Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Testosterone Test System
Product Code: 3725-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Testosterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Testosterone, (17β-Hydroxy-4-androstene-3-one), a C_{19} steroid, is the most potent naturally secreted androgen.[1] In normal post pubertal males, testosterone is secreted primarily by the testes with only a small amount derived from peripheral conversion of 4-Androstene-3, 17-dione (ASD).[2] In adult women, it has been estimated that over 50% of serum testosterone is derived from peripheral conversion of ASD secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

In the male, testosterone is mainly synthesized in the interstitial Leydig cells and the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary (the female equivalent of ICSH).[3] Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and auxiliary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, orchidectomy, estrogen therapy, Klinefelter's syndrome, hypopituitarism, and hepatic cirrhosis.[2-4]

In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women 50–60% of the daily testosterone production arises from peripheral metabolism of prohormone, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein-Leventhal syndrome), ovarian tumors, adrenal tumors and adrenal hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

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.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = 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. Testosterone Calibrators – 1ml/vial – Icons A-G
Seven (7) vials of serum reference for Testosterone at concentrations of 0 (**A**), 0.1 (**B**), 0.5 (**C**), 1.0 (**D**), 2.5 (**E**), 5.0 (**F**) and 12.0 (**G**) in ng/ml Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.47.
For example: 1ng/ml x 3.47 = 3.47 nM/L

B. Testosterone Enzyme Reagent – 6.0 ml/vial – Icon Ⓔ
One (1) ready to use vial of Testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with buffer, red dye, preservative, and binding protein inhibitors. Store 2-8°C.

C. Testosterone Biotin Reagent – 6.0 ml – Icon ∇
One (1) vial containing anti-Testosterone biotinylated purified rabbit IgG 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.

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

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

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 2-8ºC. 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 capable of delivering 0.010ml (10µl), 0.050ml(50µl), 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.50ml (50µl) .0.100ml (100 µl) 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 or (for plasma) in 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 use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20µl) 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 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.
3. **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 - 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 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 0.010 ml (10µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the ready to use Testosterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Testosterone 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 12 ng/ml 1:5 and 1:10 with Testosterone '0' ng/ml calibrator or female patient sera with a known low value for testosterone.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Testosterone 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 Testosterone concentration in ng/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 Testosterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.764) intersects the dose response curve at (0.57ng/ml) Testosterone concentration (See Figure 1).

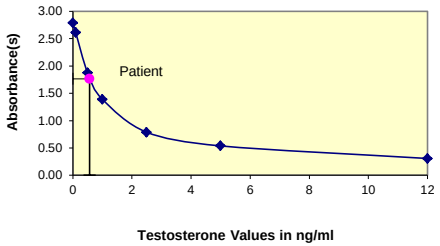
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.780	2.787	0
	B1	2.794		
Cal B	C1	2.576	2.611	0.1
	D1	2.646		
Cal C	E1	1.789	1.877	0.5
	F1	1.965		
Cal D	G1	1.391	1.392	1.0
	H1	1.393		
Cal E	A2	0.780	0.788	2.5
	B2	0.796		
Cal F	C2	0.530	0.538	5.0
	D2	0.547		
Cal G	E2	0.301	0.308	12.0
	F2	0.314		
Ctrl 1	G2	1.040	0.760	**1.61**
	H2	1.045		
Patient	A3	1.751	1.764	**0.57**
	B3	1.778		

*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 ng/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 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 producefalse 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 RANGES OF VALUES

In agreement with established reference intervals[5] for a "normal" adult population, the expected ranges for the Testosterone AccuBind® ELISA Test System are detailed in Table 1.

TABLE I
Expected Values for Testosterone EIA Test System (ng/ml)

Boys Before Puberty	0.1 – 3.7
Male	2.5 – 10.0
Female	0.2 – 0.95

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 Testosterone 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 ng/ml)

Sample	N	X	σ	C.V.
Low	22	1.63	0.16	9.8%
Normal	22	9.14	0.44	4.8%
High	22	14.22	0.79	5.6%

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

Sample	N	X	σ	C.V.
Low	24	1.72	0.16	9.1%
Normal	24	7.06	0.69	9.7%
High	24	13.08	1.03	7.9%

*As measured in several experiments in duplicate over a ten day period.

14.2 Sensitivity

The Testosterone AccuBind® ELISA Test System has a sensitivity of 0.576 pg. This is equivalent to a sample containing a concentration of 0.0576 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Testosterone AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high Testosterone level populations were used. The values ranged from 0.29 ng/ml – 21.9ng/ml. The total number of such specimens was 58. The least square regression equation and the correlation coefficient were computed for this Testosterone 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)	3.12	y= -0.265+0.944(x)	0.985
Reference (X)	3.02		

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 testosterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Testosterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Testosterone	1.0000
Androstenedione	0.0009
Dihydotestosterone	0.0178
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	<0.0001
Spirolactone	<0.0001
Progesterone	<0.0001
17α-OH Progesterone	<0.0001
DHEA sulfate	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Estriol	<0.0001
Hemolysis	<0.0001
Rubella	<0.0001
Lipemia	<0.0001

15.0 REFERENCES

1. Dorfman, RI and Shipley, RA, ED: Androgens, New York,: John Wiley and Sons, 1956.
2. Horton R, Tait JF: Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of conversion to testosterone. J.Clin Invest 45: 301-303, 1966.
3. Faiman C and Winter, JSD, Reyes, FI, Clin *Obstet Gynaecol,* 3, 467 (1976).
4. Sizonenka, PC, *Pediatrician,* 14, 191 (1987).
5. Cummings DC, Wall SR: Non sex hormone binding globulin bound testosterone as a marker for hyperandrogenism. J. Clin Endocrinol Metab. 61:873-876, 1985.
6. Lashansky, G, et. al., *J Clin Endocrinol Metab*,58, 674 (1991)
7. Tietz, NW, ED: Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, WA Saunders Co, 1995.

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MP3725 **Product Code: 3725-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	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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IVD 2° 8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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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

Thyroglobulin (Tg) Test System
Product Code: 2225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroglobulin (Tg) Concentration in Human Serum by a Microplate Enzyme immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human thyroglobulin (Tg) is a large glycoprotein (660 kD) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intrathyroid synthesis of primary thyroid hormones like *Triiodothyronine (T3) and Thyroxine (T4).*

Tg is elevated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto's thyroiditis and Graves Disease. Tg levels are found to be normal in patients with medullary thyroid carcinoma. Serial measurements of Tg is most useful in detecting recurrence of differentiated thyroid carcinoma following surgical resection or radioactive iodine ablation. Tg determination is used as an adjunct to iodine scanning but not as a replacement for it. Assessment of Tg levels aids in management of infants with congenital hypothyroidism.

Tg determination has been done with various methods using direct competitive binding RIA and double antibody sandwich IRMA or ELISA, of which latter is more useful. All these methods suffer from interference by endogenous autoantibodies to Tg. It is useful to determine the effect of autoantibodies before screening such patients for levels of Tg. *Monobind* provides Tg autoantibody ELISA to rule out such interference. ***(Please see Monobind Anti-Tg AccuBind® ELISA Test System, Product Code: 1025-300).***

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 Thyroglobulin antibody.

When monoclonal biotinylated antibody is mixed with a serum containing the Tg antigen, a reaction results between the Tg antigen and the antibody, to form an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation:

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

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

$Ag_{(Tg)} - {}^{Btn}Ab_{(m)} + \underline{Streptavidin}_{C.W.} \Rightarrow \underline{immobilized\ complex}$ (IC)
$\underline{Streptavidin}_{C.W.}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex\ (IC)}$ = 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 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.

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

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

4.0 REAGENTS

Materials Provided:

A. Thyroglobulin Calibrators – 1.0 ml/vial – Icons A - F
Six (6) vials of references for Thyroglobulin antigen (Tg) at levels of **0(A), 2.0 (B), 10.0(C), 40(D), 100(E), and 250(F) ng/ml**. A preservative has been added.
Note: There is no known, internationally accepted thyroglobulin standard available. The Tg used in the serum based calibrators is a highly purified (98+% pure) human Tg preparation that is calibrated gravimetrically against the reference material obtained from Community Bureau of Reference # CRM 457.

B. x-Tg Biotin Reagent – 13ml/vial – Icon ∇
One (1) vial contains biotinylated anti-Tg monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Tg Enzyme Reagent – 13 ml/vial - Icon
One (1) vial contains anti-thyroglobulin IgG labeled with horseradish peroxidase (HRP) in buffer, dye, and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well (break well modules) microplate coated with streptavidin and 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 contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

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

G. Stop Solution – 8ml/vial – Icon
One (1) vial contains 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: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
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 or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

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

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

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE (Time 4hr 15min)

Before proceeding with the assay, bring all reagents, serum reference calibrator 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 calibrator, control and patient sample 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 x-Tg Biotin 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 or microplate cover.
5. Incubate for 2 hours 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 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 Tg Enzyme Reagent to all wells
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover with a plastic wrap. Incubate at room temperature for 120 minutes.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and 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 used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100 µl) of substrate to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Cover with a plastic wrap or microplate cover. Incubate at room temperature for 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 fifteen (15) minutes of adding the stop solution.**

9.1 ALTERNATE PROCEDURE (Time 2hr 15min)
This procedure can be used with the help of a laboratory hematology shaker.
1. Format the microplates' wells for each calibrator, control and patient sample 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 biotin labeled monoclonal antibody to each well. **It is very important to dispense all reagents close to the bottom of the microwell and swirl to mix.**
4. Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. 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 used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the wash and repeat two (2) additional times.**
7. Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells
8. Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
9. Repeat steps 5-6 as described in the 'Test Procedure' above.
10. Follow steps 11-14 to develop color and measure.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of human thyroglobulin (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 Tg concentration in ng/ml on linear graph

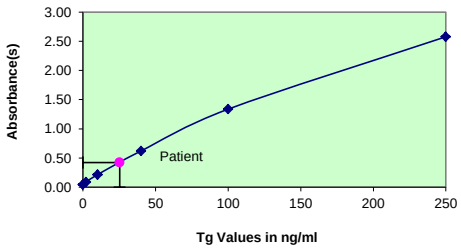
paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of Tg for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting 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 (0424) intersects the dose response curve at 25.2 ng/ml Tg 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
(Data is based on 9.0 Test Procedure - time 4 hr. 15min)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.047	0.047	0
	B1	0.047		
Cal B	C1	0.093	0.091	2
	D1	0.090		
Cal C	E1	0.221	0.217	10
	F1	0.214		
Cal D	G1	0.612	0.625	40
	H1	0.634		
Cal E	A2	1.343	1.339	100
	B2	1.335		
Cal F	C2	2.596	2.577	250
	D2	2.557		
Cont 1	E2	0.142	0.146	4.99
	F2	0.150		
Cont 2	G2	1.622	0.876	125.0
	H2	1.566		
Patient 1	A3	0.426	0.424	25.2
	B3	0.422		

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 A should be ≤ 0.06.
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. 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 samples with thyroglobulin concentrations above 250 ng/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
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.

13.0 EXPECTED RANGE OF VALUES

Based on the clinical data gathered by Monobind in concordance with the published literature a normal range was established.

Table 1: Expected Values for TG

POPULATION	RANGE
Adult	3.5 – 56 ng/ml

Tg is found to be elevated in patients with thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto's thyroiditis and Graves' disease. Low levels of Tg are an indication of thyrotoxicosis factitia.
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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within and between assay precisions of the Thyroglobulin 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 ng/ml)

Sample	N	X	σ	C.V.
Pool 1	22	6.2	0.41	6.6%
Pool 2	22	64.4	2.23	3.6%
Pool 3	22	194.1	8.17	4.2%

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

Sample	N	X	σ	C.V.
Pool 1	10	5.8	0.52	9.0%
Pool 2	10	62.2	3.82	6.1%
Pool 3	10	192.3	10.90	5.7%

*As measured in ten experiments in duplicate over seven days.

14.2 Sensitivity
The analytical 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 0.44 ng/ml.

14.3 Accuracy
The Tg AccuBind® ELISA test system was compared with a reference coated tube radioimmunoassay (IRMA) assay. Biological specimens from symptomatic and asymptomatic populations were used. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	13.6	y = 2.55 + 0.908 (x)	0.975
Reference	11.4		

The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity
The cross-reactivity of the Tg AccuBind® ELISA method 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 Thyroglobulin needed to produce the same absorbance.

Substance	Concentration	Cross Reactivity
Thyroglobulin	100 ng/ml	100.0%
Triiodothyronine	1000 ng/dl	N/D
Thyroxine	1000 ng/ml	N/D
TBG	100 ng/ml	N/D

14.5 High Dose Effect
Since the assay is sequential in design, high concentrations of Tg do not show the hook effect. Samples with concentrations over 50,000 ng/ml demonstrated extremely high levels of absorbance.

15.0 REFERENCES

1. Beever K, Bradbury J, Phillips D, et al, "Highly sensitive assays of autoantibodies to Thyroglobulin and Thyroid Peroxidase", *Clin Chem*, 35, 1949-1954 (1989).
2. Ladenson PW, "Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer", *Clin Chem*, 42, 183-187 (1996).
3. Mayo Medical Laboratories: test Catalog, Rochester, MN (1997).
4. Spencer CA, Takeucho M, Kazarosyn M, "Current status and performance goals for serum thyroglobulin assays", *Clin Chem*, 42, 164-173 (1996).
5. Tietz N. Ed: *Clinical Guide to Laboratory Tests.* 3rd Ed. Philadelphia. Saunders (1995).
6. Surks, MI, Chopra, IJ, Mariash, CN, "American Thyroid Association guidelines for use of laboratory tests in thyroid disorders", *JAMA,* 263, 1529-1532 (1990).
7. Ng, M., Rajna, A, Khalid, B, "Enzyme immunoassay for simultaneous measurements of autoantibodies against thyroglobulin and thyroid microsomes in serum", *Clin Chem,* 33, 2286-2288 (1987).
8. Spencer, CA, Takeucho M, Kazarosyn M, Wang CC, Guttler RB, Singer PA, et al, "Serum thyroglobulin autoantibodies; prevalence, influence on serum thyroglobulin measurements, and prognostic significance in patients with differentiated thyroid carcinoma", J Clin *Endocrinol Metab,* 83, 1121-27 (1998).
9. Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT, "Detection of residual and recurrent differentiated thyroid carcinoma by serum thyroglobulin measurements", *Thyroid,* 9, 435-41 (1999).
10. Schlumberger M, Baudin E, "Serum thyroglobulin determinations in the follow up of patients with differentiated thyroid carcinoma", *Eur J. Endocrinol*, **138,** 249-252 (1998).

Effective Date: 2019-Jul-16 Rev. 4 **DCO: 1353**
MP2225 **Product Code: 2225-300**

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 (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

Thyrotropin (TSH) Test System
Product Code: 325-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 Daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism.[1,2] The structure of human TSH is similar to that of the pituitary and placental gonadotropins, consisting of an 89-amino acid α-subunit which is similar or identical between these hormones and a 115-amino acid β-subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with apparent excess production of the α-subunit. The TSH molecule has a linear structure consisting of the protein core with carbohydrate side chains; the latter accounts for 16% of the molecular weight.

TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provides the means for discrimination in the hyperthyroid-euthyroid range. The functional sensitivity (<20% between assay CV) of the one-hour procedure is 0.195 µIU/ml while the two-hour procedure has a functional sensitivity of 0.095µIU/ml.[3]

In this method, TSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the streptavidin coated to the well.

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

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

3.0 PRINCIPLE

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

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

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

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

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

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

4.0 REAGENTS

Materials Provided:

A. TSH Calibrators – 1ml/vial - Icons A-G
Seven (7) vials of references for TSH Antigen at levels of 0(**A**), 0.5(**B**), 2.5(**C**), 5.0(**D**), 10(**E**), 20(**F**) and 40(**G**) µIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP 80/558.

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

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

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

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

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

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

H. Product Instructions.

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

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

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

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

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal, and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

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

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

9.0 TEST PROCEDURE

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

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the TSH Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature. ******
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

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

** For better low-end sensitivity (< 0.5µIU/ml), incubate 120 minutes at room temperature. The 40µIU/ml calibrator should be excluded since absorbance over 3.0 units will be experienced. Follow the remaining steps.

Note: Dilute samples reading over 40 µIU/ml by 1:5 and 1:10 with TSH '0' Calibrator. Multiply the results by the dilution factor to obtain accurate results.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1
2. Plot the absorbance for each duplicate serum reference versus the corresponding TSH concentration in µIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

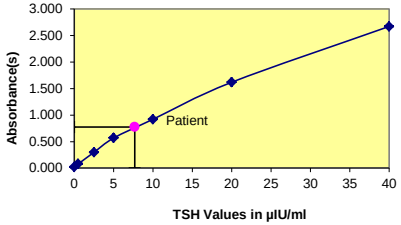
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.775) intersects the dose response curve at (7.66 µIU/ml) TSH concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs	Mean Abs	Value (µIU/ml)
Cal A	A1	0.018	0.019	0
	B1	0.021		
Cal B	C1	0.076	0.079	0.5
	D1	0.082		
Cal C	E1	0.302	0.298	2.5
	F1	0.293		
Cal D	G1	0.556	0.567	5.0
	H1	0.577		
Cal E	A2	0.926	0.921	10
	B2	0.916		
Cal F	C2	1.610	1.619	20
	D2	1.629		
Cal G	E2	2.694	2.671	40
	F2	2.647		
Control	G2	0.800	0.775	**7.66**
	H2	0.751		
Patient	A3	1. 391	1.383	**16.65**
	B3	1.375		

Figure 1



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

11.0 Q.C. PARAMETERS

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

1. The absorbance of calibrator 'G' (40 µIU/ml) should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. Patient specimens with TSH concentrations over 40µIU/ml may be diluted (1:5 or 1:10) with the '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurement and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. **Thus, thyrotropin concentration alone is not sufficient to assess clinical status.**
7. Serum TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.
8. A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine.[4]
9. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the TSH AccuBind® ELISA Test System. The number and determined range are given in Table 1. A nonparametric method (95% Percentile Estimate) was used.

TABLE I
Expected Values for the TSH ELISA Test System (in µIU/ml)

Number	139	2.5 Percentile–70% Conf Int	
Low Normal	0.39	Low Range	0.28 – 0.53
High Normal	6.16	High Range	5.60 – 6.82

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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

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

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

Sample	N	X	σ	C.V.
Pool 1	24	0.37	0.03	8.1%
Pool 2	24	6.75	0.43	6.4%
Pool 3	24	29.30	1.94	6.6%

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

Sample	N	X	σ	C.V.
Pool 1	10	0.43	0.04	9.3%
Pool 2	10	6.80	0.54	7.9%
Pool 3	10	28.40	1.67	5.9%

*As measured in ten experiments in duplicate over seven days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:

For I hr incubation = 0.078 µIU/ml
For 2 hr incubation = 0.027 µIU/ml

14.3 Accuracy

The TSH AccuBind® ELISA test system was compared with a reference immunochemiluminescence assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01µIU/ml – 61µIU/ml). The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the TSH AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	4.54	y = 0.47 + 0.968 (x)	0.995
Reference	4.21		

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

14.4 Specificity

The cross-reactivity of the TSH AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyrotropin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Follitropin (hFSH)	< 0.0001	1000ng/ml
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin(hCG)	< 0.0001	1000ng/ml

14.5 Correlation between 1 hr and 2 hr incubation

The one- (1) hr and two (2) hr (optional) incubation procedures were compared. Thirty (30) biological specimens (ranging from 0.1 – 18.5 µIU/ml) were used The least square regression equation and the correlation coefficient were computed for the 2 hr procedure (y) in comparison with the 1 hr method (x). Excellent agreement is evidenced by the correlation coefficient, slope and intercept: Y = 0.986 (x) + 0.119 Regression Correlation =0.998

15.0 REFERENCES

1. Hopton MR, & Harrap JJ, "Immunoradiometric assay of thyrotropin as a first line thyroid function test in the routine laboratory", *Clinical Chemistry,* 32, 691 (1986).
2. Caldwell, G et al, "A new strategy for thyroid function testing", *Lancet*, I, 1117 (1985).
3. Young DS, Pestaner LC, and Gilberman U, "Effects of Drugs on Clinical Laboratory Tests", *Clinical Chemistry,* 21, 3660 (1975).
4. Spencer, CA, et al, "Interlaboratory/Intermethod differences in Functional Sensitivity of Immunometric Assays of Thyrotropin (TSH) and Impact on Reliability of Measurement of Subnormal Concentrations of TSH", *Clinical Chemistry,* 41, 367 (1995).
5. Beck-Peccoz P, Persani L, "Variable biological activity of thyroid stimulating hormone", *Eur J Endocrinol,* 131, 331-340 (1994).
6. Bravermann, LE, "Evaluation of thyroid status in patients with thyrotoxicosis", *Clin Chem*, 42, 174-181 (1996).
7. Fisher, DA, "Physiological variations in thyroid hormones. Physiological and pathophysiological considerations", *Clin Chem*, 42, 135-139 (1996).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP325 Product Code: *325-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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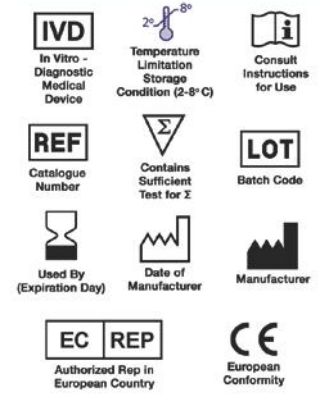
Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Anti-Müllerian Hormone (AMH) Test System
Product Code: 9725-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Anti-Müllerian Hormone (AMH) is a disulfide-linked homodimeric 140kDa glycoprotein from the trans-forming growth factor-β (TGF-β) superfamily of growth and differentiation factors. It is primarily produced by the gonads in both males and females.

In fetal males, AMH is produced by the Sertoli cells and induces regression of the Müllerian duct and therefore promotes development of the male reproductive tract.[1] Infant males have very high levels of AMH (>30 ng/ml) that slowly decreases until post-pubescence where it remains at a low level (<10 ng/ml).[2-3]

In females, AMH begins to be produced near the time of birth with levels increasing until puberty. After puberty, blood AMH levels decrease until menopause where it becomes nearly undetectable (<0.1 ng/ml). AMH concentration in female blood has repeatedly been linked to ovarian reserve, thereby giving an indication to patients' remaining reproductive lifespans.[4] Additionally, high levels of AMH (>4.7 ng/ml, 80% CI) in females are an indication of polycystic ovarian syndrome (PCOS).[4]

When AMH levels drop below 1.0 ng/ml in females, they are considered to have low ovarian reserves. Patients in these ranges are advised to not delay family planning or to undergo infertility treatments such as in vitro fertilization.[4-5]

The AccuBind® AMH test kit is a highly sensitive assay that can be used to measure blood AMH levels in order to monitor progress of patients' infertility treatments and approximate the onset of menopause.

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
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 x-AMH antibody coated on the well.

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

$$^{Enz}Ab + Ag_{AMH} + Ab_{(well)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{AMH} - Ab_{(well)}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{AMH} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{AMH} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, 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. AMH Calibrators – 1.0 ml/vial (Lyophilized) Icons [A – F]
Six (6) vials of references for AMH at levels of 0(**A**), 0.2(**B**), 0.5(**C**), 1.0(**D**), 5.0(**E**) and 15.0(**F**) ng/ml. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted calibrators are stable for 10 days at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20 °C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation and are traceable against NIBSC code 16/190.

B. AMH Controls – 1.0 ml/vial (Lyophilized) Icons [M&N]
Two (2) vials of reference controls for AMH. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted controls are stable for 10 days at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.

C. AMH Enzyme Reagent – 6 ml/vial – Icon
One (1) vial contains anti-AMH conjugate reagent. Store at 2-8 °C.

D. AMH Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with x-AMH antibody. Store at 2-8 °C.

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

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

G. Stop Solution – 8 ml/vial – Icon STOP
One (1) vial contains a strong acid (0.5 M 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.
Note 3: The above components are for one 96-well microplate. For other kit configurations, refer to table at the end of the instructions.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
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 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 EDTA/heparin containing tubes for plasma. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

If the specimen(s) cannot be assayed immediately after blood withdrawal, the sample(s) may be stored at temperatures of 2-8 °C for up to seven (7) days or -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing (a maximum of two freeze/thaw cycles prior to use). When assayed in duplicate, 0.100 ml (100 µl) 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 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.

Note: Do not use reagents that are contaminated or have bacterial 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 0.050 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 AMH 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, cover and incubate for 60 minutes at room temperature.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. 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.**
7. Add 0.100 ml (100 µl) of TMB Substrate to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
8. Incubate at room temperature for twenty (20) minutes.
9. 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.**
10. 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: For re-assaying specimens with concentrations greater than 15 ng/ml, the samples should be diluted and multiplied accordingly. Acceptable diluents are serum from postmenopausal females (<0.1 ng/ml AMH), the "0" calibrator, and other diluent solutions sold by Monobind.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of AMH in unknown specimens.
1. Plot the absorbance for each duplicate serum reference versus the corresponding AMH concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of AMH 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 (0.299) intersects the dose response curve at 1.33 ng/ml AMH concentration (See Figure 1).

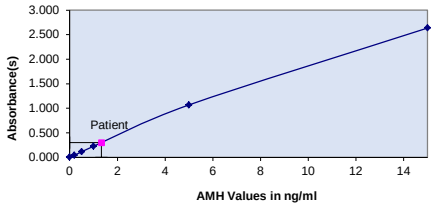
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.005	0.005	0
	B1	0.005		
Cal B	C1	0.041	0.043	0.20
	D1	0.045		
Cal C	E1	0.109	0.112	0.50
	F1	0.115		
Cal D	G1	0.215	0.225	1.00
	H1	0.234		
Cal E	A2	1.047	1.068	5.00
	B2	1.097		
Cal F	C2	2.570	2.635	15.00
	D2	2.678		
Patient	E2	0.300	0.299	**1.33**
	F2	0.298		

*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. Maximum Absorbance (Calibrator 'F') ≥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. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. 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 used in combination with clinical examination, patient's history, and, all other clinical findings.

13.0 EXPECTED RANGES OF VALUES

AMH levels were measured by the AMH Accubind® Test System in apparently normal females of different age groups. The values obtained are given in Table 2.

Table 2
Female Reference Ranges for the AMH Test System

Age Group	N	Mean (ng/ml)	Median (ng/ml)	Minimum (ng/ml)	Maximum (ng/ml)
20-29	24	5.95	5.94	1.77	12.41
30-39	80	2.83	2.47	0.11	12.67
40-49	38	1.33	0.59	0.02	9.77

The expected values for males and females of untested age groups as determined by literature sources are collected in Table 3.[6]

Table 3
Additional Reference Ranges for the AMH Test System

Males	Range (ng/ml)
<24 months	14-466
2-12 years	7.4-243
>12 years	0.7-19
Females	
<24 months	<4.7
2-12 years	<8.8
13-19 years	0.9-9.5

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 assay precision of the AMH AccuBind® Microplate EIA Test System were determined by analyses on six different levels of pool control and patient sera. The mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 4.

TABLE 4
Precision data for the AMH Test System

Sample	Mean Value (pg/ml)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Control 1	0.80	0.02	2.87	0.04	5.32
Control 2	4.54	0.12	2.60	0.24	5.24
Control 3	14.16	0.30	2.11	0.77	5.47
Patient 1	1.42	0.03	2.20	0.08	5.79
Patient 2	0.30	0.02	5.69	0.03	9.46
Patient 3	9.57	0.26	2.73	0.51	5.36

*As measured in forty experiments in duplicate over a 20 day period.

