w12	echte Goldrute			w12	Goldenrod
Wx2	Kräuter Mischung 2	w9	Spitzwegerich	Wx2	Weed Mix 2	w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w11	Salzkraut			w11	Saltwort
Wx3	Kräuter Mischung 3	w6	Beifuß	Wx3	Weed Mix 3	w6	Mugwort
		w9	Spitzwegerich			w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w20	Brennnessel			w20	Nettle
Wx4	Blumen Mischung 4	w7	Margerite	Wx4	Flower Mix 4	w7	Ox Eye Daisy
		w17	Aster			w17	Aster
		w22	Chrysantheme			w22	Chrysanthemum
		w23	Dahlie			w23	Dahlia
Wx5	Blumen Mischung 5	w30	Tulpe	Wx5	Flower Mix 5	w30	Tulip
		w35	Geranie			w35	Geranium
		w36	Primel			w36	Primerose
		w40	Hyazinthe, blau			w40	Hyacinth, blue
Wx6	Kräuter Mischung 6	w1	beifußbl. Ambrosie	Wx6	Weed Mix 6	w1	Common Ragweed
		w6	Beifuß			w6	Mugwort
		w9	Spitzwegerich			w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w11	Salzkraut			w11	Saltwort
Wx7	Kräuter Mischung 7	w6	Beifuß	Wx7	Weed Mix 7	w6	Mugwort
		w9	Spitzwegerich			w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w12	echte Goldrute			w12	Goldenrod
Wx9	Kräuter Mischung 9	w3	dreilappige Ambrosie	Wx9	Weed Mix 9	w3	Giant Ragweed
		w6	Beifuß			w6	Mugwort
		w9	Spitzwegerich			w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w15	Melde			w15	Scale
		w20	Brennnessel			w20	Nettle
Wx10	Kräuter Mischung 10	w6	Beifuß	Wx10	Weed Mix 10	w6	Mugwort
		w9	Spitzwegerich			w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w11	Salzkraut			w11	Saltwort
Wx11	Kräuter Mischung 11	w1	beifußbl. Ambrosie	Wx11	Weed Mix 11	w1	Common Ragweed
		w6	Beifuß			w6	Mugwort
		w9	Spitzwegerich			w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w19	Glaskraut 2			w19	Wall Pellitory 2
		w20	Brennnessel			w20	Nettle
Wx12	Kräuter Mischung 12	w9	Spitzwegerich	Wx12	Weed Mix 12	w9	English Plantain
		w10	Weißer Gänsefuß			w10	Lamb's Quaters
		w11	Salzkraut			w11	Saltwort
		w19	Glaskraut 2			w19	Wall Pellitory 2
Wx13	Blumen Mischung 13	w7	Margerite	Wx13	Flower Mix 13	w7	Ox Eye Daisy
		w28	Rose			w28	Rose
		w30	Tulpe			w30	Tulip
		w36	Primel			w36	Primerose
Wx14	Blumen Mischung 14	w17	Aster	Wx14	Flower Mix 14	w17	Aster
		w22	Chrysantheme			w22	Chrysanthemum
		w23	Dahlie			w23	Dahlia
		w40	Hyazinthe, blau			w40	Hyacinth, blue

Weitere Allergene auf Anfrage. Further allergens on request

Pollen

Kräuter und Blumen
Multi-Allergene

Pollens

Weeds and Flowers
Multi-Allergens

Code	Deutsch			Code	English		
Wx21	Parietaria Mischung	w19 w21	Glaskraut 2 Glaskraut 1	Wx21	Parietaria Mix	w19 w21	Wall Pellitory 2 Wall Pellitory 1
Wx22	Kräuter Mischung 22	w6 w8 w9	Beifuß Löwenzahn Spitzwegerich	Wx22	Weed Mix 22	w6 w8 w9	Mugwort Dandelion English Plantain
Wx23	Kräuter Mischung 23	w1 w6 w9 w20 w21 w29	beifußbl. Ambrosie Beifuß Spitzwegerich Brennnessel Glaskraut 1 Sonnenblume	Wx23	Weed Mix 23	w1 w6 w9 w20 w21 w29	Common Ragweed Mugwort English Plantain Nettle Wall Pellitory 1 Sunflower
Wx25	Kräuter Mischung 25	w7 w20 w28 w40 w44	Margerite Brennnessel Rose Hyazinthe, blau Lilie	Wx25	Weed Mix 25	w7 w20 w28 w40 w44	Margerite Nettle Rose Hyacinth, blue Lily
Wx26	Kräuter Mischung 26 *	w9 w10 w11 w18	Spitzwegerich weißer Gänsefuß Salzkraut Sauerampfer	Wx26	Weed Mix 26 *	w9 w10 w11 w18	English Plantain Lamb's Quaters Saltwort Sorrel
Wx27	Kräuter Mischung 27 *	w1 w6 w7 w8	beifußbl. Ambrosie Beifuß Margerite Löwenzahn	Wx27	Weed Mix 27 *	w1 w6 w7 w8	Common Ragweed Mugwort Ox Eye Daisy Dandelion
Wx28	Kräuter Mischung 28 *	w6 w9 w10 w21	Beifuß Spitzwegerich Weißer Gänsefuß Glaskraut 1	Wx28	Weed Mix 28 *	w6 w9 w10 w21	Mugwort English Plantain Lamb's Quaters Wall Pellitory 1
Wx29	Kräuter Mischung 29 *	w6 w8 w12 w13 w18	Beifuß Löwenzahn echte Goldrute Spitzklette Sauerampfer	Wx29	Weed Mix 29 *	w6 w8 w12 w13 w18	Mugwort Dandelion Goldenrod Common Cucklebur Sorrel
Wx30	Kräuter Mischung 30 *	w6 w9 w13 w18 w20 w21	Beifuß Spitzwegerich Spitzklette Sauerampfer Brennnessel Glaskraut 1	Wx30	Weed Mix 30 *	w6 w9 w13 w18 w20 w21	Mugwort English Plantain Common Cucklebur Sorrel Nettle Wall Pellitory 1
TWx1	Kräuter Mischung T1 *	w1 w5 w12 w29 w38	beifußbl. Ambrosie Wermut echte Goldrute Sonnenblume Rispenkraut	TWx1	Weed Mix T1 *	w1 w5 w12 w29 w38	Common Ragweed Wormwood Goldenrod Sunflower Marsh, elder rough
TWx3	Kräuter Mischung T3	w6 w9 w10 w12 w20	Beifuß Spitzwegerich Weißer Gänsefuß echte Goldrute Brennnessel	TWx3	Weed Mix T3	w6 w9 w10 w12 w20	Mugwort English Plantain Lamb's Quaters Goldenrod Nettle

* nur als biotinyliertes Reagenz verfügbar
* available only as biotinylated reagent

Pollen

Gräser und Getreide

Pollens

Grasses and Corn

Code	Deutsch	English	Latein/Latin
g1	Ruchgras	Sweet Vernal Grass	Anthoxanthum odoratum
g2	Hundszahngras	Bermuda Grass	Cynodon dactylon
g3	Knäuelgras	Orchard Grass	Dactylis glomerata
g4	Wiesenschwingel	Meadow fescue	Festuca elatior
g5	Lolch	Perennial Rye Grass	Lolium perenne
g6	Lieschgras	Timothy Grass	Phleum pratense
g7	Riedgras	Common Reed	Phragmites communis
g8	Wiesenrispengras	June Grass	Poa pratensis
g9	Weißes Straußgras	Creeping Bentgrass	Agrostis stolonifera
g10	Sudangras (Sorgho)	Sudan Grass	Sorghum halepense
g11	Trespe	Brome Grass	Bromus inermis
g12	Roggen	Cultivated Rye	Secale cereale
g13	Wolliges Honiggras	Velvet Grass	Holcus lanatus
g14	Hafer	Cultivated Oat	Avena sativa
g15	Weizen	Wheat	Triticum sativum
g16	Wiesenfuchsschwanz	Meadow foxtail	Alopecurus pratensis
g17	Bahiagras	Bahia Grass	Paspalum notatum
g18	Gerste	Barley	Hordeum vulgare
g19	Kammgras	Dog's Tail Grass	Cynosurus cristatus
g20	Mais	Corn	Zea mays
g21	Quecke	Couch Grass	Elymus repens
g71	Glatthafer	Oat Grass Tall	Arrenatherum elatius
g74	Rohrglanzgras	Canary Grass red	Phalaris arundinacea

Gräser und Getreide
Multi-Allergene

Grasses and Corn
Multi-Allergens

Code	Deutsch			Code	English		
Gx1	Gräser frühblühend	g3 g4 g5 g6 g8	Knäuelgras Wiesenschwingel Lolch Lieschgras Wiesenrispengras	Gx1	Grasses early	g3 g4 g5 g6 g8	Orchard Grass Meadow Fescue Perennial Rye Grass Timothy Grass June Grass
Gx2	Gräser spätblühend	g1 g5 g7 g12 g13	Ruchgras Lolch Riedgras Roggen Wolliges Honiggras	Gx2	Grasses late	g1 g5 g7 g12 g13	Sweet Vernal Grass Perennial Rye Grass Common Reed Cultivated Rye Velvet Grass
Gx3	Gräser Mischung 3	g3 g4 g5 g8	Knäuelgras Wiesenschwingel Lolch Wiesenrispengras	Gx3	Grass Mix 3	g3 g4 g5 g8	Orchard Grass Meadow Fescue Perennial Rye Grass June Grass
Gx4	Getreide Mischung 4	g12 g14 g15 g18 g20	Roggen Hafer Weizen Gerste Mais	Gx4	Corn Mix 4	g12 g14 g15 g18 g20	Cultivated Rye Cultivated Oat Wheat Barley Corn

Pollen
Gräser und Getreide
Multi-Allergene

Pollens
Grasses and Corn
Multi-Allergens

Code	Deutsch			Code	English		
Gx5	Gräser Mischung 5	g1	Ruchgras	Gx5	Grass Mix 5	g1	Sweet Vernal Grass
		g2	Hundszahngras			g2	Bermuda Grass
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g10	Sudangras			g10	Sudan Grass
Gx6	Gräser Mischung 6	g2	Hundszahngras	Gx6	Grass Mix 6	g2	Bermuda Grass
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g8	Wiesenrispengras			g8	June Grass
		g10	Sudangras			g10	Sudan Grass
Gx10	Gräser Mischung 10	g2	Hundszahngras	Gx10	Grass Mix 10	g2	Bermuda Grass
		g4	Wiesenschwingel			g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g8	Wiesenrispengras			g8	June Grass
		g14	Hafer			g14	Cultivated Oat
Gx11	Gräser Mischung 11	g3	Knäuelgras	Gx11	Grass Mix 11	g3	Orchard Grass
		g4	Wiesenschwingel			g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g8	Wiesenrispengras			g8	June Grass
		g20	Mais			g20	Corn
Gx12	Gräser Mischung 12	g1	Ruchgras	Gx12	Grass Mix 12	g1	Sweet Vernal Grass
		g2	Hundszahngras			g2	Bermuda Grass
		g9	Weißes Straußgras			g9	Creeping Bentgrass
		g10	Sudangras			g10	Sudan Grass
		g15	Weizen			g15	Wheat
Gx13	Gräser Mischung 13	g3	Knäuelgras	Gx13	Grass Mix 13	g3	Orchard Grass
		g4	Wiesenschwingel			g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g13	Wolliges Honiggras			g13	Velvet Grass
Gx15	Gräser Mischung 15	g2	Hundszahngras	Gx15	Grass Mix 15	g2	Bermuda Grass
		g3	Knäuelgras			g3	Orchard Grass
		g4	Wiesenschwingel			g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g8	Wiesenrispengras			g8	June Grass
Gx17	Gräser Mischung 17	g2	Hundszahngras	Gx17	Grass Mix 17	g2	Bermuda Grass
		g4	Wiesenschwingel			g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g10	Sudangras			g10	Sudan Grass
		g17	Bahiagras			g17	Bahia Grass
Gx18	Gräser Mischung 18	g4	Wiesenschwingel	Gx18	Grass Mix 18	g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g21	Quecke			g21	Couch Grass

Pollen
Gräser und Getreide
Multi-Allergene

Pollens
Grasses and Corn
Multi-Allergens

Code	Deutsch			Code	English		
Gx19	Gräser Mischung 19	g6	Lieschgras	Gx19	Grass Mix 19	g6	Timothy Grass
		g12	Roggen			g12	Cultivated Rye
		g14	Hafer			g14	Cultivated Oat
		g15	Weizen			g15	Wheat
		g18	Gerste			g18	Barley
		g21	Quecke			g21	Couch Grass
Gx20	Gräser Mischung 20	g1	Ruchgras	Gx20	Grass Mix 20	g1	Sweet Vernal Grass
		g3	Knäuelgras			g3	Orchard Grass
		g4	Wiesenschwingel			g4	Meadow Fescue
		g5	Lolch			g5	Perennial Rye Grass
		g8	Wiesenrispengras			g8	June Grass
TGx3	Gräser Mischung T3	g1	Ruchgras	TGx3	Grass Mix T3	g1	Sweet Vernal Grass
		g5	Lolch			g5	Perennial Rye Grass
		g6	Lieschgras			g6	Timothy Grass
		g12	Roggen			g12	Cultivated Rye
		g13	Wolliges Honiggras			g13	Velvet Grass
		w6	Beifuß			w6	Mugwort

Tierallergene
(Epithelien, Haare, Federn, Urin, Kot)

Animal Allergens
(Dander, Hair, Feathers, Urine, Droppings)

Code	Deutsch	English
e1	Katze (Epithel)	Cat (Dander)
e2	Hund (Haare)	Dog (Hair)
e3	Pferd (Epithel)	Horse (Dander)
e4	Rind (Epithel)	Cow (Dander)
e5	Hund (Epithel)	Dog (Dander)
e6	Meerschweinchen (Haare)	Guinea Pig (Hair)
e7	Taube (Kot)	Pigeon (Droppings)
e9	Kanarienvogel (Federn)	Canary (Feathers)
e10	Papagei (Federn)	Parrot (Feathers)
e11	Taube (Federn)	Pigeon (Feathers)
e12	Taube (Eiweiß)	Pigeon (Egg White)
e13	Taube (Serum)	Pigeon (Serum)
e14	Kanarienvogel (Serum)	Canary (Serum)
e15	Huhn (Serum)	Chicken (Serum)
e16	Papagei (Serum)	Parrot (Serum)
e17	Kamelhaar (Wolle)	Camel hair (Wool)
e18	Kanarienvogel (Kot)	Canary (Droppings)
e19	Gans (Kot)	Goose (Droppings)
e20	Huhn (Kot)	Chicken (Droppings)
e32	Katze (Serum)	Cat (Serum)
e33	Kaninchen (Serum)	Rabbit (Serum)
e50	Zierfink (Federn)	Finch (Feathers)
e51	Zierfink (Kot)	Finch (Droppings)
e52	Hase (Epithel)	Hare (Dander)
e70	Gans (Federn)	Goose (Feathers)
e71	Maus (Epithel)	Mouse (Dander)
e72	Maus (Urin)	Mouse (Urine)
e73	Ratte (Epithel)	Rat (Dander)
e74	Ratte (Urin)	Rat (Urine)
e75	Ratte (Serum)	Rat (Serum)
e76	Maus (Serum)	Mouse (Serum)
e77	Wellensittich (Kot)	Budgerigar (Droppings)
e78	Wellensittich (Federn)	Budgerigar (Feathers)

Tierallergene
(Epithelien, Haare, Federn, Urin, Kot)

Animal Allergens
(Dander, Hair, Feathers, Urine, Droppings)

Code	Deutsch	English
e79	Wellensittich (Serum)	Budgerigar (Serum)
e80	Ziege (Epithel)	Goat (Dander)
e81	Schaf (Epithel)	Sheep (Dander)
e82	Kaninchen (Haare)	Rabbit (Hair)
e83	Schwein (Epithel)	Pig (Dander)
e84	Goldhamster (Haare)	Gold Hamster (Hair)
e85	Huhn (Federn)	Chicken (Feathers)
e86	Ente (Federn)	Duck (Feathers)
e87	Ratte (Epithel + Protein)	Rat (Dander + Protein)
e88	Maus (Epithel + Protein)	Mouse (Dander + Protein)
e89	Maus (Kot)	Mouse (Droppings)
e90	Ratte (Kot)	Rat (Droppings)
e91	Truthahn (Federn)	Turkey (Feathers)
e97	Papagei (Kot)	Parrot (Droppings)
e98	Chinchilla (Haare)	Chinchilla (Hair)
e99	Gans (Eiweiß)	Goose (Egg White)
e100	Ente (Kot)	Duck (Droppings)
e101	BSA Rinderserum	BSA Bovine Serum albumine (Cow)
e102	Schwein (Serum)	Pig (Serum)
e103	Wildschwein (Epithel)	Wild Boar (Dander)

Tierallergene
Multi-Allergene

Animal Allergens
Multi-Allergens

Code	Deutsch			Code	English		
Ex1	Tierepithelien 1	e1	Katze (Epithel)	Ex1	Epithelia 1	e1	Cat (Dander)
		e3	Pferd (Epithel)			e3	Horse (Dander)
		e4	Rind (Epithel)			e4	Cow (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
Ex2	Tierepithelien 2	e1	Katze (Epithel)	Ex2	Epithelia 2	e1	Cat (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
		e6	Meerschweinchen (Haare)			e6	Guinea Pig (Hair)
		e84	Goldhamster (Haare)			e84	Goldhamster (Hair)
Ex3	Tierepithelien 3	e3	Pferd (Epithel)	Ex3	Epithelia 3	e3	Horse (Dander)
		e4	Rind (Epithel)			e4	Cow (Dander)
		e81	Schaf (Epithel)			e81	Sheep (Dander)
		e82	Kaninchen (Haare)			e82	Rabbit (Hair)
Ex4	Bettfedern	e70	Gans (Federn)	Ex4	Bed Feathers	e70	Goose (Feathers)
		e85	Huhn (Federn)			e85	Chicken (Feathers)
		e86	Ente (Federn)			e86	Duck (Feathers)
Ex5	Nagetiere	e6	Meerschweinchen (Haare)	Ex5	Rodents	e6	Guinea Pig (Hair)
		e71	Maus (Epithel)			e71	Mouse (Dander)
		e73	Ratte (Epithel)			e73	Rat (Dander)
		e82	Kaninchen (Haare)			e82	Rabbit (Hair)
		e84	Goldhamster (Haare)			e84	Gold Hamster (Hair)
Ex6	Federn Mischung 6	e11	Taube (Federn)	Ex6	Feathers 6	e11	Pigeon (Feathers)
		e70	Gans (Federn)			e70	Goose (Feathers)
		e85	Huhn (Federn)			e85	Chicken (Feathers)
		e86	Ente (Federn)			e86	Duck (Feathers)
Ex7	Käfigvögel Mischung 7	e14	Kanarienvogel (Serum)	Ex7	Cagebirds 7	e14	Canary (Serum)
		e16	Papagei (Serum)			e16	Parrot (Serum)
		e51	Zierfink (Kot)			e51	Finch (Droppings)
		e79	Wellensittich (Serum)			e79	Budgerigar (Serum)

Tierallergene
Multi-Allergene

Animal Allergens
Multi-Allergens

Code	Deutsch			Code	English		
Ex11	Käfigvögel Mischung 11	e9	Kanarienvogel (Federn)	Ex11	Cagebirds 11	e9	Canary (Feathers)
		e10	Papagei (Federn)			e10	Parrot (Feathers)
		e50	Zierfink (Federn)			e50	Finch (Feathers)
		e78	Wellensittich (Federn)			e78	Budgerigar (Feathers)
Ex13	Tier Mischung 13	e1	Katze (Epithel)	Ex13	Animal Mix 13	e1	Cat (Dander)
		e11	Taube (Federn)			e11	Pigeon (Feathers)
		e80	Ziege (Epithel)			e80	Goat (Dander)
		e81	Schaf (Epithel)			e81	Sheep (Dander)
Ex14	Tier Mischung 14	e1	Katze (Epithel)	Ex14	Animal Mix 14	e1	Cat (Dander)
		e3	Pferd (Epithel)			e3	Horse (Dander)
		e4	Rind (Epithel)			e4	Cow (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
		e6	Meerschweinchen (Haare)			e6	Guinea Pig (Hair)
Ex16	Tierepithelien/ Federn *	e3	Pferd (Epithel)	Ex16	Epithelia/ Feathers *	e3	Horse (Dander)
		e4	Rind (Epithel)			e4	Cow (Dander)
		e70	Gans (Federn)			e70	Goose (Feathers)
		e85	Huhn (Federn)			e85	Chicken (Feathers)
Ex17	Tierepithelien 17*	e1	Katze (Epithel)	Ex17	Epithelia 17*	e1	Cat (Dander)
		e3	Pferd (Epithel)			e3	Horse (Dander)
		e4	Rind (Epithel)			e4	Cow (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
		e70	Gans (Federn)			e70	Goose (Feathers)
		e81	Schaf (Epithel)			e81	Sheep (Dander)
		e85	Huhn (Federn)			e85	Chicken (Feathers)
Ex18	Tier Mischung 18*	e1	Katze (Epithel)	Ex18	Epithelia 18*	e1	Cat (Dander)
		e3	Pferd (Epithel)			e3	Horse (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
		e6	Meerschweinchen (Haare)			e6	Guinea Pig (Hair)
		e82	Kaninchen (Haare)			e82	Rabbit (Hair)
Ex19	Tier Mischung 19*	e1	Katze (Epithel)	Ex19	Animal Mix19*	e1	Cat (Dander)
		e4	Rind (Epithel)			e4	Cow (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
		e70	Gans (Federn)			e70	Goose (Feathers)
		e81	Schaf (Epithel)			e81	Sheep (Dander)
		e88	Maus (Epithel + Protein)			e88	Mouse (Dander + Protein)
TEx2	Tierepithelien*	e1	Katze (Epithel)	TEx2	Animal Epithelia*	e1	Cat (Dander)
		e5	Hund (Epithel)			e5	Dog (Dander)
		e6	Meerschweinchen (Haare)			e6	Guinea Pig (Hair)
		e87	Ratte (Epithel + Protein)			e87	Rat (Dander + Protein)
		e88	Maus (Epithel + Protein)			e88	Mouse (Dander + Protein)

* nur als biotinyliertes Reagenz verfügbar
* available only as biotinylated reagent

Insekten Gifte — Insects Venoms

Code	Deutsch	English	Latein/Latin
i1	Bienengift	Honey Bee Venom	Apis mellifera
i3	Wespengift	Wasp Venom	Vespula germanica
i4	Bremse	Gadfly	Tabanus spp.
i5	Gelbwespe	Yellow Hornet	Dolichovespula arenaria
i6	Küchenschabe (deutsch)	German Cockroach	Blatella germanica
i7	Hornissengift	Hornet Venom	Vespa crabro
i8	Hummelgift	Bumble Bee Venom	Bombus terrestris
i9	Reismehlkäfer	Tribolium confusum	Tribolium confusum
i10	Papierwespe	Paper Wasp	Polystes apachus
i11	Phospholipase A	Phospholipase A/Honey Bee	Phospholipase A/Apis mellifera
i12	Melittin	Melittin	
i13	Dolichovespula maculata	White (bald) faced Hornet	Dolichovespula maculata
i14	Küchenschabe (amerikanisch)	American Cockroach	Periplaneta americana
i15	Hausfliege	Housefly	Musca domestica
i70	Feuerameise	Fire Ant	Solenopsis invicta
i71	Stechmücke	Mosquito	Culex pipiens
i73	Rote Mückenlarve	Red Midge Larva	Chironomus spp.
i74	Wasserfloh	Waterflea	Daphnia spp.

Milben — Mites

Code	Latein/Latin
d1	D. pteronyssinus
d2	D. farinae
d3	Euroglyphus maynei
d4	D. microceras
d5	Blomia tropicalis
d70	Acarus siro
d71	Lepidoglyphus destructor
d72	Tyrophagus putreus
d73	Glycophagus domesticus

Multi-Allergene — Multi-Allergens

Code	Deutsch		Latein/Latin	Code	English	
Dx1	Hausstaub-/ Mehlmilbe	d1 d2	D. pteronyssinus D. farinae	Dx1	House Dust-Mites	d1 d2
Dx3	Milben-Mischung 3	d70 d71 d72 d73	Acarus siro Lepidoglyphus destructor Tyrophagus putreus Glycophagus domesticus	Dx3	Mites - Mix 3	d70 d71 d72 d73
Dx4	Milben-Mischung 4	d1 d2 d3 d4 d70 d71 d72 d73	D. pteronyssinus D. farinae Euroglyphus maynei D. microceras Acarus siro Lepidoglyphus destructor Tyrophagus putreus Glycophagus domesticus	Dx4	Mites - Mix 4	d1 d2 d3 d4 d70 d71 d72 d73

Hausstaub Mischungen — House Dust Mixes

Code	Deutsch		Code	English	
H2	Hausstaub-Mischung T/S (Hollister Stier)	e1 Katze (Epithel) e5 Hund (Epithel) d1 D. pteronyssinus d2 D. farinae m2 Cladosporium herbarum m3 Aspergillus fumigatus	H2	House Dust T/S (Hollister Stier)	e1 Cat (Dander) e5 Dog (Dander) d1 D. pteronyssinus d2 D. farinae m2 Cladosporium herbarum m3 Aspergillus fumigatus
H3	Hausstaub-Mischung M (Bencard)	e1 Katze (Epithel) e5 Hund (Epithel) d1 D. pteronyssinus d2 D. farinae m2 Cladosporium herbarum m3 Aspergillus fumigatus	H3	House Dust M (Bencard)	e1 Cat (Dander) e5 Dog (Dander) d1 D. pteronyssinus d2 D. farinae m2 Cladosporium herbarum m3 Aspergillus fumigatus
Hx1	Haus-Mischung 1	d1 D. pteronyssinus d2 D. farinae i6 Küchenschabe	Hx1	House Dust Mix 1	d1 D. pteronyssinus d2 D. farinae i6 German Cockroach
Hx2	Haus-Mischung 2	d1 D. pteronyssinus d2 D. farinae e1 Katze (Epithel) e5 Hund (Epithel)	Hx2	House Dust Mix 2	d1 D. pteronyssinus d2 D. farinae e1 Cat (Dander) e5 Dog (Dander)
HMx1	Haus-Mischung	d1 D. pteronyssinus d2 D. farinae e1 Katze (Epithel) e5 Hund (Epithel) m2 Cladosporium herbarum m3 Aspergillus fumigatus	HMx1	House Mix	d1 D. pteronyssinus d2 D. farinae e1 Cat (Dander) e5 Dog (Dander) m2 Cladosporium herbarum m3 Aspergillus fumigatus
HMx2	Hausmischung 2	d1 D. pteronyssinus e1 Katze (Epithel) e5 Hund (Epithel) m3 Aspergillus fumigatus m6 Alternaria tenuis (alternata)	HMx2	House Mix 2	d1 D. pteronyssinus e1 Cat (Dander) e5 Dog (Dander) m3 Aspergillus fumigatus m6 Alternaria tenuis (alternata)
HMx3	Hausmischung 3	d1 D. pteronyssinus d2 D. farinae i6 Küchenschabe m1 Penicillium chrysogenum (notatum) m3 Aspergillus fumigatus m5 Candida albicans m6 Alternaria tenuis (alternata)	HMx3	House Mix 3	d1 D. pteronyssinus d2 D. farinae i6 German Cockroach m1 Penicillium chrysogenum (notatum) m3 Aspergillus fumigatus m5 Candida albicans m6 Alternaria tenuis (alternata)

Parasiten — Parasites

Code	Deutsch	Code	English
p1	Ascaris	p1	Ascaris

Medikamente Drugs

Code	Deutsch	English
c1	Penicilloyl G	Penicilloyl G
c2	Penicilloyl V	Penicilloyl V
c50	Ampicillin	Ampicillin
c51*	Acetylsalicylsäure (ASS)	Acetylsalicylic Acid (ASS)
c52*	Pyrazolon (4-Amino-Antipyrin)	Pyrazolone (4-Amino-Antipyrine)
c53*	Alcuronium	Alcuronium
c54*	Cefalotin	Cefalotin
c55*	Cephalosporin	Cephalosporin
c56	Amoxycillin	Amoxycillin
c57*	TMP (Trimethoprim)	TMP (Trimethoprime)
c58*	SMZ (Sulfamethoxazol)	SMZ (Sulfamethoxazole)
c59*	Tetracyclin	Tetracycline
c60*	Gentamycin	Gentamycin
c61*	Erythromycin	Erythromycin
c62*	Doxycyclin	Doxycyclin
c64*	Piperacillin	Piperacillin
c65*	Phenylbutazon	Phenylbutazone
c66*	Streptomycin	Streptomycin
c67*	Cloxacillin	Cloxacillin
c68*	Articain	Articaine
c70*	Insulin human (Protaphane Penfill)	Insulin human (Protaphane Penfill)
c71*	Insulin human (Insuman Rapid)	Insulin human (Insuman Rapid)
c73*	Insulin human (Humalog)	Insulin human (Humalog)
c77*	Piroxicam	Piroxicam
c78*	Ibuprofen	Ibuprofen
c79*	Diclofenac	Diclofenac
c80*	Tetanus - Toxoid	Tetanus - Toxoide
c81*	Theophyllin / Aminophyllin	Theophylline / Aminophylline
c82*	Lidocain / Xylocain	Lidocaine / Xylocain
c83*	Procain	Procaine
c85*	Paracetamol	Paracetamol
c86*	Benzocain	Benzocaine
c87*	Carbocain	Carbocain
c88*	Mepivacain	Mepivacain
c89*	Bupivacain	Bupivacain
c90*	Propyphenazon	Propyphenazone
c91*	Dipyron/Metamizol	Dipyron/Metamizole
c93*	Indometacin	Indomethacine
c94*	Tobramycin	Tobramycin
c95*	Neomycin	Neomycin
c96*	Ambroxol	Ambroxole
c97*	Bromhexin	Bromhexine
c99*	L-Thyroxin	L-Thyroxine
c100*	Prilocain	Prilocaine
c103*	Isoprenalin / Orciprenalin	Isoprenalin / Orciprenalin
c104*	Clindamycin	Clindamycin
c106*	Vitamin B1 (Thiamin)	Vitamin B1 (Thiamine)
c107*	Captopril	Captoprile
c108*	Ciprofloxacin	Ciprofloxacin
c109*	Vitamin B6	Vitamin B6
c110*	Naproxen	Naproxene
c111*	Phenacetin	Phenacetine
c112*	Tartrazin	Tartrazin
c113*	Tyramin	Tyramine
c114*	Tryptophan	Tryptophan
c115*	Lincomycin	Lincomycin
c116*	Oxacillin	Oxacillin
c118*	Ofloxacin	Ofloxacin

*Zu Forschungszwecken. *For research use

Medikamente Drugs

Code	Deutsch	English
c119	Bacampicillin	Bacampicillin
c120*	Carbenicillin	Carbenicillin
c122*	Nystatin	Nystatin
c126*	Penicillamin	Penicillamin
c127*	5-Aminosalicylsäure	5-Aminosalicylicacid
c128*	Minocyclin	Minocyclin
c129*	Erythrosin-B	Erythrosin-B
c130*	Azlocillin	Azlocillin
c133*	Cyanocobalamin Vitamin B12	Cyanocobalamin Vitamin B12
c138*	Ginkgo	Ginkgo
c145*	Echinacea	Echinacea
c151*	Acetylcystein	Acetylcysteine
c152*	Chloramphenicol	Chloramphenicol
c153*	Metronidazol	Metronidazole
c154*	Prednisolon	Prednisolone
c156*	Maleinsäureanhydrid	Maleinacidanhydrid
c157*	Hexahydrophthalsäure	Hexahydrophthalicacid
c158*	Methyltetrahydrophthalsäure	Methyltetrahydrophthalicacid
c161*	Roxithromycin	Roxithromycin
c162*	Vancomycin	Vancomycin
c165*	Cefaclor	Cefaclor
c169*	Heparin	Heparin
c170*	Clarithromycin	Clarithromycin
c172*	Ketoprofen	Ketoprofen
c175*	Norfloxacin	Norfloxacin
c179*	Chymotrypsin	Chymotrypsin
c181*	Ascorbinsäure	Ascorbic acid
c186*	Hydrochlorothiazid	Hydrochlorothiazid
c194*	Azithromycin	Azithromycin
c196*	Epinephrin	Epinephrine
c200*	Clavulansäure	Clavulanic acid
c210*	Tetracain	Tetracaine
c308*	Cefuroxim	Cefuroxime
c425	Simvastatin	Simvastatin

*Zu Forschungszwecken. *For research use

Berufs-allergene

Occupational Allergens

Code	Deutsch	English	E-Nr.
k70	Grüne Kaffeebohne	Green Coffee Bean	
k71	Rhizinusbohne	Castor Bean	
k72	Isphagula	Isphagula	
k74	Rohseide (Bombyx mori)	Silk (Bombyx mori)	
k75	Isocyanat TDI	Toluene diisocyanate TDI	
k76	Isocyanat MDI	Diphenyl methane MDI	
k77	Isocyanat HDI	Hexamethylene diisocyanate HDI	
k78	Ethylenoxid	Ethyleneoxide	
k79	Phthalsäureanhydrid	Phthalic anhydride	
k80	Formaldehyd	Formaldehyde	
k81	Birkenfeige	Ficus benjamina	
k82	Latex (Hevea brasiliensis)	Latex (Hevea brasiliensis)	
k83	Guarkernmehl	Guarflour	E 412
k84	Sonnenblumensamen	Sunflower Seed	
k85	Chloramin T	Chloramine T	
k86	Trimellitsäureanhydrid	Trimellitic anhydride	
k87	Phenylendiamin	Phenylendiamine	
k88	Amyloglucosidase	Amyloglucosidase	
k89	Hemizellulase	Hemicellulase	
k90	Lipoxigenase	Lipoxigenase	
k92	Abietinsäure (Kollophonium)	Collophonium (Abietic acid)	
k93	Ammoniumpersulfat	Ammoniumpersulphate	
k94	Kollagen (tierisch, pflanzlich)	Collagen (animal, herbal)	
k95	Tragant (Astragalus spp.)	Tragacanth (Astragalus spp.)	E 413
k96	Chinolingelb	Chinolin yellow	E 104
k97	Gelborange S	Yelloworange S	E 110
k99	Amaranth	Amaranth	E 123
k102	Alkalase	Alcalase	
k104	Savinase	Savinase	
k105	Gummi Arabicum	Gum arabic	E 414
k106	Karminrot	Carmine red	
k107	Azorubin	Azorubin	E 122

Beruf und Hobby

Occupational Allergens

Code	Deutsch	English
b2	Baumwolle (bearbeitet)	Cotton (treated)
b3	Baumwollflocken (unbearbeitet)	Cotton flock (untreated)
b4	Dreschstaub	Threshing Dust
b5	Flachs	Flax
b7	Heustaub	Hay Dust
b8	Hopfen	Hop
b13	Jute	Jute
b14	Kapok	Kapok
b16	Leinen	Linen
b20	Schafwolle (bearbeitet)	Sheep Wool (treated)
b21	Schafwolle (unbearbeitet)	Sheep Wool (untreated)
b22	Seide (Bombyx mori)	Silk (Bombyx mori)

Beruf und Hobby

Occupational Allergens

Code	Deutsch	English
b23	Strohstaub	Straw Dust
b24	Tabakstaub	Tobacco Dust
b26	Weizendrusch	Wheat Threshing

Holz-/Sägespäne

Smuts

b31	Ahorn	Maple
b32	Buche	Beech
b33	Eiche	Oak
b34	Esche	Ash
b35	Fichte	Fir
b36	Kiefer	White Pine
b40	Nussbaum	Walnut Tree
b41	Obechi (Abachi)	Obechi (Abachi)
b43	Rote Zeder	Red Cedar
b44	Tanne	Silver Fir
b50	Pappel	Poplar
b52	Erle	Alder
b53	Kirschbaum	Cherry Tree
b55	Lärche	Larch

Beruf und Hobby

Multi-Allergene

Occupational Allergens

Multi-Allergens

Code	Deutsch			Code	English		
Bx2	Naturstoffe/ Fasern	b2	Baumwolle (bearb.)	Bx2	Natural Fibres	b2	Cotton (treated)
		b13	Jute			b13	Jute
		b20	Schafwolle (bearb.)			b20	Sheep´s Wool (treated)
		b22	Seide			b22	Silk
Bx3	Weichhölzer	b32	Buche	Bx3	Wood I	b32	Beech
		b36	Kiefer			b36	White Pine
		b43	Rote Zeder			b43	Red Cedar
		b44	Tanne			b44	Fir
Bx5	Stäube	b4	Dreschstaub	Bx5	Dusts	b4	Threshing Dust
		b7	Heustaub			b7	Hay Dust
		b23	Strohstaub			b23	Straw Dust
		b26	Weizendrusch			b26	Wheat Threshing
Bx7	Staub-mischung 7	b7	Heustaub	Bx7	Dust Mix 7	b7	Hay Dust
		b23	Strohstaub			b23	Straw Dust
		b24	Tabakstaub			b24	Tobacco Dust
		b26	Weizendrusch			b26	Wheat Threshing

Konservierungsstoffe Preservatives

Code	Deutsch	English	E-Nr.
Ko1*	p-Hydroxybenzoesäureethylester	p-Hydroxybenzoicacidethylester	E 214
Ko2*	p-Hydroxybenzoesäurebutylester	p-Hydroxybenzoicacidbutylester	
Ko3*	p-Hydroxybenzoesäurepropylester	p-Hydroxybenzoicacidpropylester	E 216
Ko4*	Sorbinsäure	Sorbic Acid	E 200
Ko5*	Benzoesäure	Benzoic Acid	E 210
Ko7*	p-Hydroxybenzoesäuremethylester	p-Hydroxybenzoicacidmethylester	E 218

Suchtests

Multi-Allergene

Code	Deutsch		
STx0	Suchtest multi	g6	Lieschgras
		g12	Roggen
		t3	Birke
		w6	Beifuß
		d1	D. pteronyssinus
		e1	Katze (Epithel)
		e5	Hund (Epithel)
		m2	Cladosporium herbarum
STx1	Suchtest saisonal	g6	Lieschgras
		t3	Birke
		w6	Beifuß
		m6	Alternaria tenuis (alternata)
STx2	Suchtest perennial	d1	D. pteronyssinus
		e1	Katze (Epithel)
		e5	Hund (Epithel)
		m3	Aspergillus fumigatus
STx3	Inhalations-Panel	t1	Ahorn
		t8	Ulme
		t17	Kastanie
		t28	Robinie
		w20	Brennessel
		b14	Kapok
		b23	Strohstaub
		m22	Mucor spinosus
		m36	Aspergillus terreus
STx4	Nahrungs-mittel-Panel	f7	Hafermehl
		f18	Paranuss
		f29	Banane
		f38	Spinat
		f48	Zwiebel
		f51	Sojaschrot
		f65	Linse
		f70	Schweizer Käse
		f88	Hammel/Lamm
STx5	Regionalmix	g6	Lieschgras
		w6	Beifuß
		w9	Spitzwegerich
		w21	Glaskraut 1
		t3	Birke

Screening Tests

Multi-Allergens

Code	English		
STx0	Screen multi	g6	Timothy Grass
		g12	Barley
		t3	Birch
		w6	Mugwort
		d1	D. pteronyssinus
		e1	Cat (Dander)
		e5	Dog (Dander)
		m2	Cladosporium herbarum
STx1	Screen saisonal	g6	Timothy Grass
		t3	Birch
		w6	Mugwort
		m6	Alternaria tenuis (alternata)
STx2	Screen perennial	d1	D. pteronyssinus
		e1	Cat (Dander)
		e5	Dog (Dander)
		m3	Aspergillus fumigatus
STx3	Inhalation-Panel	t1	Maple
		t8	Elm
		t17	Chestnut
		t28	Robinia
		w20	Nettle
		b14	Kapok
		b23	Straw Dust
		m22	Mucor spinosus
		m36	Aspergillus terreus
STx4	Food-Panel	f7	Oat Flour
		f18	Brazil Nut
		f29	Banana
		f38	Spinach
		f48	Onion
		f51	Soy bean (bruised grain)
		f65	Lentil
		f70	Swiss Cheese
		f88	Mutton/Lamb
STx5	Regionalmix	g6	Timothy Gras
		w6	Mugwort
		w9	English Plantain
		w21	Wall Pellitory 1
		t3	Birch