14.2 Sensitivity

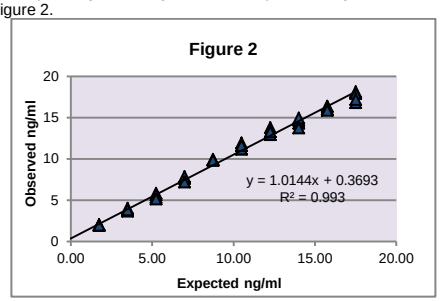
The AMH Accubind® ELISA test system has a LoB=0.024 ng/ml and a LoD=LoQ=0.044 ng/ml.

14.3 Accuracy

14.3.1 Linearity

The linearity of the AMH Accubind® ELISA test system was tested by diluting human serum samples containing high levels of AMH (2 to 17.5 ng/ml) with low AMH (<0.2 ng/ml) human serum samples or the "0" Calibrator. The system demonstrates excellent linearity through the range of the test up to 17.5 ng/ml as shown in Figure 2.

Figure 2



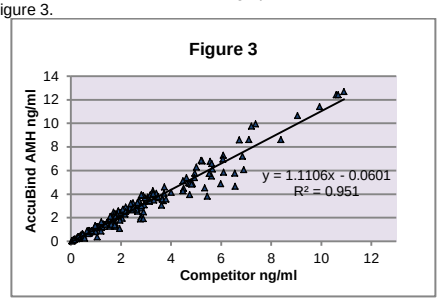
14.3.2 Recovery

The recovery of the AMH AccuBind® Microplate ELISA Test System was calculated for five patient samples spiked with 0.5, 1.0, 2.0, 4.0, and 8.0 ng/ml AMH. Recoveries were determined to be within 15% of the expected values for all samples.

14.3.3 Method Comparison

The AMH AccuBind® Microplate ELISA Test System was initially evaluated on 167 patients with known AMH concentrations calculated by a different AMH test system from a different manufacturer. Correlation between the two methods is excellent with a R^2 coefficient = 0.951. A graph of the data is shown in Figure 3.

Figure 3



14.4 Cross-Reactivity

The following analytes were tested and found to be non-reactive.

Analyte	Concentration	% Reactivity
Follicle Simulating Hormone	100 mIU/ml	<0.001
Human Chorionic Gonadotropin	1000 mIU/ml	<0.001
Leuteinizing Hormone	200 mIU/ml	<0.001
Prolactin	100 ng/ml	<0.001

15.0 REFERENCES

1. Wilson, J.D.; George, F.W.; Griffin, J.E. The hormonal control of sexual development. *Science* 1981, 211, *4488*, 1278-1284.
2. Jopling, H.; Yates, A.; Burgoyne, N.; Hayden, K.; Chaloner, C.; Tetlow, L. Paediatric Anti-Müllerian Hormone measurement: Male and Female reference intervals established… *Endocrinal Diab Metab.* 2018, 1, *4*, e00021.
3. Matuszczak, E.; Hermanowicz, A.; Komarowska, M.; Debek, W. Serum AMH in Physiology and Pathology of Male Gonads. *Int. J. of Endocrin.* 2013, 2013, Article ID 128907, 6 pgs.
4. Broer, S.L.; Broekmans, F.J.M.; Laven, J.S.E.; Fauser, B.C.J.M. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Human Reproduction Update*, 2014, 20, *5*, 688-701.
5. Lee, J.Y.; Ahn, S.; Lee, J.R.; Jee, B.C.; Kim, C.H.; Seo, S.; Suh, C.S.; Kim, S.H. Reference Values for the Revised Anti-Müllerian Hormone Generation II Assay: Infertile Population-based Study. *J Korean Med Sci* 2017, 32, 825-829.
6. Test ID: AMH Antimullerian Hormone (AMH), Serum. https://www.mayocliniclabs.com/test-catalog/Clinical and Interpretive/89711 (accessed Aug 16, 2019).

Effective Date: 2022-OCT-27 Rev. 4 **DCO: 1578**
MP9725 **Product Code: 9*725-300***

Size		96(A)	192(B)
Reagent (fill)	A)	1.0ml (dry) set	1.0ml (dry) set
	B)	1.0ml (dry) set	1.0ml (dry) 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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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

Instructions for Use

Sperm Antibody ELISA

IVD

CE

REF EIA-1826

Σ 96

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, use sólo la versión válida de las instrucciones de uso que se suministran con el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.
Utilize apenas a versão válida das Instruções de Utilização fornecidas com o kit.

Introduced modifications / Durchgeführte Änderungen / Modifiche introdotte / Modificaciones introducidas / Modifications apportées / Modificações introduzidas	
The following changes have been made in comparison to the previous version: Im Vergleich zur Vorgängerversion wurden folgende Änderungen vorgenommen: Rispetto alla versione precedente, sono state apportate le seguenti modifiche: Se han introducido los siguientes cambios en comparación con la versión anterior: Les modifications suivantes ont été apportées par rapport à la version précédente : Foram efetuadas as seguintes alterações em comparação com a versão anterior:	
Detailed editorial revision. Changed wording in several chapters. **Ausführliche redaktionelle Überarbeitung.** Geänderter Wortlaut in mehreren Kapiteln. **Revisione editoriale dettagliata.** Modificato il testo in diversi capitoli. **Revisión editorial detallada.** Se ha cambiado la redacción de algunos capítulos. **Révision éditoriale détaillée.** Modification de la formulation dans plusieurs chapitres. **Revisão editorial detalhada.** Texto alterado em vários capítulos.	
1 INTENDED USE:	More definitions for intended use and use not intended.
4.4 Reagent Preparation:	Correction for stability of diluted wash solution to 1 week (before: 2 weeks).
5.2 Samples Storage:	Sample stability at 2 °C to 8 °C changed to 2 months (before: 12 months)
6.2 Test Procedure:	**Incubation time for Enzyme Conjugate** reduced to **30 minutes** (before: 60 minutes); **Pipetting volume for Stop Solution** increased to **100 µL** (before: 50 µL);
9 PERFORMANCE CHARACTERISTICS:	Updated and additional data.
10 LIMITATIONS OF THE PROCEDURE:	Updated.

1 INTENDED USE

The **DRG Sperm Antibody ELISA** is a manual enzyme immunoassay for the **quantitative** measurement of antibodies directed against human spermatozoa (ASA) in human serum.

For *in vitro* diagnostic use. For laboratory professional use.

The device is **intended** to be used as an aid for the diagnosis of immunologically caused disorders of fertility.

The device is **not intended** to be used for the detection of poly- and monoclonal gammopathies.

1.1 Scientific Validity

Antibodies directed against spermatozoa antigens may cause infertility in women or men. The application of the Sperm Antibody ELISA is recommended for the diagnosis of immunologically caused disorders of fertility.

Unwanted childlessness is a growing problem with which up to 20% of all couples in the reproductive age are confronted temporarily or long-term. In 5-20 % of these cases the presence of anti-spermatozoa antibodies in the male or the female patient is detectable [1,2,15].

The definition of infertility according to the WHO (WHO Laboratory Manual for the Examination of Human Semen and Semen Cervical-Mucus Interaction, 1999) is the absence of a conception within 12 months of unprotected intercourse. The main cause of an immunological fertility disorder is the formation of antibodies directed against spermatozoa antigens.

Anti-spermatozoa antibodies (ASA) exert heterogeneous effects on the ability of spermatozoa to fertilize. The inhibiting effect of ASA on the motility of spermatozoa by binding to their surface and by agglutinating processes is well-known [3].

The penetration of the spermatozoa into the cervical mucus is impaired by the presence of ASA in the seminal plasma and/or in the cervical mucus [4]. ASA negatively influence the capacitation and the acrosome reaction of spermatozoa and thereby impede the interaction of the spermatozoa with the oocyte [5,6].

The interaction of the spermatozoon with the oocyte and the subsequent binding to and penetration of the zona pellucida may be inhibited by ASA. The following fusion of the oocyte and a spermatozoon may also be impaired by the presence of ASA [7,8].

The rate of pregnancies in couples with ASA on the part of the man or the woman was shown to be 38% lower compared to the control groups [9]. Furthermore, an influence on the implantation and on the early embryological development could be confirmed. An association of ASA and miscarriages is discussed. The frequency of ASA in infertile couples amounts to 20% [10,11].

ASA may occur dissolved in the ejaculate or bound to the surface of spermatozoa. In addition, ASA can be detected in serum. ASA may be found in men and in women [12]. In women, ASA may be found in cervical mucus, oviduct liquid and follicular liquid. Men having more than 50% of their spermatozoa coated with anti-spermatozoa antibodies show a conspicuously reduced rate of fertility [13].

ASA have been shown to be associated with chronic prostatitis which has a negative effect on male reproductive function [14].

2 PRINCIPLE OF THE TEST

The DRG Sperm Antibody ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **sandwich principle.**

The microtiter wells are coated with a mix of spermatozoa proteins.

During incubation, anti-spermatozoa antibodies in the samples (standards, controls, patient samples) bind to the coated surface of the wells.

A washing step removes unbound sample components.

Added enzyme conjugate binds to the immobilized antigen-antibody-complexes. The conjugate contains anti-human immunoglobulin antibodies, labelled with horseradish peroxidase (HRP).

After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is proportional to the concentration of the analyte in the sample.

A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 WARNINGS AND PRECAUTIONS

- This kit is for *in vitro* diagnostic use only. For laboratory professional use only.
- Before starting the assay, read the instructions for use completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- Do not mix or use components from kits with different lot numbers. It is advised not to interchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Do not reuse microtiter wells.
- Reagents of other manufacturers must not be used together with the reagents of this test kit.
- All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In that case, contact DRG.
- Microbial contamination of reagents or samples may give false results.
- Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay.
- All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into original vials as reagent contamination may occur.

General precautions

- Follow good laboratory practice and safety guidelines.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
- Wear lab coats and disposable latex gloves when handling samples and reagents and where necessary safety glasses.

Biohazard information

- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. However, no known test method can offer total assurance that no infectious agent is present.
- The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
- Bovine components originate from countries where BSE (Bovine spongiform encephalopathy) has not been reported.
- All materials and samples of human or animal origin must be handled as if capable of transmitting infectious diseases.
- Handling must be done in accordance with the procedures defined by appropriate national biohazard and safety guideline or regulation. Waste must be discarded according to local rules and regulations.

Information to chemical hazards and hazard classification

- Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
- Substrate Solution contains an ingredient in non-declarable concentrations which causes serious eye irritation. In case of possible contact with eyes, rinse immediately carefully and thoroughly with eye wash or water. After contact with skin, wash with plenty of water. Take-off contaminated clothing and wash it before reuse.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Chemicals and prepared or used reagents must be treated as hazardous waste according to the national safety guideline or regulation.
- This product does not contain substances which have carcinogenic, mutagenic or toxic for reproduction (CMR) properties.

All reagents of this test kit do NOT contain hazardous substances in concentrations to be declared, a classification and labelling is not required.

For detailed information, please refer to the Safety Data Sheet, which is available upon request directly from DRG.

4 MATERIALS

4.1 Materials provided with the kit

Symbol	Quantity	Description	Preparation
Microtiterwells	12 x 8 wells (break apart)	**Microtiter plate** Coated with a mix of spermatozoa proteins	Ready to use
Dilution Buffer / Zero Standard	1 x 50 mL	**Sample Dilution Buffer / Zero Standard *** Concentration: 0 U/mL	Ready to use
Standard (Standard 1 - 4)	4 x 0.5 mL	**Standards *** Concentrations: 31 – 62 – 125 – 250 U/mL	Ready to use
Quality Control	1 x 0.5 mL	**Control** * *For control values and ranges please refer to vial label or Certificate of Analysis.*	Ready to use
Enzyme Conjugate	1 x 8 mL	**Enzyme Conjugate *** Anti-human IgG antibody conjugated to horseradish peroxidase; Colored red.	Ready to use
Substrate Solution	1 x 14 mL	**Substrate Solution** Contains 3,3‘,5,5‘-tetramethylbenzidine (TMB). *Keep away from direct sun light.*	Ready to use
Stop Solution	1 x 14 mL	**Stop Solution** Contains 0.5 M H_2SO_4. *Avoid contact with the stop solution. It may cause skin irritations and burns.*	Ready to use
Wash Solution	1 x 30 mL	**Wash Solution, 40X concentrate ***	*See “Reagent Preparation“.*
Cover foil	1 x	**Cover foil**	
	1 x	**Instructions for Use**	
	1 x	**Certificate of Analysis (CoA)**	
		***** Contains non-mercury preservative.	

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Incubator for 37 °C
- Manual or automatic equipment for rinsing microtiter plate wells
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

4.3 Storage and Stability of the Kit

Unopened kits and reagents as well as **opened reagents** must be stored at 2 °C to 8 °C.

The microtiter plate contains snap-off strips. Do not open the pouch of the wells until it reaches room temperature. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch including the desiccant and used in the plate frame provided. Once the foil bag has been opened, care must be taken to close it tightly again.

Once opened, reagent vials must be closed tightly again.

	Storage Temperature	Stability
Unopened kits and unopened reagents	2 °C to 8 °C	Until the expiration date printed on the label. Do not use reagents beyond this date!
Opened kit	2 °C to 8 °C	8 weeks

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Wash Solution

Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL distilled water to a final volume of 1200 mL.

Stability after dilution:	at 20 °C to 26 °C	1 week

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any damage to the test kit or components, DRG must be informed in writing, at the latest one week after receiving the kit. Damaged single components must not be used for a test run. They have to be stored until a final solution has been found. After this, they must be disposed of according to the official regulations.

5 SAMPLE COLLECTION, STORAGE AND PREPARATION

The following sample material can be used in this test:

Human serum

Samples containing sodium azide should not be used in the assay.

In general, it should be avoided to use hemolytic, icteric, or lipemic samples. For further information refer to chapter "*Interfering Substances*".

*For the determination of anti-spermatozoa antibodies in seminal plasma please use our Sperm Antibody (seminal plasma) ELISA (*REF *EIA-4249).*

5.1 Sample Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Whole blood should not be frozen before centrifugation.

5.2 Samples Storage

Samples must be stored tightly capped prior to performing the assay. If stored frozen, freeze only once. Thawed samples must be inverted several times prior to testing.

Stability	at 2 °C to 8 °C	4 days
	at -20 °C (in aliquots)	up to 2 months

5.3 Sample Preparation

Prior to assaying, dilute each patient sample **1:100** with *Dilution Buffer.*

Example:

Dilution 1:100: 5 µL sample + 495 µL *Dilution Buffer* (mix thoroughly)

Stability of diluted samples	at 2 °C to 8 °C	4 days
	at -20 °C (in aliquots)	7 days

Note: The *Quality Control* is ready to use and must not be diluted!

6 ASSAY PROCEDURE

6.1 Procedural Notes

- All reagents and samples must be allowed to come to room temperature (20 °C to 26 °C) before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Use new disposal plastic pipette tips for each standard, control, or sample in order to avoid carry-over.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.

- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the test has been started, all steps must be completed without interruption and in the same sequence for each step.
- The enzymatic reaction is linearly proportional to time and temperature.
- Optical density is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in chapter “Test Procedure”.
- Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- During the incubation at 37 °C cover microtiter strips with foil to avoid evaporation.
- **Important note to wash procedure:**
  Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
- **Test performance using fully automated analysis devices:**
  Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.

6.2 Test Procedure

Each run must include a standard curve.

The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.

The given test procedure describes manual processing.

Important note: The accuracy of this assay is markedly influenced by the correct incubation temperature and incubation time. Pipetting the samples should not exceed 15 minutes.

1. Secure the desired number of microtiter wells in the frame holder.
2. Pipette **50 µL** of each ***Zero Standard, Standard, Quality Control,*** and **diluted sample** with new disposable tips into appropriate wells.
3. Cover with foil and incubate for **60 minutes** at **37 °C**.
4. Wash the wells as follows:
   If the wash step is performed manually:
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted *Wash Solution* per well.

   If an automated plate washer is used:
   Rinse the wells **3 times** with **400 µL** diluted *Wash Solution* per well.

   At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!
5. Add **50 µL *Enzyme Conjugate*** into each well.
6. Cover with foil and incubate for **30 minutes** at **37 °C**.
7. Wash as described in step 4.
8. Pipette **50 µL** of ***Substrate Solution*** to each well.
9. Incubate for **30 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
11. Measure the optical density (OD) of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. The concentration of the samples can be read directly from the standard curve.
   The standards are already pre-diluted, therefore the 1:100 dilution of the samples must not be taken into account for the final calculation of sample concentrations.
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and patient sample must be taken. If the two values deviate substantially from one another, DRG recommends retesting the samples.
3. Samples with concentrations exceeding the highest standard can be further diluted with *Dilution Buffer* and re-assayed as described in “Test Procedure”, or must be reported as > 250 U/mL. For the calculation of the concentrations, this dilution factor must be considered.
4. Automated method:
   The results in the instructions for use have been calculated automatically using a four-parameter logistic (4PL) curve fit. (4PL Rodbard or 4PL Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. Manual method:
   Using graph paper, construct a standard curve by plotting the (mean) OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
   Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Density (450 nm)
Zero Standard (0 U/mL)	0.147
Standard 1 (31 U/mL)	0.515
Standard 2 (62 U/mL)	0.857
Standard 3 (125 U/mL)	1.423
Standard 4 (250 U/mL)	2.127

7 REFERENCE VALUES

It is strongly recommended that each laboratory determine its own reference values.

In a study conducted with apparently healthy subjects, using the DRG Sperm Antibody ELISA the following data were observed:

Normal values 0 U/mL – 60 U/mL
Borderline 55 U/mL – 65 U/mL
Elevated values > 60 U/mL

In the case of a value in the range near the cut-off (55 U/mL to 65 U/mL) we recommend a follow-up determination using a new sample taken within the next two weeks.

The results alone should not be the only reason for any therapeutic consequences. The results must be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the Quality Control Laboratory are stated in the Certificate of Analyses (CoA) added to the kit. The values and ranges stated on the CoA always refer to the current kit lot and must be used for direct comparison of the results.
If available, it is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Apply appropriate statistical methods for analyzing control values and trends. If the results of the assay do not agree with the established acceptable ranges of control materials, patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above-mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Sensitivity

Limit of Blank (LoB)	0.490 U/mL
Limit of Detection (LoD)	3.367 U/mL
Limit of Quantification (LoQ)	9.632 U/mL
Measuring range	3.367 U/mL – 250 U/mL
Linear range	4.333 U/mL – 250 U/mL

9.2 Reproducibility

9.2.1 Within-run Precision

The within-run precision was determined with 4 patient samples covering the complete measuring range within 20 days in 2 independent runs per day. CV was calculated as mean CV of 40 runs.

Sample	n	Mean (U/mL)	CV (%)
1	40	39.68	3.9
2	40	77.99	3.0
3	40	106.16	4.2
4	40	155.95	3.9

9.2.2 Between-run Precision

The between-run precision was determined with 4 patient samples covering the complete measuring range within 20 days in 2 independent runs per day and with 2 replicates per run (20 x 2 x 2). CV was calculated from 80 determinations.

Sample	n	Mean (U/mL)	CV (%)
1	80	39.68	11.2
2	80	77.99	8.7
3	80	106.16	11.4
4	80	151.86	13.1

9.2.3 Between-lot Precision

The between-lot variation was determined by 6 measurements of different samples with 3 different kit lots.

Sample	n	Mean (U/mL)	CV (%)
1	18	17.78	9.3
2	18	26.65	10.4
3	18	34.58	3.9
4	18	55.56	14.7

9.3 Recovery

Recovery was determined by adding increasing amounts of the analyte to different patient samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (U/mL)		10.86	34.93	38.34	45.42
Average Recovery (%)		98.6	89.0	101.0	105.0
Range of Recovery (%)	**from**	90.3	86.2	92.0	99.0
	to	114.4	93.1	111.3	109.5

9.4 Linearity

Samples containing different amounts of analyte were serially diluted with *Dilution Buffer*. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (U/mL)		36.37	66.20	183.74	280.32
Average Recovery (%)		105.7	95.1	103.2	104.7
Range of Recovery (%)	**from**	103.9	92.3	99.7	98.6
	to	107.5	97.9	110.8	107.3

10 LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Hemoglobin (up to 4 mg/mL), bilirubin (up to 0.5 mg/mL) and triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Sera from patients with liver diseases should not be used.

Results may be adversely affected by certain pathologic conditions, such as poly- and monoclonal gammopathies, autoimmune diseases or by an altered immune status.

10.2 High-Dose Hook Effect

"High-Dose Hook Effect" is not detected up to 5000 U/mL of ASA.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. If there is any doubt or concern regarding a result, please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11.4 Reporting of Serious Incident

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

1 ZWECKBESTIMMUNG

Der **DRG Sperm Antibody ELISA** ein manueller Enzymimmunoassay zur **quantitativen** Messung von Antikörpern gegen Spermatozoen (ASA) in humanem Serum.

Für den Einsatz in der *In-vitro* Diagnostik. Für den beruflichen Gebrauch in Laboratorien.

Das Produkt ist als Hilfsmittel für die Diagnose von immunologisch bedingten Fruchtbarkeitsstörungen bestimmt.

Das Produkt ist **nicht** für den Nachweis von poly- und monoklonalen Gammopathien bestimmt.

1.1 Wissenschaftliche Validität

Antikörper, die gegen Spermatozoen-Antigene gerichtet sind, können Unfruchtbarkeit bei Frauen oder Männern verursachen. Die Anwendung des Sperm Antibody ELISA wird bei immunologisch begründeten Fertilitätsstörungen empfohlen.

Ungewollte Kinderlosigkeit ist ein wachsendes Problem, mit dem bis zu 20 % aller Paare im reproduktionsfähigen Lebensalter zeitweilig oder ständig konfrontiert werden. In 5% bis 20 % dieser Fälle werden Anti-Spermatozoen-Antikörper bei männlichen oder weiblichen Patienten nachgewiesen [1,2,15].

Entsprechend den Bestimmungen der WHO wird Infertilität angenommen, wenn innerhalb von 12 Monaten ungeschützten Geschlechtsverkehrs keine Empfängnis stattgefunden hat (WHO Laboratory Manual for the Examination of Human Semen and Semen Cervical-Mucus Interaction, 1999). Die Hauptursache einer immunologisch bedingten Fertilitätsstörung ist die Bildung von Antikörpern, die gegen Spermatozoen Antigene gerichtet sind.

Anti-Spermatozoen-Antikörper (ASA) wirken sich in unterschiedlicher Weise auf die Befruchtungsfähigkeit der Spermatozoen aus. Die hemmende Wirkung der Anti-Spermatozoen-Antikörper auf die Beweglichkeit der Spermatozoen durch Oberflächenbindung und Agglutinationsprozesse ist gut bekannt [3].

Das Durchdringen des Zervikalmucus durch die Spermatozoen wird durch die Anwesenheit von ASA im Seminalplasma und/oder im Zervikalmucus beeinträchtigt [4]. ASA beeinflussen die Fähigkeit zur Kapazitation und die Akrosomenreaktion der Spermatozoen und behindern dadurch die Interaktion der Spermatozoen mit der Eizelle [5,6].

Die Interaktion des Spermatozoons mit der Eizelle sowie die spätere Bindung an diese und das Durchdringen der Zona pellucida kann durch ASA verhindert werden. Auch die darauf folgende Verschmelzung des Spermatozoons mit der Eizelle kann durch die Anwesenheit von ASA beeinträchtigt werden [7,8].

Es wurde gezeigt, dass die Schwangerschaftsquote bei Paaren mit ASA auf Seiten des Mannes oder der Frau im Vergleich zu den Kontrollgruppen um 38 % vermindert ist [9. Außerdem konnte ein Einfluss auf die Einnistung des Eies in der Uterusschleimhaut und auf die frühe embryonale Entwicklung bestätigt werden. Ein Zusammenhang zwischen dem Auftreten von ASA und Fehlgeburten wird diskutiert.

Die Häufigkeit von ASA bei infertilen Paaren liegt bei 20 % [10,11].

Diese Antikörper können ungebunden im Ejakulat auftreten oder an die Oberfläche von Spermatozoen gebunden sein. Weiterhin können sie im Serum nachgewiesen werden. Anti-Spermatozoen-Antikörper können sowohl bei Männern als auch bei Frauen gefunden werden [12]. Bei Frauen werden ASA in Zervikalmucus, Eileiterflüssigkeit und Follikelflüssigkeit nachgewiesen. Männer, deren Spermatozoen zu mehr als 50 % mit Anti-Spermatozoen-Antikörpern bedeckt sind, weisen eine deutlich verringerte Fertilitätsrate auf [13].

Es wurde gezeigt, dass ASA mit chronischer Prostatitis assoziiert sind, was einen negativen Einfluss auf die männliche Fortpflanzungsfunktion hat [14].

2 TESTPRINZIP

Der DRG **Sperm Antibody ELISA** ist ein Festphasen-Enzymimmunoassay, der auf der **Sandwichtechnik** basiert.

Die Wells der Mikrotiterplatte sind mit einer Mischung humaner Spermatozoen-Antigene beschichtet.

Während der Inkubation binden sich Anti-Spermatozoen-Antikörper in der Probe (Standards, Kontrollen, Patientenproben) an die beschichtete Oberfläche der Vertiefungen.

In einem Waschschritt werden ungebundene Probenbestandteile entfernt.

Zugegebenes Enzymkonjugat bindet an die immobilisierten Antigen-Antikörper-Komplexe. Das Konjugat enthält anti-humane Immunoglobulin-Antikörper, die mit Meerrettichperoxidase markiert sind.

Nach einem Waschschritt, um alle ungebundenen Substanzen zu entfernen, wird die feste Phase mit der Substratlösung inkubiert. Die Farbreaktion wird durch die Zugabe der Stopplösung beendet und die optische Dichte (OD) des resultierenden gelben Produktes gemessen. Die Intensität der Farbe ist proportional zur Konzentration des Analyten in der Probe.

Durch Auftragen der OD-Werte gegen die Konzentrationen der Standards wird eine Standardkurve erstellt, und die Konzentrationen der unbekannten Proben werden anhand dieser Standardkurve bestimmt.

3 WARNUNGEN UND VORSICHTSMAßNAHMEN

- Dieser Kit ist nur für den Einsatz in der In-vitro Diagnostik bestimmt. Nur für den professionellen Gebrauch in Laboratorien.
- Bevor Sie mit dem Test beginnen, lesen Sie die Gebrauchsanweisung vollständig und sorgfältig durch. Verwenden Sie nur die gültige, im Testkit enthaltene, Gebrauchsanweisung. Stellen Sie sicher, dass Sie alles verstanden haben.
- Komponenten aus Kits mit unterschiedlichen Chargennummern dürfen nicht gemischt oder zusammen verwendet werden. Vertiefungen verschiedener Platten, auch aus derselben Charge, sollten nicht untereinander ausgetauscht werden. Die Kits können unter unterschiedlichen Bedingungen transportiert oder gelagert worden sein, so dass die Bindungscharakteristik der Platten leichte Unterschiede aufweisen kann.
- Reagenzien nicht über das auf den Kit-Etiketten angegebene Verfallsdatum hinaus verwenden.
- Mikrotitervertiefungen nicht wiederverwenden.
- Reagenzien anderer Hersteller dürfen nicht zusammen mit den Reagenzien dieses Testkits verwendet werden.
- Alle Reagenzien dieses Kits sind klare Lösungen, die Substratlösung ist klar und farblos. Veränderungen des Aussehens können die Durchführung des Tests beeinträchtigen. In diesem Fall wenden Sie sich bitte an DRG.
- Eine mikrobielle Kontamination von Reagenzien oder Proben kann zu falschen Ergebnissen führen.
- Lassen Sie die Reagenzien vor Testbeginn Raumtemperatur (20 °C bis 26 °C) erreichen. Die Temperatur wirkt sich auf die Messungen der optischen Dichte des Assays aus.
- Alle im Kit-Protokoll angegebenen Volumina müssen genau eingehalten werden. Optimale Ergebnisse können nur durch Verwendung kalibrierter Pipetten und Mikrotiterplatten-Lesegeräte erreicht werden.
- Behältnisse jeweils nur für ein einziges Reagenz verwenden. Dies gilt insbesondere für die Substrat-Behälter. Die Verwendung eines Behälters zum Pipettieren der Substratlösung, der zuvor für die Konjugatlösung verwendet wurde, kann zu einer Verfärbung der Lösung führen. Geben Sie keine Reagenzien zurück in die Originalfläschchen, da es zu einer Kontamination der Reagenzien kommen kann.

Allgemeine Vorsichtsmaßnahmen

- Befolgen Sie die gute Laborpraxis und die Sicherheitsrichtlinien.
- Niemals mit dem Mund pipettieren und den Kontakt von Reagenzien und Proben mit Haut und Schleimhäuten vermeiden.
- In Bereichen, in denen mit Kitbestandteilen oder Proben gearbeitet wird, nicht rauchen, essen, trinken oder Kosmetika verwenden.
- Beim Umgang mit Proben und Reagenzien sind Laborkittel und Einweg-Latexhandschuhe sowie, falls erforderlich, eine Schutzbrille zu tragen.

Informationen zur biologischen Gefährdung

- Alle Bestandteile dieses Testkits, die humanes Serum oder Plasma enthalten, wurden mit FDA-geprüften Methoden auf HIV I/II, HbsAg und HCV getestet und als negativ bestätigt Kein bekanntes Testverfahren kann jedoch mit absoluter Sicherheit ausschließen, dass kein Infektionserreger vorhanden ist.
- Das Produkt enthält Material tierischen Ursprungs, das nachweislich frei von infektiösen oder ansteckenden Krankheiten und schädigenden Parasiten ist.
- Komponenten von Rindern stammen aus Ländern, in denen keine BSE (Bovine Spongiforme Enzephalopathie) gemeldet wurde.
- Alle Materialien und Proben menschlichen oder tierischen Ursprungs müssen so behandelt werden, als ob sie ansteckende Krankheiten übertragen könnten.
- Die Handhabung muss in Übereinstimmung mit den Verfahren erfolgen, die in den entsprechenden nationalen Richtlinien oder Vorschriften für Biogefährdung und Sicherheit festgelegt sind. Abfälle müssen gemäß den lokalen Regeln und Vorschriften entsorgt werden.

Informationen zu chemischen Gefahren und zur Gefahreneinstufung

- Einige Reagenzien enthalten Konservierungsmittel in nicht kennzeichnungspflichtiger Konzentrationen. Bei Kontakt der Reagenzien mit den Augen oder der Haut dennoch sofort mit ausreichend Wasser spülen.
- Die Substratlösung enthält einen Inhaltsstoff in nicht kennzeichnungspflichtiger Konzentration, der schwere Augenreizungen verursacht. Bei möglichem Kontakt mit den Augen sofort sorgfältig und gründlich mit Augenspülung oder Wasser spülen. Bei Berührung mit der Haut mit reichlich Wasser abwaschen. Kontaminierte Kleidung ausziehen und vor Wiederverwendung waschen.
- Kontakt mit der Stopplösung (*Stop Solution*) vermeiden, da sie 0,5 M H_2SO_4 enthält. Schwefelsäure kann Hautreizungen und Verbrennungen verursachen.
- Chemikalien und zubereitete oder gebrauchte Reagenzien müssen als gefährlicher Abfall gemäß den nationalen Sicherheitsrichtlinien oder -vorschriften behandelt werden.
- Dieses Produkt enthält keine Stoffe, die krebserregende, erbgutverändernde oder fortpflanzungsgefährdende Eigenschaften (CMR) haben.

Alle Reagenzien dieses Testkits enthalten KEINE gefährlichen Stoffe in deklarationspflichtigen Konzentrationen, eine Einstufung und Kennzeichnung ist nicht erforderlich.

Ausführliche Informationen entnehmen Sie bitte dem Sicherheitsdatenblatt, das Sie auf Anfrage direkt bei DRG erhalten.

4 MATERIALIEN

4.1 Im Kit mitgelieferte Materialien

Symbol	Anzahl/Menge	Beschreibung	Vorbereitung
Microtiterwells	12 x 8 Wells (einzeln brechbar)	**Mikrotiterplatte** Mit Spermatozoen-Antigenen beschichtet.	Gebrauchsfertig
Dilution Buffer / Zero Standard	1 x 50 mL	**Probenverdünnungsmedium / Nullstandard) *** Konzentration: 0 U/mL	Gebrauchsfertig
Standard (Standard 1 - 4)	4 x 0,5 mL	**Standards *** Konzentrationen: 31 – 62 – 125 – 250 U/mL	Gebrauchsfertig
Quality Control	1 x 0,5 mL	**Kontrolle *** *Kontrollwerte und -bereiche entnehmen Sie bitte dem Fläschchenetikett oder dem CoA.*	Gebrauchsfertig
Enzyme Conjugate	1 x 8 mL	**Enzymkonjugat *** Anti-Human-IgG-Antikörper mit Meerrettichperoxidase konjugiert; rot gefärbt	Gebrauchsfertig
Substrate Solution	1 x 14 mL	**Substratlösung** Enthält 3,3',5,5'-Tetramethylbenzidin (TMB). *Von direktem Sonnenlicht fernhalten.*	Gebrauchsfertig
Stop Solution	1 x 14 mL	**Stopplösung** Enthält 0,5 M H_2SO_4. *Kontakt mit der Stopplösung vermeiden! Kann Hautreizungen und Verbrennungen verursachen.*	Gebrauchsfertig
Wash Solution	1 x 30 mL	**Waschlösung, 40X-Konzentrat ***	*Siehe "Vorbereitung der Reagenzien".*
Cover foil	1 x	**Abdeckfolie**	
	1 x	**Gebrauchsanweisung (IFU)**	
	1 x	**Analysenzertifikat (CoA)**	
		***** Enthält quecksilberfreies Konservierungsmittel.	

4.2 Erforderliche, aber nicht enthaltene Materialien

- Kalibriertes Mikrotiterplattenlesegerät (450 nm, mit Referenzwellenlänge bei 620 nm bis 630 nm)
- Kalibrierte variable Präzisions-Mikropipetten
- Inkubator für 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Saugfähiges Papier
- Destilliertes Wasser
- Laborwecker
- Millimeterpapier oder Software zur Datenauswertung

4.3 Lagerung und Haltbarkeit des Kits

Ungeöffnete Kits und Reagenzien sowie **geöffnete Reagenzien** müssen bei 2 °C bis 8 °C gelagert werden.

Die Mikrotiterplatte enthält abbrechbare Streifen. Öffnen Sie den Beutel mit den Vertiefungen erst, wenn er Raumtemperatur erreicht hat. Ungenutzte Vertiefungen müssen bei 2 °C bis 8 °C im verschlossenen Folienbeutel mit dem Trockenmittel gelagert und in dem mitgelieferten Plattenrahmen verwendet werden. Nach dem Öffnen des Folienbeutels muss darauf geachtet werden, dass dieser wieder dicht verschlossen wird.

Einmal geöffnete Reagenzfläschchen müssen wieder fest verschlossen werden.

	Lagerungstemperatur	Stabilität
Ungeöffneter Kit und ungeöffnete Reagenzien	2 °C bis 8 °C	Bis zu dem auf dem Etikett aufgedruckten Verfallsdatum. Reagenzien nach Ablauf dieses Datums nicht mehr verwenden!
Geöffneter Kit	2 °C bis 8 °C	8 Wochen

4.4 Vorbereitung der Reagenzien

Alle Reagenzien und die benötigte Anzahl der Mikrotiterstreifen vor dem Gebrauch auf Raumtemperatur (20 °C bis 26 °C) bringen.

Wash Solution

Fügen Sie der 40-fach konzentrierten Waschlösung (*Wash Solution*) destilliertes Wasser hinzu.

30 mL der konzentrierten Waschlösung mit 1170 mL destilliertem Wasser auf ein Endvolumen von 1200 mL verdünnen.

Stabilität nach Verdünnung:	bei 20 °C bis 26 °C	1 Woche

4.5 Entsorgung des Kits

Die Entsorgung des Kits und aller verwendeten Materialien / Reagenzien muss gemäß den nationalen gesetzlichen Vorschriften erfolgen. Spezielle Informationen für dieses Produkt finden Sie im Sicherheitsdatenblatt, Abschnitt 13.

4.6 Beschädigte Testkits

Im Falle einer Beschädigung des Testkits oder der Komponenten muss die Firma DRG in schriftlicher Form spätestens eine Woche nach Erhalt des Kits informiert werden. Beschädigte Einzelkomponenten dürfen nicht für den Testlauf verwendet werden. Sie müssen aufbewahrt werden, bis eine endgültige Lösung gefunden wurde. Danach sollten Sie gemäß den offiziellen Richtlinien entsorgt werden.

5 ENTNAHME, LAGERUNG UND VORBEREITUNG DER PROBEN

Das folgende Probenmaterial kann in diesem Test eingesetzt werden:

Humanes Serum

Proben, die Natriumazid enthalten, sollten nicht verwendet werden.

Generell sollte die Verwendung von hämolytischen, ikterischen oder lipämischen Proben vermieden werden. Weitere Informationen finden Sie im Kapitel „*Interferenzen*“.

Zur Bestimmung von anti-Spermatozoen-Antikörpern in Seminalplasma verwenden Sie bitte den Sperm Antibody (seminal plasma) ELISA (REF EIA-4249).

5.1 Probenentnahme

Serum: Blut durch Venenpunktion entnehmen (z.B. mit Sarstedt Monovette für Serum), gerinnen lassen und das Serum durch Zentrifugation bei Raumtemperatur abtrennen. Vor der Zentrifugation muss die Gerinnung vollständig abgeschlossen sein. Bei Patienten, die Antikoagulantien erhalten, kann die Gerinnungszeit länger dauern.

Vollblut sollte vor der Zentrifugation nicht eingefroren werden.

5.2 Probenlagerung

Die Proben müssen bis zur Durchführung des Tests fest verschlossen aufbewahrt werden. Wenn sie gefroren gelagert werden, nur einmal einfrieren. Aufgetaute Proben müssen vor dem Test mehrmals geschwenkt werden.

Stabilität:	bei 2 °C bis 8 °C	4 Tage
	bei -20 °C (in Aliquoten)	bis zu 2 Monate

5.3 Probenvorbereitung

Vor Einsatz im Test müssen die Patientenproben **1:100** mit *Dilution Buffer* verdünnt werden.

Beispiel:

Verdünnung 1:100: 5 µL Probe + 495 µL *Dilution Buffer* gründlich mischen)

Stabilität der verdünnten Probe:	bei 2 °C bis 8 °C	4 Tage
	bei -20 °C (in Aliquoten)	7 Tage

Beachten: Die Kontrolle (*Quality Control)* ist gebrauchsfertig und muss nicht verdünnt werden.

6 TESTDURCHFÜHRUNG

6.1 Hinweise zur Durchführung

- Alle Reagenzien und Proben müssen vor Gebrauch auf Raumtemperatur (20 °C bis 26 °C) gebracht werden.
- Alle Reagenzien müssen ohne Schaumbildung gemischt werden.
- Die Kappen der Reagenzfläschchen dürfen nicht vertauscht werden, um Kreuzkontaminationen zu vermeiden.
- Für jeden Standard, jede Kontrolle oder Probe eine neue Einweg-Plastikspitze verwenden, um Verschleppungen zu vermeiden.
- Mischen Sie den Inhalt der Mikrotiterplatten-Vertiefungen gründlich, um gute Testergebnisse zu gewährleisten.
- Kavitäten während der Testdurchführung nicht trocknen lassen; Reagenzien unmittelbar nach Ende des Waschschritts hinzufügen.
- Sobald der Test begonnen wurde, müssen alle Schritte ohne Unterbrechung und in der gleichen Reihenfolge für jeden Schritt abgeschlossen werden.

- Die enzymatische Reaktion ist linear proportional zu Zeit und Temperatur.
- Die optische Dichte ist eine Funktion der Inkubationszeit und -temperatur. Die in Kapitel "Testverfahren" angegebenen Inkubationszeiten und -temperaturen müssen eingehalten werden.
- Es wird empfohlen, vor Beginn der Testdurchführung alle Reagenzien in einen arbeitsbereiten Zustand zu bringen, die Deckel der Fläschchen zu öffnen, alle benötigten Wells in den Halter zu setzen, usw. Nur eine solche Vorbereitung garantiert für jeden Pipettierschritt gleiche Zeiten ohne Unterbrechung.
- Während der Inkubation bei 37 °C die Mikrotiterstreifen mit Folie abdecken, um Verdunstung zu vermeiden.
- **Wichtiger Hinweis zum Waschvorgang:**
  Das Waschen ist entscheidend. Unsachgemäß gewaschene Kavitäten führen zu fehlerhaften Ergebnissen. Die Sensitivität und Präzision dieses Assays wird erheblich beeinflusst von der korrekten Durchführung des Waschschrittes!
- **Testdurchführung mit vollautomatischen Analysegeräten:**
  Eine automatisierte Testdurchführung mit vollautomatischen, systemoffenen Analysegeräten ist möglich. Die Kombination muss jedoch vom Anwender validiert werden.

6.2 Testdurchführung

Jeder Lauf muss eine Standardkurve beinhalten.
Die Kontrollen dienen der internen Überprüfung der Zuverlässigkeit des Testverfahrens. Sie müssen bei jedem Testdurchlauf gemessen werden.
Das angegebene Testverfahren beschreibt die manuelle Abarbeitung.

Wichtiger Hinweis: Die Genauigkeit dieses Tests wird maßgeblich durch die korrekte Inkubationstemperatur und -zeit beeinflusst! Das Pipettieren der Proben sollte nicht länger als 15 Minuten dauern.

1. Die benötigte Anzahl der Mikrotiter-Wells in der Halterung befestigen.
2. **Je 50 µL *Zero Standard, Standard, Quality Control*** und **verdünnte Probe** mit neuen Plastikspitzen in die entsprechenden Wells pipettieren.
3. Mit Folie abdecken und **60 Minuten** bei **37 °C** inkubieren.
4. Die Vertiefungen folgendermaßen waschen:
   Wenn der Waschschritt manuell durchgeführt wird:
   Den Inhalt der Wells kräftig ausschütteln.
   Wells **3-mal** mit **300 µL** verdünnter *Wash Solution* pro Well waschen.

   Bei Verwendung eines Waschautomaten:
   Wells **3-mal** mit **400 µL** verdünnter *Wash Solution* pro Well waschen.

   Am Ende des Waschschritts die Vertiefungen immer kräftig auf saugfähigem Papier ausklopfen, um verbliebene Flüssigkeit zu entfernen.
5. **50 µL *Enzyme Conjugate*** in jedes Well zugeben.
6. Mit Folie abdecken und **30 Minuten** bei **37 °C** inkubieren.
7. Waschschritt durchführen wie in Schritt 4 beschrieben.
8. **50 µL *Substrate Solution*** in jedes Well pipettieren.
9. **30 Minuten** bei Raumtemperatur inkubieren.
10. Die enzymatische Reaktion durch Zugabe von **100 µL *Stop Solution*** in jedes Well abstoppen.
11. Die Optische Dichte (OD) der Lösung in jedem Well bei **450 nm (Messung) und 620 nm bis 630 nm (Abzug des Hintergrundes, empfohlen)** mit einem Mikrotiterplattenleser bestimmen.
    Es wird empfohlen, die Vertiefungen **innerhalb von 10 Minuten** nach Zugabe der Stopplösung abzulesen.

6.3 Berechnung der Ergebnisse

1. Die Konzentration der Proben kann direkt von der Standardkurve abgelesen werden.
   Die Standards sind bereits vorverdünnt, daher darf die 1:100-Verdünnung der Proben bei der endgültigen Berechnung der Probenkonzentrationen nicht berücksichtigt werden.
2. Bei Doppelbestimmungen muss für jeden Standard, jede Kontrolle und Patientenproben der Mittelwert der beiden OD-Werte verwendet werden. Weichen die beiden Werte erheblich voneinander ab, empfiehlt die DRG, die Proben erneut zu testen.
3. Proben mit Konzentrationen, die den höchsten Standard überschreiten, können mit *Dilution Buffer* weiter verdünnt und wie unter "Testdurchführung " beschrieben erneut gemessen werden oder müssen als > 250 U/mL angegeben werden. Bei der Berechnung der Konzentrationen muss dieser Verdünnungsfaktor berücksichtigt werden.
4. Automatische Methode:
   Die in der Gebrauchsanweisung angegebenen Ergebnisse wurden automatisch mit Hilfe der 4-Parameter-Gleichung bestimmt. (4-Parameter-Rodbard oder 4-Parameter-Marquardt sind die bevorzugten Methoden.) Andere Auswertungsfunktionen können leicht abweichende Werte ergeben.

5. Manuelle Methode:
   Erstellen Sie unter Verwendung von Millimeterpapier eine Standardkurve, indem Sie die (mittlere) OD jedes Standards gegen seine Konzentration auftragen, wobei der OD-Wert auf der vertikalen (Y) Achse und die Konzentration auf der horizontalen (X) Achse liegt. Bestimmen Sie die entsprechende Probenkonzentration anhand der Standardkurve, indem Sie den (mittleren) OD-Wert für jede Probe verwenden.

6.3.1 Beispiel einer typischen Standardkurve

Die folgenden Daten dienen nur zur Orientierung und dürfen **nicht** anstelle der Datengenerierung zum Zeitpunkt des Tests verwendet werden.

Standard		Optische Dichte (450 nm)
Zero Standard	(0 U/mL)	0,147
Standard 1	(31 U/mL)	0,515
Standard 2	(62 U/mL)	0,857
Standard 3	(125 U/mL)	1,423
Standard 4	(250 U/mL)	2,127

7 REFERENZWERTE

Es wird dringend empfohlen, dass jedes Labor seine eigenen Referenzwerte ermittelt.

In einer Studie mit dem DRG Sperm Antibody ELISA wurden die Proben von scheinbar gesunden Probanden untersucht. Dabei ergaben sich folgende Werte:

Normalbereich 0 U/mL – 60 U/mL
Grenzbereich 55 U/mL – 65 U/mL
Erhöhte Werte > 60 U/mL

Bei einem Wert im Grenzbereich (55 U/mL bis 65 U/mL) empfehlen wir eine Nachbestimmung mit einer neuen Probe, die innerhalb der nächsten zwei Wochen entnommen wird.

Die mit diesem Testkit erzielten Ergebnisse sollten niemals als alleinige Grundlage für therapeutische Konsequenzen dienen. Die Ergebnisse müssen zusammen mit anderen klinischen Befunden und diagnostischen Tests des Patienten interpretiert werden.

8 QUALITÄTSKONTROLLE

Gute Laborpraxis erfordert, dass mit jeder Standardkurve Kontrollen mitgeführt werden. Eine statistisch signifikante Anzahl von Kontrollen sollte gemessen werden, um Mittelwerte und Akzeptanzbereiche zu ermitteln und damit eine korrekte Testdurchführung zu gewährleisten.
Es wird empfohlen, die Kontrollproben gemäß den nationalen gesetzlichen Bestimmungen einzusetzen. Durch die Verwendung von Kontrollproben wird eine Tag-zu-Tag-Überprüfung der Ergebnisse erzielt. Es sollten Kontrollen sowohl mit normalem als auch pathologischem Level eingesetzt werden.
Die Kontrollen mit den entsprechenden Ergebnissen des QC-Labors sind im Analysenzertifikat (CoA), das dem Kit beiliegt, aufgeführt. Die im CoA angegebenen Werte und Bereiche beziehen sich stets auf die aktuelle Kitcharge und sollen zum direkten Vergleich der Ergebnisse verwendet werden.
Falls verfügbar, wird ebenfalls empfohlen, an nationalen oder internationalen Qualitätssicherungsprogrammen teilzunehmen, um die Genauigkeit der Ergebnisse zu sichern.
Es sollten geeignete statistische Methoden zur Analyse von Kontrollwerten und Trends angewendet werden. Wenn die Ergebnisse des Assays nicht mit den angegebenen Akzeptanzbereichen des Kontrollmaterials übereinstimmen, sollten die Patientenergebnisse als ungültig eingestuft werden.
In diesem Fall überprüfen Sie bitte die folgenden Bereiche: Pipetten und Zeitnehmer, Photometer, Verfallsdaten der Reagenzien, Lagerungs- und Inkubationsbedingungen, Absaug- und Waschmethode.
Sollten nach Überprüfung der vorgenannten Bereiche keine Fehler erkennbar sein, setzen Sie sich bitte mit Ihrem Lieferanten oder direkt mit der Firma DRG in Verbindung.

9 LEISTUNGSMERKMALE

9.1 Sensitivität

„Limit of Blank" (LoB)	0,490 U/mL
Nachweisgrenze (LoD)	3,367 U/mL
Quantifizierungsgrenze (LoQ)	9,632 U/mL
Messbereich	3,367 U/mL - 250 U/mL
Linearer Bereich	4,333 U/mL - 250 U/mL

Die Daten zu:

9.2 Reproduzierbarkeit (Präzision)

9.3 Wiederfindung

9.4 Linearität

entnehmen Sie bitte der ausführlichen englischen Version der Gebrauchsanweisung.

10 GRENZEN DES VERFAHRENS

Zuverlässige und reproduzierbare Ergebnisse werden erzielt, wenn das Testverfahren mit vollständigem Verständnis der Gebrauchsanweisung und unter Einhaltung der guten Laborpraxis durchgeführt wird.

Jede unsachgemäße Handhabung der Proben oder eine Modifikation dieses Tests kann die Ergebnisse beeinflussen.

10.1 Störsubstanzen

Hämoglobin (bis zu 4 mg/mL), Bilirubin (bis zu 0,5 mg/mL) und Triglyceride (bis zu 7,5 mg/mL) haben keinen Einfluss auf das Testergebnis.

Seren von Patienten mit Lebererkrankungen sollten nicht verwendet werden.

Die Ergebnisse können durch bestimmte pathologische Zustände, wie poly- und monoklonale Gammopathien, Autoimmunerkrankungen oder durch einen veränderten Immunstatus beeinträchtigt werden.

10.2 High-Dose-Hook-Effekt

Ein Hook-Effekt tritt in diesem Test bis zu einer Konzentration von 5000 U/mL an ASA nicht auf.

11 RECHTLICHE GRUNDLAGEN

11.1 Zuverlässigkeit der Ergebnisse

Der Test muss exakt gemäß der Testanleitung des Herstellers abgearbeitet werden. Darüber hinaus muss der Anwender die Regeln der GLP (Gute Laborpraxis) oder andere anwendbare nationale Normen und/oder Gesetze strikt einhalten. Dies betrifft besonders den Gebrauch der Kontrollreagenzien. Es ist sehr wichtig, bei der Testdurchführung stets eine ausreichende Anzahl Kontrollen zur Überprüfung der Genauigkeit und Präzision mitzuführen.
Die Testergebnisse sind nur gültig, wenn alle Kontrollen in den vorgegebenen Bereichen liegen, und wenn alle anderen Testparameter die vorgegebenen Spezifikationen für diesen Assay erfüllen.
Wenn bezüglich eines Ergebnisses Zweifel oder Bedenken bestehen, setzen Sie sich bitte mit der Firma DRG in Verbindung.

11.2 Therapeutische Konsequenzen

Therapeutische Konsequenzen sollten keinesfalls nur aufgrund von Laborergebnissen erfolgen, selbst dann nicht, wenn alle Testergebnisse mit den in 11.1 genannten Voraussetzungen übereinstimmen. Jedes Laborergebnis ist nur ein Teil des klinischen Gesamtbildes eines Patienten.
Nur in Fällen, in denen die Laborergebnisse in akzeptabler Übereinstimmung mit dem allgemeinen klinischen Bild des Patienten stehen, sollten therapeutische Konsequenzen eingeleitet werden.

Das Testergebnis allein sollte niemals als alleinige Grundlage für die Einleitung therapeutischer Konsequenzen dienen.

11.3 Haftung

Jegliche Veränderungen des Testkits und/oder Austausch oder Vermischung von Komponenten unterschiedlicher Chargen von einem Testkit zu einem anderen, können die gewünschten Ergebnisse und die Gültigkeit des gesamten Tests negativ beeinflussen. Solche Veränderungen und/oder Austausch haben den Ausschluss jeglicher Ersatzansprüche zur Folge.
Reklamationen, die aufgrund von Falschinterpretation von Laborergebnissen durch den Kunden gemäß Punkt 11.2 erfolgen, sind ebenfalls abzuweisen. Im Falle jeglicher Reklamation ist die Haftung des Herstellers maximal auf den Wert des Testkits beschränkt. Jegliche Schäden, die während des Transports am Kit entstanden sind, unterliegen nicht der Haftung des Herstellers.

11.4 Meldung von schwerwiegenden Vorkommnissen

Jedes schwerwiegende Vorkommnis im Zusammenhang mit dem Produkt ist dem Hersteller und der zuständigen Behörde des Mitgliedstaats, in dem der Anwender und/oder der Patient niedergelassen ist, zu melden.

1 DESTINAZIONE D'USO

DRG **Sperm Antibody ELISA** è un test immunoenzimatico manuale per la misurazione **quantitativa** di anticorpi anti-spermatozoi umani (ASA) nel siero umano.

Per uso diagnostico *in vitro*. Per uso professionale di laboratorio.

Per ulteriori informazioni sulla destinazione d'uso, consultare le istruzioni per l'uso in inglese.

1.1 Validità scientifica

Gli anticorpi specifici per antigeni degli spermatozoi possono causare infertilità in donne e uomini. L'utilizzo del test Sperm Antibody ELISA è raccomandato per la diagnosi di disordini alla fertilità di origine immunologia.

L'incapacità a procreare è un problema crescente che riguarda fino al 20 % di coppie in età riproduttiva in modo temporaneo o a lungo termine. Nel 5 % - 20 % di questi casi si è evidenziata la presenza di anticorpi anti-spermatozoi nei pazienti di sesso maschile o femminile [1,2,15].

La definizione d'infertilità secondo il WHO (WHO Laboratory Manual for the Examination of Human Semen and Semen Cervical-Mucus Interaction, 1999) è l'assenza di concepimento per un periodo di 12 mesi di rapporti non protetti. La causa più importante di un disordine della fertilità di origine immunologica è la formazione di anticorpi contro antigeni degli spermatozoi.

Gli anticorpi anti-spermatozoi (ASA) esercitano una serie eterogenea di effetti sulla capacità degli spermatozoi di fecondare. L'effetto inibitore di ASA sulla mobilità degli spermatozoi a causa del legame sulla loro superficie e i processi di agglutinazione è ben noto [3].

La penetrazione degli spermatozoi nel muco cervicale è ostacolata dalla presenza di anticorpi anti-spermatozoi nel liquido seminale e/o nel muco cervicale [4]. ASA influenzano negativamente la capacitazione e la reazione acrosomiale degli spermatozoi e quindi impedisce la interazione degli spermatozoi con l'ovocita [5,6].

L'interazione dello spermatozoo con l'ovocita e il successivo legame e penetrazione della zona pellucida possono essere inibiti da ASA. Anche la fusione derivante dell'ovocita e dello spermatozoo può essere pregiudicata dalla presenza di ASA [7,8].

Secondo Crosignani et al. [9] la percentuale di gravidanze in coppie con anticorpi anti-spermatozoi nell'uomo o nella donna è del 38 % inferiore a quella di gruppi di controllo. Inoltre è stata confermato un effetto sull'impianto dell'ovulo e sul primo sviluppo embrionale. È attualmente in discussione una relazione tra anticorpi anti-spermatozoi e aborto.

La presenza di anticorpi anti-spermatozoi in coppie infertili ammonta al 20 % [10,11].