*Zu Forschungszwecken. *For research use

Suchtests

Multi-Allergene

Code	Deutsch		
STx6	Inhalations-Panel 6	d2	D. farinae
		e1	Katze (Epithel)
		e5	Hund (Epithel)
		e3	Pferd (Epithel)
		m6	Alternaria tenuis (alternata)
STx7	Inhalations-Mix	g2	Hundszahngras
		g4	Wiesenschwingel
		g5	Lolch
		t11	Platane
		t14	Pappel
		t15	Esche
		t36	Akazie
		w9	Spitzwegerich
		w18	Sauerampfer
STx8	Inhalations-Mix	d1	D. pteronyssinus
		d2	D. farinae
		ex6	Federn Mischung 6
		e1	Katze (Epithel)
		e5	Hund (Epithel)
		m2	Cladosporium herbarum
		m3	Aspergillus fumigatus
		m6	Alternaria tenuis (alternata)
STx9	Pollen/ Schimmel-Pilze*	m3	Aspergillus fumigatus
		m6	Alternaria tenuis (alternata)
		g12	Roggen
		g15	Weizen
STx10	Suchtest Multi 10	e1	Katze (Epithel)
		e5	Hund (Epithel)
		d1	D. pteronyssinus
		d3	Euroglyphus maynei
		m2	Cladosporium herbarum
		m3	Aspergillus fumigatus
STx32	Suchtest Multi 32*	d1	D. pteronyssinus
		d2	D. farinae
		e1	Katze (Epithel)
		e5	Hund (Epithel)
		g6	Lieschgras
		t3	Birke
		t14	Pappel
		w6	Beifuß
		w15	Melde
		m6	Alternaria tenuis (alternata)

Screening Tests

Multi-Allergens

Code	English		
STx6	Inhalation-Panel 6	d2	D. farinae
		e1	Cat (Dander)
		e5	Dog (Dander)
		e3	Horse (Dander)
		m6	Alternaria tenuis (alternata)
STx7	Inhalation-Mix	g2	Bermuda Grass
		g4	Meadow fescue
		g5	Perennial Rye Grass
		t11	Plane
		t14	Poplar
		t15	Ash
		t36	Acacia
		w9	English Plantain
		w18	Sorrel
STx8	Inhalation-Mix	d1	D. pteronyssinus
		d2	D. farinae
		ex6	Feathers 6
		e1	Cat (Dander)
		e5	Dog (Dander)
		m2	Cladosporium herbarum
		m3	Aspergillus fumigatus
		m6	Alternaria tenuis (alternata)
STx9	Pollen/ Molds *	m3	Aspergillus fumigatus
		m6	Alternaria tenuis (alternata)
		g12	Cultivated Rye
		g15	Wheat
STx10	Screen Multi 10	e1	Cat (Dander)
		e5	Dog (Dander)
		d1	D. pteronyssinus
		d3	Euroglyphus maynei
		m2	Cladosporium herbarum
		m3	Aspergillus fumigatus
STx32	Screen Multi 32*	d1	D. pteronyssinus
		d2	D. farinae
		e1	Cat (Dander)
		e5	Dog (Dander)
		g6	Timothy Grass
		t3	Birch
		t14	Poplar
		w6	Mugwort
		w15	Scale
		m6	Alternaria tenuis (alternata)

* nur als biotinyliertes Reagenz verfügbar
* available only as biotinylated reagent

Nahrungsmittel Foods

Code	Deutsch	English	Latein/Latin
f1	Eiklar	Egg White	
f2	Kuhmilch (roh)	Cow's milk (raw)	
f3	Dorsch (Kabeljau)	Codfish	Gadus morhua
f4	Weizenmehl	Wheat flour	Triticum aestivum
f5	Roggenmehl	Rye flour	Secale cereale
f6	Gerstenmehl	Barley flour	Hordeum vulgare
f7	Hafermehl	Oat flour	Avena sativa
f8	Maismehl	Corn flour	Zea mays
f9	Reis	Rice	Oryza sativa
f10	Sesamschrot	Sesame (bruised grain)	Sesamum indicum
f11	Buchweizenmehl	Buckwheat flour	Fagopyrum esculentum
f12	Erbse	Pea	Pisum sativum
f13	Erdnuss	Peanut	Arachis hypogaea
f14	Sojabohne	Soybean	Glycine max.
f15	Bohne (weiß)	White Bean	Phaseolus vulgaris
f16	Walnuss	Walnut	Juglans regia
f17	Haselnuss	Hazelnut	Corylus avellana
f18	Paranuss	Brazil Nut	Bertholletia excelsa
f19	Esskastanie	Sweet Chestnut	Castanea sativa
f20	Mandel	Almond	Amygdalus communis
f21	Hering	Herring	Clupea harengus
f22	Forelle	Trout	Oncorhynchus mykiss (Salmo gairdnieri)
f23	Krabbe	Crab	Cancer pagurus
f24	Garnele	Shrimp	Pandalus borealis
f25	Tomate	Tomato	Solanum lycopersicum
f26	Schweinefleisch	Pork	Sus spp.
f27	Rindfleisch	Beef	Bos spp.
f29	Banane	Banana	Musa spp.
f30	Birne	Pear	Pyrus communis
f31	Karotte	Carrot	Daucus carota
f32	Zitrone	Lemon	Citrus limon
f33	Orange	Orange	Citrus sinensis
f34	Mandarine	Tangerine	Citrus reticulata
f35	Kartoffel	Potato	Solanum tuberosum
f36	Kokosnuss	Coconut	Cocos nucifera
f37	Miesmuschel	Blue mussel	Mytilus edulis
f38	Spinat	Spinach	Spinachia oleracea
f39	Kohl	Cabbage	Brassica oleracea var. capitata
f40	Thunfisch	Tuna	Thunnus albacares
f41	Lachs	Salmon	Salmo salar
f42	Sauerampfer	Sorrel	Rumex acetosella
f43	Brauereihefe	Saccharomyces carlsbergensis	Saccharomyces carlsbergensis
f44	Erdbeere	Strawberry	Fragaria ananassa
f45	Bäckerhefe	Yeast	Saccharomyces cerevisiae
f46	Paprika	Paprika	Capsicum spp.
f47	Knoblauch	Garlic	Allium sativum
f48	Zwiebel	Onion	Allium cepa
f49	Apfel	Apple	Malus sylvestris
f50	Weintraube	Grape	Vitis vinifera
f51	Sojaschrot	Soy (bruised grain)	Glycine max.
f52	Schokolade	Chocolate	Theobroma cacao
f53	Pfirsich	Peach	Prunus persica
f54	Stutenmilch	Maremilk	
f55	Aal	Eel	Anguilla anguilla
f56	Rotbarsch	Rosefish	Sebastus marinus
f57	Entenfleisch	Duck meat	Anas spp.
f58	Gänsefleisch	Goose meat	Anser spp.
f61	Blumenkohl roh	Cauliflower raw	Brassica oleracea

Nahrungsmittel Foods

Code	Deutsch	English	Latein/Latin
f62	Blumenkohl gekocht	Cauliflower boiled	Brassica oleracea
f63	Rindfleisch gekocht	Beef boiled	Bos spp.
f64	Kresse	Cress	Lepidium sativum
f65	Linse	Lentil	Lens esculenta
f66	Porree	Leek	Melium porrum
f67	Ovalbumin	Ovalbumin	Ovalbumina
f68	Ovomucoid	Ovomucoid	Ovomucoida
f70	Schweizer Käse	Swiss Cheese	
f71	Languste	Spiny Lobster	Palinurus spp.
f72	Ananas	Pineapple	Ananas comosus
f73	Kirsche	Cherry	Prunus spp.
f74	Maiskorn	Corn (grain)	Zea mays
f75	Eigelb	Egg Yolk	
f76	Alpha - Lactalbumin	Alpha - Lactalbumin	Alpha - Lactalbumina
f77	Beta - Lactoglobulin	Beta - Lactoglobulin	Beta - Lactoglobulina
f78	Casein	Casein	Caseina
f79	Gluten	Gluten	Gluten
f80	Hummer	Lobster	Homarus spp.
f81	Cheddarkäse	Cheddar Cheese	
f82	Schimmelkäse	Mould Cheese	
f83	Hühnerfleisch	Chicken meat	Gallus spp.
f84	Kiwi	Kiwi	Actinidia deliciosa
f85	Sellerie	Celeriac	Apium graveolens
f86	Petersilie	Parsley	Petroselinum crispum
f87	Melone	Melon	Citrullus lanatus
f88	Hammel/Lamm	Mutton/Lamb	Ovis spp.
f89	Senf	Mustard	Sinapis spp.
f90	Malz	Malt	
f91	Mango	Mango fruit	Mangifera indica
f92	Grapefruit	Grapefruit	Citrus paradisi
f93	Roquefort	Roquefort Cheese	
f94	Camembert	Camembert Cheese	
f95	Kaffee	Coffee	Coffea spp.
f96	Kamillentee	Camomile Tea	Chamomilla
f97	Kakao	Cacao	Theobroma cacao
f98	Leinsamenschrot	Flax Seed (bruised grain)	
f99	Schwarzer Tee	Black Tea	
f100	Kopfsalat	Lettuce	Lactuca sativa
f101	Venusmuschel	Clam Shell	Ruditapes spp.
f102	Kohlrabi	Kohlrabi	Brassica oleracea var. gongylodes
f103	Pecannuss	Pecan Nut	Carya illinoensis
f108	Rosenkohl	Brussels sprout	Brassica oleracea var. gemmifera
f114	Sonnenblumenkerne	Sunflowergrain	Helianthus spp.
f122	Olive grün	Olive green	Olea europea
f124	Feldsalat	Lamb's lettuce	Valerianella
f126	Pfefferminze	Peppermint	Mentha piperita
f127	Champignon	Mushroom	Agaricus hortensis
f128	Mohn	Poppy	Papaver somniferum
f129	Makadamianuss	Macadamia Nut	
f130	Truthahn	Turkey	
f131	Avocado	Avocado	Persea americana
f132	Grüne Bohne	Green bean	Phaseolus vulgaris
f133	Gurke	Cucumber	Cucumis sativus
f134	Broccoli	Broccoli	Brassica oleracea var. italica
f136	Rote Beete	Beet Root	Beta vulgaris
f137	Spargel	Asparagus	Asparagus officinalis
f138	Emmentalerkäse	Emmentaler Cheese	
f140	Hirse	Millet	Panicum miliaceum

Nahrungsmittel Foods

Code	Deutsch	English	Latein/Latin
f142	Schwarzwurzel	Black Salsify	Scorzonera hispanica
f144	Pistazienkerne	Pistachio Nut	Pistacia vera
f145	Feige	Fig	Ficus carica
f146	Hartweizengries	Semolina	
f147	Hibiscustee	Hibiscus Tea	Hibiscus
f148	Pflaume	Plum	Prunus domestica
f149	Papaya	Papaya	Carica papaya
f150	Edamer Käse	Edam Cheese	
f151	Zucchini	Zucchini	Cucurbita pepo subsp. pepo convar. giromontiina
f152	Aprikose	Apricot	Prunus armeniaca
f153	Weizenkleie	Wheat Clay	Triticum clia
f154	Johannisbrot	Carob	Ceratonia siliqua
f155	Vanille	Vanilla	Vanilla planifolia
f156	Himbeere	Raspberry	Rubus idaeus
f157	Kabeljau	Codfish	Gadus morhua
f158	Cashew - Kerne	Cashew Nut	Anacardium occidentale
f159	Kichererbse	Chick-pea	Cicer arietinum
f160	Sardine	Anchovy	Sardinops melanosticta
f161	Tintenfisch	Squid	Loligo spp.
f162	Seezunge	Sole	Solea solea
f163	Hecht	Pike	Merluccius merluccius
f164	Schwertfisch	Swordfish	Xiphias gladius
f165	Kalbfleisch	Veal	
f166	Fenchel	Fennel	Foeniculum vulgare
f167	Kaninchen	Rabbit	Oryctolagus cuniculus
f168	Milchpulver	Milk powder	
f169	Milch, gekocht	Milk, cooked	
f170	Nektarine	Nectarine	
f171	Johannisbeere, rot	Redcurrant	Ribes spicatum
f172	Artischocke	Artichoke	Cynara scolymus
f173	Knurrhahn	Gurnard	Trigla spp.
f174	Makrele	Mackerel	Scomber scombrus
f175	Brombeere	Blackberry	Rubus fruticosus
f176	Aubergine	Aubergine	Solanum melongena
f177	Auster	Oyster	Ostrea edulis
f178	Schellfisch	Haddock / White Fish	Gadus aeglefinus
f179	Steingarnele	Prawn	Palaemon squilla
f180	Karpfen	Carp	Cyprinus carpio
f182	Preiselbeere	Cranberry	Vaccinicum vitis-idaea
f183	Dinkel	Dinkel	Triticum spelta
f184	Pferdefleisch	Horsemeat	Cavallus spp.
f185	Rotkohl	Red cabbage	Brassica gemmifera
f186	Scholle	Plaice	Pleuronectes platessa
f187	Schafskäse	Sheep´s milk cheese	
f188	Sojamehl	Soy flour	
f191	Kürbis	Pumpkin	Cucurbita pepo
f192	Wachtelfleisch	Quail Meat	Coturnix coturnix
f193	Grünkern	Green rye	
f194	Peperoni	Chili	Capiscum annuum
f195	Sojamilch	Soymilk	
f196	Heilbutt	Halibut	Hippoglossus hippoglossus
f197	Pinienkerne	Pine nut, pignoles	Pinus edulis
f198	Goudakäse	Gouda Cheese	
f199	Rosinen	Raisin	
f200	Steinpilz	Boletus	Boletus edulis
f201	Pfifferling	Chanterelle	Cantharellus cibarius
f202	Gelatine (Schwein)	Gelatin (Pork)	
f203	Granatapfel	Grenadine	Punica granatum

Nahrungsmittel Foods

Code	Deutsch	English	Latein/Latin
f205	Ziegenkäse	Goat‘s milk cheese	Capra hircus
f206	Rote Kidney Bohnen	Red Kidney Bean	Phaseolus vulgaris
f207	Fencheltee	Fennel Tea	Foeniculum vulgare
f208	Chinakohl	Chinese Cabbage	Brassica chinensis
f209	Salbeitee	Sage Tea	Salvia officinale
f210	Weizenschrot	Wheat (bruised grain)	Triticum sativum
f211	Maracuja	Maracuja	
f212	Johannisbeere schwarz	Black Currant	Ribes nigrum
f213	Rhabarber	Rhubarb	Rheum officinale
f214	Radieschen	Red radish	Raphanus vadicula
f215	Maisstärke	Corn Starch	Zea mays
f217	Sojaeiweiß	Soy white	
f219	Ziegenmilch	Goat´s milk	Capra hircus
f220	Sardelle	Anchovis	Engraulidae
f221	Bambussprossen	Bamboo‘s sprouts	
f222	Kürbiskerne	Pumpkin seed	Cucurbita pepo
f223	Alpha-Amylase	Alpha-Amylase	
f224	Runkelrübe	Beet (Root)	Beta vulgaris
f226	Flugente	Muscovy duck	Cairina moschata
f227	Reh	Deer	Capreolus capreolus
f228	Wildschwein	Wild Boar	Sus scrofa
f229	Heidelbeere	Blueberry	Vaccinium myrtilleus
f230	Kaviar (schwarz)	Caviare (black)	
f231	Lychee	Lychee	Litchi chinensis
f232	Seeteufel	Monk Fish	Lophius piscatorius
f233	Grünkohl	Green cabbage	Brassia spp.
f234	Chicorée	Chicory	Cichorium intybus
f235	Stachelbeere	Gooseberry	Ribes grossularia
f236	Mangold	Mangel	Beta cicla
f237	Quitte	Quince	Cydonia oblonga
f238	Kartoffelmehl	Potato flour	Solanum tuberosum
f239	Rettich	White radish	Raphanus sativus
f240	Aspartam	Aspartam	
f241	Rinderleber	Beefliver	Bos primijenius taurus
f242	Wels	Cat fish	Silurus glanis
f243	Hopfen	Hop	Humulus lupulus
f244	Gartenbohne	Garden bean	Phaseolus vulgaris
f245	Guave	Guava	Psidium guajava
f246	Schafsmilch	Sheep´s milk	
f247	Zander	Pike perch	Sander lucioperca
f248	Dattel	Date	
f249	Seelachs	Pollack	Pollachius virens
f250	Joghurt	Yoghurt	
f251	Parmesan	Parmesan	
f252	Vollei	Egg (White & Yolk)	
f253 *	Meerrettich	Horseradish	Armoracia rusticana
f254	Roggenkorn	Rye corn	Secale cereale
f255	Weizenkorn	Wheat corn	Triticum aestivum
f256	Kokosmilch	Coconut milk	
f257	Eisbergsalat	Iceberg lettuce	
f258	Kapern	Caper	Capparis spinosa
f259	Limette	Limette	
f260	Tofu	Tofu	
f264	Leerdamerkäse	Leerdam Cheese	
f265	Appenzellerkäse	Appenzell Cheese	
f266	Grüner Tee	Green Tea	
f267	Tilsiterkäse	Tilsit Cheese	
f268	Wirsingkohl	Savoy cabbage	Brassica oleracea var. sabauda

Nahrungsmittel Foods

Code	Deutsch	English	Latein/Latin
f269	Rucola	Rocket	Eruca vesicaria
f281	Hagebutte	Rose hip	Rosa canina
f283	Römischer Salat	Roman lettuce	
f284	Radicchio	Radicchio	
f285	Zitronenmelisse	Lemon balm	Melissa officinalis
f286	Kaki	Kaki	Diospyros kaki
f287	Hase	Hare	Leporidae
f288	Hirsch	Deer	Cervidae
f289	Fasan	Pheasant	Phasianus colchicus
f291	Chesterkäse	Chester Cheese	
f292	Krebsfleisch	Crab meat	
f293	Alpha - Lactalbumin (gekocht)	Alpha - Lactalbumin (boiled)	
f294	Beta - Lactoglobulin (gekocht)	Beta - Lactoglobulin (boiled)	
f295	Casein (gekocht)	Casein (boiled)	
f298	Petersilienwurzel	Parsley root	Petroselium crispum subsp. tuberosum
f300	Honigmelone	Honeydew melon	Cucumis melo
f301	Weintraube (blau)	Grape (blue)	
f302	Austernpilz	Chinese mushroom	Pleurotus ostreatus
f315	Amaranth	Amaranth	
f320	Gerstenkorn	Barley (bruised grain)	Hordeum vulgare
f321	Haferkorn	Oat (bruised grain)	Avena sativa
f323	Kaviar (rot)	Caviare (red)	
f326	Bärlauch	Wild Garlic	Allium ursinum
f328	Rooibos Tee	Rooibos Tea	
f341	Steinbutt	Turbot	Scophthalmus maximus
f342	Mirabelle	Mirabelle	Prunus domestica subsp. syriaca
f344	Süßlupinen (Mehl)	Sweet Lupines (Flour)	
f348	Olive schwarz	Olive black	
f352	Zackenbarsch	Goliath Grouper	Epinephelus itajara
f353	Seebarsch	Bass	Atractoscion nobilis
f354	Seehecht	Hake	Merluccius merluccius
f355	Dorade	Gilthead	Sparus auratus
f357	Zitronengras	Lemon Grass	Cymbopogon citratus
f358	Sauerkirsche	Sour cherry	Prunus cerasus
f359	Physalis	Cape gooseberry	Physalis peruviana
f360	Pangasius	Thai catfish	Pangasianodon hypophthalmus