Gli anticorpi anti-spermatozoi possono essere presenti in soluzione nell'eiaculato o legati alla superficie degli spermatozoi. Inoltre, possono essere rilevati nel siero. ASA possono ritrovarsi sia nell'uomo che nella donna [12]. Nella donna ASA possono trovarsi nel muco cervicale, nel liquido del dotto ovarico e nel liquido follicolare. Gli uomini con presenza di anticorpi anti-spermatozoi in oltre il 50 % dei loro spermatozoi presentano un quoziente di fertilità notevolmente ridotto [13].

È stato dimostrato che gli anticorpi anti-spermatozoi (ASA) sono associati alla prostatite cronica che ha un effetto negativo sulla funzione riproduttiva maschile [14].

2 PRINCIPIO DEL TEST

Il test DRG Sperm Antibody ELISA è un dosaggio immuno-assorbente legato a un enzima a fase solida (ELISA) basato sul **principio sandwich**.

I micropozzetti sono ricoperti con una miscela di proteine di spermatozoi.

Durante l'incubazione, gli anticorpi anti-spermatozoi umani nel campione del paziente si legano alla superficie rivestita dei pozzetti.

Una fase di lavaggio rimuove i componenti del campione non legati.

Il coniugato enzimatico aggiunto si lega ai complessi antigene-anticorpo immobilizzati. Il coniugato contiene anticorpi anti-immunoglobulina umana marcati con perossidasi di rafano (HRP).

Dopo una fase di lavaggio per rimuovere tutte le sostanze non legate, la fase solida viene incubata con la soluzione di substrato. La reazione colorimetrica viene bruscamente interrotta con l'aggiunta di soluzione di arresto e viene misurata la densità ottica (DO) del prodotto giallo risultante. L'intensità della colorazione è proporzionale alla concentrazione dell'analita nel campione.

Una curva standard viene costruita tracciando i valori di DO rispetto alle concentrazioni di standard; le concentrazioni di campioni sconosciuti vengono determinate usando questa curva standard.

3 AVVERTENZE E PRECAUZIONI

- Questo kit è solo per uso diagnostico in vitro. Solo per uso professionale di laboratorio.
- Prima di avviare il dosaggio, leggere completamente e attentamente le istruzioni per l'uso. Utilizzare la versione valida delle istruzioni per l'uso fornita con il kit. Assicurarsi che tutto sia stato compreso.
- Non miscelare o utilizzare componenti provenienti da kit con un diverso numero di lotto. Si raccomanda di non scambiare pozzetti di piastre diverse, anche se dello stesso lotto. I kit potrebbero essere stati spediti o conservati in condizioni differenti e le caratteristiche di legame delle piastre potrebbero essere leggermente diverse.
- Non utilizzare reagenti oltre la data di scadenza riportata sulle etichette del kit.
- Non riutilizzare i pozzetti di microtitolazione.
- Non usare reagenti di altri produttori in combinazione con i reagenti di questo kit di test.
- Tutti i reagenti di questo kit sono liquidi trasparenti; la soluzione di substrato è trasparente e incolore. Modifiche nell'aspetto possono influenzare le prestazioni del test. In questo caso, contattare DRG.
- La contaminazione microbica dei reagenti o dei campioni può dare risultati falsi.
- Prima di avviare il test, attendere che i reagenti raggiungano la temperatura ambiente (da 20 °C a 26 °C). La temperatura influenza le letture della densità ottica del dosaggio. Tuttavia, i valori relativi ai campioni dei pazienti non verranno influenzati.
- Usare i volumi indicati secondo quanto previsto dal protocollo. I risultati ottimali del test si ottengono solo utilizzando pipette calibrate e lettori di piastre per microtitolazione.
- Utilizzare serbatoi solo per reagenti singoli. Ciò vale in particolare per i serbatoi per il substrato. L'utilizzo di un serbatoio per l'erogazione di una soluzione di substrato precedentemente usato per la soluzione di coniugato potrebbe causare una colorazione della soluzione. Non versare nuovamente i reagenti nelle fiale originali, poiché potrebbe verificarsi una contaminazione.

Precauzioni generali

- Seguire le linee guida relative alle buone prassi e alla sicurezza in laboratorio.
- Non pipettare mai a bocca ed evitare il contatto dei reagenti e dei campioni con la pelle e le mucose.
- Non fumare, mangiare, bere o applicare cosmetici nelle aree dove vengono manipolati campioni o reagenti del kit.
- Quando si maneggiano campioni e reagenti, indossare camici da laboratorio e guanti in lattice monouso e occhiali di sicurezza ove necessario.

Informazioni sul rischio biologico

- Tutti i reagenti di questo kit che contengono siero o plasma umano sono stati testati e confermati negativi rispetto a HIV I/II, HBsAg e HCV usando procedure approvate dalla FDA. Tuttavia, nessun metodo noto può garantire con certezza assoluta che non sia presente alcun agente infettivo.
- Il dispositivo contiene materiale di origine animale, certificato come apparentemente privo di malattie infettive o contagiose e parassiti nocivi.
- I componenti bovini provengono da paesi in cui non è stata segnalata la BSE (Encefalopatia spongiforme bovina).
- Maneggiare tutti i materiali e i campioni di origine umana o animale come potenziali fonti di malattie infettive.
- Manipolare in conformità con le procedure definite dalle linee guida o dai regolamenti nazionali in materia di rischio biologico e sicurezza. Smaltire i rifiuti secondo le norme e i regolamenti locali.

Informazioni sul rischio chimico e sulla classificazione dei pericoli

- Alcuni reagenti contengono conservanti in concentrazioni non dichiarabili. Tuttavia, in caso di contatto con gli occhi o la pelle, sciacquare immediatamente con acqua.
- La soluzione di substrato contiene un ingrediente in concentrazioni non dichiarabili che provoca grave irritazione oculare. In caso di contatto con gli occhi, sciacquare subito accuratamente ed abbondantemente con una soluzione di lavaggio oculare o acqua. Dopo il contatto con la pelle, lavare abbondantemente con acqua. Togliere gli indumenti contaminati e lavarli prima di riutilizzarli.
- Evitare il contatto con la soluzione di arresto contenente 0,5 M H_2SO_4. Può provocare irritazioni e ustioni alla pelle.
- Trattare i prodotti chimici e i reagenti preparati o usati come rifiuti pericolosi secondo le linee guida o i regolamenti nazionali sulla sicurezza.
- Questo prodotto non contiene sostanze con proprietà cancerogene, mutagene o tossiche per la riproduzione (CMR).

Tutti i reagenti di questo kit di test NON contengono sostanze pericolose in concentrazioni da dichiarare; non è richiesta una classificazione ed etichettatura.

Per informazioni dettagliate fare riferimento alla Scheda di Sicurezza, disponibile su richiesta direttamente da DRG.

4 MATERIALI

4.1 Materiali forniti nel kit

Simbolo	Quantità	Descrizione	Preparazione
Microtiterwells	12 x 8 pozzetti (separabili)	**Piastra per microtitolazione** Rivestita con antigene spermatico.	Pronto all'uso
Dilution Buffer / Zero Standard	1 x 50 mL	**Diluente dei campioni / Standard zero** Concentrazione: 0 U/mL	Pronto all'uso
Standard (Standard 1 - 4)	4 x 0,5 mL	**Standard *** Concentrazioni: 31 – 62 – 125 – 250 U/mL	Pronto all'uso
Quality Control	1 x 0,5 mL	**Controllo** * *Per gli intervalli e i valori di controllo vedere l'etichetta della fiala o il certificato di analisi (CoA).*	Pronto all'uso
Enzyme Conjugate	1 x 8 mL	**Coniugato enzimatico *** Anticorpo IgG anti-umano coniugato con perossidasi di rafano, Colorata di rosso	Pronto all'uso
Substrate Solution	1 x 14 mL	**Soluzione di substrato** Contiene 3,3',5,5'-tetrametilbenzidina (TMB). *Conservare al riparo dalla luce solare diretta.*	Pronto all'uso
Stop Solution	1 x 14 mL	**Soluzione di arresto** Contiene 0,5 M H_2SO_4. *Evitare il contatto con la soluzione di arresto. Potrebbe causare irritazioni cutanee e ustioni.*	Pronto all'uso
Wash Solution	1 x 30 mL	**Soluzione di lavaggio, Concentrato 40X ***	*Vedere "Preparazione dei reagenti".*
Cover foil	1 x	**Pellicola sigillante**	
	1 x	**Istruzioni per l'uso (IFU)**	
	1 x	**Certificato di analisi (CoA)**	
		***** Contiene un conservante privo di mercurio.	

4.2 Materiali necessari ma non forniti

- Lettore di piastre per microtitolazione calibrato (450 nm, con lunghezza d'onda di riferimento tra 620 nm e 630 nm)
- Micropipette a precisione variabile, calibrate
- Incubatore a 37 °C
- Dispositivo di lavaggio manuale o automatico per piastre per microtitolazione
- Carta assorbente
- Acqua distillata
- Timer
- Carta millimetrata o software per il calcolo dei dati

4.3 Conservazione e stabilità del kit

I kit e i reagenti non aperti e **i reagenti aperti** devono essere conservati a una temperatura compresa tra 2 °C e 8 °C.

La piastra per microtitolazione contiene strisce staccabili. Non aprire il sacchetto dei pozzetti finché non raggiunge la temperatura ambiente. I pozzetti inutilizzati devono essere conservati tra 2 °C e 8 °C nel sacchetto di alluminio sigillato con dentro l'essiccante e devono essere utilizzati nel telaio fornito. Dopo l'apertura, il sacchetto di alluminio deve essere chiuso ermeticamente e con la massima cura.

Dopo l'apertura, le fiale di reagente devono essere nuovamente chiuse ermeticamente.

	Temperatura di conservazione	Stabilità
Kit non aperto e reagenti non aperti	2 °C a 8 °C	Fino alla data di scadenza stampata sull'etichetta. Non utilizzare i reagenti dopo questa data!
Kit aperti	2 °C a 8 °C	8 settimane

4.4 Preparazione dei reagenti

Prima dell'uso portare tutti i reagenti e il numero necessario di pozzetti a temperatura ambiente (20 °C a 26 °C).

Soluzione di lavaggio

Aggiungere acqua distillata alla soluzione di lavaggio con concentrazione di 40X.

Diluire 30 mL soluzione di lavaggio concentrata con 1170 mL di acqua distillata fino a un volume finale di 1200 mL.

Stabilità dopo la diluizione:	da 20 °C a 26 °C	1 settimana

4.5 Smaltimento del kit

Lo smaltimento del kit e di tutti i materiali/reagenti usati deve essere effettuato nel rispetto delle normative nazionali. Informazioni specifiche su questo prodotto sono riportate nella Scheda di Sicurezza, sezione 13.

4.6 Kit di test danneggiati

In caso di danni al kit del test o ai componenti, DRG deve essere informato per iscritto, al più tardi una settimana dopo la ricezione del kit. I singoli componenti danneggiati non devono essere utilizzati per i test. Devono essere invece conservati fino a quando non è stata individuata una soluzione definitiva. Successivamente potranno essere smaltiti secondo le norme in vigore.

5 PRELIEVO, CONSERVAZIONE E PREPARAZIONE DEI CAMPIONI

In questo test è possibile utilizzare il seguente materiale campione:

Siero umano

Campioni contenenti azoturo di sodio non devono essere utilizzati nel dosaggio.

In generale si dovrebbe evitare l'uso di campioni emolitici, itterici o lipemici. Per ulteriori informazioni consultare il capitolo "*Sostanze interferenti*".

Per la determinazione degli anticorpi anti-spermatozoi nel plasma seminale si prega di utilizzare il Sperm Antibody (seminal plasma) ELISA (REF EIA-4249).

5.1 Prelievo dei campioni

Siero: Prelevare il sangue mediante venipuntura (ad es. Sarstedt Monovette per siero), far coagulare e separare il siero centrifugando a temperatura ambiente. Non centrifugare prima che la coagulazione sia completata. Campioni di pazienti in terapia anticoagulante potrebbero richiedere più tempo per la coagulazione.

Il sangue intero non deve essere congelato prima della centrifugazione.

5.2 Conservazione dei campioni

I campioni devono essere conservati ben tappati prima di eseguire il dosaggio. Se vengono conservati in congelatore, congelarli solo una volta. I campioni scongelati devono essere invertiti più volte prima di eseguire il test.

Stabilità:	da 2 °C a 8 °C	4 giorni
	a -20 °C (in aliquote)	fino a 2 mesi

5.3 Preparazione dei campioni

Prima del dosaggio, diluire ogni campione paziente **1:100** con il tampone di diluizione.

Esempio:

diluizione 1:100: 5 µL campione + 495 µL *Dilution Buffer* (miscelare accuratamente)

Stabilità di campioni diluiti:	da 2 °C a 8 °C	4 giorni
	a -20 °C (in aliquote)	7 giorni

Nota: Il controllo (*Quality Control*) è pronto all'uso e non deve essere diluito!

6 PROCEDURA DEL DOSAGGIO

6.1 Note sulla procedura

- Portare tutti i reagenti e i campioni a temperatura ambiente (tra 20 °C e 26 °C) prima dell'uso.
- Miscelare tutti i reagenti senza formare schiuma.
- Non scambiare tra loro i tappi delle fiale di reagente per evitare contaminazioni incrociate.
- Per ogni componente, standard, controllo o campione è necessario utilizzare un nuovo puntale di pipettaggio in plastica monouso per evitare il carry-over.
- Per evitare la contaminazione incrociata e risultati falsamente elevati, pipettare i campioni dei pazienti e dispensare il coniugato accuratamente sul fondo dei pozzetti senza produrre schizzi.
- Per garantire risultati ottimali del test, mescolare accuratamente il contenuto dei pozzetti della piastra per microtitolazione.

- Non lasciare asciugare i pozzetti durante il dosaggio; aggiungere i reagenti subito dopo aver completato le fasi di risciacquo.
- Dopo l'avvio del test, completare tutti i passaggi senza interruzioni e seguendo la stessa sequenza per ogni passaggio.
- La reazione enzimatica è linearmente proporzionale al tempo e alla temperatura.
- La densità ottica è una funzione del tempo di incubazione e della temperatura. Rispettare i tempi e le temperature di incubazione come indicato nel capitolo "Procedura del test".
- Prima di avviare il dosaggio, è raccomandato fare in modo che tutti i reagenti siano pronti, i tappi rimossi, tutti i pozzetti necessari fissati sul supporto ecc. Questo garantirà un tempo trascorso identico per ogni fase di pipettaggio senza interruzioni.
- Durante l'incubazione a 37 °C coprire le strisce di microtitolazione con un foglio per evitare l'evaporazione.
- **Nota importante sulla procedura di lavaggio:**
  Il lavaggio è fondamentale. I pozzetti lavati in modo improprio daranno risultati errati. La sensibilità e la precisione di questo dosaggio sono notevolmente influenzate dalla corretta esecuzione della procedura di lavaggio!
- **Prestazioni del test utilizzando dispositivi di analisi completamente automatizzati:**
  È possibile eseguire test automatizzati utilizzando dispositivi di analisi a sistema aperto completamente automatizzati. Tuttavia, la combinazione deve essere convalidata dall'utente.

6.2 Procedura del test

Ogni analisi deve includere una curva standard.

I controlli servono come controlli interni per la valutazione dell'affidabilità della procedura del test. Essi devono essere dosati a ogni esecuzione del test.

La procedura del test indicata descrive l'elaborazione manuale.

Nota importante: l'accuratezza di questo dosaggio è notevolmente influenzata dalla corretta temperatura e dal tempo di incubazione. Il pipettaggio dei campioni non deve superare i 15 minuti.

1. Fissare il numero desiderato di pozzetti di microtitolazione nel telaio di supporto.
2. Pipettare **50 µL** di ogni ***Zero Standard, Standard, Quality Control,*** e **campione diluiti** nei pozzetti appropriati, utilizzando puntali monouso.
3. Coprire con un foglio e incubare per **60 minuti** a **37 °C**.
4. Lavare i pozzetti nel modo seguente:
   Qualora la fase di lavaggio venga eseguita manualmente:
   Agitare energicamente il contenuto dei pozzetti.
   Risciacquare ogni pozzetto **3 volte** con **300 µL** di soluzione di lavaggio diluita.

   Qualora si usi un dispositivo di lavaggio di micropiastre automatizzato:
   Risciacquare ogni pozzetto **3 volte** con **400 µL** di soluzione di lavaggio diluita.

   Al termine della fase di lavaggio, scuotere sempre energicamente i pozzetti su carta assorbente per rimuovere le gocce residue.
5. Aggiungere **50 µL** di ***Enzyme Conjugate*** in ogni pozzetto.
6. Coprire con un foglio e incubare per **30 minuti** a **37 °C**.
7. Lavare come descritto al punto 4.
8. Pipettare **50 µL** di ***Substrate Solution*** in ogni pozzetto.
9. Incubare per **30 minuti** a temperatura ambiente.
10. Arrestare la reazione enzimatica aggiungendo **100 µL** di ***Stop Solution*** in ogni pozzetto.
11. Misurare la densità ottica (DO) della soluzione in tutti i pozzetti a **450 nm (lettura) e tra 620 e 630 nm (sottrazione del fondo, consigliata)** utilizzando un lettore per piastre per microtitolazione.
    Si consiglia di effettuare la lettura dei pozzetti **entro 10 minuti** dall'aggiunta della soluzione di arresto.

6.3 Calcolo dei risultati

1. La concentrazione dei campioni può essere letta direttamente dalla curva standard.
   Gli standard sono già pre-diluiti, pertanto la diluizione 1:100 dei campioni non deve essere presa in considerazione per il calcolo finale delle concentrazioni dei campioni.
2. Per le misurazioni duplicate, è necessario considerare la media dei due valori di densità ottica (DO) per ogni standard, controllo e campione di paziente Se i due valori si discostano sostanzialmente l'uno dall'altro, DRG raccomanda di ritestare i campioni.
3. I campioni con concentrazioni superiori a quelle dello standard più elevato possono essere ulteriormente diluiti con Dilution Buffer e analizzati nuovamente secondo quanto descritto in "Procedura del test"; in alternativa, devono essere refertati come > 250 U/mL. Per il calcolo delle concentrazioni è necessario considerare questo fattore di diluizione.
4. Metodo automatizzato:
   I risultati nelle istruzioni per l'uso sono stati calcolati automaticamente utilizzando un adattamento della curva logistica a quattro parametri (4PL). (I metodi preferiti sono 4PL Rodbard o 4PL Marquardt.) Altre funzioni di riduzione dei dati potrebbero dare risultati leggermente diversi.

5. Metodo manuale:
   Utilizzando carta millimetrata, costruire una curva standard tracciando la (media) DO ottenuta da ogni standard contro la rispettiva concentrazione con il valore DO sull'asse verticale (Y) e la concentrazione sull'asse orizzontale (X). Determinare la concentrazione del campione corrispondente dalla curva standard utilizzando il valore OD (medio) per ogni campione.

6.3.1 Esempio di una curva standard tipica

I seguenti dati vengono riportati a scopo esclusivamente dimostrativo e **non possono** sostituire i dati generati al momento di esecuzione del dosaggio.

Standard		Densità ottica (450 nm)
Zero Standard	(0 U/mL)	0,147
Standard 1	(31 U/mL)	0,515
Standard 2	(62 U/mL)	0,857
Standard 3	(125 U/mL)	1,423
Standard 4	(250 U/mL)	2,127

7 VALORI DI RIFERIMENTO

È fortemente consigliato che ogni laboratorio determini i propri valori di riferimento.

In uno studio condotto su soggetti apparentemente sani, usando il test DRG Sperm Antibody ELISA, sono stati osservati i seguenti risultati:

Valori normali	0 - 60 U/mL
Intervallo limite	55 U/mL - 65 U/mL
Valori elevati	> 60 U/mL

Se il valore è nell'intervallo limite (da 55 U/mL a 65 U/mL), si consiglia di ripetere la determinazione con un nuovo campione raccolto entro le due settimane successive.

I risultati da soli non dovrebbero essere l'unico motivo per eventuali conseguenze terapeutiche. Correlare i risultati ad altre osservazioni cliniche e test diagnostici.

8 CONTROLLO DI QUALITÀ

Secondo le buone prassi di laboratorio, i controlli devono essere eseguiti per ogni curva standard. Un numero statisticamente significativo di controlli dovrebbe essere analizzato per stabilire i valori medi e gli intervalli accettabili per garantire prestazioni adeguate.
Si raccomanda di usare campioni di controllo secondo quanto previsto dalle norme locali o nazionali. Si consiglia di utilizzare campioni di controllo per garantire la validità giornaliera dei risultati. Utilizzare controlli sia a livelli normali che a livelli patologici.
I controlli e i corrispondenti risultati del Laboratorio di controllo qualità sono riportati nel Certificato di Analisi (CoA) inserito nel kit. I valori e gli intervalli indicati sul Certificato di analisi si riferiscono sempre al lotto del kit corrente e devono essere utilizzati per il confronto diretto dei risultati.
Se disponibili, si raccomanda inoltre di partecipare ai programmi nazionali o internazionali della valutazione della qualità per assicurare la precisione dei risultati.
Per analizzare i valori di controllo e gli andamenti, utilizzare metodi statistici appropriati. Se i risultati del dosaggio non si adattano agli intervalli di riferimento stabiliti per i controlli, i risultati dei pazienti non possono essere considerati validi.
In tal caso, verificare le seguenti aree tecniche: Dispositivi di pipettaggio e temporizzazione; fotometro, date di scadenza dei reagenti, condizioni di conservazione e incubazione, metodi di aspirazione e lavaggio.
Dopo aver verificato le voci sopra indicate senza riscontrare alcun errore, contattare il proprio distributore o direttamente DRG.

9 CARATTERISTICHE PRESTAZIONALI

9.1 Sensibilità

Limite del bianco (LoB)	0,490 U/mL
Limite del bianco (LoD)	3,367 U/mL
Limite di quantificazione (LoQ)	9,632 U/mL
Intervallo di misurazione	3,367 U/mL - 250 U/mL
Intervallo lineare	4,333 U/mL - 250 U/mL

I dati relativi a:

9.2 Riproducibilità (precisione)

9.3 Recupero

9.4 Linearità

sono riportati nella versione inglese dettagliata delle istruzioni per l'uso.

10 LIMITAZIONI DELLA PROCEDURA

Se si esegue la procedura del dosaggio con una completa comprensione delle istruzioni per l'uso e nel rispetto delle buone prassi di laboratorio, i risultati ottenuti saranno affidabili e riproducibili.

Qualsiasi manipolazione impropria dei campioni o modifica di questo test potrebbe influenzare i risultati.

10.1 Sostanze interferenti

L'emoglobina (fino a 4 mg/mL), bilirubina (fino a 0,5 mg/mL) e i trigliceridi (fino a 7,5 mg/mL) non influiscono in alcun modo sui risultati del dosaggio.

Sieri da pazienti con patologia epatiche non devono essere analizzati.

I risultati potrebbero essere sensibilmente alterati da certe condizioni patologiche, quali le gammopatie poli- e monoclonali, le malattie autoimmuni o gli alterati stati immunitari.

10.2 Effetto gancio a dose elevata

Non viene rilevato alcun "Effetto gancio a dose elevata" fino a 5000 U/mL di anticorpi anti-spermatozoi.

11 ASPETTI LEGALI

11.1 Affidabilità dei risultati

Il test deve essere eseguito esattamente secondo quanto previsto dalle istruzioni d'uso del produttore. Inoltre, l'utente deve attenersi rigorosamente alle norme delle buone prassi di laboratorio o ad altri standard nazionali e/o leggi in vigore. Questo è particolarmente importante per l'uso dei reagenti di controllo. È importante includere sempre, all'interno della procedura del test, un numero sufficiente di controlli per convalidare l'accuratezza e la precisione del test.

I risultati del test sono validi solo se tutti i controlli sono compresi negli intervalli specificati e se anche tutti gli altri parametri del test rientrano nelle specifiche del dosaggio. In caso di dubbi o preoccupazioni in relazione a un risultato, contattare DRG.

11.2 Conseguenze terapeutiche

Le conseguenze terapeutiche non devono mai basarsi esclusivamente sui risultati di laboratorio, anche qualora tutti i risultati dei test concordino con gli elementi come indicato al punto 11.1. Qualsiasi risultato di laboratorio costituisce solo una parte del quadro clinico complessivo di un paziente.

Si dovrebbero trarre conseguenze terapeutiche solo nei casi in cui i risultati di laboratorio concordino in modo accettabile con il quadro clinico complessivo del paziente.

Il risultato del test, di per sé, non deve mai essere l'unico fattore determinante per una decisione terapeutica.

11.3 Responsabilità legali

Qualsiasi modifica del kit di test e/o scambio o miscela di qualsiasi componente di lotti diversi da un kit di test a un altro potrebbe influenzare negativamente i risultati previsti e la validità del test nel suo complesso. Tali modifiche e/o scambi rendono nulla qualsiasi richiesta di sostituzione.

Anche i reclami presentati a causa di un'errata interpretazione da parte del cliente dei risultati di laboratorio indicati al punto 11.2 non saranno ritenuti validi.

In ogni caso, in caso di reclamo, la responsabilità del produttore non potrà superare il valore del kit di test. Il produttore non sarà responsabile di eventuali danni causati al kit di test durante il trasporto.

11.4 Segnalazione di incidenti gravi

Tutti gli incidenti gravi relativi a questo prodotto devono essere notificati al fabbricante e all'autorità competente dello Stato membro di residenza dell'utente e/o del paziente.

1 FINALIDAD PREVISTA

El **DRG Sperm Antibody ELISA** es un inmunoensayo enzimático manual para realizar diagnósticos **cuantivativos** de anticuerpos dirigidos contra espermatozoides humanos (AAE) en suero humano.

Para uso diagnóstico *in vitro*. Para uso profesional de laboratorio.

Para obtener más información sobre el uso previsto, consulte la versión en inglés de las instrucciones de uso.

1.1 Validez científica

Los anticuerpos dirigidos contra antígenos de espermatozoides pueden causar infertilidad en mujeres y hombres. Se recomienda el uso del Sperm Antibody ELISA para el diagnóstico de desórdenes de infertilidad provocados por auto inmunidad.

El no tener descendencia de manera no deseada es un problema en crecimiento con el que más del 20 % de las parejas en edad reproductiva se enfrentan temporalmente o de manera más duradera. En el 5 % - 20 % de estos casos se detecta la presencia de anticuerpos anti-espermatozoide en el paciente masculino o femenino. ([1,2,15].

De acuerdo con la WHO (WHO Manual de laboratorio para el Examen de Semen Humano y la interacción del Semen y el mucus cervical, 1999), la definición de infertilidad es la ausencia de una concepción en 12 meses de relaciones sexuales sin protección. La principal causa de un desorden de infertilidad inmunológico es la formación de anticuerpos dirigidos contra antígenos espermáticos.

Los anticuerpos anti-espermatozoides (AAE) ejercen efectos heterogéneos en la habilidad de los espermatozoides para fertilizar. Se conoce el efecto inhibitorio de los anticuerpos anti-espermatozoides en la movilidad de estos debido a su unión a la superficie y por procesos de aglutinación [3].

La penetración de los espermatozoides en el mucus cervical se debilita en presencia de AAE en el plasma seminal y/o en el mucus cervical [4]. AAE producen una influencia negativa en la capacitación y en la reacción del acrosoma del espermatozoide y por ello impiden la interacción del espermatozoide con el oocito [5,6].

La interacción del espermatozoide con el oocito y la subsiguiente unión y la penetración de la zona pelúcida puede ser inhibida por AAE. La subsiguiente fusión del oocito y el espermatozoide puede ser impedida también por la presencia de AAE [7,8].

De acuerdo con Crosignani *et al*. [9] la razón de embarazos en parejas con anticuerpos anti-espermatozoides por parte del hombre o la mujer es un 38 % menor que los grupos control. Más aún, también se confirma una influencia en la implantación e en el desarrollo temprano del embrión. Se discute la asociación de anticuerpos anti-espermatozoides y el aborto espontáneo.

La frecuencia de AAE en parejas infértiles asciende al 20 % [10,11].

AAE pueden aparecer disueltos en el eyaculado o unidos a la superficie de los espermatozoides. Además, se pueden detectar en suero. Pueden encontrarse en hombres y en mujeres [12]. En mujeres, AAE pueden encontrares en el mucus cervical, en el líquido del oviducto y el líquido folicular. Los hombres que tienen más del 50 % de sus espermatozoides recubiertos con AAE muestran una llamativa reducción de la razón de fertilidad [13].

Se ha demostrado que los anticuerpos antiespermatozoides (AAE) están asociados con la prostatitis crónica, que tiene un efecto negativo en la función reproductiva masculina [14].

2 PRINCIPIO DEL TEST

El DRG Sperm Antibody ELISA es un ensayo inmunoabsorbente ligado a enzimas (ELISA) en fase sólida basado en el **principio de sándwich.**

Los pocillos de las placas están recubiertos con una mezcla de proteínas de espermatozoides.

Durante la incubación, los anticuerpos anti-espermatozoides de las muestras (estándares, controles, muestra del paciente) se unen a la superficie recubierta de los pocillos.

Un paso de lavado elimina los componentes de la muestra no ligados.

El conjugado enzimático añadido se liga a los complejos antígeno-anticuerpo inmovilizados. El conjugado enzimático contiene anticuerpos de immunoglobulina antihumana, marcados con peroxidasa de rábano (HRP).

Tras un proceso de lavado para eliminar cualquier sustancia sin unir, la fase sólida se incuba con la solución de sustrato. La reacción colorimétrica se detiene añadiendo una solución de parada, y se realiza una medición de la densidad óptica (DO) del producto amarillo resultante.

La intensidad del color es proporcional a la concentración del analito en la muestra.

Se crea una curva estándar cotejando los valores de DO con las concentraciones de estándares, y las concentraciones de las muestras desconocidas se determinan usando esta curva estándar.

3 ADVERTENCIAS Y PRECAUCIONES

- Este kit es exclusivo para diagnóstico *in vitro*. Para uso profesional exclusivo de laboratorio.
- Antes de iniciar el ensayo, lea todas las instrucciones de uso detenidamente. Utilice la versión en vigor de las instrucciones de uso suministradas junto con el kit. Asegúrese de que todo está claro.
- No mezcle ni utilice componentes de kits con números de lote distintos. No es aconsejable intercambiar pocillos de placas diferentes, aun cuando pertenezcan al mismo lote. Puede que los kits se hayan enviado o almacenado en unas condiciones distintas y existe la posibilidad de que las características de unión de las placas sean ligeramente diferentes.
- No use los reactivos una vez superada la fecha de caducidad indicada en las etiquetas del kit.
- No reutilice los pocillos de microtítulo.
- No use reactivos de otros fabricantes junto con los reactivos de este kit de prueba.
- Todos los reactivos incluidos en este kit son líquidos transparentes. La solución de sustrato es transparente e incolora. Cualquier cambio en su apariencia podría afectar al rendimiento de la prueba. Si así es, póngase en contacto con DRG.
- Una contaminación microbiana de los reactivos o de las muestras podría arrojar resultados falsos.
- Antes de iniciar la prueba, deje que los reactivos alcancen la temperatura ambiente (entre 20 °C y 26 °C). La temperatura afectará a las lecturas de densidad óptica del ensayo.
- Todos los volúmenes indicados se deben respetar siguiendo el protocolo. Solo se obtendrán unos resultados de prueba óptimos si se usan pipetas calibradas y lectores de placas de microtítulo.
- Utilice depósitos solo con reactivos únicos. Esto es especialmente cierto en el caso de los depósitos de sustratos. Si se usa un depósito para dispensar una solución de sustrato que ya se usó previamente con la solución de conjugado, la solución podría acabar tiznada. No vierta reactivo de nuevo a su vial original, ya que podría producirse una contaminación del reactivo.

Precauciones generales

- Siga las buenas prácticas de laboratorio y las instrucciones de seguridad.
- No pipetee nunca con la boca y evite el contacto con los reactivos y las muestras con la piel y las membranas mucosas.
- No fume, coma, beba ni aplique sustancias cosméticas en las áreas de manipulación de muestras o reactivos del kit.
- Utilice batas de laboratorio y guantes de látex desechables al manipular reactivos y, si fuera necesario, gafas protectoras también.

Información de riesgo biológico

- Todos los reactivos de este kit de prueba que contienen plasma o suero humano se han analizado y se ha confirmado su negativo en HIV I/II, HBsAg y HCV mediante procedimientos aprobados de la FDA. Pese a ello, no existe ningún método de prueba conocido que ofrezca garantía total de no presencia de agentes infecciosos.
- El producto contiene materia de origen animal certificado como aparentemente libre de enfermedades contagiosas o infecciosas y parásitos nocivos.
- Los componentes bovinos proceden de países en los que no se han notificado casos de EEB (encefalopatía espongiforme bovina).
- Todas las materias y muestras de origen humano o animal deben tratarse como si existiera la posibilidad de transmisión de enfermedades infecciosas.
- La manipulación de material debe realizarse siguiendo los procedimientos establecidos según la normativa o instrucción de seguridad y riesgo biológico nacional pertinente. Los residuos deben desecharse respetando las normativas o regulaciones locales correspondientes.

Información sobre riesgos químicos y la clasificación de riesgos

- Algunos reactivos contienen conservantes con niveles de concentración no declarables. Aún así, en caso de contacto con los ojos o la piel, enjuague de inmediato con agua.
- La solución de sustrato contiene un ingrediente con niveles de concentración no declarables que puede provocar irritaciones de los ojos graves. En caso de posible contacto con los ojos, enjuáguelos concienzudamente de inmediato con agua o con algún colirio. Tras un contacto con la piel, lave con abundante agua. Quítese la ropa contaminada y lávela antes de volver a utilizarla.
- Evite el contacto con una solución de parada que contenga 0,5 M H_2SO_4. Puede provocar irritación cutánea o quemaduras.
- Los componentes químicos y los reactivos preparados o usados se deben tratar como desecho peligroso siguiendo la normativa o instrucción de seguridad nacional pertinente.
- Este producto no contiene sustancias con propiedades carcinogénicas, mutagénicas o tóxicas para la reproducción (CMR).

Los reactivos de este kit de prueba NO contienen sustancias peligrosas con niveles de concentración que deban declararse. No es necesario clasificarlo o etiquetarlo a tal efecto.

Para obtener información detallada, consulte la ficha de datos de seguridad, que puede solicitar directamente a DRG.

4 MATERIAL

4.1 Material suministrado junto con el kit

Símbolo	Cantidad	Descripción	Preparación
Microtiterwells	12 x 8 pocillos (por separado)	**Placa de microtítulo** Recubierta con proteínas de espermatozoides	Listo para usar
Dilution Buffer / Zero Standard	1 x 50 mL	**Solución para dilución de la muestra / Estándar cero *** Concentración: 0 U/mL	Listo para usar
Standard (Standard 1 - 4)	4 x 0,5 mL	**Estándares *** Concentraciones: 31 – 62 – 125 – 250 U/mL	Listo para usar
Quality Control	1 x 0,5 mL	**Control** * *Para obtener los intervalos y valores de control, consulte la etiqueta del vial o el certificado de análisis (CoA).*	Listo para usar
Enzyme Conjugate	1 x 8 mL	**Conjugado enzimático*** Anticuerpo anti-IgG humana conjugado con peroxidasa de rábano; coloreado en rojo.	Listo para usar
Substrate Solution	1 x 14 mL	**Solución de sustrato** Contiene 3,3‘,5,5‘-tetrametilbenzidina (TMB). *Mantener lejos de la luz solar directa.*	Listo para usar
Stop Solution	1 x 14 mL	**Solución de parada** Contiene 0,5 M H_2SO_4. *Evite el contacto con la solución de parada. Puede provocar irritación cutánea o quemaduras.*	Listo para usar
Wash Solution	1 x 30 mL	**Solución de lavado, Concentrado 40X***	*Ver «Preparación de los reactivos».*
Cover foil	1 x	**Lámina de cubierta**	
	1 x	**Instrucciones de uso (IFU)**	
	1 x	**Certificado de análisis (CoA)**	
		* Contiene conservante sin mercurio.	

4.2 Materiales necesarios no suministrados

– Un lector de placas de microtítulo calibrado (450 nm, con una longitud de onda de referencia de entre 620 nm y 630 nm)
– Micropipetas de precisión variable calibradas
– Incubadora a 37 °C
– Equipamiento manual o automático para lavar los pocillos de placa de microtítulo
– Papel absorbente
– Agua destilada
– Cronómetro
– Papel cuadriculado o software para la reducción de datos

4.3 Almacenamiento y estabilidad del kit

Los reactivos y kits sin abrir, así como **los reactivos abiertos**, se deben almacenar a una temperatura entre 2 °C y 8 °C.

La placa de microtítulo contiene bandas desprendibles. No abra la bolsa de los pocillos hasta que esté a temperatura ambiente. Los pocillos sin usar se deben almacenar a una temperatura entre 2 °C y 8 °C en la bolsa de aluminio sellada junto con el desecante, y deben utilizarse en el bastidor indicado. Una vez abierta la bolsa de aluminio, hay que tener cuidado para volver a cerrarla herméticamente.

Una vez abiertos, los viales de reactivo se deben volver a cerrar herméticamente.

	Temperatura de almacenamiento	Estabilidad
Kit y reactivos sin abrir	2 °C a 8 °C	Hasta la fecha de caducidad indicada en la etiqueta impresa. No use los reactivos una vez superada esta fecha.
Kit abierto	2 °C a 8 °C	8 semanas

4.4 Preparación de los reactivos

Antes de usarlos, espere a que todos los reactivos y la cantidad de bandas necesaria estén a temperatura ambiente (20 °C a 26 °C).

Solución de lavado

Añada agua destilada a la solución de lavado concentrada a 40X (*Wash Solution*).

Diluya 30 mL de solución de lavado concentrada con 1170 mL de agua destilada hasta llegar a un volumen final de 1200 mL.

Estabilidad tras la dilución:	entre 20 °C y 26 °C	1 semana

4.5 Eliminación del kit

El kit y todo los materiales/reactivos usados deberán desecharse siguiendo la regulación nacional correspondiente. En el apartado 13 de la ficha de datos de seguridad encontrará información general relativa a este producto.

4.6 Kits de prueba dañados

En caso de que el kit de prueba o alguno de sus componentes estén dañados, se deberá comunicar a DRG por escrito como máximo una semana después de la recepción del kit. Los componentes dañados no deben usarse en ninguna serie de prueba. Deberán guardarse hasta que el asunto se resuelva. Tras ello, deberán desecharse siguiendo la regulación oficial en vigor.

5 TOMA DE LA MUESTRA, ALMACENAMIENTO Y PREPARACIÓN

En este ensayo pueden ser usados los tipos de muestra detallados a continuación:

Suero humano.

En el ensayo no deben usarse muestras que contengan azida de sodio.

En términos generales, absténgase de usar muestras hemolíticas, lipémicas o con ictericia. Para obtener más información, consulte el capítulo «*Sustancias interferentes*».

Para la determinación de anticuerpos anti-espermatozoides en plasma seminal, utilice nuestro Sperm Antibody (seminal plasma) ELISA (REF EIA-4249)

5.1 Toma de muestras

Suero: Extraiga sangre mediante venipuntura (p. ej., Sarstedt Monovette para suero), deje que coagule y separe el suero mediante centrifugado a temperatura ambiente. No centrifugue hasta que la coagulación se realice por completo. Puede que las muestras de los pacientes sometidos a terapia anticoagulante necesiten más tiempo para coagularse.

La sangre total no debe congelarse antes del centrifugado.

5.2 Almacenamiento de muestras

Las muestras deben almacenarse cerradas herméticamente antes de realizar el ensayo. Si se van a almacenar congeladas, se pueden congelar solo una vez. Las muestras descongeladas se deben invertir varias veces antes de analizarlas.

Estabilidad	entre 2 °C y 8 °C	4 días
	a -20 °C (en alícuotas)	hasta 2 meses

5.3 Preparación de las muestras

Antes de ensayar, diluya la muestra de cada paciente **1:100** con tampón de dilución (*Dilution Buffer*).

Ejemplo:

Dilución 1:100: 5 µL muestra + 495 µL *Dilution Buffer* (mezclar totalmente)

Estabilidad de las muestras diluidas	entre 2 °C y 8 °C	4 días
	a -20 °C (en alícuotas)	7 días

Nota: El control (*Quality Control*) está listo para usar do y no debe ser diluido!

6 PROCEDIMIENTO DEL ENSAYO

6.1 Notas sobre el procedimiento

- Todos los reactivos y muestras deben estar a temperatura ambiente (entre 20 °C y 26 °C) antes de usarlos.
- Todos los reactivos deben mezclarse sin que generen espuma.
- No intercambie los tapones de los viales de reactivo para evitar posibles contaminaciones cruzadas.
- Use puntas de pipeta de plástico desechables nuevas con cada estándar, control o muestra para evitar posibles contaminaciones por arrastre.
- Para evitar posibles contaminaciones cruzadas y resultados engañosamente elevados, pipetee las muestras de paciente y dispense conjugado sin salpicar de forma minuciosa en el fondo de los pocillos.
- Mezcle bien el contenido de los pocillos de la placa de microtítulo para procurar que los resultados de la prueba sean correctos.
- No deje que los pocillos se sequen durante el ensayo; añada reactivo inmediatamente una vez acabados los pasos de enjuagado.

- Una vez iniciada la prueba, todos los pasos se deben completar de forma ininterrumpida y en la misma secuencia de cada paso.
- La reacción enzimática es linealmente proporcional al tiempo y la temperatura.
- La densidad óptica es una función del tiempo y la temperatura de incubación. Es importante respetar los tiempos y las temperaturas de incubación que se indican en el capítulo «Procedimiento de la prueba».
- Antes de iniciar el ensayo, se recomienda tener todos los reactivos listos, los tapones quitados, todos los pocillos necesarios fijados en el soporte, etc. De este modo, se asegurará de que el tiempo que va a transcurrir en cada paso de pipeteo es el mismo y sin interrupción alguna.
- Durante la incubación a 37 °C, cubra las bandas de microtítulo con lámina de cubierta para evitar la evaporación.
- **Nota importante sobre el procedimiento de lavado:**
  El lavado es tremendamente importante. Con unos pocillos mal lavados se obtendrán resultados engañosos. La sensibilidad y la precisión de este ensayo dependen enormemente de un correcto rendimiento del procedimiento de lavado.
- **Rendimiento de pruebas mediante aparatos de análisis completamente automáticos:**
  Se puede obtener un rendimiento de pruebas automático usando aparatos de análisis de sistema abierto y completamente automáticos. Sin embargo, la combinación debe estar validada por el usuario.

6.2 Procedimiento de la prueba

Cada serie debe incluir una curva estándar.

Los controles actúan como controles internos de la fiabilidad del procedimiento del test. Se deben analizar en cada serie de prueba.

El procedimiento de prueba aquí indicado describe un procesamiento manual.

Nota importante: La precisión de este ensayo depende enormemente de que haya una temperatura y un tiempo de incubación correctos.
El pipeteo de las muestras no debe superar los 15 minutos.

1. Fije la cantidad de pocillos de microtítulo que desee en el soporte del bastidor.
2. Pipetee **50 µL** de cada ***Zero Standard, Standard***, cada ***Quality Control*** y cada **muestra diluida** con puntas nuevas desechables en los pocillos correspondientes.
3. Cubrir con un folio e incubar durante **60 minutes** a **37 °C**.
4. Lave los pocillos del siguiente modo:
   Si el paso de lavado se efectúa manualmente:
   Agite enérgicamente el contenido de los pocillos.
   Enjuague los pocillos **3 veces** con **300 µL** de solución de lavado diluida por pocillo.

   Si se usa un aparato de lavado automático de placas:
   Enjuague los pocillos **3 veces** con **400 µL** de solución de lavado diluida por pocillo.

   Al término de cada paso de lavado, frote bien los pocillos con papel absorbente para eliminar las gotas residuales!
5. Añada **50 µL** de ***Enzyme Conjugate*** en cada pocillo.
6. Cubrir con un folio e incube durante **30 minutos** a a **37 °C**.
7. Lavar como se describe en el paso 4.
8. Pipetee **50 µL** de ***Substrate Solution*** en cada pocillo.
9. Incube durante **30 minutos** a temperatura ambiente.
10. Detenga la reacción enzimática añadiendo **100 µL** de ***Stop Solution*** a cada pocillo.
11. Mida la densidad óptica (DO) de la solución a **450 nm (lectura) y entre 620 nm y 630 nm (reducción del fondo, recomendada)** con un lector de placas de microtítulo. Se recomienda realizar la lectura de los pocillos **en los 10 minutos** siguientes a la incorporación de la solución de parada.

6.3 Cálculo de los resultados

1. La concentración de las muestras se puede leer directamente de la curva estándar.
   Los estándares ya están prediluidos, por lo que no se ha tenido en cuenta la dilución de 1:100 de las muestras para el cálculo final de las concentraciones de las muestras.
2. Para determinar los duplicados, se debe hacer la media de los dos valores de densidad óptica (DO) de cada estándar, cada control y cada muestra de paciente. Si estos dos valores se desvían considerablemente el uno de otro, DRG recomienda volver a analizar las muestras.
3. Las muestras con concentraciones por encima del estándar más alto se pueden seguir diluyendo con Dilution Buffer y volver a analizarse según se describe en «Procedimiento de la prueba », o bien comunicarse como > 250 U/mL.
   En el cálculo de las concentraciones se debe tener en cuenta este factor de dilución.
4. Método automático:
   Los resultados de estas instrucciones de uso se han calculado automáticamente mediante un ajuste de curva de una función logística de cuatro parámetros (4PL). (Los métodos de preferencia son 4PL Rodbard o 4PL Marquardt.) Otras funciones de reducción de datos podrían arrojar resultados ligeramente distintos.

5. Método manual:
Usando un papel cuadriculado, construya una curva estándar trazando el valor medio de densidad óptica obtenido de cada estándar en comparación con su concentración con un valor de densidad óptica en el eje vertical (Y) y la concentración en el eje horizontal (X). Determine la concentración de muestra correspondiente de la curva estándar usando el valor medio de densidad óptica de cada muestra.

6.3.1 Ejemplo de curva estándar típica

Los siguientes datos se proporcionan únicamente a título ilustrativo y **no** se pueden usar como reemplazo de las generaciones de datos en el momento de realizar el ensayo.

Estándares		Densidad óptica (450 nm)
Zero Standard	(0 U/mL)	0,147
Standard 1	(31 U/mL)	0,515
Standard 2	(62 U/mL)	0,857
Standard 3	(125 U/mL)	1,423
Standard 4	(250 U/mL)	2,127

7 VALORES DE REFERENCIA

Se recomienda encarecidamente que cada laboratorio establezca sus propios valores de referencia.

Un estudio realizado con sujetos aparentemente sanos en el que se usó DRG Sperm Antibody ELISA reveló lo siguiente:

Valores normales 0 - 60 U/mL
Límite 55 U/mL - 65 U/mL
Valores elevados > 60 U/mL

Si el valor se encuentra en el rango límite (55 U/mL a 65 U/mL), recomendamos repetir la determinación con una nueva muestra tomada en las dos semanas siguientes.

Los resultados no deben ser el único motivo que justifique la aplicación de una terapia. Los resultados deben estar vinculados con otras observaciones clínicas y pruebas diagnósticas.

8 CONTROL DE CALIDAD

Unas buenas prácticas de laboratorio requieren el uso de controles en cada curva estándar. Es conveniente analizar una cantidad de controles estadísticamente significativa para poder establecer unos valores de media y unos intervalos aceptables que favorezcan un rendimiento adecuado.
Se recomienda usar las muestras de control según las regulaciones estatales y federales. El uso de muestras de control es aconsejable para garantizar la validez de los resultados cada día. Use controles en niveles tanto normales como patológicos.
Los controles y los resultados correspondientes del laboratorio de control de calidad figuran en el certificado de cnálisis (CoA) incluido con el kit. Los valores e intervalos reflejados en un CoA siempre se corresponden con el lote de kit actual, y deben usarse para cotejar los resultados de forma directa.
Si los hay, también es recomendable participar en programas de control de calidad nacionales o internacionales para garantizar la precisión de los resultados.
Emplee unos métodos estadísticos adecuados para analizar las tendencias y los valores de control. Si los resultados del ensayo no coinciden con los intervalos aceptables establecidos del material de control, los resultados de paciente deben considerarse como no válidos.
En tal caso, compruebe las siguientes cuestiones técnicas: Aparatos de pipeteo y temporizadores; fotómetro, fechas de caducidad de los reactivos, condiciones de almacenamiento e incubación, métodos de aspiración y lavado.
Si, una vez comprobadas todas estas cuestiones, sigue sin encontrar errores, póngase en contacto su distribuidor o directamente con DRG.

9 CARACTERÍSTICAS DE FUNCIONAMIENTO

9.1 Sensibilidad

Límite de blanco (LoB)	0,490 U/mL
Límite de detección (LoD)	3,367 U/mL
Límite de cuantificación (LoQ)	9,632 U/mL
Intervalo de medición	3,367 U/mL - 250 U/mL
Intervalo lineal	4,333 U/mL - 250 U/mL

Encontrará información sobre lo siguiente:

9.2 Reproducibilidad (precisión)

9.3 Recuperación

9.4 Linealidad

en la versión en inglés detallada de las instrucciones de uso.

10 LIMITACIONES DEL PROCEDIMIENTO

Se obtendrán unos resultados fiables y reproducibles si el procedimiento de ensayo se lleva a cabo habiendo comprendido completamente las instrucciones de uso y poniendo en marcha unas buenas prácticas de laboratorio.

Manipular las muestras de forma indebida o alterar esta prueba podría influir en los resultados.

10.1 Sustancias interferentes

La hemoglobina (hasta 4 mg/mL), la bilirrubina (hasta 0,5 mg/mL) y los triglicéridos (hasta 7,5 mg/mL) no tienen efecto alguno en los resultados del ensayo.

No debe utilizarse suero de pacientes con enfermedades de hígado.

Los resultados pueden estar afectados severamente por ciertas condiciones patológicas, como gammapatías poli- y monoclonal, enfermedades auto inmunes o por un estado inmune alterado.

10.2 Efecto gancho en concentraciones elevadas

No se aprecia ningún efecto gancho en concentraciones elevadas de hasta 5000 U/mL de AAE.

11 CUESTIONES LEGALES

11.1 Fiabilidad de los resultados

La prueba se debe realizar siguiendo exactamente las instrucciones de uso del fabricante. Es más, el usuario debe cumplir estrictamente las reglas de buenas prácticas de laboratorio o cualquier norma o legislación nacional en vigor. Esto es especialmente relevante al usar reactivos de control. Es importante incluir siempre en el procedimiento de la prueba una cantidad suficiente de controles que validen la precisión de la prueba.

Los resultados de la prueba serán válidos únicamente si todos los controles están dentro de los intervalos especificados y si todos los demás parámetros de la prueba están dentro también de las especificaciones del ensayo pertinentes. Si existe alguna duda o reparo en relación con un resultado, póngase en contacto con DRG.

11.2 Aplicación de terapias

La aplicación de una terapia no debe estar justificada únicamente por los resultados de laboratorio, aun cuando todos los resultados de la prueba coincidan con lo establecido en el punto 11.1. Un resultado de laboratorio es solo una parte del cuadro clínico total de un paciente.

La aplicación de una terapia solo estará justificada en aquellos casos en los que los resultados de laboratorio coincidan con el cuadro clínico general del paciente.

El resultado de la prueba en sí no debe tomarse como único factor determinante de la aplicación de una terapia.

11.3 Responsabilidad

Cualquier alteración del kit de prueba y/o intercambio o mezcla de componentes de lotes distintos entre un kit de prueba y otro podría afectar negativamente a los resultados previstos y a la validez de la prueba en general. Tal alteración o intercambio invalidará cualquier reclamación de sustitución.

Tampoco serán válidas las reclamaciones enviadas con motivo de una mala interpretación por parte del cliente de los resultados de laboratorio según el punto 11.2. Con independencia de todo esto, en caso de reclamación, la responsabilidad del fabricante no superará el valor del kit de prueba. Cualquier daño causado al kit de prueba durante su transporte quedará fuera de la responsabilidad del fabricante.

11.4 Información de incidentes graves

Cualquier incidente grave relacionado con el producto deberá comunicarse al fabricante y a la autoridad competente del Estado miembro en el que estén establecidos el usuario y/o el paciente.

1 DESTINATION

Le **DRG Sperm Antibody ELISA** est un dosage immunoenzymatique manuel pour la mesure **quantitative** des anticorps anti-spermatozoïdes (AAS) humains dans le sérum humain.

Destiné à une utilisation de diagnostic in vitro. Destiné à un usage professionnel en laboratoire.

Pour de plus amples informations sur l'usage prévu, veuillez vous reporter à la version anglaise du mode d'emploi.

2 PRINCIPE DU TEST

Le DRG Sperm Antibody ELISA est un dosage d'immunoabsorption par enzyme (ELISA) en phase solide reposant sur **le principe de sandwich**.

Les puits de microtitration sont recouverts d'un mélange de protéines de spermatozoïdes.

Pendant l'incubation, les anticorps anti-spermatozoïdes présents dans les échantillons (étalons, contrôles, échantillons de patients) se lient à la surface enduite des puits.

Une étape de lavage élimine les composants de l'échantillon non liés.

Le conjugué enzymatique ajouté se lie aux complexes antigène-anticorps immobilisés. Le conjugué contient des anticorps anti-immunoglobulines humaines, marqués à la peroxydase de raifort (HRP).

Après une étape de lavage pour éliminer toutes les substances non liées, la phase solide est incubée avec la solution de substrat. La réaction colorimétrique est arrêtée par l'ajout d'une solution d'arrêt, et la densité optique (DO) du produit jaune résultant est mesurée. L'intensité de couleur est proportionnelle à la concentration de l'analyte dans l'échantillon.

Une courbe standard est construite en traçant les valeurs de DO en fonction des concentrations des standards, et les concentrations des échantillons inconnus sont déterminées en utilisant cette courbe standard.

3 AVERTISSEMENTS ET PRECAUTIONS

- Ce kit est destiné exclusivement à une utilisation de diagnostic in vitro. Destiné uniquement à un usage professionnel en laboratoire.
- Lire attentivement toutes les instructions avant de commencer le dosage. Utiliser la version valide de la notice d'utilisation fournie avec la trousse. S'assurer que tout a bien été compris.
- Ne pas mélanger les composants des trousses et ne pas utiliser de composants de trousses portant des numéros de lot différents. Il est recommandé de ne pas intervertir les puits de différentes plaques, même s'ils appartiennent au même lot. Les trousses ont peut-être été expédiés ou conservés dans des conditions différentes et les caractéristiques de liaison des plaques peuvent légèrement différer.
- Ne pas utiliser de réactifs au-delà de la date d'expiration indiquée sur les étiquettes de la trousse.
- Ne pas réutiliser les puits de microtitration.
- Les réactifs d'autres fabricants ne doivent pas être utilisés avec les réactifs de cette trousse de test.
- Tous les réactifs de cette trousse sont des liquides clairs, la solution de substrat est claire et incolore. Des variations dans son apparence peuvent affecter la performance du test. Dans un tel cas, contactez DRG.
- La contamination microbienne des réactifs ou des échantillons peut entraîner des résultats erronés.
- Laisser les réactifs atteindre la température ambiante (20 °C à 26 °C) avant de commencer le test. La température affecte la lecture de la densité optique du test.
- Tous les volumes indiqués doivent être réalisés conformément au protocole. Des résultats de tests optimaux ne sont possibles qu'avec des pipettes et des lecteurs de microplaques calibrés.
- N'utilisez les réservoirs que pour des réactifs uniques. Ceci s'applique particulièrement aux réservoirs de substrat. L'utilisation d'un réservoir pour distribuer une solution de substrat qui avait été précédemment utilisé pour la solution de conjugué peut colorer la solution. Ne pas reverser les réactifs dans les flacons d'origine, car cela pourrait contaminer les réactifs.