Nahrungsmittel Foods

Multi-Allergene Multi-Allergens

Code	Deutsch			Code	English		
Fx1	Nüsse 1	f13	Erdnuss	Fx1	Nuts 1	f13	Peanut
		f16	Walnuss			f16	Walnut
		f17	Haselnuss			f17	Hazelnut
		f20	Mandel			f20	Almond
Fx2	Mehle 2	f4	Weizenmehl	Fx2	Flours 2	f4	Wheat Flour
		f5	Roggenmehl			f5	Rye Flour
		f7	Hafermehl			f7	Oat Flour
		f79	Gluten			f79	Gluten
Fx3	Schalentiere/ Fische	f3	Dorsch / Kabeljau	Fx3	Crustaceae/ Fish	f3	Codfish
		f24	Garnele			f24	Shrimp
		f37	Miesmuschel			f37	Blue Mussel
		f40	Thunfish			f40	Tuna
		f41	Lachs			f41	Salmon
Fx4	Nahrungs- mittel 4	f1	Eiklar	Fx4	Foods 4	f1	Egg White
		f2	Kuhmilch (roh)			f2	Cow´s milk (raw)
		f4	Weizenmehl			f4	Wheat Flour
		f13	Erdnuss			f13	Peanut
		f14	Sojabohne			f14	Soybean
Fx5	Gemüse 5	f12	Erbse	Fx5	Vegetable 5	f12	Pea
		f15	Weiße Bohne			f15	White Bean
		f31	Karotte			f31	Carrot
		f35	Kartoffel			f35	Potato
Fx6	Gemüse 6	f25	Tomate	Fx6	Vegetable 6	f25	Tomato
		f38	Spinat			f38	Spinach
		f39	Kohl			f39	Cabbage
		f46	Paprika			f46	Paprika
Fx7	Gemüse 7	f14	Sojabohne	Fx7	Vegatable 7	f14	Soybean
		f48	Zwiebel			f48	Onion
		f85	Sellerie			f85	Celeriac
		f127	Champignon			f127	Mushroom
Fx8	Fleisch Mischung 8	f26	Schweinefleisch	Fx8	Meat 8	f26	Pork
		f27	Rindfleisch			f27	Beef
		f88	Hammel/Lamm			f88	Mutton / Lamb
Fx9	Früchte 9	f29	Banane	Fx9	Fruit 9	f29	Banana
		f33	Orange			f33	Orange
		f49	Apfel			f49	Apple
		f53	Pfirsich			f53	Peach
Fx10	Früchte 10	f30	Birne	Fx10	Fruits 10	f30	Pear
		f32	Zitrone			f32	Lemon
		f44	Erdbeere			f44	Strawberry
		f72	Ananas			f72	Pineapple
Fx11	Käse 11	f70	Schweizer Käse	Fx11	Cheese 11	f70	Swiss Cheese
		f81	Cheddarkäse			f81	Cheddar Cheese
		f82	Schimmelkäse			f82	Mold Cheese
		f150	Edamer Käse			f150	Edam Cheese
Fx12	Geflügelfleisch	f57	Ente	Fx12	Poultry	f57	Duck
		f58	Gans			f58	Goose
		f83	Huhn			f83	Chicken
		f130	Truthahn			f130	Turkey
Fx13	Nahrungs- mittel 13	f1	Eiklar	Fx13	Foods 13	f1	Egg White
		f2	Kuhmilch (roh)			f2	Cow´s milk (raw)
		f13	Erdnuss			f13	Peanut
		f85	Sellerie			f85	Celeriac

Nahrungsmittel

Multi-Allergene

Foods

Multi-Allergens

Code	Deutsch			Code	English		
Fx14	Mehle 14	f4	Weizenmehl	Fx14	Flours 14	f4	Wheat Flour
		f7	Hafermehl			f7	Oat Flour
		f8	Maismehl			f8	Corn Flour
		f10	Sesamschrot			f10	Sesame (bruised grain)
		f11	Buchweizenmehl			f11	Buckwheat Flour
Fx15	Nüsse 15	f13	Erdnuss	Fx15	Nuts 15	f13	Peanut
		f17	Haselnuss			f17	Hazelnut
		f18	Paranuss			f18	Brazil Nut
		f20	Mandel			f20	Almond
		f36	Kokosnuss			f36	Coconut
Fx16	Fleisch Mischung 16	f26	Schweinefleisch	Fx16	Meat Mix 16	f26	Pork
		f27	Rindfleisch			f27	Beef
		f83	Hühnerfleisch			f83	Chicken
		f88	Hammel/ Lamm			f88	Mutton / Lamb
Fx17	Fische 17	f3	Dorsch	Fx17	Fish 17	f3	Codfish
		f21	Hering			f21	Herring
		f174	Makrele			f174	Mackerel
		f186	Scholle			f186	Plaice
Fx19	Früchte 19	f32	Zitrone	Fx19	Fruit 19	f32	Lemon
		f33	Orange			f33	Orange
		f34	Mandarine			f34	Tangerine
		f92	Grapefruit			f92	Grapefruit
Fx20	Nahrungsmittel Screen	f1	Eiklar	Fx20	Food Screen	f1	Egg White
		f2	Kuhmilch (roh)			f2	Cow's milk (raw)
		f3	Dorsch			f3	Codfish
		f4	Weizenmehl			f4	Wheat Flour
		f13	Erdnuss			f13	Peanut
		f14	Sojabohne			f14	Soybean
		f44	Erdbeere			f44	Strawberry
		f85	Sellerie			f85	Celeriac
Fx23	Nüsse 23	f16	Walnuss	Fx23	Nuts 23	f16	Walnut
		f17	Haselnuss			f17	Hazelnut
		f20	Mandel			f20	Almond
		f52	Schokolade			f52	Chocolate
Fx25	Milch-komponenten	f76	Alpha-Lactalbumin	Fx25	Milk-components	f76	Alpha-Lactalbumin
		f77	Beta-Lactoglobulin			f77	Beta-Lactoglobulin
		f78	Casein			f78	Casein
Fx26	Mehle 26	f4	Weizenmehl	Fx26	Flours 26	f4	Wheat Flour
		f7	Hafermehl			f7	Oat Flour
		f8	Maismehl			f8	Corn Flour
		f9	Reis			f9	Rice
		f11	Buchweizenmehl			f11	Buckwheat Flour
Fx27	Fische 27	f3	Dorsch (Kabeljau)	Fx27	Fish 27	f3	Codfish
		f40	Thunfisch			f40	Tuna
		f41	Lachs			f41	Salmon
Fx28	Nüsse 28	f16	Walnuss	Fx28	Nuts 28	f16	Walnut
		f17	Haselnuss			f17	Hazelnut
		f18	Paranuss			f18	Brazil Nut
		f20	Mandel			f20	Almond
		f36	Kokosnuss			f36	Coconut
Fx29	Gemüse 29	f12	Erbse	Fx29	Vegetable 29	f12	Pea
		f25	Tomate			f25	Tomato
		f31	Karotte			f31	Carrot
		f35	Kartoffel			f35	Potato
		f85	Sellerie			f85	Celeriac

Nahrungsmittel

Multi-Allergene

Foods

Multi-Allergens

Code	Deutsch			Code	English		
Fx30	Früchte 30	f29	Banane	Fx30	Fruits 30	f29	Banana
		f30	Birne			f30	Pear
		f33	Orange			f33	Orange
		f44	Erdbeere			f44	Strawberry
		f49	Apfel			f49	Apple
		f53	Pfirsich			f53	Peach
		f131	Avocado			f131	Avocado
Fx34	Nüsse 34	f13	Erdnuss	Fx34	Nuts 34	f13	Peanut
		f16	Walnuss			f16	Walnut
		f17	Haselnuss			f17	Hazelnut
		f20	Mandel			f20	Almond
		f36	Kokosnuss			f36	Coconut
Fx35	Schalentiere Mischung	f24	Garnele	Fx35	Crustaceae	f24	Shrimp
		f80	Hummer			f80	Lobster
Fx36	Fisch-mischung 36	f40	Thunfisch	Fx36	Fish Mix 36	f40	Tuna
		f41	Lachs			f41	Salmon
		f163	Hecht			f163	Hake
Fx37	Fisch-mischung 37	f24	Garnele	Fx37	Fish Mix 37	f24	Shrimp
		f40	Thunfisch			f40	Tuna
		f41	Lachs			f41	Salmon
		f80	Hummer			f80	Lobster
Fx38	Obst- und Gemüse 38	f14	Sojabohne	Fx38	Fruits & Vegetables 38	f14	Soybean
		f25	Tomate			f25	Tomato
		f29	Banane			f29	Banana
		f31	Karotte			f31	Carrot
		f33	Orange			f33	Orange
		f49	Apfel			f49	Apple
		f74	Maiskorn			f74	Corn
Fx40	Zitrusfrüchte*	f32	Zitrone	Fx40	Fruits*	f32	Lemon
		f33	Orange			f33	Orange
		f92	Grapefruit			f92	Grapefruit
Fx50	Obst-Birkenpollen Ass.*	f17	Haselnuss	Fx50	Fruit-Birch Pollen Ass.*	f17	Hazelnut
		f49	Apfel			f49	Apple
		f53	Pfirsich			f53	Peach
		f73	Kirsche			f73	Cherry
		f148	Pflaume			f148	Plum
Fx51	Obst Latex Ass.*	f29	Banane	Fx51	Fruit Latex Ass.*	f29	Banana
		f84	Kiwi			f84	Kiwi
		f91	Mango			f91	Mango
		f131	Avocado			f131	Avocado
		f149	Papaya			f149	Papaya
Fx52	Nahrungsmittel (Fleisch) *	f26	Schwein	Fx52	Meat Mix*	f26	Pork
		f27	Rind			f27	Beef
		f75	Eigelb			f75	Egg Yolk
		f83	Huhn			f83	Chicken
		f130	Truthahn			f130	Turkey
Fx54	Nahrungs-mittel 54	f1	Eiklar	Fx54	Food 54	f1	Egg White
		f2	Kuhmilch (roh)			f2	Cow's milk (raw)
		f4	Weizenmehl			f4	Wheat Flour
		f52	Schokolade			f52	Chocolate
		f144	Pistazie			f144	Pistachio Nut
Fx55	Nahrungs-mittel 55	f1	Eiklar	Fx55	Food 55	f1	Egg White
		f27	Rindfleisch			f27	Beef
		f44	Erdbeere			f44	Strawberry
		f83	Huhn			f83	Chicken
		f144	Pistazienkerne			f144	Pistachio Nut

Nahrungsmittel

Multi-Allergene

Code	Deutsch		
Fx56	Nahrungs-mittel 56	f1	Eiklar
		f25	Tomate
		f29	Banane
		f48	Zwiebel
		s26	Grüner Pfeffer
Fx57	Nahrungs-mittel 57	f25	Tomate
		f31	Karotte
		f45	Bäckerhefe
		f47	Knoblauch
		f48	Zwiebel
		f85	Sellerie
Fx58	Nahrungs-mittel 58	f29	Banane
		f53	Pfirsich
		f72	Ananas
		f84	Kiwi
		f87	Melone
Fx90	Früchte 90*	f30	Birne
		f49	Apfel
		f53	Pfirsich
		f73	Kirsche
		f148	Pflaume
Fx114	Käse 114*	f70	Schweizer Käse
		f81	Cheddarkäse
		f82	Schimmelkäse
		f150	Edamer Käse
		f198	Gouda
Fx128	Mehle 128*	f4	Weizenmehl
		f6	Gerstenmehl
		f7	Hafermehl
		f8	Maismehl
		f9	Reis
		f14	Sojabohne
Fx129	Mehle 129*	f4	Weizenmehl
		f5	Roggenmehl
		f6	Gerstenmehl
		f7	Hafermehl
		f8	Maismehl
		f14	Sojabohne
		f79	Gluten

Foods

Multi-Allergens

Code	English		
Fx56	Food 56	f1	Egg White
		f25	Tomato
		f29	Banana
		f48	Onion
		s26	Green Pepper
Fx57	Food 57	f25	Tomato
		f31	Carrot
		f45	Yeast
		f47	Garlic
		f48	Onion
		f85	Celeriac
Fx58	Food 58	f29	Banana
		f53	Peach
		f72	Pineapple
		f84	Kiwi
		f87	Melon
Fx90	Fruits 90 *	f30	Pear
		f49	Apple
		f53	Peach
		f73	Cherry
		f148	Plum
Fx114	Cheese 114*	f70	Swiss Cheese
		f81	Cheddar Cheese
		f82	Mold Cheese
		f150	Edam Cheese
		f198	Gouda Cheese
Fx128	Flours 128*	f4	Wheat Flour
		f6	Barley Flour
		f7	Oat Flour
		f8	Corn Flour
		f9	Rice
		f14	Soybean
Fx129	Flours 129*	f4	Wheat Flour
		f5	Rye Flour
		f6	Barley Flour
		f7	Oat Flour
		f8	Corn Flour
		f14	Soybean
		f79	Gluten

* nur als biotinyliertes Reagenz verfügbar
* available only as biotinylated reagent

Gewürze

Spices

Code	Deutsch	English	Latein/Latin
s1	Anis	Aniseed	Pimpinella anisum
s2	Curry	Curry	
s3	Kümmel	Caraway	Lavum carvi
s4	Lorbeerblatt	Laurel	Laurus nobilis
s5	Muskatnuss	Nutmeg	Myristica fragrans
s6	Paprika	Paprika	Capsicum spp.
s7	Schwarzer Pfeffer	Black Pepper	Piper nigrum
s8	Zimt	Cinnamon	Cinnamomum spp.
s9	Oregano	Origan	Origanum vulgare
s10	Basilikum	Basil	Ocimum basilicum
s11	Dill	Dill	Anethum graveolens
s12	Schnittlauch	Chives	Allium shoenoprasum
s13	Thymian	Thyme	Thymus vulgaris
s14	Majoran	Marjoram	Origanum majorana
s15	Chili	Chili	Capsicum frutescens
s16	Gewürznelke	Clove	Syzygium aromaticum
s17	Koriander	Coriander	Coriandrum sativum
s18	Salbei	Sage	Salvia officinalis
s19	Melisse	Balm	Melissa officinalis
s20	Liebstöckel	Lovage	Levisticum officinale
s21	Wacholderbeeren	Juniper berry	Juniperus communis
s22	Bohnenkraut	Beanstalk	Satureja hortensis
s23	Kerbel	Chervil	Anthriscus cerefolium
s24	Rosmarin	Rosemary	Rosmarinus spp.
s25	Ingwer	Ginger	Zingiber officinale
s26	Grüner Pfeffer	Green Pepper	Piper spp.
s27	Estragon	Tarragon	Artemisia dracunculus
s28	Kardamom	Cardamom	Elettaria cardamomum
s29	Roter Pfeffer	Red Pepper	Piper nigrum
s30	Curcuma	Curcuma	Curcuma
s31	Muskatblüte	Mace	Myristica fragrans
s32	Piment	Piment	Pimentum
s33	Weißer Pfeffer	White Pepper	Piper spp.

Multi-Allergene

Multi-Allergens

Code	Deutsch			Code	English		
Sx1	Gewürze 1	s1	Anis	Sx1	Spices 1	s1	Aniseed
		s2	Curry			s2	Curry
		s3	Kümmel			s3	Caraway
		f47	Knoblauch			f47	Garlic
Sx2	Gewürze 2	s4	Lorbeerblatt	Sx2	Spices 2	s4	Laurel
		s6	Paprika			s6	Paprika
		s7	Schwarzer Pfeffer			s7	Black Pepper
		f89	Senf			f89	Mustard
Sx3	Gewürze 3	s5	Muskatnuss	Sx3	Spices 3	s5	Nutmeg
		s6	Paprika			s6	Paprika
		s7	Schwarzer Pfeffer			s7	Black Pepper
		f79	Gluten			f79	Gluten
Sx4	Gewürze 4	s1	Anis	Sx4	Spices 4	s1	Aniseed
		s2	Curry			s2	Curry
		s3	Kümmel			s3	Caraway
Sx5	Gewürze 5	s5	Muskatnuss	Sx5	Spices 5	s5	Nutmeg
		s6	Paprika			s6	Paprika
		s7	Schwarzer Pfeffer			s7	Black Pepper
Sx16	Gewürze 16*	s1	Anis	Sx16	Spices 16*	s1	Aniseed
		s2	Curry			s2	Curry
		s3	Kümmel			s3	Caraway
		s6	Paprika			s6	Paprika
		f47	Knoblauch			f47	Garlic
Sx71	Gewürze 71*	s3	Kümmel	Sx71	Spices 71*	s3	Caraway
		s5	Muskat			s5	Nutmeg
		s16	Nelke			s16	Clove
		s28	Kardamom			s28	Cardamom

* nur als biotinyliertes Reagenz verfügbar * available only as biotinylated reagent

Schimmelpilze Molds

Code	Latein/Latin
m1	Penicillium chrysogenum (notatum)
m2	Cladosporium herbarum
m3	Aspergillus fumigatus
m4	Mucor racemosus
m5	Candida albicans
m6	Alternaria tenuis (alternata)
m7	Botrytis cinerea
m8	Helminthosporium halodes
m9	Gibberella fujikuroi (Syn. Fusarium moniliforme)
m10	Stemphylium botryosum
m11	Rhizopus nigricans
m12	Aureobasidium pullulans
m13	Phoma betae
m14	Epicoccum purpurascens
m15	Trichoderma viride
m16	Curvularia lunata
m19	Aspergillus versicolor
m20	Mucor mucedo
m22	Mucor spinosus
m23	Neurospora sitophila
m24	Paecilomyces spp.
m25	Penicillium brevicompactum
m28	Penicillium expansum
m30	Penicillium roqueforti
m32	Cladosporium spp.
m33	Aspergillus niger
m34	Serpula lacrymans (Syn. Merulius lacrymans)
m37	Trichophyton mentagrophytes (Var. interdigitale)
m40	Aspergillus amstelodami
m41	Cephalosporium acremonium
m43	Saccharomyces carlsbergensis (Brauereihefe)
m44	Saccharomyces cerevisiae (Bäckerhefe)
m45	Chaetomium globosum
m46	Saccharomyces ellipsoideus (Weinhefe)
m47	Aspergillus flavus
m48	Aspergillus oryzae
m49	Aspergillus nidulans
m52	Thermoactinomyces vulgaris
m55	Penicillium digitatum
m56	Microsporum canis
m57	Epidermophyton floccosum
m58	Thermoactinomyces candidus

Schimmelpilze Molds

Multi-Allergene Multi-Allergens

Code	Deutsch		Latein/Latin	Code	English	
Mx1	Schimmelpilz-Mischung 1	m1	Penicillium chrysogenum (notatum)	Mx1	Mold Mix 1	m1
		m2	Cladosporium herbarum			m2
		m3	Aspergillus fumigatus			m3
		m6	Alternaria tenuis (alternata)			m6
Mx2	Schimmelpilz-Mischung 2	m11	Rhizopus nigricans	Mx2	Mold Mix 2	m11
		m12	Aureobasidium pullulans			m12
		m22	Mucor spinosus			m22
		m23	Neurospora sitophila			m23
Mx3	Schimmelpilz-Mischung 3	m14	Epicoccum purpurascens	Mx3	Mold Mix 3	m14
		m20	Mucor mucedo			m20
		m45	Chaetomium globosum			m45
Mx4	Schimmelpilz-Mischung 4	m13	Phoma betae	Mx4	Mold Mix 4	m13
		m24	Paecilomyces spp.			m24
Mx5	Schimmelpilz-Mischung 5	m4	Mucor racemosus	Mx5	Mold Mix 5	m4
		m11	Rhizopus nigricans			m11
		m20	Mucor mucedo			m20
		m22	Mucor spinosus			m22
Mx6	Schimmelpilz-Mischung 6	m3	Aspergillus fumigatus	Mx6	Mold Mix 6	m3
		m40	Aspergillus amstelodami			m40
		m49	Aspergillus nidulans			m49
Mx8	Schimmelpilz-Mischung 8	m1	Penicillium chrysogenum (notatum)	Mx8	Mold Mix 8	m1
		m25	Penicillium brevicompactum			m25
		m28	Penicillium expansum			m28
		m30	Penicillium roqueforti			m30
Mx11	Schimmelpilz-Mischung 11	m1	Penicillium chrysogenum (notatum)	Mx11	Mold Mix 11	m1
		m3	Aspergillus fumigatus			m3
		m5	Candida albicans			m5
Mx12	Schimmelpilz-Mischung 12	m1	Penicillium chrysogenum (notatum)	Mx12	Mold Mix 12	m1
		m2	Cladosporium herbarum			m2
		m3	Aspergillus fumigatus			m3
		m5	Candida albicans			m5
		m6	Alternaria tenuis (alternata)			m6
Mx14	Schimmelpilz-Mischung 14	m1	Penicillium chrysogenum (notatum)	Mx14	Mold Mix 14	m1
		m2	Cladosporium herbarum			m2
		m3	Aspergillus fumigatus			m3
		m4	Mucor racemosus			m4
		m5	Candida albicans			m5
Mx15	Schimmelpilz-Mischung 15	m6	Alternaria tenuis (alternata)	Mx15	Mold Mix 15	m6
		m7	Botrytis cinerea			m7
		m8	Helminthosporium halodes			m8
		m9	Fusarium moniliforme			m9
		m16	Curvularia lunata			m16
Mx17	Schimmelpilz-Mischung 17	m1	Penicillium chrysogenum (notatum)	Mx17	Mold Mix 17	m1
		m3	Aspergillus fumigatus			m3
		m5	Candida albicans			m5
		m47	Aspergillus flavus			m47
		m56	Microsporium canis			m56
TMx9	Schimmel-pilze TM9	m1	Penicillium chrysogenum (notatum)	TMx9	Mold TM9	m1
		m2	Cladosporium herbarum			m2
		m3	Aspergillus fumigatus			m3
		m5	Candida albicans			m5
		m6	Alternaria tenuis (alternata)			m6
		m8	Helminthosporium halodes			m8

Rekombinante (R) und native (N) Allergene

Recombinant (R) and native (N) allergens

Code	Deutsch	English
ND11	D. pteronyssinus (Der p 1)*	D. pteronyssinus (Der p 1)*
ND12	D. pteronyssinus (Der p 2)*	D. pteronyssinus (Der p 2)*
RD 110	D. pteronyssinus (Der p 10)*	D. pteronyssinus (Der p 10)*
RD 123	D. pteronyssinus (Der p 23)*	D. pteronyssinus (Der p 23)*
ND21	D. farinae (Der f 1)*	D. farinae (Der f 1)*
ND22	D. farinae (Der f 2)*	D. farinae (Der f 2)*
RE11	Katze (Fel d 1)*	Cat (Fel d 1)*
NF24	Tropomyosin Garnele*	Tropomyosin Shrimp*
F67	Hühnerei (Gal d 2)*	Hen's egg (Gal d 2)*
F68	Hühnerei (Gal d 1)*	Hen's egg (Gal d 1)*
NF103	Hühnerei (Gal d 3)*	Hen's egg (Gal d 3)*
F76	Kuhmilch (Bos d4)*	Cow's milk (Bos d 4)*
F77	Kuhmilch (Bos d 5)*	Cow's milk (Bos d 5)*
F78	Kuhmilch (Bos d 8/9/10)*	Cow's milk (Bos d 8/9/10)*
NF131	Erdnuss (Ara h 1)*	Peanut (Ara h 1)*
NF132	Erdnuss (Ara h 2)*	Peanut (Ara h 2)*
NF133	Erdnuss (Ara h 3)*	Peanut (Ara h 3)*
NF136	Erdnuss (Ara h 6)*	Peanut (Ara h 6)*
RF138	Erdnuss (Ara h 8)*	Peanut (Ara h 8)*
RF139	Erdnuss (Ara h 9)*	Peanut (Ara h 9)*
RF171	Haselnuss (Cor a 1)*	Hazelnut (Cor a 1)*
RF178	Haselnuss (Cor a 8)*	Hazelnut (Cor a 8)*
RF179	Haselnuss (Cor a 9)*	Hazelnut (Cor a 9)*
RF1714	Haselnuss (Cor a 14)*	Haselnut (Cor a 14)*
RF180	Parvalbumin Karpfen (Cyp c 1)*	Parvalbumin Carp (Cyp c 1)*
RF311	Karotte (Dau c 1)*	Carrot (Dau c 1)*
RF491	Apfel (Mal d 1)*	Apple (Mal d 1)*
RF493	Apfel (Mal d 3)*	Apple (Mal d 3)*
RF441	Erdbeere (Fra a 1)*	Strawberry (Fra a 1)*
RF443	Erdbeere (Fra a 3)*	Strawberry (Fra a 3)*
RF531	Pfirsich (Pru p 1)*	Peach (Pru p 1)*
RF533	Pfirsich (Pru p 3)*	Peach (Pru p 3)*
RF534	Pfirsich (Pru p 4)*	Peach (Pru p 4)*
NFgal	α-Gal*	α-Gal*
NF253	CCD Meerrettich*	CCD Horseradish*
RG601	Lieschgras (Phl p 1)*	Timothy Grass (Phl p 1)*
RG605	Lieschgras (Phl p 5)*	Timothy Grass (Phl p 5)*
RG607	Lieschgras (Phl p 7)*	Timothy Grass (Phl p 7)*
RG612	Lieschgras (Phl p 12)*	Timothy Grass (Phl p 12)*
RG620	Lieschgras (Phl p 1/Phl p 5)*	Timothy Grass (Phl p 1/Phl p 5)*
RG621	Lieschgras (Phl p 7/Phl p 12)*	Timothy Grass (Phl p 7/Phl p 12)*
RI101	Bienengift (Api m 1)*	Honey Bee Venom (Api m 1)*
RI102	Bienengift (Api m 2)*	Honey Bee Venom (Api m 2)*
RI110	Bienengift (Api m 10)*	Honey Bee Venom (Api m 10)*
RI305	Wespengift (Ves v 5)*	Wasp Venom (Ves v 5)*
RK825	Latex (Hev b 5)*	Latex (Hev b 5)*
RK826	Latex (Hev b 6)*	Latex (Hev b 6)*
RK827	Latex (Hev b 7)*	Latex (Hev b 7)*
RK828	Latex (Hev b 8)*	Latex (Hev b 8)*
NW101	Ambrosia (Amb a 1)*	Common ragweed (Amb a 1)*
RW601	Beifuß (Art v 1)*	Mugwort (Art v 1)*
RM601	Alternaria alternata (Alt a 1)*	Alternaria alternata (Alt a 1)*
RT201	Hasel (Cor a 1)*	Hazel (Cor a 1)*
RT301	Birke (Bet v 1a)*	Birch (Bet v 1a)*
RT302	Birke (Bet v 2)*	Birch (Bet v 2)*
RT304	Birke (Bet v 4)*	Birch (Bet v 4)*

* nur als biotinyliertes Reagenz verfügbar
* available only as biotinylated reagent

Weitere Allergene auf Anfrage. Further allergens on request

Pollenflugkalender für Deutschland*

Allergen	Code	Jan.	Feb.	Mär.	Apr.	Mai	Jun.	Jul.	Aug.	Sep.	Okt.	Nov.	Dez.
Bäume													
Ahorn	t1			■	■	■	■						
Birke	t3			■	■	■							
Buche	t5				■	■	■	■					
Eibe	t37			■	■	■	■						
Eiche	t7			■	■	■	■						
Erle	t2	■	■	■	■								
Esche	t15			■	■	■	■	■					
Fichte	t35				■	■	■						
Flieder	t21				■	■							
Hainbuche	t46			■	■	■							
Hasel	t4		■	■	■								
Holunder	t26					■	■	■	■				
Rosskastanie	t17				■	■	■						
Kiefer	t16				■	■	■	■	■	■			
Kirsche	t29			■	■								
Liguster	t20					■	■	■					
Linde	t27						■	■	■				
Pappel	t14			■	■	■							
Platane	t11			■	■	■							
Robinie	t28					■	■						
Tanne	t38					■	■						
Thuja (Koniferen)	t43			■	■	■	■	■	■				
Ulme	t8			■	■								
Walnuss	t10				■	■	■						
Weide	t12		■	■	■	■							
Kräuter													
Ambrosie	w1								■	■	■		
Beifuß	w6						■	■	■	■	■		
Berennnessel	w20					■	■	■	■	■	■		
Gänsefuß	w10						■	■	■	■	■		
Goldrute	w12						■	■	■	■			
Löwenzahn	w8				■	■	■	■	■	■			
Raps	w32				■	■	■	■	■				
Sauerampfer	w18					■	■	■	■				
Spitzwegerich	w9				■	■	■	■	■				
Gräser													
Gerste	g18					■	■	■					
Glatthafer	g71					■	■	■	■	■			
Hafer	g14					■	■	■	■				
Honiggras	g13					■	■	■	■	■			
Kammgras	g19					■	■	■	■				
Knäuelgras	g3					■	■	■	■				
Lieschgras	g6					■	■	■	■	■			
Lolch	g5					■	■	■	■	■			
Mais	g20						■	■	■				
Roggen	g12					■	■	■					
Rohrglanzgras	g74					■	■	■	■				
Ruchgras	g1				■	■	■	■	■	■			
Straußgras	g9						■	■	■				
Weizen	g15					■	■	■					
Wiesenfuchsschwanzgras	g16				■	■	■	■					
Wiesenrispengras	g8					■	■	■	■				
Wiesenschwingel	g4					■	■	■	■	■			
		Jan.	Feb.	Mär.	Apr.	Mai	Jun.	Jul.	Aug.	Sep.	Okt.	Nov.	Dez.

* aufgrund der regionalen Unterschiede im Pollenflugverhalten wurde auf die Angabe von Vor,- Haupt- und Nachblütezeit verzichtet.

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Rev: 05-2019

HBc IgM

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

- for "in vitro" diagnostic use only -

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REF BCM.CE
96 Tests

HBc IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibodies to Hepatitis B Virus core Antigen in human plasma and sera with the "capture" system.
The kit is intended for the classification of the viral agent and for the follow-up of chronic patients under therapy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of Hepatitis B virus (or HBV).
Particles have a size of 27nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBcAg. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles ; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg IgM antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, together or slightly later than HBsAg, the viral surface antigen.
Anti HBcAg IgM titers, very high during the acute phase, decrease along the illness, as IgG antibodies appear, down to undetectable levels in convalescent patients.
In chronic hepatitis, however, spikes of anti HBcAg IgM synthesis are present, confirming reactivation of HBV in hepatocites and giving origin to permanent IgM low titers.
The determination of anti HBcAg IgM antibodies has become very important for the fast classification of the virus, of the phase of the illness and for the monitoring of patients under treatment with interferon.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of recombinant HBcAg, labelled with a monoclonal antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified anti human IgM specific mouse monoclonal antibody, post-coated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on the HBcIgM reference preparation supplied by Paul Erlich Institute (HBc-Referenzserum-IgM 84), ranging: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 U/ml // CAL 5 = 50 U/ml // CAL 6 = 100 U/ml.
It contains chemical inactivated HBcIgM positive human plasma, 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The Calibration Curve is coded with blue alimentary dye.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme Conjugate (Immunocomplex) : CONJ
1x16.0 ml/vial. Ready-to-use solution. Contains an immunocomplex formed by a specific mouse monoclonal antibody, labelled with HRP, and a purified recombinant HBcAg.
The reagent is dissolved into a buffer solution 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives. The component is red colour coded.

5. Specimen Diluent : DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples; it contains 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 2% Casein, 0.045% ProClin 300 and 0.09% sodium azide as preservatives. The component is blue color coded.

6. Control Serum : CONTROL …ml
1 vial. Lyophilized. Contains fetal bovine serum, human HBcIgM positive human plasma calibrated at 20 ± 10% PEI U/ml. 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Important Notes
1. The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .
2. Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

7. Chromogen/Substrate : SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Plate sealing foils: n° 2

10. Package insert: n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

Calibration Curve:
Ready to use. Mix well on vortex before use.

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

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

Specimen Diluent
Ready to use. Mix on vortex before use.

Control Serum
Dissolve the content of the vial with EIA grade water as reported in the label. Mix well on vortex before use. The dissolved control serum is ready to use.
Note: The control after dissolution is not stable. Store frozen in aliquots at –20°C.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

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

Legenda:

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

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

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

M. ASSAY PROCEDURE

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

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

M.1 Quantitative analysis

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

2. 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 dilute the Calibrators and the dissolved Control Serum as they are ready-to-use.
3. Leave the A1+B1 wells empty for blanking purposes.
4. Pipette 100 µl of the Calibrators in duplicate, 100 µl dissolved Control Serum in duplicate followed by 100 µl of diluted 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.
5. Incubate the microplate **for 60 min at +37°C**.

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

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

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

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

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

10. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction.. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M.2 Qualitative analysis

1 Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.
2 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 dilute the Calibrators as they are ready-to-use.
3 Leave the A1 well empty for blanking purposes.
4 Pipette 100 µl Calibrator 0 U/ml in duplicate, 100 µl Calibrator 10 U/ml in duplicate and 100 µl Calibrator 100 U/ml in single. Then dispense 100 µl diluted samples in proper sample wells. Check that Calibrators and samples have been correctly added.
5 Incubate the microplate **for 60 min at +37°C**.

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

6 When the first incubation is finished, wash the microwells as previously described (section I.3)
7 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 inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

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

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

10 Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11 Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12 Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management*

N. ASSAY SCHEME

The assay protocol can be summarized in the table below:

Calibrators & diluted samples & dissolved Control Serum	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100ul
3rd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm /620-630nm

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

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S1									
B	BLK	CAL4	S2									
C	CAL1	CAL5	S3									
D	CAL1	CAL5	S4									
E	CAL2	CAL6	S5									
F	CAL2	CAL6	S6									
G	CAL3	CS	S7									
H	CAL3	CS	S8									

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

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

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL3	S 6	S 14									
E	CAL3	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

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

O. INTERNAL QUALITY CONTROL

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

Parameter	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 PEI U/ml coefficient of variation	< 0.150 OD450nm after blanking < 30%
Calibrator 5 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 5SD and anyway > OD450nm Cal 0 U/ml + 0.100
Calibrator 10 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 0.200
Calibrator 100 PEI U/ml	> 1.000 OD450nm
Control Serum	OD450nm = OD450nm of the Calibrator 20 U/ml ± 10%

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

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

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 U/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of Cal 0); 4. that no contamination of the Cal O, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator 5 U/ml < CAL 0 + 5SD or < CAL 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 10 U/ml < CAL 0 + 0.200	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 100 U/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the calibrator; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

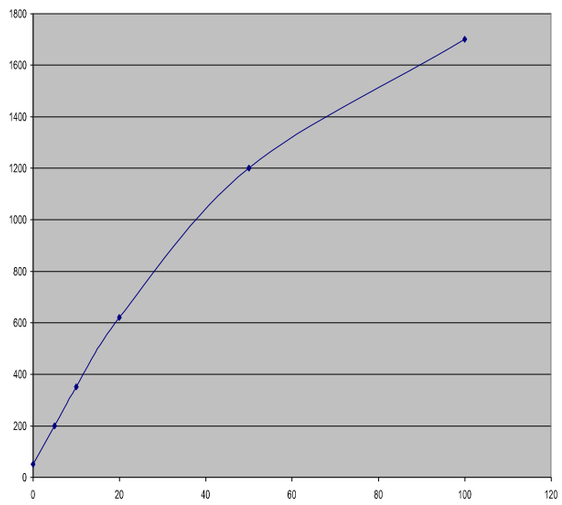
If any of the above problems have occurred, report the problem to the supervisor for further actions.

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

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm/620-630nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBc IgM antibody in samples.
An example of Calibration curve is reported below.



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

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 U/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the the reading step described in the section M, point 12).

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

Calibrator 0 U/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 10 U/ml: 0.350 – 0.330 OD450nm
Mean Value: 0.340 OD450nm
Higher than Cal 0 + 0.200 – Accepted
Calibrator 100 U/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Q.1 Qualitative results
For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of HBc IgM ≥ 10 PEI U/ml.
Test results are therefore interpreted as a ratio of the sample OD450nm and the OD450nm/620-630nm of the Cal 10 PEI U/ml (or S/Co) according to the following table:

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

Q.2 Quantitative results
The calibration curve is used to determine the concentration of IgM antibodies to HBcAg in samples.
Samples with a concentration lower than 5 PEI U/ml are considered negative for HBcIgM.
Samples with a concentration between 5 and 10 PEI U/ml are considered in a gray-zone.
In the follow up of chronic hepatitis, however, values higher of 5 PEI U/ml may be considered positive for HBcIgM, when in presence of other clinical signs.
Samples with a concentration higher than 10 PEI U/ml are considered positive for HBcIgM.

Important general notes:
1. *When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to produce the calibration curve, calculate sample concentration and generate the correct interpretation of results.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
3. *A positive result is indicative of HBV infection and therefore the patient should be treated accordingly.*
4. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
5. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

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

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

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

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

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
PEI U/ml	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
100	2.752	**8.9**	2.883	**9,7**	2.911	**9.1**
50	1.917	**6.2**	1.972	**6.7**	2.053	**6.4**
20	0.980	**3.2**	0.914	**3.1**	1.095	**3.4**
10	0.544	**1.8**	0.513	**1.7**	0.592	**1.8**
5	0.310	**1.0**	0.296	**1.0**	0.321	**1.0**
2.5	0.155	0.5	0.149	0.5	0.161	0.5
1.25	0.084	0.3	0.084	0.3	0.093	0.3
negative	0.040		0.035		0.044	

BBI Accurun # 113 lot # 48-9999-0621

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
BBI 113	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1 x	3.336	**10.8**	3.195	**10.4**	3.269	**10.3**
2 x	2.472	**8.0**	2.385	**7.8**	2.385	**7.5**
4 x	1.467	**4.7**	1.413	**4.6**	1.429	**4.5**
8 x	0.865	**2.8**	0.807	**2.6**	0.856	**2.7**
16 x	0.430	**1.4**	0.427	**1.4**	0.410	**1.3**
32 x	0.234	0.8	0.234	0.8	0.248	0.8
64 x	0.129	0.4	0.133	0.4	0.122	0.4
128 x	0.086	0.3	0.082	0.3	0.089	0.3
negative	0.040		0.040		0.052	

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

BBI – Panel code PHE 102

	Lot # 0103	Lot # 0103/2	Lot # 0303	Sorin EIA
Member	S/Co	S/Co	S/Co	S/Co
01	**6.7**	**6.3**	**6.5**	**2.0**
02	**11.3**	**10.0**	**10.7**	**6.1**
03	**9.5**	**7.2**	**8.4**	**3.0**
04	**5.8**	**3.4**	**4.1**	**2.1**
05	**11.3**	**11.4**	**11.2**	**3.1**
06	**12.1**	**11.6**	**11.8**	**4.1**
07	**0.1**	**0.1**	**0.1**	**0.2**
08	**9.2**	**8.5**	**8.8**	**2.3**
09	**12.2**	**11.7**	**11.9**	**4.2**
10	**11.7**	**10.2**	**10.8**	**2.8**
11	**5.9**	**5.8**	**5.8**	**2.1**
12	**12.7**	**11.4**	**11.7**	**5.2**
13	**11.6**	**11.0**	**11.3**	**3.6**
14	**7.0**	**6.3**	**6.6**	**2.3**
15	**12.4**	**11.5**	**11.8**	**4.5**

2. Diagnostic Sensitivity:
It is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
The diagnostic sensitivity has been tested internally and externally in a qualified Clinical Laboratory on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different patients and from different HBV pathologies (acute and chronic hepatitis).
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
A Seroconversion panel produced by BBI, USA, code # PHM 935A, havs also been studied; results are reported below with reference to two commercial kits (BBI's results).

BBI Panel PHM 935A

	Lot # 0103	Abbott EIA	DiaSorin EIA
Member #	S/Co	S/Co	S/Co
01	0.2	0.1	0.1
02	0.2	0.1	0.1
03	0.2	0.1	0.1
04	0.1	0.1	0.1
05	0.2	0.1	0.1
06	0.2	0.1	0.1
07	0.2	0.1	0.1
08	0.1	0.1	0.1
09	0.1	0.1	0.1
10	0.1	0.1	0.1
11	0.2	0.1	0.1
12	0.2	0.1	0.1
13	**2.8**	**3.7**	0.7
14	**5.0**	**6.4**	0.9
15	**> 12**	**6.2**	**4.5**
16	**> 12**	**5.6**	**4.5**
17	**> 12**	**5.5**	**4.3**
18	**> 12**	**4.8**	**4.3**
19	**> 12**	**> 6.6**	**4.4**
20	**> 12**	**> 6.6**	**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 panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit.
A total number of more than 400 negative specimens were tested. A diagnostic specificity > 98% has been found.
Moreover, the diagnostic specificity was assessed by testing more than 50 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.).
No interference was observed in the study.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

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

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

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.055	0.053	0.051	0.053
Std.Deviation	0.005	0.006	0.005	0.006
CV %	9.9	12.3	10.7	10.9

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.324	0.308	0.321	0.318
Std.Deviation	0.022	0.018	0.024	0.021
CV %	6.8	5.7	7.5	6.7

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.109	2.048	2.052	2.070
Std.Deviation	0.101	0.088	0.136	0.109
CV %	4.8	4.3	6.7	5.2

BCM.CE: lot # 0103/2
Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.057	0.053	0.054	0.055
Std.Deviation	0.005	0.005	0.004	0.004
CV %	8.3	9.0	7.3	8.2

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.332	0.331	0.322	0.328
Std.Deviation	0.017	0.018	0.016	0.017
CV %	5.0	5.5	4.9	5.1

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.311	2.208	2.212	2.244
Std.Deviation	0.110	0.090	0.095	0.098
CV %	4.7	4.1	4.3	4.4

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

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.043	0.042	0.040	0.042
Std.Deviation	0.004	0.005	0.004	0.004
CV %	10.3	11.1	10.9	10.8

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
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	7.6

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.150	2.163	2.092	2.135
Std.Deviation	0.057	0.067	0.076	0.067
CV %	2.6	3.1	3.6	3.1

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

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
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CE
0318

HBcAb

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

- for “in vitro” diagnostic use only -

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REF. BCAB.CE
96 Tests

HBcAb

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis B core Antigen in human plasma and sera.
The kit is intended for the screening of blood units and the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

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

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

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

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

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

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

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

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

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of HBV.
HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregating the core particles; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, slightly later than HBsAg, the viral surface antigen.
Anti HBcAg antibodies are produced usually at high titers and their presence is detectable even years after infection. Isolated HBcAb, in absence of other HBV markers, have been observed in infected blood units, suggesting the use of this test for screening HBV, in addition of HBsAg.
The determination of HBcAb has become important for the classification of the viral agent, together with the detection of the other markers of HBV infection, in sera and plasma.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with a monoclonal antibody for a fixed amount of antigen on the solid phase.
A purified recombinant HBcAg is coated to the microwells.
The patient's serum/plasma is added to the microwell together with an additive able to block interferences present in the sample.
In the second incubation after washing, a monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and specific for HBcAg is added and binds to the free rec-HBcAg coated on the plastic.
After incubation, microwells are washed to remove any unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product.
The color intensity is inversely proportional to the amount of antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

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

2. Negative Control CONTROL -
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, anti HBcAg antibodies at a concentration of about 10 PEI U/ml, (calibrated on PEI HBc Reference Material 82), 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The positive control is green color coded.