Précautions générales

- Suivre les bonnes pratiques de laboratoire et les directives de sécurité.
- Ne jamais les pipeter à la bouche et éviter tout contact des réactifs et des échantillons avec la peau ou les muqueuses.
- Ne pas fumer, boire, manger ni utiliser des cosmétiques dans les zones de manipulation des échantillons ou de réactifs de la trousse.
- Porter des blouses de laboratoire et des gants en latex jetables lors de la manipulation des échantillons et des réactifs et, si nécessaire, des lunettes de sécurité.

Informations sur les risques biologiques

- Tous les réactifs de cette trousse de test contenant du sérum ou du plasma humain ont été testés et confirmés négatifs pour le VIH I/II, le HBsAg et le VHC par les procédures approuvées par la FDA. Cependant, aucune méthode d'essai connue ne peut offrir une garantie totale qu'aucun agent infectieux n'est présent.
- Le dispositif contient des matières d'origine animale, qui sont certifiées apparemment exemptes de maladies infectieuses ou contagieuses et de parasites nuisibles.
- Les composants bovins proviennent de pays où l'ESB (encéphalopathie spongiforme bovine) n'a pas été signalée.
- Tous les matériaux et échantillons d'origine humaine ou animale doivent être manipulés comme susceptibles de transmettre des maladies infectieuses.
- La manipulation doit être conforme aux procédures définies par les directives ou règlements nationaux concernant la sécurité et les déchets à risque biologique. Les déchets doivent être mis au rebut conformément aux règles et réglementations locales.

Informations sur les risques chimiques et classification des risques

- Certains réactifs contiennent des agents de conservation à des concentrations non soumises à une obligation de déclaration. Toutefois, en cas de contact avec les yeux ou la peau, rincer immédiatement à l'eau.
- La solution de substrat contient un ingrédient à des concentrations non soumises à une obligation de déclaration, qui provoque une grave irritation des yeux. En cas de contact possible avec les yeux, rincer immédiatement et soigneusement au moyen d'une douche oculaire ou à l'eau. En cas de contact avec la peau, rincer abondamment à l'eau. Enlever les vêtements contaminés et les laver avant de les porter de nouveau.
- Éviter le contact avec la solution d'arrêt, qui contient 0,5 M de H_2SO_4. Elle pourrait provoquer une irritation de la peau et des brûlures.
- Les produits chimiques et les réactifs préparés ou utilisés doivent être considérés comme des déchets dangereux conformément à la réglementation ou aux directives de sécurité nationales.
- Ce produit ne contient pas de substances ayant des propriétés cancérigènes, mutagènes ou toxiques pour la reproduction (CMR).

AUCUN des réactifs de cette trousse de test ne contient de substances dangereuses à des concentrations soumises à une obligation de déclaration; une classification et un étiquetage n'est pas requis.

Pour des informations détaillées, veuillez consulter la fiche de données de sécurité, disponible sur demande directement auprès de DRG.

4 MATÉRIAUX

4.1 Matériaux fournis avec la trousse

Symbole	Quantité	Description	Préparation
Microtiterwells	12 x 8 puits (divisibles)	**Microplaque** Recouvert d'un mélange de protéines de spermatozoïdes	Prêt à l'emploi
Dilution Buffer / Zero Standard	1 x 50 mL	**Tampon de dilution de l'échantillon / Standard zéro *** Concentration : 0 U/mL	Prêt à l'emploi
Standard (Standard 1 - 4)	4 x 0,5 mL	**Standards *** Concentrations: 31 – 62 – 125 – 250 U/mL	Prêts à l'emploi
Quality Control	1 x 0,5 mL	**Contrôle *** *Pour les valeurs de contrôle et les plages de valeurs, veuillez vous référer à l'étiquette du flacon ou au certificat d'analyse (CoA).*	Prêts à l'emploi
Enzyme Conjugate	1 x 8 mL	**Conjugué enzymatique *** Anticorps anti-IgG humaine conjugué à de la peroxydase de raifort. Coloré en rouge.	Prêt à l'emploi
Substrate Solution	1 x 14 mL	**Solution de substrat** Contient du 3,3‘,5,5‘-tétraméthylbenzidine (TMB). *Tenir à l'écart de la lumière directe du soleil.*	Prêt à l'emploi
Stop Solution	1 x 14 mL	**Solution d'arrêt** Contient 0,5 M de H_2SO_4. *Eviter les contacts avec la solution stop. Cela pourrait engendrer des irritations ou brûlures de la peau.*	Prêt à l'emploi
Wash Solution	1 x 30 mL	**Solution de lavage, concentré 40X ***	*Voir « Préparation des réactifs ».*
Cover foil	1 x	**Feuille de couverture**	
	1 x	**Notice d'utilisation (IFU)**	
	1 x	**Certificat d'analyse (CoA)**	
		***** Contient un agent de conservation sans mercure.	

4.2 Matériel nécessaire mais non fourni

- Un lecteur de microplaques calibré (450 nm, avec une longueur d'onde de référence de 620 nm à 630 nm)
- Micropipettes calibrées à précision variable
- Incubateur pour 37 °C
- Équipement manuel ou automatique pour le rinçage des puits de microplaques
- Papier absorbant
- Eau distillée
- Minuterie
- Papier graphique ou logiciel pour la réduction des données

4.3 Stockage et stabilité du kit

Les kits et réactifs non ouverts ainsi que **les réactifs ouverts** doivent être conservés à une température entre 2 °C et 8 °C.

La microplaque contient des bandes détachables. Ne pas ouvrir la poche des puits avant qu'elle n'ait atteint la température ambiante. Les puits inutilisés doivent être conservés à une température comprise entre 2 °C et 8 °C dans une poche en aluminium scellée, avec l'absorbeur d'humidité fourni, et être utilisés avec le cadre fourni. Une fois la poche en aluminium ouverte, s'assurer de la refermer hermétiquement.

Une fois ouverts, les flacons de réactifs doivent être refermés hermétiquement.

	Température de stockage	Stabilité
Kit non ouvert et réactifs non ouverts	2 °C à 8 °C	Jusqu'à la date d'expiration imprimée sur l'étiquette. Ne pas utiliser les réactifs au-delà de cette date !
Kit ouvert	2 °C à 8 °C	8 semaines

4.4 Préparation des réactifs

Amener tous les réactifs et le nombre requis de bandes à température ambiante (20 °C à 26 °C) avant de les utiliser.

Solution de lavage

Ajouter l'eau distillée à la solution de lavage concentrée à 40x (*Wash Solution*).

Diluer 30 mL de solution de lavage concentrée avec 1170 mL d'eau distillée pour un volume final de 1200 mL.

Stabilité après dilution:	entre 20 °C et 26 °C	1 semaine

4.5 Élimination du kit

L'élimination du kit et de tout le matériel/tous les réactifs doit être conforme aux réglementations nationales. Des informations spécifiques au produit sont indiquées dans la fiche de données de sécurité, section 13.

4.6 Kits de tests endommagés

En cas de dommage du kit de tests ou de ses composants, DRG doit en être informé par écrit, au plus tard une semaine après réception du kit. Les composants endommagés ne doivent pas être utilisés pour le test. Ils doivent être stockés jusqu'à ce qu'une solution adaptée ait été trouvée. Après cela, ils doivent être éliminés conformément à la réglementation en vigueur.

5 PRÉLÈVEMENT, STOCKAGE ET PRÉPARATION DES ÉCHANTILLONS

Le matériau d'échantillon suivant peut être utilisé dans ce test:

Sérum humain

Les échantillons contenant de l'azoture de sodium ne doivent pas être utilisés dans le test.

En général, il faut éviter d'utiliser des échantillons hémolytiques, ictériques ou lipémiques. Pour de plus amples informations, se reporter au chapitre « *Substances interférentes* ».

Pour la détermination des anticorps anti-spermatozoïdes dans le plasma séminal, veuillez utiliser notre test Sperm Antibody (seminal plasma) ELISA (REF EIA-4249)

5.1 Prélèvement des échantillons

Sérum: Prélever le sang par ponction veineuse (ex. Sarstedt Monovette pour le sérum), laisser coaguler et extraire le sérum par centrifugation à température ambiante. Ne pas centrifuger avant la coagulation complète. Le temps de coagulation peut être plus long chez les patients sous traitement anticoagulant.

Le sang total ne doit pas être congelé avant la centrifugation.

5.2 Stockage des échantillons

Les échantillons doivent être conservés hermétiquement fermés avant d'effectuer le dosage. S'ils sont conservés au congélateur, ne les congeler qu'une seule fois. Les échantillons décongelés doivent être retournés plusieurs fois avant le test.

Stabilité:	entre 2 °C et 8 °C	4 jours
	à -20 °C (en aliquotes)	jusqu'à 2 mois

5.3 Préparation des échantillons

Avant de procéder au dosage, diluer chaque échantillon de patient au **1:100** avec le tampon de dilution (*Dilution Buffer)*.

Exemple :

Dilution 1:100 : 5 µL d'échantillon + 495 µL de *Dilution Buffer* (mélanger soigneusement).

Stabilité des échantillons dilués	entre 2 °C et 8 °C	4 jours
	à -20 °C (en aliquotes)	7 jours

Note : Le contrôle de qualité (*Quality Control*) est prêt à l'emploi et ne doit pas être dilué !

6 PROCÉDURE DE DOSAGE

6.1 Notes de procédure

- Tous les réactifs et échantillons doivent être amenés à température ambiante (entre 20 °C et 26 °C) avant d'être utilisés.
- Tous les réactifs et échantillons doivent être mélangés sans mousse.
- Ne pas interchanger les bouchons des flacons de réactifs pour éviter toute contamination croisée.
- Utiliser des embouts de pipette en plastique neufs pour chaque standard, contrôle ou échantillon afin d'éviter tout transfert.
- Pour éviter la contamination croisée et des résultats faussement élevés, pipeter les échantillons du patient et distribuer le conjugué sans éclabousser précisément le fond des puits.
- Mélanger soigneusement le contenu des puits de la microplaque pour garantir de bons résultats.
- Ne pas laisser les puits sécher pendant le dosage ; ajouter les réactifs immédiatement après avoir terminé les étapes de rinçage.

- Une fois le test lancé, toutes les étapes doivent être réalisées sans interruption et dans le même ordre pour chaque étape.
- La réaction enzymatique est linéairement proportionnelle au temps et à la température.
- La densité optique dépend du temps d'incubation et de la température. Respecter les temps et températures d'incubation indiqués dans le chapitre « Procédure de test ».
- Avant de commencer le dosage, il est recommandé que tous les réactifs soient prêts, les bouchons retirés, tous les puits nécessaires fixés dans le support, etc. Cela permet de garantir un temps égal pour chaque étape de pipetage sans interruption.
- Pendant l'incubation à 37 °C, recouvrir les bandes de microtitration avec un film pour éviter l'évaporation.
- **Remarque importante pour la procédure de lavage:**
  Le lavage est essentiel. Des puits mal lavés donneront des résultats erronés. La sensibilité et la précision de ce dosage sont fortement influencées par l'exécution correcte de la procédure de lavage!
- **Réalisation de tests avec des dispositifs d'analyse entièrement automatisés:**
  Il est possible d'effectuer des tests automatisés au moyen de dispositifs d'analyse entièrement ouverts automatisés. Toutefois, la combinaison doit être validée par l'utilisateur.

6.2 Procédure de test

Chaque cycle doit inclure une courbe standard.

Les contrôles servent de contrôles internes pour la fiabilité de la procédure de test. Ils doivent être dosés lors de chaque cycle de tests.

La procédure de test décrite correspond à un traitement manuel.

Remarque importante: La précision de ce test est fortement influencée par une température et un temps d'incubation correcte. Le pipetage des échantillons ne doit pas dépasser 15 minutes.

1. Fixer le nombre souhaité de puits de microtitration dans le support du cadre.
2. Pipeter **50 µL** de chaque ***Zero Standard, Standard***, ***Quality Control*** et **échantillon dilué** avec de nouveaux embouts jetables dans les puits correspondants.
3. Couvrir avec une feuille et incuber pendant **60 minutes** à **37 °C**
4. Laver les puits comme suit:
   Si l'étape de lavage est effectuée à la main:
   Agiter énergiquement le contenu des puits.
   Rincer les puits à **3 reprises** avec **300 µL** de solution de lavage diluée par puits.

   Si un laveur de plaques automatique est utilisé:
   Rincer les puits à **3 reprises** avec **400 µL** de solution de lavage diluée par puits.

   À la fin de l'étape de lavage, toujours frapper énergiquement les puits sur du papier absorbant pour éliminer les gouttelettes résiduelles.
5. Ajouter **50 µL** de conjugué enzymatique (***Enzyme Conjugate***) dans chaque puits.
6. Couvrir avec une feuille et incuber pendant **30 minutes** à **37 °C**.
7. Laver comme décrit à l'étape 4.
8. Pipeter **50 µL** de solution de substrat (***Substrate Solution***) dans chaque puits.
9. Incuber pendant **30 minutes** à température ambiante.
10. Arrêter la réaction enzymatique en ajoutant **100 µL** de solution d'arrêt (***Stop Solution***) dans chaque puits.
11. Mesurer la densité optique (DO) de la solution dans chaque puits **à 450 nm (lecture) et à 620 nm à 630 nm (soustraction des bruits de fond, recommandé)** avec un lecteur de microplaques.
    Il est recommandé de lire les puits dans un délai de **10 minutes** après l'ajout de la solution d'arrêt.

6.3 Calcul des résultats

1. La concentration des échantillons peut être lue directement à partir de la courbe standard.
   Les étalons sont déjà pré-dilués, donc la dilution des échantillons au 1:100 ne doit pas être prise en compte pour le calcul final des concentrations des échantillons.
2. Pour les déterminations en double, prendre la moyenne des deux valeurs de densité optique (DO) pour chaque standard, contrôle et échantillon de patient. Si les deux valeurs s'écartent considérablement l'une de l'autre, DRG recommande de tester à nouveau les échantillons.
3. Les échantillons dont la concentration est supérieure à celle de l'étalon le plus élevé peuvent être dilués davantage avec Dilution Buffer et dosés à nouveau comme décrit dans la section « Procédure de test» ou doivent être signalés comme > 250 U/mL. Pour le calcul des concentrations, ce facteur de dilution doit être pris en compte.
4. Méthode automatisée:
   Les résultats figurant dans les instructions d'utilisation ont été calculés automatiquement en utilisant un ajustement de la courbe logistique à quatre paramètres (4 PL). (Les méthodes privilégiées sont les modèles logistiques à quatre paramètres [4 PL] de Rodbard ou de Marquardt.) D'autres fonctions de réduction des données peuvent donner des résultats légèrement différents.

5. Méthode manuelle:
   Avec du papier graphique, construire une courbe standard en traçant la DO (moyenne) obtenue à partir de chaque standard en fonction de sa concentration avec la valeur de la DO sur l'axe vertical (Y) et la concentration sur l'axe horizontal (X). Déterminer la concentration correspondante de l'échantillon à partir de la courbe standard en utilisant la valeur (moyenne) de la DO pour chaque échantillon.

6.3.1 Exemple de courbe standard caractéristique

Les données suivantes ont uniquement une fin de démonstration et **ne peuvent pas** être utilisées à la place des générations de données au moment du dosage.

Standard		Densité optique (450 nm)
Zero Standard	(0 U/mL)	0,147
Standard 1	(31 U/mL)	0,515
Standard 2	(62 U/mL)	0,857
Standard 3	(125 U/mL)	1,423
Standard 4	(250 U/mL)	2,127

7 VALEURS DE RÉFÉRENCE

Il est fortement recommandé à chaque laboratoire de déterminer ses propres valeurs de référence.

Dans une étude menée sur des sujets apparemment sains, à l'aide du test Sperm Antibody ELISA de DRG, les valeurs suivantes ont été observées :

Valeurs normales	0 U/mL – 60 U/mL
Limites	55 U/mL – 65 U/mL
Valeurs élevées	> 60 U/mL

En cas de valeur proche du seuil (55 U/mL à 65 U/mL), nous recommandons une détermination de suivi à l'aide d'un nouvel échantillon prélevé dans les deux semaines suivantes.

Les résultats ne doivent pas être utilisés seuls pour déterminer les décisions thérapeutiques. Ils doivent être corrélés avec d'autres observations cliniques et tests diagnostiques.

8 CONTRÔLE DE QUALITÉ

Les bonnes pratiques de laboratoire exigent que des contrôles soient effectués avec chaque courbe standard. Un nombre statistiquement significatif de contrôles doit être analysé afin d'établir les valeurs moyennes et les plages acceptables pour garantir une bonne performance.
Il est recommandé d'utiliser les échantillons de contrôle conformément aux réglementations locales et nationales. L'utilisation d'échantillons de contrôle est conseillée pour assurer la validité jour par jour des résultats. Utiliser les contrôles au niveau normal et au niveau pathologique.
Les contrôles et les résultats correspondants du laboratoire de contrôle de la qualité sont indiqués dans le certificat d'analyse (CoA) joint au kit. Les valeurs et les plages indiquées sur le « CoA » se rapportent toujours au lot actuel du kit et doivent être utilisées pour une comparaison directe des résultats.
En cas de disponibilité, il est également recommandé de participer à des programmes nationaux ou internationaux d'évaluation de la qualité afin d'assurer l'exactitude des résultats.
Appliquer les méthodes statistiques appropriées pour l'analyse des valeurs de contrôle et des tendances. Si les résultats du dosage ne concordent pas avec les intervalles acceptables établis du matériel de contrôle, les résultats de patient doivent être considérés comme invalides.
Dans ce cas, veuillez vérifier les domaines techniques suivants: Dispositifs de pipetage et de chronométrage; photomètre, dates d'expiration des réactifs, conditions de stockage et d'incubation, méthodes d'aspiration et de lavage. Si aucune erreur n'est révélée par l'examen des éléments susmentionnés, veuillez contacter votre distributeur ou DRG directement.

9 CARACTERISTIQUES EN MATIERE DE PERFORMANCES

9.1 Sensibilité

La limite du blanc (LoB)	0,490 U/mL
La limite de détection (LoD)	3,367 U/mL
La limite de quantification (LoQ)	9,632 U/mL
Plage de mesure	3,367 U/mL - 250 U/mL
Plage linéaire	4,333 U/mL - 250 U/mL

Les données pour:

9.2 Reproductibilité (précision)

9.3 Récupération

9.4 Linéarité

se trouvent dans la version anglaise détaillée du mode d'emploi.

10 LIMITES DE LA PROCÉDURE

Les résultats seront fiables et reproductibles si la procédure de dosage est effectuée dans le respect le plus strict de la notice d'utilisation et des bonnes pratiques de laboratoire.

Toute manipulation incorrecte des échantillons ou toute modification de ce test peut affecter les résultats.

10.1 Substances interférentes

L'hémoglobine (jusqu'à 4 mg/mL), la bilirubine (jusqu'à 0,5 mg/mL) et les triglycérides (jusqu'à 7,5 mg/mL) n'ont aucune influence sur les résultats du dosage.

Les sérums de patients souffrant de maladies du foie ne doivent pas être utilisés.

Les résultats peuvent être affectés par certains états pathologiques, tels que les gammapathies poly- et monoclonales, les maladies auto-immunes ou par un statut immunitaire altéré.

10.2 Effet crochet

Aucun effet crochet n'a été observé pour ce test jusqu'à une concentration de 5000 U/mL de AAS.

11 ASPECTS JURIDIQUES

11.1 Fiabilité des résultats

Le test doit être effectué exactement selon la notice d'utilisation du fabricant. En outre, l'utilisateur doit adhérer strictement aux règles de bonnes pratiques de laboratoire (ou GLP pour Good Laboratory Practice) et aux autres normes et/ou lois nationales applicables. Ceci s'applique en particulier dans le cadre de l'utilisation des réactifs de contrôle. Il est important de toujours inclure dans la procédure de test un nombre suffisant de contrôles pour valider l'exactitude et la précision du test.
Les résultats de tests ne sont valides que si tous les contrôles se situent dans l'intervalle spécifié et que tous les autres paramètres de test correspondent également aux spécifications du dosage. En cas de doute ou de préoccupation relative à un résultat, veuillez contacter DRG.

11.2 Décisions thérapeutiques

Les décisions thérapeutiques ne doivent jamais s'appuyer uniquement sur les résultats de laboratoire, même si tous les résultats de tests sont conformes aux critères définis au point 11.1. Tout résultat de laboratoire ne représente qu'une partie du tableau clinique global d'un patient.
Des décisions thérapeutiques ne peuvent être prises que dans les cas où les résultats de laboratoire sont en accord avec le tableau clinique global du patient.
Le résultat de test lui-même ne doit jamais être le seul critère déterminant la prise de décisions thérapeutiques.

11.3 Responsabilité

Toute modification du kit de test et/ou échange ou mélange de composants de différents lots de kits pourrait avoir un impact négatif sur les résultats escomptés et sur la validité du test global. De telles modifications et/ou de tels échanges invalident toute demande de remplacement.
Les réclamations dues à une mauvaise interprétation des résultats de laboratoire par le client selon le point 11.2 sont également invalides. Quoi qu'il en soit, en cas de réclamation, la responsabilité du fabricant ne doit pas excéder la valeur du kit de test. Tout dommage causé au kit de test lors du transport ne relève pas de la responsabilité du fabricant.

11.4 Notification des incidents graves

Tout incident grave survenu en lien avec le dispositif fait l'objet d'une notification au fabricant et à l'autorité compétente de l'État membre dans lequel l'utilisateur et/ou le patient est établi.

12 LITERATURE

1. Lahteenmaki A et a.: Hum Reprod (1995) 10, 2824-28
2. Nagy ZP et al. Hum Reprod (1995) 10, 1775-80.
3. Zouari R et al. Fertil Steril (1993) 59, 606-12
4. Eggert-Kruse W et al. Hum Reprod (1993) 8, 1025-31
5. Francavilla F et al. Front Biosci (1999) 4, 9-25
6. Bohring C et al. Hum Reprod (2001) 7,113-8
7. Mazumdar S et al. Fertil Steril (1998) 70, 799-810
8. Kutteh WH. Hum Reprod, (1999) 14, 2426-9
9. Vegetti Wet al. Hum Reprod (1998) 13, 1796-800
10. Lahteenmaki A et al. Hum Reprod (1995) 10, 2824-28
11. Nagy ZP et al. Hum Reprod (1995) 10, 1775-80
12. Clarke GN et al. Am J Reprod Immunol Microbiol (1985) 7, 143-7
13. Abshagen K et al. Fertil Steril (1998) 70, 355-6
14. Jiang Y et al. J Reprod Immunol (2016) 118:85-91
15. Lu SM et al. Asian J Androl (2019) 21(5):473-477

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsan-weisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter la notice d'utilisation	Consultar as instruções de utilização
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico in vitro	Dispositif médical de diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
LOT	Batch code *	Fertigungslosnumm er, Charge *	Lotto no	Número de lote	No. de lot	Código do lote
Σ	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperatur-begrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Date of Manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches	
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur	Distribuidor
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement	Conteúdo
Volume/No.	Volume / No.	Volumen / Anzahl	Volume / Quantità	Volumen / Número	Volume / Quantité	Volume / Quantidade
Microtiterwells	Microtiter plate	Mikrotiterplatte	Piastra per microtitolazione	Placa de microtítulo	Microplaque	Placa de microtitulação
Antiserum	Antiserum	Antiserum	Antisiero	Antisuero	Antisérum	
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Coniugato enzimatico	Conjugado enzimático	Conjugué enzymatique	Conjugado Enzimático
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complesso enzimatico	Compuesto enzimático	Complexe enzymatique	Complexo Enzimático
Substrate Solution	Substrate Solution	Substratlösung	Soluzione di substrato	Solución de sustrato	Solution de substrat	Solução de Substrato
Stop Solution	Stop Solution	Stopplösung	Soluzione d´ arresto	Solución de parada	Solution d'arrêt	Solução de Paragem
Zero Standard	Zero Standard	Nullstandard	Standard zero	Estándar cero	Zero Standard	Padrão Zero
Standard	Standard	Standard	Standard	Estándar	Standard	Padrão
Control	Control	Kontrolle	Controllo	Control	Contrôle	Controlo
Assay Buffer	Assay Buffer	Assaypuffer	Tampone del dosaggio	Tampón de ensayo	Tampon d'essai	Tampão de Teste
Wash Solution	Wash Solution	Waschlösung	Soluzione di lavaggio	Solución de lavado	Solution de lavage	Solução de Lavagem
1 N NaOH	1 N NaOH	1 N NaOH	1 N NaOH	1 N NaOH	1 N NaOH	1 N NaOH
1 N HCl	1 N HCl	1 N HCl	1 N HCl	1 N HCl	1 N HCl	1 N HCl
Sample Diluent	Sample Diluent	Probenver-dünnungsmedium	Diluente per campioni	Diluyente de muestras	Diluant d'échantillon	Diluente de Amostra
Conjugate Diluent	Conjugate Diluent	Konjugatver-dünnungsmedium	Diluente coniugato	Diluyente de conjugados	Diluant de conjugué	Diluente de Conjugado

* Definition according to DIN EN ISO 15223-1

Instructions for Use

β-HCG ELISA

IVD

REF EIA-1911

Σ 96

CE

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, use sólo la versión válida de las instrucciones de uso que se suministran con el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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1 INTENDED USE

The **DRG β-HCG ELISA Kit** is an enzyme immunoassay for the quantitative *in vitro* diagnostic measurement of total human chorionic gonadotropin (hCG and β-hCG) in serum.

This kit is NOT intended to be used for the risk evaluation of trisomy 21.

1.1 Summary and Explanation

Chorionic Gonadotropin (hCG) is a glycoprotein hormone which is normally produced by the placenta during pregnancy. After conception, the hCG concentration increases rapidly to reach a peak near the end of the first trimester. High concentrations are observed throughout pregnancy. After delivery, hCG levels fall rapidly and become undetectable after a few days.

Structurally intact hCG molecules are composed of an alpha and a beta subunit. The alpha subunit is nearly identical to the alpha subunits of other glycoprotein hormones, such as Thyroid Stimulating Hormone (TSH), Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH). The differences in the beta subunit of the respective hormones account for their biological specificity and immunochemical distinctiveness.

Monoclonal antibodies recognizing unique sites on the beta chain of the ß-hCG/hCG molecule are essential for differentiation between hCG and LH, FSH and TSH.

Specific assays for beta-hCG permit the early detection of pregnancy.

In addition to the elevated hCG levels during pregnancy, high concentrations of βhCG/hCG may be associated with neoplasms of trophoblastic and nontrophoblastic origin such as hydatiform mole, chorionepithelioma, embryonal cell carcinoma,seminoma and many others.

2 PRINCIPLE OF THE TEST

The DRG β-HCG ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **sandwich principle.**

The microtiter wells are coated with a monoclonal antibody [mouse] directed towards a unique antigenic site on a β-HCG molecule. An aliquot of patient sample containing endogenous β-HCG and/or HCG is incubated in the coated well with enzyme conjugate, which is an anti- β-HCG antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of β-HCG/HCG in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of β-HCG/HCG in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for *in vitro* diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Prodin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. *Microtiterwells*, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti-β-HCG antibody (monoclonal).
2. *Standard (Standard 0-5)*, 6 vials (lyophilized), 1 mL;
   Concentrations: 0, 5; 25; 50; 100; 200 mIU/mL
   Conversion: 1 pg/mL = 0.00916 mIU/mL
   The standards are calibrated against the following reference material: 5th WHO International Standard Chorionic Gonadotrophin, NIBSC code: 07/364.
   See „Preparation of Reagents";
   Contain non-mercury preservative.
3. *Sample Diluent*, 1 vial, 10 mL, ready to use,
   Contains non-mercury preservative.
4. *Enzyme Conjugate*, 1 vial, 11 mL, ready to use,
   Anti-β-HCG antibody conjugated to horseradish peroxidase;
   Contains non-mercury preservative.
5. *Substrate Solution*, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
6. *Stop Solution*, 1 vial, 14 mL, ready to use,
   contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

Note: Additional *Sample Diluent* for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of each vial with 1 ml distilled water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Note: The reconstituted standards are stable for 2 months at 2 °C - 8 °C.
For longer storage aliquot and freeze at -20 °C.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum should be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.

In general, it should be avoided to use haemolytic, icteric, or lipaemic specimens. For further information refer to chapter "*Interfering Substances*".

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 7 days at 2 °C - 8 °C prior to assaying.

Specimens held for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10:	10 µL Serum + 90 µL *Sample Diluent* (mix thoroughly)
b) dilution 1:100:	10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly).
c) dilution 1:1000	10 µL dilution b) 1:100 + 90 µL *Sample Diluent* (mix thoroughly).

NOTE:

Sera of pregnant women must be diluted 1/100 in Sample Diluent before starting the assay.

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure (quantitative method)

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **25 µL** of each *Standard, Control* and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** *Enzyme Conjugate* into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature.
5. Briskly shake out the contents of the wells. Rinse the wells 5 times with distilled water (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **15 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
9. Determine the optical density of the solution in each well at **450 nm (reading) and at 620 - 630 nm (background subtraction, recommended)** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results (quantitative)

1. Calculate the average optical density (OD) values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 200 mIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Density (450 nm)
Standard 0 (0 mIU/mL)	0.04
Standard 1 (5 mIU/mL)	0.15
Standard 2 (25 mIU/mL)	0.28
Standard 3 (50 mIU/mL)	0.53
Standard 4 (100 mIU/mL)	0.94
Standard 5 (200 mIU/mL)	1.50

6.4 Assay Procedure (qualitative method)

This procedure differentiates positive (pregnant) from negative samples by comparing the sample beta hCG levels with the *Standard 0* (0 mIU/mL) and *Standard 3* (50 mIU/mL).

Patient samples are run with the Standard 0 and the 50 mIU/mL Standard. The assay procedure is identical with the Quantitative Method, but step 9 and 10 is omitted.

6.5 Calculation of Results (qualitative)

For a qualitative analysis of the β-hCG level the color development of the specimen is compared with the color of the 0 mIU/mL and 50 mIU/mL standards.

If the blue color is less intense than the color of the 50 mIU/mL standard, the sample is considered as negative.

If the blue color is more intense than or equal to the color of the 50 mIU/mL standard the sample is considered as positive.

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

7.1 Normal healthy adults, non-pregnant

In a study conducted with apparently normal healthy adults, using the DRG β-HCG ELISA the following values are observed:

Population	Age (years)	Valid N	β-hCG [mIU/mL]
men	< 50	40	< 5
	> 50	10	< 5
women	< 50	42	< 5
	> 50	7	< 5

7.2 Pregnant women in the 2nd trimester

Week of pregnancy	Valid samples	5% Percentile [mIU/mL]	95% Percentile [mIU/mL]
14	103	10 303	71 980
15	96	9 246	51 666
16	91	5 266	36 947
17	14	4 632	24 033

CAUTION:

1. For the detection of pregnancy in serum, a qualitative assay is used with a cut-off point of 50 mIU/mL. Negative or borderline results should be repeated on a fresh specimen obtained at least 48 hours after the first specimen.
2. It has been shown that immunological pregnancy tests may yield false results in cases of several medications and diseases such as rheumatoid arthritis. In such cases, the interpretation of the pregnancy test should be done carefully.

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.44 – 200 mIU/mL.

9.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross reactivity of the assay:

Protein	Concentration	Produced Colour Intensity Equivalent to β-HCG in serum (mIU/mL)
hLH	300 mIU/mL	6
hLH	200 mIU/mL	< 5
hLH	80 mIU/mL	< 5
TSH	75 µIU/mL	8
TSH	50 µIU/mL	< 5
TSH	25 µIU/mL	< 5
FSH	200 mIU/mL	< 5
FSH	50 mIU/mL	< 5

9.3 Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of the Standard 0 and was found to be 0.44 mIU/mL.

9.4 Reproducibility

9.4.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (mIU/mL)	CV (%)
1	20	5.9	9.4
2	20	18.6	4.0
3	20	147.9	3.5

9.4.2 Inter Assay Variation

The between assay variability is shown below:

Sample	Mean (mIU/mL)	CV (%)
1	5.6	9.9
2	17.1	7.2
3	140.0	6.9

9.5 Recovery

Samples have been spiked by adding β-HCG solutions with known concentrations.

The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

		Sample 1	Sample 2	Sample 3
Concentration (mIU/mL)		30.9	69.0	115.2
Average Recovery (%)		94.4	91.3	90.7
Range of Recovery (%)	from	91.3	88.6	89.0
	to	99.3	94.2	92.8

9.6 Linearity

Samples were measured undiluted and in serial dilutions with standard 0. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

		Sample 1	Sample 2	Sample 3
Concentration (mIU/mL)		39.7	75.6	128.4
Average Recovery (%)		102.0	95.5	96.8
Range of Recovery (%)	from	90.9	86.2	92.9
	to	115.0	109.3	101.3

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of β-HCG in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 15 800 mIU/mL of β-HCG.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid.

Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES / LITERATURE

1. Sancken, U., Bartels, I., Der sogenannte Triple-Test. Reproduktionsmedizin 15:276-284, 1999
2. Cole L.A. Clinical Chemistry 43:12, 2233-2243, 1997
3. Iles R.K., Javid M.K., Gunn L.K., Chard T. Clinical Chemistry 45:4, 532-538, 1999
4. Iles R.K., Javid M.K., Gunn L.K., Chard T. Clinical Chemistry 40:3, 484-485, 1994
5. Korhonen J., Alfthan H., Ylöstalo P., Veldhuis J., Stenman U.-H. Clinical Chemistry 43:11, 2155-2163, 1997

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AccuBind
ELISA Microwells

Free ß-Subunit Human Chorionic Gonadotropin (Free Beta hCG) Test System

Product Code: 2025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Beta (ß) Chorionic Gonadotropin Subunit Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic tumors. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and the potential diagnosis *of early* pregnancy disorders. Free ß-subunit hCG testing has improved the diagnostic probability of abnormal pregnancy/disease states.[1]

Patients with trophoblastic diseases produce ordinary and irregular forms of hCG; e.g. nicked hCG, hCG missing the *ß-subunit* C-terminal segment, hyperglycosylated hCG and free *ß* subunit. On the other hand, common epithelial tumors of the urogenital tract frequently express the free ß-Subunit of hCG with no concomitant expression of its heterodimer partner, the common α-subunit of the glycoprotein hormone. While most hCG assays do a very good job of monitoring the normal pregnancies, still there needs to be a system of differential diagnosis of ovarian tumors, epithelial tumors and trophoblastic malfunctions. That is where determination of free α-subunit, free β-subunit, nicked hCG and nonnicked hCG etc are of individual value.

Although Free ß-Subunit hCG normally constitutes less than 1% of the total hCG concentrations in normal pregnancy, it constitutes a significant part (as much as 26% of hCG) in trophoblast disease.[2,3] There is also increasing evidence that free beta subunit may be better than total hCG measurement in assessing Down's Syndrome.[4]

In this method, Free Beta hCG calibrator, patient specimen or control is first added to an anti-Free Beta hCG coated well. Assay Buffer is added and the reactants mixed. Reaction between the antibody and native Free Beta hCG forms complex that binds with the antibodies coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to Free Beta hCG is added to the wells. The enzyme labeled antibody binds to the Free Beta hCG already immobilized on the well. 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 Free Beta hCG in the sample.

The employment of several serum references of known Free Beta Chorionic Gonadotropin 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 Free Beta hCG concentration.

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.

Upon mixing assay buffer and a serum containing the native antigen, reaction results between the native antigen and the coated antibody, forming an antibody-antigen complex. This interaction is illustrated below:

$$Ag + Ab_{(C)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{(C)}$$

$Ab_{(C)}$ = Coated Antibody (Excess Quantity)
Ag = Native Antigen (Variable Quantity)
$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

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.

$$AgAb_{(C)} + {}^{Enz}Ab \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab - Ag - Ab_{(c)}$$

^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

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.

4.0 REAGENTS

Materials Provided:

A. Free Beta hCG Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references fß-hCG Antigen at levels of 0(**A**), 10(**B**), 25(**C**), 50(**D**), 100(**E**) and 250(**F**) ng/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO $1P^{stP}$ IRP (75/551).

Conversion to mass units = One (1) mIU/ml is equivalent to 1 ng/ml

B. Assay Buffer – 7 ml/vial - Icon Ⓑ
One (1) vial containing buffer, dye, surfactants and preservatives. Store at 2-8°C.

C. Free Beta hCG Enzyme Reagent – 13 ml/vial - Icon Ⓔ
One (1) vial containing Enzyme (HRP) labeled monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

D. Free Beta hCG Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with anti-fßhCG mouse IgG and 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 A – 7 ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

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

H. Stop Solution – 8 ml/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: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate. For other kit configurations, refer to table at the end of the instructions.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025ml (25µl), 0.050ml (50µl) 0.100 ml (100 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.50 ml (50 µl), 0.100 ml (100 µl) and 0.350 ml (0.350 µl) 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 microplates cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, 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 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, 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 serum reference, control or specimen into the assigned well.
3. Add 0.050ml of Assay Buffer to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350 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 the Free Beta hCG Enzyme Reagent to each well.
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 15 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 0.350 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.**
12. Add 0.100 ml (100 µl) 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 PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. 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.**
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 250 ng/ml, see 12.1(#9) "Assay Performance" for dilution details.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Free Beta hCG in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free Beta hCG 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 Free Beta hCG 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 (0.366) intersects the dose response curve at 14.7 ng/ml Free Beta hCG concentration (See Figure 1).

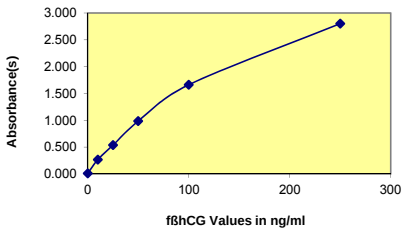
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Position	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.008	0.008	0
	B1	0.008		
Cal B	C1	0.290	0.266	10
	D1	0.242		
Cal C	E1	0.557	0.536	25
	F1	0.513		
Cal D	G1	1.021	0.984	50
	H1	0.946		
Cal E	A2	1.695	1.662	100
	B2	1.630		
Cal F	C2	2.910	2.803	250
	D2	2.695		
Control	E2	1.414	1.343	79.4
	F2	1.271		
Patient	G2	0.362	0.366	14.7
	H2	0.370		

*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. Maximum Absorbance (Calibrator 'F') = >1.3
2. Maximum Absorbance (Calibrator 'A') = U≤U 0.1
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product 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. The reagents for the test system procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin.Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
5. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
6. 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.
7. Plate readers measure vertically. Do not touch the bottom of the wells.
8. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
9. Use components from the same lot. No intermixing of reagents from different batches.
10. Patient specimens with Free Beta hCG concentrations above 250 ng/ml may be diluted (for example 1/10) with normal male serum (Free Beta hCG < 1 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
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 procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of Immunoassays (Boscato LM, Stuart MC ''Heterophilic antibodies: a problem for all immunoassays'' *Clin. Chem* (1988:3427-33). For diagnostic purposes, the results from this assay should be 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 RANGES OF VALUES

Serum Free beta hCG (as intact hCG) increases rapidly in normal pregnancy reaching maximum levels of approximately 60ng/ml (which is equivalent to 60 mIU/ml) at eight-nine weeks of gestation. This is followed by a gradual decline during the next eleven to twelve weeks. The ration of Free Beta hCG to intact hCG reaches 1% at five weeks of pregnancy and remains constant at approximately 0.5% (w/w) for the remaining twenty-two weeks.[2]

The use of Free Beta hCG in combination with AFP levels as a screening protocol for Down syndrome (Trisomy 21) has been promoted to achieve high detection efficiency with low false positive rates.[5]

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 Free Beta hCG AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

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

Sample	N	X	σ	C.V.
Level 1	20	3.5	0.15	4.3%
Level 2	20	10.5	0.55	5.2%
Level 3	20	49.6	1.50	3.0%

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

Sample	N	X	σ	C.V.
Level 1	10	3.1	0.17	5.5%
Level 2	10	11.2	0.71	6.3%
Level 3	10	48.5	1.75	3.6%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The Free Beta hCG AccuBind® ELISA test system has a sensitivity of 0.0007 mIU. This is equivalent to a sample containing 0.026 ng/ml Free Beta hCG concentration.

14.3 Specificity

The cross-reactivity of the Free Beta hCG AccuBind® ELISA test method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Chorionic Gonadotropin needed to produce the same absorbance.

Substance	Cross Reactivity
fß-hCG subunit	1.0000
Intact Chorionic Gonadotropin (hCG)	< 0.0001
Follicle Stimulating Hormone (FSH)	< 0.0001
Luteinizing Hormone (LH)	< 0.0001
Thyroid Stimulating Hormone (TSH)	< 0.0001

15.0 REFERENCES

1. Henry JB, *Clinical Diagnosis and Management by Laboratory Methods*, WB Saunders Company, 486 (1996).
2. Osturk M, et al, *Endocrinology*, 120, 549 (1987).
3. Cole LA, et al, *Serono Symposia Publ*, 65, 59-78 (1989).
4. Macri JN, and Spencer K, *Am J Obstet Gynecol*, 174, 1668-69 (1996).
5. Macri JN, et al, *Am J Obstet Gynecol*, 163, 1248 (1990).
6. Cole LA, "β core fragment (β-core, IUGP or UGF)", *Tumor Marker Update*, 6, 69-75 (1994).
7. Yamanaka N, Kawabata G, Morisue K, Hazama M, Nishimura R,"Urinary hCG β-core fragment as a tumor marker for bladder cancer", *Nippon Hinyokika Gakkai Zasshi*, 84, 700-706 (1993).
8. Kinugasa M, Nishimura R, Hasegawa K, Okamura M, Kimura A, Ohtsu F", Assessment of urinary β-core fragment of hCG as a tumor marker of cervical cancer", *Acta Obstet Gynecol Jpn*, 11, 188-94 (1992).
9. Cole LA, "Stability of hCG free β-subunit in urine", *Prenat Diagnosis*, 17, 185-89 (1996).
10. Sancken U, Bahner D, "The effect of thermal instability of intact human chorionic gonadotropin on the application of its free β-subunit as a serum marker of Down's syndrome screening", *Prenat Diagnosis*, 15, 731-738 (1995).
11. Javadpour N, "Current status of tumor markers in testicular cancer", *Eur Urol*, 21, 34-36 (1992).
12. Canick JA, Kellner DN Jr, Palomaki GE, Walker RP, Osathanondh R, "Second trimester levels of maternal urinary gonadotropin peptide in Down syndrome pregnancy", *Prenat Diagnosis*, 15, 752-759 (1995).

Effective Date: 2024-Jun-21 Rev. 3 DCO: 1678
MP2025 Product Code: 2025-300

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (7ml)	2 (7ml)
	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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IVD 2° 8° CE

EC REP **CEpartner4U,** Esdoornlaan 13
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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	

GOLD STANDARD DIAGNOSTICS

NovaLisa®

Aspergillus fumigatus IgG

ELISA

CE

Only for in-vitro diagnostic use

Instructions for use / Gebrauchsanweisung / Notice d'utilisation / Istruzioni per l'uso / Instrucciones de uso / Instruções de utilização

REF ASPG0680 (96 Determinations)

ENGLISH

1. INTENDED USE

The Aspergillus fumigatus IgG ELISA is intended for the qualitative determination of IgG class antibodies against Aspergillus fumigatus in human serum or plasma (citrate, heparin).

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

3. MATERIALS

3.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Aspergillus fumigatus antigens; in resealable aluminium foil.
- **DIL:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **SOLN STOP:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **WASH BUF 20x:** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **SUB TMB:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 11.1.

3.2. Materials supplied

- 1 Cover foil
- 1 Instructions for use (IFU)

3.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

5.1. Microtiterplate

The break-apart snap-off strips are coated with Aspergillus fumigatus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

5.2. WASH BUF 20x

Dilute WASH BUF 20x 1 + 19; e. g. 10 mL WASH BUF 20x + 190 mL distilled water. The diluted buffer (WASH BUF 1x) is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g., in a water bath. Mix well before dilution.

5.3. SUB | TMB

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. SUB | TMB should be colourless or could have a slight blue tinge. If SUB | TMB turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise, they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with DIL. Dispense 10 µL sample and 1 mL DIL into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

Please read the instructions for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instructions for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of WASH | BUF | 1x from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 60 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of WASH | BUF | 1x. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL SUB | TMB into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL SOLN | STOP into all wells in the same order and at the same rate as for SUB | TMB, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of SOLN | STOP.

7.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay run to be considered valid, these instructions for use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

8.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU

8.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

8.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

9.1. Precision

Intraassay	**n**	**Mean (OD)**	**CV (%)**
#1	24	0.408	5.21
#2	24	1.181	3.01
#3	24	0.945	1.52
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	29.72	6.25
#2	12	22.93	4.67
#3	12	4.93	4.98

9.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 95.65% (95% confidence interval: 78.05% - 99.89%).

9.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100% (95% confidence interval: 83.16% - 100%).

9.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

9.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters (rheumatoid factors and antibodies to Candida albicans) did not reveal evidence of false-positive results due to cross-reactions.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and 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. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

11.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 3.1).
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated clothing and wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to 3.1).
Therefore, the following hazard and precautionary statements apply.

Warning	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	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.

Further information can be found in the safety data sheet.

11.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

For information about the packaging materials refer to PACKAGING MATERIALS.

12. ORDERING INFORMATION

REF	ASPG0680	Aspergillus fumigatus IgG	(96 Determinations)

DEUTSCH

1. VERWENDUNGSZWECK

Der Aspergillus fumigatus IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Aspergillus fumigatus in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

2. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerrettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

3. MATERIALIEN

3.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit Aspergillus fumigatus Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **DIL:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; gelb gefärbt; gebrauchsfertig; weiße Verschlusskappe; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **SOLN STOP:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **WASH BUF 20x:** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe; 0,2% (w/v) 5-Brom-5-nitro-1,3-dioxan.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugierten Antikörpern gegen humanes IgG in Phosphatpuffer (10 mM); blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **SUB TMB:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig. ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig. ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 11.1.

3.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Gebrauchsanweisung

3.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

4. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

5. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

5.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit Aspergillus fumigatus Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

5.2. WASH | BUF | 20x

WASH | BUF | 20x ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL WASH | BUF | 20x + 190 mL destilliertes Wasser. Der verdünnte Puffer (WASH | BUF | 1x) ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

5.3. SUB | TMB

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. SUB | TMB ist farblos, kann aber auch leicht hellblau sein. Sollte SUB | TMB blau sein, ist es kontaminiert und kann nicht im Test verwendet werden.

6. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden! Hitzeinaktivierung der Proben wird nicht empfohlen.

6.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit DIL verdünnen, z. B. 10 µL Probe und 1 mL DIL in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

7. TESTDURCHFÜHRUNG

Gebrauchsanweisung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Gebrauchsanweisung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf bis zu fünf und das WASH | BUF | 1x -Volumen von 300 µL auf 350 µL zu erhöhen. Kapitel 11 beachten. Vor Testbeginn die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL WASH | BUF | 1x waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL SUB | TMB in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL SOLN | STOP in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei der Zugabe von SUB | TMB pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe von SOLN | STOP messen.

7.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben notieren.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

8. BERECHNUNG DER ERGEBNISSE

8.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

8.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86 : 2 = 0,43
Cut-off = 0,43

8.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

8.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

8.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion

9. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

9.1. Präzision

Intraassay	n	Mittelwert (OD)	Vk (%)
#1	24	0,408	5,21
#2	24	1,181	3,01
#3	24	0,945	1,52

Interassay	n	Mittelwert (NTU)	Vk (%)
#1	12	29,72	6,25
#2	12	22,93	4,67
#3	12	4,93	4,98

9.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 95,65% (95% Konfidenzintervall: 78,05% - 99,89%).

9.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 100% (95% Konfidenzintervall: 83,16% - 100%).

9.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL für Hämoglobin, von 5 mg/mL Triglyceride und von 0,5 mg/mL für Bilirubin keine Interferenzen im vorliegenden ELISA.

9.5. Kreuzreaktivität

Die Untersuchung eines Panels mit potenziell kreuzreagierenden Proben (Rheumafaktoren und Antikörper gegen Candida albicans) ließ keine Anhaltspunkte für falsch-positive Ergebnisse aufgrund von Kreuzreaktivitäten erkennen.

10. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

11. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Gebrauchsanweisung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

11.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 3.1).
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen.

Die Reagenzien können 5-Brom-5-nitro-1,3-dioxan enthalten (siehe 3.1).
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H315	Verursacht Hautreizungen.
H319	Verursacht schwere Augenreizung.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P305+P351+P338	BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.
P337+P313	Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

11.2. Entsorgungshinweise

Rückstände von Chemikalien und Zubereitungen werden im Allgemeinen als gefährliche Abfälle betrachtet. Die Entsorgung dieser Art von Abfällen wird durch nationale und regionale Gesetze und Vorschriften geregelt. Wenden Sie sich an Ihre örtlichen Behörden oder an Abfallentsorgungsunternehmen, die Sie über die Entsorgung von Sondermüll beraten.

Informationen zu den Verpackungsmaterialien finden Sie unter VERPACKUNGSMATERIALIEN.

12. BESTELLINFORMATIONEN

REF	ASPG0680	Aspergillus fumigatus IgG	(96 Bestimmungen)

FRANÇAIS

1. INDICATION D'UTILISATION

La trousse Aspergillus fumigatus IgG ELISA est prévue pour la détection qualitative des anticorps IgG anti-Aspergillus fumigatus dans le sérum humain ou plasma (citrate, héparine).

2. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaques de Microtitrage sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un photomètre de Plaques de Microtitrage ELISA.

3. MATERIEL

3.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Aspergillus fumigatus; en sachets d'aluminium refermables.
- **DIL:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; prêt à l'emploi; couleur jaune; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **SOLN STOP:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchon rouge.
- **WASH BUF 20x:** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc; 0,2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- **Conjugué:** 1 flacon contenant 20 mL d'anticorps IgG anti-humaines conjugués à peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur bleue, bouchon noir.
- **SUB TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1%; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 11.1.

3.2. Matériel fourni

- 1 couvercle autocollante
- 1 notice d'utilisation

3.3. Matériel et équipement requis

- Photomètre de Plaque de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaque de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

4. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8 °C.

5. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20...25 °C) et les mélanger avant de commencer le test!

5.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigène de Aspergillus fumigatus. Immédiatement après avoir prélevé les barrettes nécessaires, les barrettes restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

5.2. WASH | BUF | 20x

Diluer WASH | BUF | 20x 1+19; par exemple 10 mL WASH | BUF | 20x + 190 mL d'eau distillée. Le Tampon diluée (WASH | BUF | 1x) est stable pendant 5 jours à la température ambiante (20…25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

5.3. SUB | TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. SUB | TMB doit être incolore ou pourrait avoir une légère couleur bleu clair. Si SUB | TMB devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

6. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum ou plasma (citrate, héparine) pour ce test. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

6.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec DIL. Diluer 10 µL d'échantillon avec 1 mL DIL dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

7. PROCEDE DE TESTE

Lire attentivement la notice d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du WASH | BUF | 1x de 300 à 350 µL. Faites attention au chapitre 11. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le Blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL WASH | BUF | 1x. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape!

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats.
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de SUB | TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL SOLN | STOP dans tous les puits dans le même ordre et à la même vitesse que pour la SUB | TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de SOLN | STOP.

7.1. Mesure

Réglez le Photomètre de Plaque de Microtitrages ELISA **à zéro** en utilisant **le Blanc substrat.**

Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrages ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance**.

8. RESULTATS

8.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ce notice d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés:

- **Blanc Substrat:** Valeur d'absorbance **< 0,100**
- **Contrôle Négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off:** Valeur d'absorbance **0,150 – 1,300**
- **Contrôle Positif:** Valeur d'absorbance **> contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

8.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du contrôle Cut-off.

Exemple: 0,44 DO contrôle Cut-off + 0,42 DO contrôle Cut-off = 0,86 : 2 = 0,43
Cut-off = 0,43

8.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l'échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

8.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il y a eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limitée dans le cas des patients immunodéprimés et des nouveaux-nés.		

8.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec un faible titre d'IgG: → suggère une infection très récente ou aigüe Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire de l'anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne

9. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

9.1. Précision

Intra-essai	**n**	**moyenne (E)**	**CV (%)**
#1	24	0,408	5,21
#2	24	1,181	3,01
#3	24	0,945	1,52

Inter-essai	**n**	**moyenne (NTU)**	**CV (%)**
#1	12	29,72	6,25
#2	12	22,93	4,67
#3	12	4,93	4,98

9.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 95,65% (95% Intervalle de confiance: 78,05% - 99,89%).

9.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 100% (95% Intervalle de confiance: 83,16% - 100%).

9.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL d'hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

9.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents (Facteur Rhumatoïde et Candida albicans anticorps) n'a pas révélé de preuves de résultats faussement positifs dus à des réactions croisées.

10. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongelation répétés de l`échantillon peuvent affecter les valeurs d'absorption.

11. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice utilisation, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

11.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3:1) ou du MIT (voir chapitre 3.1).
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attention	H317	Peut provoquer une allergie cutanée.
	P261	Éviter de respirer les aérosols.
	P280	Porter des gants de protection/ des vêtements de protection.
	P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
	P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
	P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

Les réactifs peuvent contenir du 5-Bromo-5-nitro-1,3-dioxane (voir chapitre 3.1).
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attention	H315	Provoque une irritation cutanée.
	H319	Provoque une sévère irritation des yeux.
	P280	Porter des gants de protection/ des vêtements de protection.
	P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
	P305+P351+P338	EN CAS DE CONTACT AVEC LES YEUX: Rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer.
	P337+P313	Si l'irritation oculaire persiste: Consulter un médecin.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

11.2. Elimination des déchets

Les résidus de produits chimiques et de préparations sont généralement considérés comme des déchets dangereux. L'élimination de ce type de déchets est réglementée par des lois et règlements nationaux et régionaux. Contactez les autorités locales ou les entreprises de gestion des déchets qui vous donneront des conseils sur la manière d'éliminer les déchets dangereux.

Pour plus d'informations sur les matériaux d'emballage, reportez-vous à la section MATÉRIELS D'EMBALLAGE.

12. INFORMATION POUR LES COMMANDES

REF	ASPG0680	Aspergillus fumigatus IgG	(96 déterminations)

ITALIANO

1. USO PREVISTO

Il Aspergillus fumigatus IgG ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgG per Aspergillus fumigatus nel siero o plasma (citrato, eparina) umano.

2. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestite con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu. L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm è letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

3. MATERIALI

3.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni dell'Aspergillus fumigatus; dentro una busta d'alluminio richiudibile.
- **DIL:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; colore giallo; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **SOLN STOP:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **WASH BUF 20x:** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco; 0,2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- **Coniugato:** 1 flacone contenente 20 mL di anticorpi IgG anti-umani, coniugati a perossidasi in tampone fosfato (10 mM); colore azzurro; pronto all'uso; tappo nero.
- **SUB TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1%; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 11.1.