4. Calibrator CAL ….
n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, human antibodies to HBcAg at a concentration of 2 PEI U/ml +/-10% (calibrated on PEI HBc Reference Material 82) and 0.045% ProClin 300 as preservative.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate CONJ
1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated mouse monoclonal antibody to HBcAg in presence of 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300. as preservatives. The component is red colour coded.

7. Chromogen/Substrate SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.6 +/-0.1, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide
Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent DILSPE
4x3ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference. The component is blue colour coded.

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

9. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foil n° 2

11. Instruction manual n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

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

Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.*

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

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

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

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

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

Legenda:

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

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right

dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.

4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of (620-630nm, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

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

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

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

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

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

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

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

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

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator*

may be used only when a laboratory internal quality control is required by the management.

N. ASSAY SCHEME

Specimen Diluent	50 ul
Controls&calibrator and samples	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

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

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

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm/620-630nm or Co/S values have been matched in the analysis.
Ensure that the following parameters are met:

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

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

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

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

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

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

P. RESULTS

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

Cut-Off = (NC + PC) / 5

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

Q. INTERPRETATION OF RESULTS

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

Results are interpreted according to the following table:

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

A negative result indicates that the patient has not been infected by HBV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
The blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

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

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 12):

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

Negative Control: 2.000 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than 0.200 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440

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

Sample 1: 0.028 OD450nm
Sample 2: 1.890 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

R. PERFORMANCES

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

1. LIMIT OF DETECTION:

The sensitivity of the assay has been calculated by means of the reference preparation for HBcAb supplied by Paul Erlich Institute (PEI HBc Reference Material 82). The assay shows a sensitivity of about 1.25 PEI U/ml.
The table below reports the Co/S values shown by the PEI standard diluted as suggested by the manufacturer to prepare a limiting dilution curve in Fetal Calf Serum (FCS).

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

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

Accurun 1 – series 3000

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

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

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

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5179 unselected donors, including 1st time donors, 206 samples from hospitalized patients and 164 potentially interfering specimen were examined, the diagnostic specificity was recently assessed by testing a total of 1498 negative samples on seven different lots. A value of specificity of 100% was observed. In addition to the above population, 189 potentially interfering samples (other liver diseases,pregnant women, hemolized, lipemic, RF positives) have been tested and found negative, confirming a 100% of specificity of the device.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
In addition to the first Performance Evaluation Study, in order to further evaluate the diagnostic sensitivity of the device, a total of 262 positive samples were recently evaluated. The respective results, collected from seven different lots of the device show a diagnostic sensitivity of 100%.

3. PRECISION

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

BCAB.CE: lot # 1202

Negative Control (N = 16)

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

Calibrator (N = 16)

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

BCAB.CE: lot # 0702

Negative Control (N = 16)

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

Calibrator (N = 16)

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

BCAB.CE: lot # 0702/2

Negative Control (N = 16)

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

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.193	0.190	0.199	0.134
Std.Deviation	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/S	2.4	2.2	2.2	2.3

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

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

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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

Manufacturer:
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CE
0318

HBsAb

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

- for “in vitro” diagnostic use only -

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REF SAB.CE
96 Tests

HBs Ab

A. INTENDED USE

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

B. INTRODUCTION

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

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

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

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

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

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

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

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

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV).
This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.
Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.
Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.
The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.
After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.
The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.
The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.
Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin and sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.
Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. Standards are blue coloured.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme conjugate : CONJ
1x16.0 ml/vial. Ready-to-use solution and red color coded.
It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

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

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

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

8. Control Serum: CONTROL …ml
1 vial. Lyophilized.
Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
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.
6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

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

2. Calibration Curve
Ready to use. Mix well on vortex before use.

3. Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

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

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

6. Specimen Diluent:
Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

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

Legenda:

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

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections “Validation of Test” and “Assay Performances”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

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

M. ASSAY PROCEDURE

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

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

M.1 Quantitative analysis

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

Important note: *This additive is added before distributing samples and controls into specific wells and is particularly intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.*

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

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

3. Wash the microplate as reported in section I.3.

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

Important notes:

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

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

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

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

M.2 Qualitative analysis

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

Important notes:

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

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

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

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

Important general notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME (standard procedure)

Specimen Diluent Calibrators Control Serum Samples	50 ul 100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

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

Microplate

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

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

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

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL5	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

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

O. INTERNAL QUALITY CONTROL

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

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

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

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 mIU/ml > 0.200 coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of standards is carried out; 4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

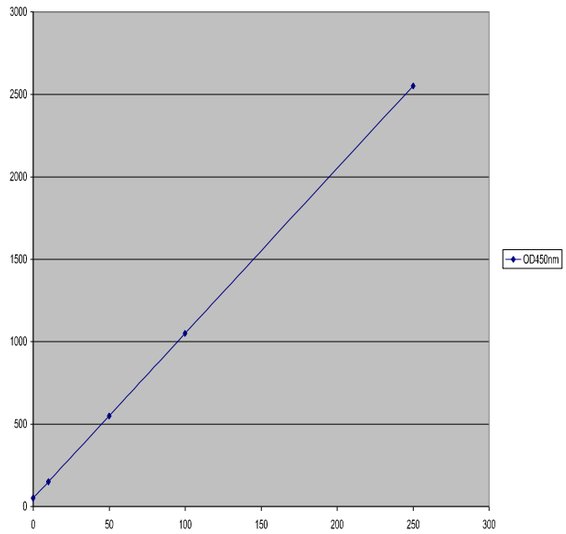
Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4.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

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 7.

P. RESULTS

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

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the reading step described in the section M, point 7).

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

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

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

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

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

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*

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

R. PERFORMANCES

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

1. LIMIT OF DETECTION:

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

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

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

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

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
More than 500 negative specimens were tested, internally and externally, against a European company.
A diagnostic specificity of 98.8% was assessed. .
Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.
More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

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

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

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

Calibrator 10 mIU/ml (N = 16)

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

Calibrator 250 mIU/ml (N = 16)

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

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

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

Calibrator 10 mIU/ml (N = 16)

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

Calibrator 250 mIU/ml (N = 16)

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

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

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

Calibrator 10 mIU/ml (N = 16)

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

Calibrator 250 mIU/ml (N = 16)

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

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

4. ACCURACY

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

Important note:

The performance data have been obtained proceeding as the reading step described in the section M, point 7.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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Manufacturer:
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CE
0318

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Anti-Thyroid Peroxidase (Anti-TPO) Test System

Product Code: 1125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroid Peroxidase (TPO) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric.
Measurements of TPO autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroid peroxidase have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), idiopathic myedema (90%) and Graves Disease (80%)[1]. In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction.[2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Measurements of antibodies to TPO have been done in the past by Passive Hemaglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroid Peroxidase Antigen (TPO) is added, and then the reactants are mixed. Reaction results between the autoantibodies to TPO and the biotinylated TPO to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential ELISA Method (TYPE 1)
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroid peroxidase antigen.

Upon mixing the biotinylated antigen and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(X\text{-}TPO)} + {}^{Btn}Ag_{(TPO)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$$

$^{Btn}Ag_{(TPO)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}TPO)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

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

$h\text{-}Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)} + \underline{\text{Streptavidin}}_{C.W.} \Rightarrow$ Immobilized complex (IC)

$\underline{\text{Streptavidin}}_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex (IC) = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

$I.C._{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$

$I.C._{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained

4.0 REAGENTS

Materials Provided

A. Anti-TPO Calibrators – 1ml/vial Icons A-F
Six (6) vials of references for anti-TPO at levels of 0(A), 25(B), 50(C), 100(D), 250(E) and 500(F) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the Medical Research Council (MRC) International Standard 66/387 for anti thyroid microsome.

B. TPO Biotin Reagent – 13ml/vial – Icon ▽
One (1) vial of biotinylated thyroid peroxidase antigen stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C

C. Anti-TPO Enzyme Reagent – 13ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a bufferred matrix. A preservative has been added. Store at 2-8°C

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

E. Serum Diluent – 20ml/vial
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-8°C.

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

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

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

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

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

Required But Not Provided:
1. Pipette capable of delivering 0.010ml (10µl), 0.025ml (25µl), and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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

Samples may be refrigerated at 2-8ºC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples(s) may be stored at temperatures of -20ºC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
   Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
   Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** – Stable for one (1) year.
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
4. **Patient Sample Dilution (1/100)**
   Dispense 0.010ml (10µl) of each patient specimen into 1ml (1000µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of the TPO Biotin Reagent
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the x-TPO Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate for thirty (30) minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 500 IU/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-TPO in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-TPO activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-TPO activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.323) intersects the dose response curve at 200 IU/ml anti-TPO concentration (See Figure 1).

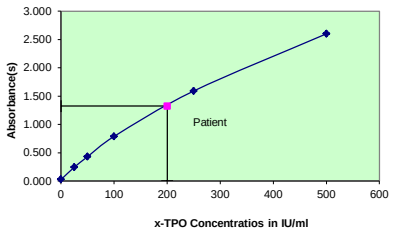
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (IU/ml)
Cal A	A1	0.022	0.026	0
	B1	0.030		
Cal B	C1	0.240	0.244	25
	D1	0.247		
Cal C	E1	0.437	0.430	50
	F1	0.422		
Cal D	G1	0.795	0.788	100
	H1	0.782		
Cal E	A2	1.610	1.590	250
	B2	1.572		
Cal F	C2	2.659	2.600	500
	D2	2.533		
Patient	E2	1.294	1.323	**200**
	F2	1.351		

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

Figure 1



11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-TPO in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

The presence of autoantibodies to TPO is confirmed when the serum level exceeds 40 IU/ml. The clinical significance of the result, coupled with anti-thyroglobulin activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the anti-TPO AccuBind® ELISA test system. The number (n), mean (x) and standard deviation (σ) are given in Table 1. Values in excess of 40IU/ml are considered positive for the presence of anti-TPO autoantibodies.

TABLE I
Expected Values for the Anti-TPO ELISA Test System (In IU/ml)

Number	**100**
Mean	17.6
Standard deviation	10.8
Upper 95% (+2σ) level	39.2

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the anti-TPO AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	25.5	1.5	5.7%
Pool 2	20	120.5	4.6	3.8%
Pool 3	20	352.4	14.8	4.2%

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

Sample	N	X	σ	C.V.
Pool 1	10	26.5	1.8	6.8%
Pool 2	10	118.5	5.3	4.5%
Pool 3	10	365.4	22.5	6.2%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The anti-TPO AccuBind® ELISA test system has a sensitivity of 0.92 IU/ml. The sensitivity (detection limit) was ascertained by determining the variablility of the '0 IU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The anti-TPO AccuBind® ELISA test system was compared with a reference anti-TPO ELISA microplate. Biological specimens from normal and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 82. The least square regression equation and the correlation coefficient were computed for the anti-TPO AccuBind® ELISA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	122.9	y = 1.02 (x) – 5.1	0.989
Reference	127.0		

14.4 Specificity

Interferences from ANA, DNA, thyroglobulin (TPO) and rheumatoid antibodies were found to be insignificant

15.0 REFERENCES

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10. Degroot LJ, "Heterogeneity of human antibodies to TPO Thyroperoxidase", *Thyroid Autoimmunity,* **207,**177-182 (1990).

Revision: 4 Date: 2019-JUL-16 DCO: 1353
MP1125 Product Code: *1125-300*

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

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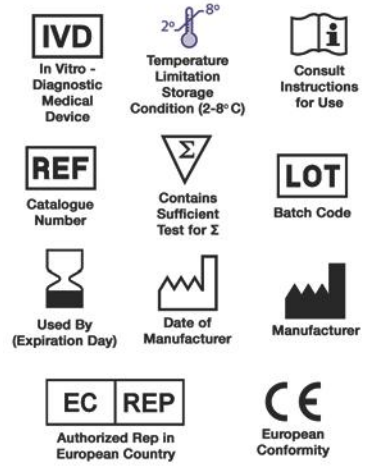
Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Thyroglobulin Ab (Anti-Tg) Test System
Product Code: 1025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric. Measurements of Tg autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroglobulin have been shown to be characteristically present from patients with thyroiditis and primary thyrotoxicosis.[1,2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Passive Hemaglutination (PHA) methods have been employed in the past for measurements of antibodies to Tg. PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to Tg. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated thyroglobulin (Tg) is added, and then the reactants are mixed. Reaction results between the autoantibodies to Tg and the biotinylated Tg to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential Sandwich ELISA Method (TYPE 1)
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroglobulin antigen.

Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$\text{h-Ab}_{(X\text{-}Tg)} + {}^{Btn}\text{Ag}_{(Tg)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \text{h-Ab}_{(X\text{-}Tg)} - {}^{Btn}\text{Ag}_{(Tg)}$$

$^{Btn}Ag_{(Tg)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}Tg)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}Tg)} - {}^{Btn}Ag_{(Tg)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:
$h\text{-}Ab_{(X\text{-}Tg)} - {}^{Btn}Ag_{(Tg)} + \underline{\text{Streptavidin}}_{C.W.} \Rightarrow \underline{\text{immobilized complex}}$ (IC)
$\underline{\text{Streptavidin}}_{C.W.}$ = Streptavidin immobolized on well
$\underline{\text{Immobilized complex (IC)}}$ = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.
$I.C._{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$
$I.C._{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Anti-Tg Calibrators – 1ml/vial Icons A-F
Six (6) vials of references for anti-Tg at levels of 0(**A**), 50(**B**), 125(**C**), 500(**D**), 1000(**E**), and 2000(**F**) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using the 1st International Reference Preparation, which was assayed against the Medical Research Council (MRC) Research Standard A 65/93 for anti-thyroglobulin activity.

B. Tg Biotin Reagent – 13ml/vial – Icon ∇
One (1) vial of biotinylated thyroglobulin stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C**.**

C. x-Tg Enzyme Reagent – 13ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a bufferred matrix. A preservative has been added. Store at 2-8°C**.**

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

E. Serum Diluent – 20ml/vial
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-8°C.

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

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

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

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

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.0101ml (10.1µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminate devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** – Stable for one (1) year.
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
4. **Patient Sample Dilution (1/100)**
Dispense 0.0101ml (10.1µl) of each patient specimen into 1ml (1000µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of Tg Biotin Reagent.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of x-Tg Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITON
9. Cover and incubate for thirty (30) minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITON
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 2000 IU/ml, dilute the sample an additional 1:5 or 1:10 using

the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-Tg in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-Tg activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-Tg activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.387) intersects the dose response curve at 790 IU/ml anti-Tg concentration (See Figure 1).

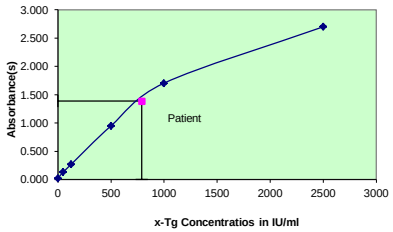
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (IU/ml)
Cal A	A1	0.022	0.025	0
	B1	0.028		
Cal B	C1	0.135	0.133	50
	D1	0.131		
Cal C	E1	0.280	0.270	125
	F1	0.261		
Cal D	G1	0.962	0.949	500
	H1	0.936		
Cal E	A2	1.709	1.703	1000
	B2	1.698		
Cal F	C2	2.730	2.698	2000
	D2	2.667		
Patient	E2	1.390	1.387	790
	F2	1.383		

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

Figure 1



11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator 'F' should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-Tg in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. Samples, which are contaminated microbiologically, should not be used.
11. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
12. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
13. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
14. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. The presence of autoantibodies to Tg is confirmed when the serum level exceeds 125 IU/ml. The clinical significance of the result, coupled with anti-thyroid peroxidase activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.
8. The cost benefits should be considered in the use of thyroglobulin antibodies testing when performed in concert with anti- thyroid peroxidase (TPO). The widespread practice of performing both tests has been questioned.[4]

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the Anti-Tg AccuBind® test system. The number (n) mean (X) and standard deviation (σ) are given in Table 1. Values in excess of 125IU/ml are considered positive for the presence of anti-Tg autoantibodies.

TABLE I
Expected Values for Anti-Tg AccuBind® ELISA Test System (In IU/ml)

Number	**100**
Mean	74.3
Standard deviation	25.2
Upper 95% (+2σ) level	124.7

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Anti-Tg AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value(X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	65.5	3.3	5.0%
Pool 2	20	385.5	15.5	4.0%
Pool 3	20	1554.4	55.4	3.6%

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

Sample	N	X	σ	C.V.
Pool 1	10	66.8	3.6	5.3%
Pool 2	10	374.2	18.5	4.9%
Pool 3	10	1625.5	65.2	4.0%

*As measured in ten experiments in duplicate.

12.2Sensitivity

The Anti-Tg AccuBind® ELISA has a sensitivity of 1.94 IU/ml. The sensitivity was ascertained by determining the variability of the '0 IU/ml' calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

12.3 Accuracy

The Anti-Tg AccuBind® ELISA test system was compared with a reference method. Biological specimens from normals, and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 181. The least square regression equation and the correlation coefficient were computed for the anti-Tg AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	415.6	y = 9.79 + 0.969 (x)	0.995
Reference	419.2		

Only slight amounts of bias between the anti-Tg AccuBind® ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system.

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Revision: 5 Date: 2019-JUL-16 DCO: 1353
MP1025 Product Code: *1025-300*

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

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Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

2° 8° — Temperature Limitation Storage Condition (2-8°C)

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cancer Antigen 125 (CA-125) Test System

Product Code: 3025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 125 (CA-125) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight entity (MB_{rB} > *200*,000). High concentrations of this antigen are associated with ovarian cancer and a range of benign and malignant diseases. Although the specificity and sensitivity of CA-125 assays are somewhat limited, especially in early diagnosis of ovarian cancer, the assay has found widespread use in the differential diagnosis of adnexal masses, in monitoring disease progression and response to therapy in ovarian cancer, and in the early detection of recurrence after surgery or chemotherapy for ovarian cancer. Published literature has shown that elevated serum CA-125 levels can be observed in patients with serious endometroid, clear cell and undifferentiated ovarian carcinoma. The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including, but not limited to, first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

In this method, CA-125 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of CA-125) are added and the reactants mixed. Reaction between the various CA-125 antibodies and native CA-125 forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CA-125 antibody bound conjugate is separated from the unbound enzyme-CA-125 conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of CA-125 levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CA-125 concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA-125 antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{CA\text{-}125} + {}^{Btn}Ab_{(m)} \underset{k\text{-}a}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{CA\text{-}125}$ = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ Immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CA-125 Calibrators - 1ml/vial- Icons A-F
Six (6) vials of references CA-125 Antigen at levels of 0(**A**), 15(**B**), 50(**C**), 100(**D**), 200(**E**) and 400(**F**) U/ml. A preservative has been added. Store at 2-8°C.

Note: The human serum based standards were made using a >99% pure affinity purified preparation of CA-125. The preparation was calibrated against Centocor CA-125 IRMA test.

B. CA-125 Enzyme-Reagent – 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

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

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

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

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

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

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

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

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and elevated ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

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

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CA-125 Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

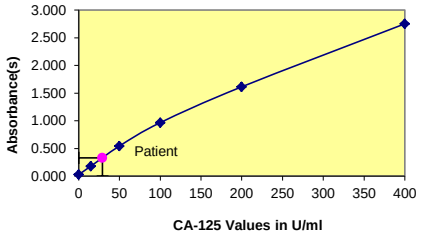
A dose response curve is used to ascertain the concentration of CA-125 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA-125 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CA-125 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.331l) intersects the dose response curve at 29.3U/ml CA-125 concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.035	0.029	0
	B1	0.022		
Cal B	C1	0.186	0.182	15
	D1	0.178		
Cal C	E1	0.536	0.545	50
	F1	0.554		
Cal D	G1	0.985	0.967	100
	H1	0.949		
Cal E	A2	1.615	1.615	200
	B2	1.616		
Cal F	C2	2.749	2.753	400
	D2	2.758		
Patient	A3	0.336	0.331	**29.3**
	B3	0.325		

Figure 1



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

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CA-125 concentrations above 400 U/ml may be diluted (for example 1/10 or higher) with normal male serum (CA-125 < 5 U/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. CA-125 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA-125 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGE OF VALUES

The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for CA-125 AccuBind® ELISA Test System

Healthy and non-pregnant subjects	U≤U 35 U/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CA-125 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

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

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.44	11.8%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA-125 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CA-125 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 121. The least square regression equation and the correlation coefficient were computed for CA-125 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	5.67	y = -0.116 +1.032x	0.998
Reference (Y)	5.75		

14.4 Specificity

In order to test the specificity of the antibody pair used, massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. In addition some widely used, over-the-counter, drugs and some cytotoxic drugs (10 fold the normal dose) were tested in the assay. No cross reaction was found. Percent recoveries for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Amount Added	% Recovery
Bilirubin	1 mMol/L	98 – 103%
Hemoglobin	1 mMol/L	100 – 106%
Triglycerides	10 mMol/L	96 – 110 %
RF	1000 kIU/L	97 – 107%
Biotin	25 µg/L	99 – 103%

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP3025 Product Code: *3025-300*

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

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device	2°-8° — Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF — Catalogue Number	Σ — Contains Sufficient Test for Σ	LOT — Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP — Authorized Rep in European Country	CE — European Conformity	

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cancer Antigen 15-3 (CA 15-3) Test System
Product Code: 5625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen (CA 15-3) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Although multiple serum based tumor markers have been described for breast cancer, such as CA 15-3, BR 27-29, carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen, and HER-2 (the extracellular domain), the most widely used are CA 15-3 and CEA. CA 15-3 is considered to be one of the first circulating prognostic factors for breast cancer.[1] Preoperative concentrations thus might be combined with prognostic factors for predicting outcome in patients with newly diagnosed breast cancer.[2] At present the most important clinical application of CA 15-3 is in monitoring therapy in patients with advanced breast cancer that is not accessible by existing clinical or radiologic procedures.[3]

The CA 15-3 assay measures the protein product of *MUC1* gene. *MUC1* protein is a large transmembrane glycosylated molecule containing three main domains, a large extracellular region, a membrane spanning sequence, and a cytoplasmic domain.[4] Although the physiologic function of *MUC1* is unclear, the glycoprotein has been implicated in cell adhesion, immunity and metastasis. Compared with healthy breast tissue, *MUC1* is present in higher concentrations but less glycosylated in breast carcinoma.[5-8]

In this method, a prediluted CA15-3 calibrator diluted patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for CA15-3) is added and the reactants mixed. Reaction between the CA15-3 antibodies and native CA15-3 forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled antibody specific for a different epitopic recognition of CA15-3 is added to the wells. The enzyme labeled antibody binds to the CA15-3 already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the CA15-3 in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA15-3 antibody.

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

$$Ag_{(CA15\text{-}3)} + {}^{Btn}Ab_{(m)} \underset{k_a}{\overset{k_a}{\rightleftharpoons}} Ag_{(CA15\text{-}3)} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(CA15\text{-}3)}$ = Native Antigen (Variable Quantity)
$Ag_{(CA15\text{-}3)}$ - $^{Btn}Ab_{(m)}$ = Antigen-antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(CA15\text{-}3)}$ - $^{Btn}Ab_{(m)}$ + $Streptavidin_{CW} \Rightarrow$ Immobilized complex (IC)
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex (IC) = Ag-Ab bound to the well

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

$$(IC) + {}^{Enz}Ab_{(x\text{-}CA15\text{-}3)} \underset{k_b}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}CA15\text{-}3)} - IC$$

$^{Enz}Ab_{(x\text{-}CA15\text{-}3)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(x\text{-}CA15\text{-}3)}$ - IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. CA 15-3 Calibrators – 1.0 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 10 (**B**), 40 (**C**), 100 (**D**), 200 (**E**) and 400 (**F**) U/ml. Store at 2-8°C. A preservative has been added.
Note 1: The calibrators are provided prediluted.
Note 2: The calibrators, human serum based, were made using a purified preparation of CA 15-3. The preparation was calibrated against Centocor CA 15-3 IRMA test.

B. CA 15-3 Biotin Reagent – 12 ml/vial – Icon ▽
One (1) vial contains biotinylated anti-human CA15-3 mIgG in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. CA15-3 Enzyme Reagent – 12 ml/vial - Icon E
One (1) vial contains horseradish peroxidase incorporated anti-human CA15-3 mIgG in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

F. CA 15-3 Dilution Matrix – 50 ml
One (1) vial of serum diluent contains buffer salts, protein, surfactants. Store at 2-8°C.

G. Substrate Solution – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (0.5M H_2SO_4) Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (25µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Pipette (1000µl) used for serum diluent in patient dilutions.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit componenets must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or heparanised plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

2. **Patient Sample Dilution (1:21)**
Dispense 0.025ml (25µl) of each control and/or patient specimen into 0.50ml (500µl) of CA 15-3 dilution matrix appropriately labeled, clean container(s) and mix thoroughly before use. Store refrigerated at 2-8°C for up to 48 hours.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional.*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µl) of the appropriate diluted calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the biotinylated labeled antibody to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the Ca15-3 Enzyme Reagent to each well.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 60 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for twenty (20) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

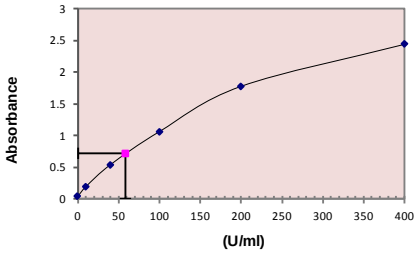
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of CA15-3 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA 15-3 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of CA 15-3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.721) intersects the dose response curve at (58.4U/ml) CA 15-3 concentration (See Figure 1).

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.044	0.043	0
	B1	0.042		
Cal B	C1	0.204	0.198	10
	D1	0.191		
Cal C	E1	0.560	0.543	40
	F1	0.525		
Cal D	G1	1.103	1.064	100
	H1	1024		
Cal E	A2	1.784	1.777	200
	B2	1.770		
Cal F	C2	2.431	2.438	400
	D2	2.445		
Patient	A3	0.737	0.721	**58.4**
	B3	0.705		

Figure 1



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

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens (diluted) with CA 15-3 concentrations above 400 U/ml may be further diluted (1/10 or higher) with CA15-3 diluted serum diluent and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurement and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CA 15-3 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA 15-3 value alone is not of diagnostic value as a test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.**

13.0 EXPECTED RANGES OF VALUES

The serum CA 15-3 is elevated in 2% of normal healthy women and 7% of patients with non-neoplastic conditions. Also, it has been reported to be elevated in cases of liver, lung, ovarian and colorectal cancers. No definitive ranges have been reported for those conditions.

TABLE I
Expected Values for the CA 15-3 Elisa Test System

Healthy Females	≤ 37 U/ml

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the CA 15-3 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	20.9	1.91	9.1%
Level 2	20	61.7	2.03	3.3%
Level 3	20	96.9	2.67	2.8%

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

Sample	N	X	σ	C.V.
Level 1	10	22.2	2.0	9.1%
Level 2	10	58.5	3.85	6.6%
Level 3	10	104.6	9.33	8.9%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA 15-3 procedure has a analytical sensitivity of 0.2 U/ml at three (3) SD from the zero calibrator. The functional sensitivity (20% CV) was found to be 1.25U/ml.

14.3 Accuracy

The CA 15-3 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 43. The least square regression equation and the correlation coefficient were computed for the CA 15-3 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	180.2	y=-0.219+1.008(x)	0.99
Reference (x)	178.6		

14.4 Specificity

In order to test the specificity of the antibody pair used massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. No cross reaction was found. Percent cross-reactions for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Concentration	Interference
CA 15-3	-	1.000
CA 125	10000 U/ml	0.001
CA 19-9	5000 U/ml	0.001
PSA	1000 ng/ml	0.026
AFP	30,000 ng/ml	ND*
CEA	5,000ng/ml	ND*
HCG	125,000mIU/ml	ND*
RF	12,500 IU/ml	0.001
Bilirubin	200 µg/ml	ND*
Hemolysis	30 µl/ml	ND*
Lipids	50 µg/ml	-0.009

15.0 REFERENCES

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5. Duffy MJ, 'Biochemical markers in breast cancer: which ones are clinically useful', *Clin Biochem*; 34, 347-352 (2001).
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12. NCCLS. 'Assessment of Laboratory Tests When Proficiency Testing is Not Available; Approved Guidelines.'2008.

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (12ml)	2 (12ml)
	C)	1 (12ml)	2 (12ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (50ml)	2 (50ml)
	G)	1 (12ml)	2 (12ml)
	H)	1 (8ml)	2 (8ml)

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IVD 2° 8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cancer Antigen 19-9 (CA 19-9) Test System

Product Code: 3925-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 19-9 (CA 19-9) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

A mucin type Sialyl Lewis Antigens group of glycoproteins (SLA) such as CA 19-9, 19-5 have been recognized as circulating cancer associated antigens for gastrointestinal cancer. The discovery of a monoclonal antibody clone (1116NS 19-9), which exhibited selective reactivity with human gastrointestinal carcinomas through the recognition of a carbohydrate determinant (CA 19-9) defined as a sialyl lacto-N-flucopenrose II, resulted in the successful purification and thus, determination of human gastrointestinal tumor associated glycoprotein antigen expressing CA 19-9 from colorectal carcinoma cell lines. Recently, reports indicate that serum CA 19-9 level is frequently elevated in the circulation of patients with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas. Together with CEA, elevated CA 19-9 is suggestive of gallbladder disease. The tumor associated antigen may also be associated in some malignant conditions. Research studies demonstrate that serum CA 19-9 values may have utility in monitoring subjects with the above mentioned diagnosed malignancies.

In this method, CA 19-9 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for CA 19-9) is added and the reactants mixed. Reaction between the CA 19-9 antibodies and native CA 19-9 forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to CA 19-9 is added to the wells. The enzyme labeled antibody binds to the CA 19-9 already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the CA 19-9 in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA19-9 antibody.

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

$$Ag_{(CA19\text{-}9)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(CA19\text{-}9)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(CA19\text{-}9)}$ = Native Antigen (Variable Quantity)
$Ag_{(CA19\text{-}9)}$ - ${}^{Btn}Ab_{(m)}$ = Antigen-antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(CA19\text{-}9)}$ - ${}^{Btn}Ab_{(m)}$ + $Streptavidin_{CW} \Rightarrow$ Immobilized complex (IC)
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex (IC) = Ag-Ab bound to the well

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

$$(IC) + {}^{Enz}Ab_{(x\text{-}CA19\text{-}9)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}CA19\text{-}9)} - IC$$

${}^{Enz}Ab_{(x\text{-}CA19\text{-}9)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(x\text{-}CA19\text{-}9)}$ - IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. CA 19-9 Calibrators – 1ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 10 (**B**), 50 (**C**), 100 (**D**), 250 (**E**) and 500 (**F**) U/ml. A preservative has been added. Store at 2-8°C.
Note: The standards, human serum based, were made using a >99% pure affinity purified preparation of CA 19-9. The preparation was calibrated against Centocor CA 19-9 IRMA test.

B. CA 19-9 Biotin Reagent – 13ml/vial ∇
One (1) vial of Anti-Human CA19-9 (MoAb)-Biotin reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. CA 19-9 Enzyme Reagent – 13ml/vial - Icon Ⓔ
One (1) vial of Anti-Human CA19-9-HRP conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

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

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

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

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

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

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

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

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

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

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the biotinylated labeled antibody to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see "Reagent Preparation"), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of the CA19-9 Enzyme Reagent labeled antibody to each well.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 60 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

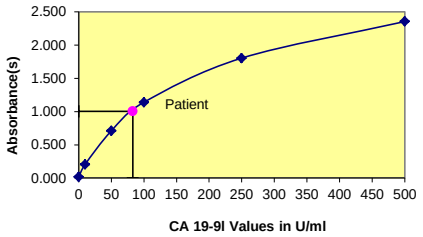
A dose response curve is used to ascertain the concentration of CA19-9 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA 19-9 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CA 19-9 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.004) intersects the dose response curve at 82.9U/ml CA 19-9 concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.013	0.014	0
	B1	0.014		
Cal B	C1	0.210	0.208	10
	D1	0.212		
Cal C	E1	0.754	0.708	50
	F1	0.662		
Cal D	G1	1.128	1.140	100
	H1	1.152		
Cal E	A2	1.850	1.805	250
	B2	1.760		
Cal F	C2	2.310	2.355	500
	D2	2.400		
Patient	A3	1.009	1.004	**82.9**
	B3	0.999		

Figure 1



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

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CA 19-9 concentrations above 500U/ml may be diluted (for example 1/10 or higher) with CA19-9 zero calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CA 19-9 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA 19-9 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGES OF VALUES

The serum CA 19-9 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for CA 19-9 AccuBind® ELISA Test System

Healthy and non-pregnant subjects	**≤ 40 U/ml**

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the CA 19-9 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

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

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.34	9.2%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA 19-9 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CA 19-9 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 136. The least square regression equation and the correlation coefficient were computed for the CA 19-9 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	18.62	x = 1.4577+0.8837(y)	0.955
Reference (Y)	19.43		

14.4 Specificity

In order to test the specificity of the antibody pair used massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. No cross reaction was found. Percent cross-reactions for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Concentration	Percent (%) Cross Reaction
CA 19-9	-	100
CA 125	10000 U/ml	0.001
CA 15-3	1000 U/ml	ND*
PSA	5000 ng/ml	ND*
AFP	10000 ng/ml	ND*
CEA	10000 ng/ml	ND*
HCG	10000 mIU/ml	ND*
RF	1000 kIU/ml	ND*

15.0 REFERENCES

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9. De Bruijn HWA, Van Der Zee AGJ & Alders JG, "The value of Cancer Antigen 125 (CA 19-9) during treatment and follow up of patients with ovarian cancer", *Curr Opin Gynecol*, 9, 8-13 (1997).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP3925 Product Code: *3925-300*

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

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Calcitonin Test System
Product Code: 9325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Calcitonin Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Calcitonin is a 32 amino acid alpha helix produced by the follicular cells of the thyroid gland. A cleavage product of procalcitonin (PCT), calcitonin is a product of the CALC1 gene in humans and provides support in regulating calcium homeostasis, lowering serum calcium concentrations and preventing hypercalcemia. Calcitonin is characterized by an N-terminal disulfide bridge, which contributes to its biological activity, and a C-terminal proline residue.

Calcitonin plays a role in calcium metabolism, with osteoclasts the most significant homeostatic targets. Calcitonin binds to CT receptors (CTRs) on osteoclasts, halting calcium resorption via prevention of cell differentiation and motility. CTR receptors are also found in the kidneys and hypothalamus, providing an excretion route for excess serum calcium. Calcitonin modulates calcium absorption via CTR receptors on renal tubules, preventing excess calcium uptake. CTR receptors belong to the family of G-protein coupled receptors, utilizing cAMP messengers to amplify and transduce signals initiated by calcitonin-CTR binding.

Calcitonin has emerged as a therapeutic avenue for hypercalcemia patients, utilized as a biomarker PCT for its rapid biomarker for medullary carcinoma of the thyroid (MCT), providing a facile and direct measurement of carcinogenic activity. Calcitonin levels are typically low in normal populations, and elevated levels suggest the presence of hypercalcemia or potential loss of thyroid function.

Medullary thyroid carcinoma is typically associated with elevated levels of calcitonin. Parafollicular C cells containing mutations in the RET gene will display elevated expression of calcitonin and the presence of nodules in the lymph nodes, potentially disrupting calcium homeostasis.

3.0 PRINCIPLE

Sandwich Equilibrium Method (Type 2):
The calcitonin immunoassay is an adapted two-site sandwich ELISA. In this assay, standards and patient samples are simultaneously incubated with the enzyme labeled detection antibody and antibody on a coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of calcitonin in the sample. Standards are used to generate a dose response curve of absorbance unit vs. concentration. Concentrations of calcitonin present in the controls and patient samples are determined directly from this curve.

The essential reagents required for a sandwich equilibrium assay include high affinity and specificity antibodies (signal and capture), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the calibrator, control or patient sample is added to the wells coated with anti-calcitonin antibody. Calcitonin from the sample binds to the anti-calcitonin (MoAb) on the wells. Subsequently an enzyme labeled anti-calcitonin is added to the wells. Calcitonin from the sample forms a sandwich between the two antibodies. Excess enzyme and sample is removed via a wash step. The interaction is illustrated by the following equation:

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

$Ab_{(m)}$ = Anti-calcitonin (MoAb) (On the Microwells in Excess Quantity)
Ag_{CT} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(CT)}$ = Enzyme labeled Mouse α CT (P) (Excess Quantity)
$^{Enz}Ab_{(CT)} - Ag_{CT} - Ab_{(m)}$ = Ag-Antibodies Sandwich complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

A suitable substrate is added to the wells to generate color in varying intensity depending upon the concentration of calcitonin in the wells. The intensity of the color in the sample can be visually compared to the known calibrators to obtain qualitative results or the color development can be read with the help of a microplate spectrophotometer to obtain quantitative results.

4.0 REAGENTS

Materials Provided:

A. Calcitonin Calibrators – 1.0 ml/vial (Dried) – Icons A-F
Six (6) vials of references for Calcitonin at levels of 0(**A**), 10(**B**), 40(**C**), 150(**D**), 400(**E**) and 1000(**F**) pg/ml. Store at 2-8°C. ***Reconstitute each vial with 1ml of distilled or deionized water***. The reconstituted calibrators are stable for 4 hours at 2-8°C. A preservative has been added. For longer periods after reconstitution, aliquot into smaller portions and freeze (<-20°C) for up to 3 months. Freeze and thaw cycles should be minimized to one time only.
Note: The calibrators are traceable against the WHO 1st International Standard NIBSC Code 89/620. Values in pg/ml can be converted to µIU/ml by multiplying by 0.19. For example, 40 pg/ml x 0.19 = 7.6 µIU/ml calcitonin.

B. Calcitonin Control M – 1.0 ml/vial (Dried) – Icon M
One (1) vial of reference control for Calcitonin .Store at 2-8 °C. ***Reconstitute each vial with 1ml of distilled or deionized water***. The reconstituted control is stable for 4 hours at 2-8°C. A preservative has been added. For longer periods after reconstitution, aliquot into smaller portions and freeze (<-20°C) for up to 3 months. Freeze and thaw cycles should be minimized to one time only.

C. Calcitonin Enzyme Reagent – 6 ml/vial – Icon (E)
One (1) vial containing anti-Calcitonin monoclonal antibody coupled to HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store at 2-8°C.

D. PCT Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with anti-PCT/Calcitonin monoclonal antibody, packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

F. Substrate Reagent – 12 ml/vial – Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide in buffer. Store at 2-8°C.

G. Stop Solution – 8 ml/vial – Icon (STOP)
One (1) vial containing a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not expose reagents to heat, sun, or strong light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C, unless otherwise specified. Kit and component stability are identified on label.**
Note 3: The above components are for a single 96-well microplate. For other kit configurations, refer to table at the end of insert.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml (50µl), 0.100ml (100µl), and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8 °C for a maximum period of 24 hours. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of wash solution concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix (500 – 600 rpm) and cover.
5. Incubate 60 minutes (1 hour) at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100 µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for twenty (20) minutes.
10. Add 0.050 ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note 1: **For re-assaying specimens with concentrations greater than 1000 pg/ml, dilution should be performed.**
Note 2: **Do not use reagents that are contaminated or have bacterial growth.**
Note 3: **Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.**
Note 3: **It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the microwells at an angle while touching the side of the well.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Calcitonin in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding calcitonin concentration in pg/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of calcitonin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

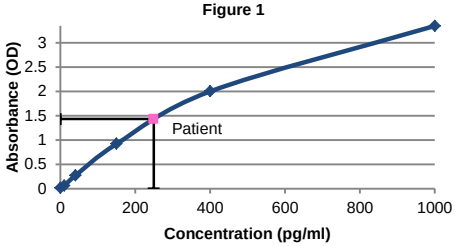
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Conc. (pg/ml)	Mean Abs
Cal A	0	0.016
Cal B	10	0.062
Cal C	40	0.268
Cal D	150	0.772
Cal E	400	2.150
Cal F	1000	3.347
Control M	80	0.365

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

Figure 1



*If the absorbance readout is off-scale or higher than the average absorbance of the highest calibrator, sample should be repeated with dilution.

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator F (1000 pg/ml) should be ≥1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problematic for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. The Calcitonin ELISA kit has exhibited no high dose hook effect with samples spiked with 1,000,000 pg/ml of calcitonin. Samples with calcitonin levels greater than the highest calibrator, however, should be diluted and re-assayed for correct values.

13.0 EXPECTED RANGES OF VALUES

Calcitonin levels were measured in apparently normal individuals (N=246). The values obtained ranged from 0.455 to 12.932 pg/ml. Based on statistical tests for skewness and kurtosis, the population follows the normal or Gaussian distribution as shown in histograms. The geometric mean ± 2 standard deviations of the mean (>95% Confidence) were calculated and found to be 6.2 ± 5.6 pg/ml.

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Calcitonin AccuBind® ELISA test system were determined by analysis of three different levels of pool control sera. The number (N), mean (X) value, standard deviation (σ) and coefficient of variation (C.V.) of each of these control sera are presented in Table 1 and Table 2.

TABLE 1
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V. %
Low	20	26.23	2.58	9.9
Normal	20	65.50	3.67	5.57
High	20	318.101	7.88	2.51

TABLE 2
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.%
Low	20	26.03	3.81	14.62
Normal	20	65.97	12.24	18.55
High	20	313.73	31.02	9.89

14.2 Sensitivity

The Calcitonin AccuBind® ELISA test system has a LoB = 1.84 pg/ml and LoD = LoQ = 2.15 pg/ml.

Table: Cross-Reactivity

SUBSTANCE	Amount Tested	Cross Reactivity
Procalcitonin	100 ng/ml	<0.001
Katacalcin	25 ng/ml	<0.001
a-CGRP	30 ng/ml	<0.001
b-CGRP	30 ng/ml	<0.001
Parathyroid Hormone	10 ng/ml	<0.001

15.0 REFERENCES

1. Felsenfeld, A. J.; Levine, B.S. "Calcitonin, the forgotten hormone: does it deserve to be forgotten?" *Clin, Kidney Jour.*, 8: 180-187, 2015.
2. Mallete, L.E., Gagel, R.F.: "Parathyroid Hormone and Calcitonin". In: Murray J.F. (ed) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 65-69, 1990.
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5. Masi, L.; Brandi, M.L. "Calcitonin and calcitonin receptors". Clin Cases Miner Bone Metab. 4(2): 117-122, 2007

Effective Date: 2023-Feb-06 Rev. 3 **DCO: 1600**
MP9325 **Product Code: 9325-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml (Dried) set	1ml (Dried) set
	B)	1ml (Dried) set	1ml (Dried) set
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

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

Product Code: 4625-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

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

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

In this method, CEA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of CEA) are added and the reactants mixed. Reaction between the various CEA antibodies and native CEA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CEA antibody bound conjugate is separated from the unbound enzyme-CEA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known carcinoembryonic antigen (CEA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CEA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CEA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

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

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

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

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

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

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

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

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

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

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

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

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

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

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

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

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 -27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CEA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Carcinoembryonic antigen in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CEA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 0.320 ng/ml intersects the dose response curve at 18.1 ng/ml CEA concentration (see Figure 1).

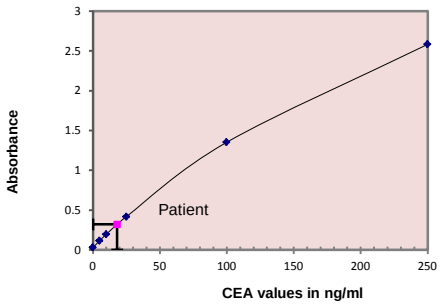
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

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

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

Figure 1



11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CEA concentrations above 250 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (CEA < 5 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CEA value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

13.0 EXPECTED RANGES OF VALUES

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

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

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

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CEA Next Generation AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	2.6	0.25	9.6%
Level 2	20	12.5	1.01	8.1%
Level 3	20	24.1	1.35	5.6%

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

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

*As measured in ten experiments in duplicate.

14.2 Sensitivity

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

14.3 Accuracy

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

TABLE 4

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

E. Specificity:

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

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

14.5 Linearity & Hook Effect:

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

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

15.0 REFERENCES

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

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

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cortisol Test System
Product Code: 3625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Cortisol Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid produced by the human adrenal cortex. As with other adrenal steroids, cortisol is synthesized from cholesterol, through a series of enzymatically mediated steps, by the adrenal cortex.[1,2] The first and rate-limiting step in adrenal steroidogenesis, conversion of cholesterol to pregnenolone, is stimulated by pituitary adrenocorticotropic hormone (ACTH) which is, in turn, regulated by hypothalamic corticotropin releasing factor (CRF). ACTH and CRF secretion are inhibited by high cortisol levels. In plasma, the major portion of cortisol is bound with high affinity to corticosteroid-binding globulin (CBG, transcortin), with most of the remainder loosely bound to albumin. Physiologically effective in anti-inflammatory activity and blood pressure maintenance, cortisol is also involved in gluconeogenesis. Cortisol acts through specific intracellular receptors and has effects in numerous other physiologic systems, including immune function, glucose-counter regulation, vascular tone, substrate utilization and bone metabolism.[1-3] Cortisol is excreted primarily in urine in an unbound (free) form.

Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factors controlling this circadian rhythm are not completely defined. The circadian rhythm of ACTH/cortisol secretion matures gradually during early infancy, and is disrupted in a number of physical and psychological conditions.[4] Furthermore, increased amounts of ACTH and cortisol are secreted independently of the circadian rhythm in response to physical and psychological stress.[4,5]

Elevated cortisol levels and lack of diurnal variation have been identified in patients with Cushing's disease (ACTH hyper secretion).[2,6] Elevated circulating cortisol levels have also been identified in patients with adrenal tumors.[7] Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, congenital adrenal hyperplasia, Addison's disease) and in ACTH deficiency.[1,2,8,9] Due to the normal circadian variation of cortisol levels, distinguishing normal and abnormally low cortisol levels can be difficult. Therefore, various tests to evaluate the pituitary-adrenal (ACTH-cortisol) axis, including insulin-induced hypoglycemia, short- and long-term ACTH stimulation, CRF stimulation and artificial blockage of cortisol synthesis with metronome have been performed.[8-10] Cortisol response characteristics for each of these procedures have been reported.

The Monobind Cortisol EIA Kit uses a specific monoclonal anti-cortisol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally-occurring steroids is low.

The employment of several serum references of known cortisol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with cortisol concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

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

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

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{\text{immobilized complex}}$
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Cortisol Calibrators – 1ml/vial – Icons A-F
Six (6) vials of serum reference for Cortisol at concentrations of 0 (**A**), 1.0 (**B**), 4.0 (**C**), 10.0 (**D**), 20.0 (**E**) and 50.0 (**F**) µg/dl. Store at 2-8°C. A preservative has been added.

B. Cortisol Enzyme Reagent – 7.0 ml/vial – Icon Ⓔ
One (1) ready to use vial containing Cortisol (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with buffer, red dye, preservative and binding protein inhibitors. Store at 2-8°C.

C. Cortisol Biotin Reagent – 7.0 ml – Icon ∇
One (1) vial containing anti-cortisol biotinylated mIgG conjugate in buffer, dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

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

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

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

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025ml (25µl), 0.050ml, (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml (50µl) 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted reagent can be stored at 2-30°C for up to 60 days.

2. **Working Substrate Solution –** Stable for 1 year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 30°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the ready to use Cortisol Enzyme Reagent to all wells
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Cortisol Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
11. Incubate at room temperature for fifteen (15) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 50 µg/dl 1:5 and 1:10 with cortisol '0' µg/dl patient serum.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of cortisol in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding cortisol concentration in µg/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of cortisol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.071) intersects the dose response curve at (10.2 µg/dl) cortisol concentration (See Figure 1).

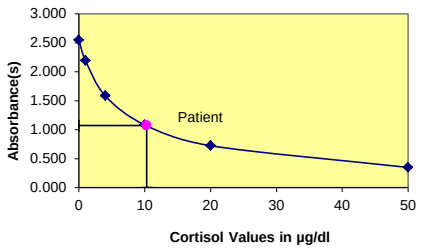
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µg/dl)
Cal A	A1	2.483	2.543	0
	B1	2.575		
Cal B	C1	2.150	2.194	1.0
	D1	2.186		
Cal C	E1	1.573	1.585	4.0
	F1	1.597		
Cal D	G1	1.103	1.084	10
	H1	1.065		
Cal E	A2	0.726	0.725	20
	B2	0.724		
Cal F	C2	0.347	0.350	50
	D2	0.353		
Ctrl 1	E2	1.624	1.617	**3.74**
	F2	1.611		
Ctrl 2	G2	0.770	0.760	**18.57**
	H2	0.749		
Patient	A3	1.056	1.071	**10.24**
	B3	1.086		

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

Figure 1



11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator 0 µg/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Total serum cortisol values may be dependent upon conditions such as time of the day for sampling or administration of prednisolone or prednisone (structurally related to cortisol). Caution must be exercised while interpreting cortisol levels for patients undergoing therapy with these and other structurally related corticosteroids such as cortisone or corticosterone.

13.0 EXPECTED RANGES OF VALUES

A study of normal adult population was undertaken to determine expected values for the Cortisol AccuBind® ELISA Test System. The mean (R) values, standard deviations (**σ**) and expected ranges (±2**σ**) are presented in Table 1.

TABLE I
Expected Values for the cortisol EIA Test System (in µg/dl)

Population	Morning	Afternoon
Adult	5 - 23 µg/dl	3 -13 µg/dl
Child	3 - 21 µg/dl	3 -10 µg/dl
Newborn	1 - 24 µg/dl	

Please note: Normal results may vary from lab to lab

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Cortisol AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.
Low	16	3.4	0.28	8.2%
Normal	16	14.2	0.91	6.4%
High	16	36.5	2.23	6.1%

TABLE 3
Between Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.
Low	10	3.1	0.30	9.7%
Normal	10	15.1	1.06	7.0%
High	10	37.4	2.71	7.3%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Cortisol AccuBind® ELISA Test System has a sensitivity of 91.5 pg. This is equivalent to a sample containing a concentration of 0.366 µg/dl. The sensitivity was ascertained by determining the variability of the 0 µg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Cortisol AccuBind® ELISA Test System was compared with a coated tube radioimmunoassay method. Biological specimens from low, normal and high cortisol level populations were used. The values ranged from 0.4 µg/dl – 95µg/dl. The total number of such specimens was 202. The least square regression equation and the correlation coefficient were computed for this cortisol EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	16.6	y = -0.228+1.0186(x)	0.984
Reference (X)	16.8		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross-reactivity of the cortisol antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of cortisol needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Cortisol	1.0000
Androstenedione	0.0004
Cortisone	0.2300
Corticosterone	0.1800
11-Deoxycortisol	0.0550
Dexamethasone	0.0001
Progesterone	0.0002
17α-OH Progesterone	ND
DHEA	ND
Estradiol	ND
Estrone	ND
Danazol	ND
Testosterone	ND

15.0 REFERENCES

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Effective Date: 2019-Jul-16 Rev. 4 **DCO: 1353**
MP3625 **Product Code: 3625-300**

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

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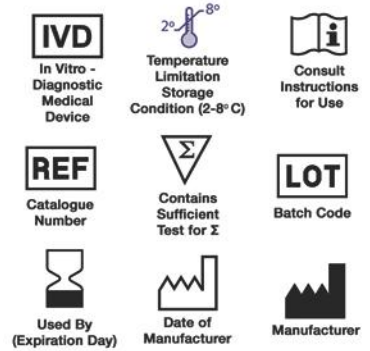
Glossary of Symbols
(EN 980/ISO 15223)



EC REP **Authorized Rep in European Country**

CE **European Conformity**

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

C-Peptide Test System
Product Code: 2725-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one third of them do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a significant role. The disease is marked by deficiencies in the body's ability to produce and properly use insulin. The most common forms of diabetes are type 1, in which the body's ability to produce insulin is destroyed, and type 2, in which the body is resistant to insulin even though some amount of insulin may be produced.

In-vitro determination of insulin and *C-Peptide* levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and *C-Peptide* are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic β-cells and is split into a 31 amino acid connecting peptide (*C-Peptide;* MW 3600) and insulin (MW 6000). *C-Peptide* is devoid of any biological activity but appears to be necessary to maintain the structural integrity of insulin. Although insulin and *C-Peptide* are secreted into portal circulation in equimolar concentrations, fasting levels of *C-Peptide* are 5-10 fold higher than those of insulin owing to the longer half-life of *C-Peptide*. The liver does not extract *C-Peptide* however; it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence urine *C-Peptide* levels correlate well with fasting *C-Peptide* levels in serum. The glucagon stimulated *C-Peptide* determination is often used for differential diagnosis of insulin-dependent from non-insulin-dependent diabetic patients.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (Ab). (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen (Ag). In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal C-peptide antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(M)} + Ag_{C\text{-}Pep} + {}^{Btn}Ab_{(M)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$$

$^{Btn}Ab_{(M)}$ = Biotinylated Monoclonal Ab (Excess Quantity)
$Ag_{C\text{-}Pep}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(M)}$ = Enzyme labeled Monoclonal Ab (Excess Quantity)
$^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$ = Antigen-Antibodies complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$
+ Streptavidin $_{CW.} \Rightarrow$ Immobilize complex
Streptavidin$_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS AND MATERIALS PROVIDED

Materials Provided:

A. C-Peptide Calibrators – 2 ml/vial (Lyophilized) - Icons A-F
Six (6) vials of references for C-Peptide antigen at levels of 0(**A**), 0.2(**B**), 1.0(**C**), 2.0(**D**), 5.0(**E**), and 10.0(**F**) ng/ml. Reconstitute each vial with 2.0ml of distilled or deionized water. The reconstituted calibrators should be assayed immediately and can be stored for 8 hours at 2-8°C, then discarded. In order to store for a longer period of time, aliquot the reconstituted calibrators in cryo vials and store at -20°C for up to 30 days. Single use only. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 84/510.

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

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

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

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

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

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

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of two (2) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

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

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the C-Peptide Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 120 minutes at room temperature (20-25°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

NOTE: Always add reagents in the same order to minimize reaction time differences between wells.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of C-Peptide in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding C-Peptide concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of C-Peptide for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.433) intersects the dose response curve at 1.03 ng/ml for the C-Peptide concentration (See Figure 1).

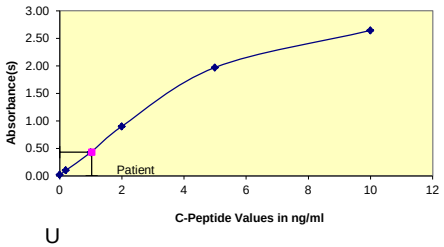
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.022	0.022	0
	B1	0.023		
Cal B	C1	0.097	0.103	0.2
	D1	0.107		
Cal C	E1	0.421	0.429	1
	F1	0.439		
Cal D	G1	0.889	0.901	2
	H1	0.910		
Cal E	A2	1.976	1.971	5
	B2	1.966		
Cal F	C2	2.717	2.643	10
	D2	2.570		
Ctrl 1	E2	0.429	0.433	1.03
	F2	0.437		
Ctrl 2	G2	1.861	1.887	4.64
	H2	1.913		
Patient 1	A3	0.388	0.405	0.82
	B3	0.421		

Figure 1



U

11.0 QC PARAMETERS

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

1. The absorbance (OD) of calibrator 'A' should be ≤ 0.07
2. The absorbance (OD) of calibrator 'F' should be ≥ 1.3
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED VALUES

C-Peptide values are consistently higher in plasma than in serum; Monobind advises that a serum sample be used for accurate determination. Compared with fasting values in non-obese non-diabetic individuals, C-Peptide levels are higher in obese non-diabetic subjects and lower in trained athletes.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

It is important to keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

Adult (Normal)	**0.7 – 1.9 ng/ml**

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the C-Peptide AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value(X), standard deviation (**σ**) and coefficient of variation (C.V.) for each of these control sera are presented in Table 1 and Table 2.

TABLE 1
Within Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	1.43	0.11	7.7%
Pool 2	20	5.07	0.46	9.0%
Pool 3	20	7.81	0.73	9.3%

TABLE 2
Between Assay Precision* (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	1.27	0.12	9.7%
Pool 2	20	5.40	0.54	9.9%
Pool 3	20	8.18	0.50	6.1%

*As measured in ten experiments in duplicate over ten days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.020 ng/ml.

14.3 Accuracy

The C-Peptide AccuBind® ELISA test system was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.2 ng/ml – 11.8ng/ml). The total number of such specimens was 124. The data obtained is displayed in Table 4 (see next column).

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	1.068	y = 0.2079 + 0.8036(x)	0.962
Reference (x)	1.066		

Only slight amounts of bias between the C-Peptide AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the C-Peptide AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentration(s). The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of C-Peptide needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
C-Peptide	1.000	-
Proinsulin	0.120	100 ng/ml
Insulin	non-detectable	1.0 mIU/ml
Glucagon	non-detectable	150 ng/ml

15.0 REFERENCES

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7. Kahn CR, Rosenthal AS, "Immunologic reactions to insulin, insulin allergy, insulin resistance and autoimmune insulin syndrome". *Diabetes Care* 2, 283-295 (1979).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP2725 Product Code: 2725-300

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

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

2°–8° — Temperature Limitation Storage Condition (2-8°C)

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity

HBe Ag&Ab

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

- for “in vitro” diagnostic use only -

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REF. HBE.CE
96 Tests

HBe Ag&Ab

A. INTENDED USE

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

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.

For “in vitro” diagnostic use only.

B. INTRODUCTION

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

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

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

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

C. PRINCIPLE OF THE TEST

HBeAg:

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

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

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

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

HBeAb

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

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

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

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

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

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

2. Negative Control: CONTROL -

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

3. Antigen Positive Control: CONTROL + Ag

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

The positive control is green color coded.

4. Antibody Positive Control: **:** CONTROL + Ab

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

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG …ml

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

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

6. Antibody Calibrator: CALAB …ml

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

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

7. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

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

8. Enzyme conjugate: CONJ

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

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

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

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Ready-to-use component. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.

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

11. Sulphuric Acid: H_2SO_4 O.3 M

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

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

12. Plate sealing foils n°2

13. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

3. Antigen Positive Control:
Ready to use. Mix well on vortex before use.

4. Antibody Positive Control:
Ready to use. Mix well on vortex before use.

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

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

6. Antibody Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

7. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

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

9. HBe Antigen:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

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

Legenda:

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

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

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

M. ASSAY PROCEDURE

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

A) HBe Antigen:

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

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

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

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

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

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

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

B) HBe Antibody:

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

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

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

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator Samples	50 ul 50 ul
Neutralising antigen	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate

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

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

O. INTERNAL QUALITY CONTROL

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

Control that the following data are matched:

HBe Antigen

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

HBe Antibody

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

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

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

HBeAg

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

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

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

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

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

P. CALCULATION OF THE CUT-OFF
The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

NC + 0.100 = Cut-Off (Co)

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

HBeAb:

(NC + PC) / 3 = Cut-Off (Co)

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

Q. INTERPRETATION OF RESULTS
Results are interpreted as follows:

HBeAg:

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

HBeAb:

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

Note:
S = OD450nm/620-630nm of the sample
Co = cut-off value

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

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

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.500 – Accepted
Cut-Off = 0.025+0.100 = 0.125
Calibrator: 0.520 - 0.540 OD450nm
Mean value: 0.530 OD450nm S/Co = 4.2
S/Co higher than 2.0 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

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

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

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 3 = 0.733
Calibrator: 0.720-0.760 OD450nm
Mean value: 0.740 OD450nm
OD450nm < NC/1.5 – Accepted

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

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

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

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

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

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

BBI's Accurun 51 (S/Co)

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

2. Diagnostic Sensitivity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.
Data are reported below and compared with those reported by BBI for two other commercial products.

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

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

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2
11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:
The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved kit.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV and HAV) and non viral pathologies of the liver that may interfere with the test were examined.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

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

HBE.CE: lot # 0103

Negative Control (N = 16)

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

PEI 1 U/ml (N = 16)

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

HBE.CE: lot # 0103/2

Negative Control (N = 16)

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

PEI 1 U/ml (N = 16)

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

HBE.CE: lot # 0303

Negative Control (N = 16)

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

PEI 1 U/ml (N = 16)

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

B) HBe Antibody

1. Limit of detection

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

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

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

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

Accurun 52 (Co/S)

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

2. Diagnostic sensitivity:

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

Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

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

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

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

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

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

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

3. Diagnostic specificity:

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

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

4. Precision:

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

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

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

PEI 0.25 U/ml (N = 16)

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

HBE.CE: lot # 0103/2

Negative Control (N = 16)

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

PEI 0.25 U/ml (N = 16)

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

HBE.CE: lot #0303

Negative Control (N = 16)

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

PEI 0.25 U/ml (N = 16)

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

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

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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

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