3.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzioni per l'uso

3.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37 °C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

4. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

5. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescolare prima di iniziare il test.

5.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con gli antigeni della Aspergillus fumigatus. Immediatamente dopo la rimozione delle strisce necessarie, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

5.2. WASH | BUF | 20x

Diluire WASH | BUF | 20x 1+19; per esempio. 10 mL WASH | BUF | 20x + 190 mL di acqua distillata. Il Tampone diluito (WASH | BUF | 1x) è stabile per 5 giorni a temperatura ambiente (20…25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

5.3. SUB | TMB

La soluzione sta pronta ll'uso e deve essere conservata a 2 ... 8 °C, al riparo dalla luce. SUB | TMB deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se SUB | TMB diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

6. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/ scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

6.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con DIL. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL DIL e mescolare bene (Vortex).

7. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume del WASH | BUF | 1x da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 11. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato). Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1°C.**
4. Al termine dell'incubazione, togliere la pellicola ed aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL WASH | BUF | 1x. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo!

   Attenzione: Il lavaggio è una fase molto importante! Da lavaggio insufficiente risulta una bassa precisione e risultati falsi.
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL SUB | TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL SOLN | STOP in tutti i pozzetti, nello stesso ordine della SUB | TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta SOLN | STOP.

7.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e registra i valori di assorbanza per ogni standard/controllo e campione.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

8. RISULTATI

8.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo:** Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

8.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

8.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

8.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

8.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: →suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata

9. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

9.1. Precisione

Intradosaggio	n	Media (E)	CV (%)
#1	24	0,408	5,21
#2	24	1,181	3,01
#3	24	0,945	1,52

Interdosaggio	n	Media (NTU)	CV (%)
#1	12	29,72	6,25
#2	12	22,93	4,67
#3	12	4,93	4,98

9.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici. La specificità diagnostica è 95,65% (95% intervallo di confidenza: 78,05% - 99,89%).

9.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici. La sensibilità diagnostica è 100% (95% intervallo di confidenza: 83,16% - 100%).

9.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

9.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti (Fattore reumatoide e anticorpi della Candida albicans) non ha rivelato alcuna evidenza di risultati falsamente positivi dovuto a reattività crociata.

10. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

11. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

11.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 3.1).
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione

H317	Può provocare una reazione allergica cutanea.
P261	Evitare di respirare gli aerosol.
P280	Indossare guanti/ indumenti protettivi.
P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

I reagenti possono contenere 5-Bromo-5-nitro-1,3-dioxane (vedi capitolo 3.1).
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza

Attenzione

H315	Provoca irritazione cutanea.
H319	Provoca grave irritazione oculare.
P280	Indossare guanti/ indumenti protettivi.
P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
P305+P351+P338	IN CASO DI CONTATTO CON GLI OCCHI: Sciacquare accuratamente per parecchi minuti. Togliere le eventuali lenti a contatto se è agevole farlo. Continuare a sciacquare.
P337+P313	Se l'irritazione degli occhi persiste: Consultare un medico.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

11.2. Smaltimento

I residui di prodotti chimici e preparati sono generalmente considerati come rifiuti pericolosi. Lo smaltimento di questo tipo di rifiuti è regolato da leggi e regolamenti nazionali e regionali. Contattare le autorità locali o le società di gestione dei rifiuti che daranno consigli su come smaltire i rifiuti pericolosi.

Per informazioni sui materiali d'imballaggio fare riferimento a MATERIALI D'IMBALLAGGIO.

12. INFORMAZIONI PER GLI ORDINI

REF	ASPG0680	Aspergillus fumigatus IgG	(96 determinazioni)

ESPAÑOL

1. USO PREVISTO

El enzimoinmunoensayo Aspergillus fumigatus IgG ELISA se utiliza para la determinación cualitativa de anticuerpos IgG específicos contra Aspergillus fumigatus en suero o plasma (citrato, heparina) humano.

2. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimático cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualizó añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. Se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placas de Microtitulación ELISA.

3. MATERIALES

3.1. Reactivos suministrados

- **Placa de Microtitulación:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Aspergillus fumigatus en bolsa de aluminio.
- **DIL:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; color amarillo; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **SOLN STOP:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **WASH BUF 20x:** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca; 0,2% (w/v) 5-Bromo-5-nitro-1,3-dioxano.
- **Conjugado:** 1 botella de 20 mL de conjugado de anticuerpos IgG anti-humano con peroxidasa en tampón de fosfato (10 mM); color azul; tapa negra; listo para ser utilizado.
- **SUB TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB),< 0,1%; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 11.1.

3.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso

3.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático para Placa de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

4. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

5. PREPARACIÓN DE LOS REACTIVOS

¡Es muy importante llevar Todos los reactivos y las muestras para a la temperatura ambiente (20...25 °C) y mezclarlos antes de serem utilizados!

5.1. Placa de Microtitulación

Las tiras rompibles están recubiertas con antígenos de Aspergillus fumigatus. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2...8 °C.

5.2. WASH | BUF | 20x

Diluir WASH | BUF | 20x 1+19; por ejemplo 10 mL WASH | BUF | 20x + 190 mL de agua destilada. El Tampón diluido (WASH | BUF | 1x) es estable durante 5 días a temperatura ambiente (20…25 °C). En caso de aparecer cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

5.3. SUB | TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. SUB | TMB debe ser incolora o podría tener un color ligeramente azul claro. Si SUB | TMB se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

6. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas de 2...8 °C, en caso contrario deben ser alícuotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

6.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con DIL, por ejemplo 10 µL de la muestra con 1 mL DIL, mezclar bien con la mezcladora Vortex.

7. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de WASH | BUF | 1x de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 11. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado). Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1°C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1°C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL WASH | BUF | 1x. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: ¡El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados.
5. Pipetar 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µL SUB | TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de SOLN | STOP en el mismo orden y mismo intervalo de tiempo como con SUB | TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir SOLN | STOP.

7.1. Medición

Ajustar el fotómetro de Placa de Microtitulación Elisa **al cero** utilizando **el Blanco.**

¡Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se pueder ajustar a cero utilizando el Blanco, el valor de la absorbancia desto debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

8. CALCULO DE LOS RESULTADOS

8.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off:** valor de la extinción **0,150 – 1,300**
- **Control Positivo**: valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

8.2. Calculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de los dos controles Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43

Cut-off = 0,43

8.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra} \times 10}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

8.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

8.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundario del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada

9. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

9.1. Precisión

Intra ensayo	**n**	**Promedio (E)**	**CV (%)**
#1	24	0,408	5,21
#2	24	1,181	3,01
#3	24	0,945	1,52
Inter ensayo	**n**	**Promediot (NTU)**	**CV (%)**
#1	12	29,72	6,25
#2	12	22,93	4,67
#3	12	4,93	4,98

9.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 95,65% (95% Intervalo de confianza: 78,05% - 99,89%).

9.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 100% (95% Intervalo de confianza: 83,16% - 100%).

9.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

9.5. Reactividad cruzada

Pruebas realizadas con un panel de muestras con distinta actividad de anticuerpos para estudiar parámetros de reactividad no dieron falsos positivos debidos a reactividad cruzada.

10. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

11. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales di origen humana o animal deveram ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice, GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

11.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 3.1).
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H317	Puede provocar una reacción alérgica en la piel.
P261	Evitar respirar el aerosol.
P280	Llevar guantes/ prendas de protección.
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Los reactivos pueden contener 5-Bromo-5-nitro-1,3-dioxano (consulte el cap. 3.1).
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H315	Provoca irritación cutánea.
H319	Provoca irritación ocular grave.
P280	Llevar guantes/ prendas de protección.
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P305+P351+P338	EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337+P313	Si persiste la irritación ocular: Consultar a un médico.

Se puede encontrar más información en la ficha de datos de seguridad.

11.2. Indicaciones para la eliminación de residuos

Los residuos de productos químicos y preparados se consideran generalmente como residuos peligrosos. La eliminación de este tipo de residuos está regulada por leyes y reglamentos nacionales y regionales. Póngase en contacto con las autoridades locales o con las empresas de gestión de residuos, que le asesorarán sobre cómo eliminar los residuos peligrosos.

Para obtener información sobre los materiales de embalaje, consulte MATERIALES DE EMBALAJE.

12. INFORMACIONES PARA PEDIDOS

REF	ASPG0680	Aspergillus fumigatus IgG	(96 determinaciones)

PORTUGUÊS

1. UTILIZAÇÃO PRETENDIDA

O kit Aspergillus fumigatus IgG ELISA destina-se à determinação qualitativa de anticorpos da classe IgG contra Aspergillus fumigatus no soro ou plasma (citrato, heparina) humanos.

2. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligada, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo. Absorvância a 450/620 nm é lida utilizando um fotômetro de Placas de Microtitulação ELISA.

3. MATERIAIS

3.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénio do Aspergillus fumigatus, em bolsas de folha de alumínio com fecho.
- **DIL:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; de cor amarela; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **SOLN STOP:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **WASH BUF 20x:** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca; 0,2% (w/v) 5-Bromo-5-nitro-1,3-dioxano.
- **Conjugado:** 1 frasco contendo 20 mL de anticorpo IgG anti-humana conjugado com peroxidase no tampão fosfato (10 mM); de cor azul, pronto a usar; tampa preta.
- **SUB TMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1%; pronto a usar; tampa amarela.
- **Controle Positivo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa vermelha. ≤ 0,02% (v/v) MIT.
- **Controle Cut-off:** 1 frasco contendo 3 mL controle; de cor amarela; pronto a usar; tampa verde. ≤ 0,02% (v/v) MIT.
- **Controle Negativo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa azul; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para advertências de perigo e recomendaçaões de prudência ver capitulo 11.1.

3.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de utilização

3.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulação ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem de Placas de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

4. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

5. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) e misturálos antes de iniciar o teste!

5.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antigénio Aspergillus fumigatus. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C.

5.2. WASH | BUF | 20x

Diluir WASH | BUF | 20x 1+19; por exemplo. 10 mL WASH | BUF | 20x + 190 mL de água destilada. O Tampão diluído (WASH | BUF | 1x) é estavel durante 5 dias à temperatura ambiente (20…25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

5.3. SUB | TMB

A Solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. SUB | TMB deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se SUB | TMB se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

6. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

6.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com DIL. Dispensar 10 µL de amostra e 1 mL DIL em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

7. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de utilização **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de utilização, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume WASH | BUF | 1x de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 11. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL WASH | BUF | 1x. Evitar que os poços de reacção transbordem.O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL SUB | TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL SOLN | STOP em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a SUB | TMB, desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição SOLN | STOP.

7.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato.**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

8. RESULTADOS

8.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas instruções de utilização devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato:** Valor de Absorvância **< 0,100**
- **Controle Negativo:** Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off:** Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo:** Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

8.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86 : 2 = 0,43
Cut-off = 0,43

8.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra} \times 10}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

8.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

8.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada

9. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

9.1. Precisão

Intra ensaio	**n**	**Média (E)**	**CV (%)**
#1	24	0,408	5,21
#2	24	1,181	3,01
#3	24	0,945	1,52
Inter ensaio	**n**	**Média (NTU)**	**CV (%)**
#1	12	29,72	6,25
#2	12	22,93	4,67
#3	12	4,93	4,98

9.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico. É de 95,65% (95% intervalo de confiança: 78,05% - 99,89%).

9.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico. É de 100% (95% intervalo de confiança: 83,16% - 100%).

9.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

9.5. Reação cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reação cruzada (fator remautoide e anticorpos de Candida albicans) e não revelou nenhuma evidencia de resultados falso-positivos devido a reações transversais.

10. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

11. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções de utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

11.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) ou MIT (ver capitulo 3.1).
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção	H317	Pode provocar uma reacção alérgica cutânea.
	P261	Evitar respirar os aerossóis.
	P280	Usar luvas de protecção/ vestuário de protecção.
	P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
	P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
	P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Os reagentes podem conter 5-Bromo-5-nitro-1,3-dioxano (ver capitulo 3.1).
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção	H315	Provoca irritação cutânea.
	H319	Provoca irritação ocular grave.
	P280	Usar luvas de protecção/ vestuário de protecção.
	P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
	P305+P351+P338	SE ENTRAR EM CONTACTO COM OS OLHOS: Enxaguar cuidadosamente com água durante vários minutos. Se usar lentes de contacto, retire-as, se tal lhe for possível. Continue a enxaguar.
	P337+P313	Caso a irritação ocular persista: Consulte um médico.

Mais informações podem ser encontradas na ficha de dados de segurança.

11.2. Considerações de Eliminação

Os resíduos de produtos químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos é regulamentada através de leis e regulamentos nacionais e regionais. Contacte as suas autoridades locais ou empresas de gestão de resíduos que darão conselhos sobre como eliminar os resíduos perigosos.

Para informações sobre os materiais de embalagem, consulte MATERIAIS DE EMBALAGEM.

12. INFORMAÇÃO DE PEDIDO

REF	ASPG0680	Aspergillus fumigatus IgG	(96 Determinações)

ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

PACKAGING MATERIALS / VERPACKUNGSMATERIALIEN / MATÉRIELS D'EMBALLAGE / MATERIALI D'IMBALLAGGIO / MATERIALES DE EMBALAJE / MATERIAIS DE EMBALAGEM

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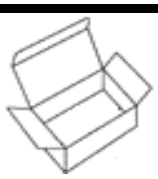
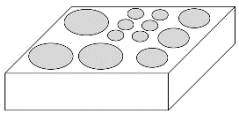
<tr><td> 
PAP 21</td><td> 
PAP 21</td><td> 
PAP 22</td></tr>
<tr><td colspan="2">SOLN | STOP   WASH | BUF | 20x   SUB | TMB   DIL
CONJ   CONTROL | +   CONTROL | -   CUT OFF</td><td>MTP</td></tr>
<tr><td> 
HDPE 2</td><td> 
PP 5</td><td> 
PET / ALU / LDPE 90</td></tr>
</table>

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE marking / CE-Kennzeichnung / Marquage CE / Marchio CE / Marca CE / Marca CE / CE-markering
UDI	Unique Device Identifier / Eindeutige Produktidentifizierung / identification unique des dispositifs / identificazione unica del dispositivo / identificación única del producto / identificação única dos dispositivos
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Gebrauchsanweisung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulacións / Placa de Microtitulação
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle Négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle / Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL	Sample Dilution Buffer / Probenverdünnungspuffer / Tampon de Dilution d'Échantillon / Tampone di Diluizione del Campione / Tampón de Dilución de Muestras / Tampão de Diluição de Amostra
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante / Solución de Parada /Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución Substrato TMB / Solução Substrato TMB
WASH | BUF | 20x	"Washing Buffer (20x concentrated)"; REF W0000 Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampone di Lavaggio concentrado x20 / Tampão de Lavagem concentrada 20x
WASH | BUF | 1x	20-fold dilution of WASH | BUF | 20x / 20-fach Verdünnung von WASH | BUF | 20x / Dilution 20 fois du WASH | BUF | 20x / Diluizione 20 volte del WASH | BUF | 20x / Dilucíon de 20 veces del WASH | BUF | 20x / Diluição de 20 dobras do WASH | BUF | 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Aspergillus fumigatus IgG

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of of **WASH BUF 1x**					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of of **WASH BUF 1x**					
SUB TMB	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
SOLN STOP	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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ASPG0680_IFU_rev01_fromLot_055N

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Unconjugated Estriol (uE3) Test System
Product Code: 5025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Unconjugated (Free) Estriol Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

The last few years have seen the development of screening for fetal Down Syndrome by measurement of multiple markers in maternal blood [1]. Although amniocentesis has been widely available for more than 40 years it can only be selectively used to diagnose the disorder because of the hazard to fetus. Of most employed for differential diagnosis the commonly used procedures are AFP, hCG, free beta-HCG and unconjugated estriol.[2]

Unconjugated estriol in the serum of pregnant women originates almost exclusively from precursors in the fetus, via the placenta.[3] The clinical evidence shows that in uncomplicated pregnancies, the production of estriol increases steadily throughout the last trimester; however, in pregnancies complicated by placental insufficiency the synthesis of estriol decreases rapidly. For many years the most commonly used method for monitoring estriol synthesis (as an index to fetal stress) has been to measure estriol and estriol conjugates in a 24 hr urine sample[4] However, changes in renal clearance and diurnal variations can make the results of these determinations suspect. In recent years investigators have found the determinations of unconjugated estriol in pregnancy plasma, as an alternative to the urinary assay, to be a better marker of fetal stress.[6] Abnormally low levels of estriol in a pregnant woman may indicate a problem with the development of the child. Levels of estriol in non-pregnant women do not change much after menopause, and levels are not significantly different from levels in men.[7]

The Monobind unconjugated estriol EIA Kit uses a specific anti-estriol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally-occurring and structurally related steroids is low.

The employment of several serum references of known Estriol 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 Estriol concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for a 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 following 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.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow$ <u>immobilized complex</u>
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
<u>Immobilized complex</u> = 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

A. uE3 Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for unconjugated estriol at concentrations of 0 (**A**), 0.4 (**B**), 2.0 (**C**), 5.0 (**D**), 15 (**E**), and 30.0 (**F**) in ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by the conversion factor 3.45. For example: 1ng/ml x 3.45 = 3.45 nM/L

B. uE3 Enzyme Reagent – 6.0 ml/vial – Icon Ⓔ
One (1) vial contains Estriol (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

C. uE3 Biotin Reagent – 6.0 ml - Icon ▽
One (1) vial contains anti-unconjugated Estriol biotinylated purified rabbit IgG conjugate in buffer, blue 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 contains 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 contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

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

H. Stop Solution – 8ml/vial - Icon STOP
One (1) vial contains 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 0.025ml (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. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
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 components 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. 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 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 buffer 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 - 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 calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the uE3 Enzyme Reagent to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of uE3 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. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the sample, suspected of concentrations higher than 30ng/ml, by diluting 1:2 and/or 1:5 with unconjugated estriol '0' ng/ml calibrator or male patient sera with a known low value for estriol. Multiply the result by the dilution factor of 2 or 5 as required to obtain the concentration of the sample.

10.0 CALCULATION OF RESULTS

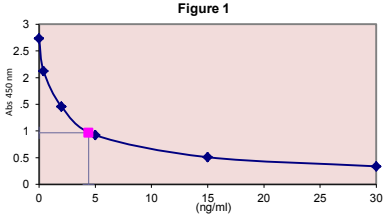
A dose response curve is used to ascertain the concentration of unconjugated estriol in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding unconjugated estriol concentration in ng/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of unconjugated estriol 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 (0.967) intersects the dose response curve at (4.71 ng/ml) unconjugated estriol concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.742	2.732	0
	B1	2.722		
Cal B	C1	2.155	2.123	0.4
	D1	2.091		
Cal C	E1	1.492	1.456	2.0
	F1	1.420		
Cal D	G1	0.940	0.921	5.0
	H1	0.903		
Cal E	A2	0.523	0.508	15.0
	B2	0.493		
Cal F	C2	0.342	0.336	30.0
	D2	0.330		
Ctrl 1	G2	1.557	1.532	***1.82***
	H2	1.507		
Patient	A3	0.991	0.967	***4.71***
	B3	0.943		

Figure 1



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

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 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 Unconjugated E3 concentrations above 30 ng/ml may be diluted (1/2,1/5 or higher) with Unconjugated E3 '0' calibrator 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. ***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 RANGES OF VALUES

In agreement with established reference intervals[5] for a "normal" adult population, the expected ranges for the Unconjugated Estriol AccuBind® ELISA Test System are detailed in Table 1.

TABLE I
Expected Values for the uE3 EIA Test System (ng/ml)

Male & Non-Pregnant Female	**< 1.0 ng/ml**

During pregnancy the Unconjugated E3 serum levels rise rapidly till the end of third trimester. (See Table 2 from published Literature).[6]

TABLE 2

Gestation Week	Expected Range (ng/ml)	Gestation Week	Expected Range (ng/ml)	Twin Pregnancy (ng/ml)
12	0.3-1.0	22	2.7-16.0	3.0-18.0
14	0.4-1.6	26	3.0-18.0	4.0-21.0
16	1.4-6.5	32	4.6-23.0	5.0-25.0
18	1.6-8.5	36	7.2-29.0	7.0-39.0
20	2.1-13.0	40	8.0-39.0	13.0-40.0

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 uE3 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 3 and Table 4.

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

Sample	N	X	σ	C.V.
Low	24	1.58	0.13	8.3%
Normal	24	5.17	0.37	7.1%
High	24	9.06	0.59	6.5%

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

Sample	N	X	σ	C.V.
Low	10	1.47	0.14	9.5%
Normal	10	4.93	0.39	7.9%
High	10	8.99	0.54	6.0%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The uE3 AccuBind® ELISA Test System has a sensitivity of 2.9 pg/T. This is equivalent to a sample containing a concentration of 0.115 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The uE3 AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal and high Unconjugated Estriol level populations were used; the values ranged from 0.15 – 29.1 ng/ml. The total number of specimens was 158. The least square regression equation and the correlation coefficient were computed for this uE3 in comparison with the reference method. The data obtained is displayed in Table 5.

TABLE 5

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	3.84	y= -0.174+0.979(x)	0.952
Reference (X)	3.74		

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 Estriol antibody to selected substances, for determination of Unconjugated Estriol, was evaluated by adding the interfering substance to a serum matrix at massive concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of uE3 needed to displace the same amount of labeled analog.

Substance	% Cross Reactivity
Estriol	100.0000
Androstenedione	0.0001
Cortisol	<0.0001
Cortisone	<0.0001
Corticosterone	<0.0001
DHEA-S	<0.0001
Dihydotestosterone	0.0001
Estradiol	0.0040
Estriol Glucouronide	<0.0001
Estriol Sulfate	0.6200
Estrone	0.0004
Prednisone	<0.0001
Progesterone	<0.0001
Spirolactone	<0.0001
Testosterone	<0.0001

15.0 REFERENCES

1. Goebelsman, U, Katagiri, H, Stanczyk et al., Estriol assays in obstetrics. *J. Steroid Biochemistry.* 6, 703-709 (1975).
2. Reynolds T, Penny M, "The mathematical basis of multivariate risk screening, with special reference to screening for Down Syndrome associated pregnancy", *Ann Clin Biochem* , 27:452-458 (1990).
3. Kastagiri,H., Stanczyk, F and Goebelsman,U, "Estriol in pregnancy.III Development, comparison and use of a specific antisera for rapid radioimmunoassay of unconjugated estriol in pregnancy plasma", *Steroids,* 24, 225 (1974).
4. Brown CH, Saffan, BD, Howard C.M and Preedy JR. "The renal clearance of endogenous estrogens in late pregnancy", *J Clinical Investigation,* .43,295 (1964).
5. Tietz, NW, ED: *Clinical Guide to Laboratory Tests*, 3rd ed. Philadelphia, WA Saunders Co, 1995.
6. Cohen M, and Cohen H, "A radioimmunoassay for plasma unconjugated estrogens in normal pregnancy", *Am J. Obstet. Gynecology,* 118, 200 (1974).
7. Wright JV, Schliesman B, Robinson L, "Comparative measurements of serum estriol, estradiol and estrone in non-pregnant, postmenopausal women; a preliminary investigation:, *Altern Med Rev* , 4 266-70 (1999).
8. NIH State-of-the-Science Conference Statement on Management of Menopause-Related Symptoms. NIH Consensus State Sci Statements. 2005. Mar 21-23; 22(1)1-38.

Rev 4 Date: 2019-Jul-16 DCO: 1353
MP5025 Product Code: 5025-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	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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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

Pregnancy Associated Plasma Protein-A (PAPP-A) Test System

Product Code: 12625-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Trisomy 21, 18, and 13 are three conditions that can cause ill effects to a fetus and, therefore, an infant. Screening for markers and inherent trends has been of interest over the years. One of the proteins of interest for these trisomy disorders is PAPP-A, pregnancy associated plasma protein A. This protein can be found in the serum of every individual, but the concentration rises in maternal serum as gestation time progresses. In studies, a correlation has been shown between the decreased level of PAPP-A and the occurrence of trisomy disorders, particularly Trisomy 21, also known as Down's Syndrome. Along with a few other markers like AFP, uE3, and hCG, trends in PAPP-A have been found to elude to trisomy disorders.[1,2,3,4,5,6]

PAPP-A is produced primarily by the placenta during pregnancy. This glycoprotein has a molecular weight of 740,000 and tends to exist as a heterotetrameric dimer with ProMBP, proform major basic protein. The concentration of PAPP-A in maternal blood increases over the time of the pregnancy as the placenta and fetus grows because it is a product of the trophoblast. In general, the concentration level of this protein in maternal serum is indicative of threatened abortion, preterm birth, intrauterine growth restriction, ectopic gravidity, preeclampsia or diabetes mellitus. When tested during the first trimester of pregnancy, PAPP-A is the major marker for Down's syndrome.[3,7,8,9]

The Monobind PAPP-A test system is designed specifically for the testing of the heterotetrameric form important during pregnancy. Another form of PAPP-A also exists in serum, but it is a dimeric form associated with coronary and cardiac conditions. Tests developed for use on pregnancy patients are not designed to test for this dimeric form. When evaluation of this protein is done, it is often compared to the Multiple of Medians (MoM) and represented as a percent of an in-house establish median. Without easy access to an IRP for PAPP-A, this method allows an easier way to compare results between laboratories and testing methods, allowing for a type of reference value to be established.[2,5,8,9]

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- *PAPP-A* 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. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation:

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

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{I(PAPP\text{-}A)}$ = Native Antigen (Variable Quantity)
$Ag_{(PAPP\text{-}A)} - {}^{Btn}Ab_{(m)}$= Antigen-Antibody complex (Variable Quan.))
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

$Ag_{(PAPP\text{-}A)} - {}^{Btn}Ab_{(m)} + \underline{Streptavidin}_{C.W.} \Rightarrow$ immobilized complex (IC)
$\underline{Streptavidin}_{C.W.}$ = 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 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.

$$(IC) + {}^{Enz}Ab_{(PAPP\text{-}A)} \underset{k_b}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(PAPP\text{-}A)} - IC$$

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

4.0 REAGENTS

Materials Provided:

A. PAPP-A Calibrators – 0.5 ml/vial – Icons A-F
Six (6) vials of serum reference for PAPP-A at concentrations of 0 (**A**), 0.64 (**B**), 1.6 (**C**), 3.2 (**D**), 9.6 (**E**) and 32 (**F**) in µg/ml. A preservative has been added. Store at 2-8°C.
Note: To convert µg/ml to mIU/L, multiply by 156.25
Ex. 32 µg/ml x 156.25 = 5000 mIU/L

B. PAPP-A Enzyme Reagent– 12 ml/vial-Icon
One (1) vial of PAPP-A Antibody-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with a preservative. Store at 2-8°C.

C. PAPP-A Biotin Reagent – 12 ml/vial – Icon ∇
One (1) vial containing biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
Note: Patients must be fasting from biotin therapy/supplements for 8 hours to prevent interference in the test

D. PAPP-A Control – 0.5 ml/vial – Icon M
One (1) vials of serum reference for PAPP-A at concentration established (exact value listed on label). A preservative has been added. Store at 2-8°C.

E. PAPP-A Diluent – 5.0 ml/vial – Icon U
One (1) vial of human serum based buffer with salts, surfactants, and preservatives. Store at 2-8°C.

F. 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.

G. 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.

H. 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.

I. Stop Solution – 8ml/vial - Icon
One (1) vial contains of a strong acid (0.5M H_2SO_4). 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 label.**
Note 3: Above reagents are for a single 96-well microplate**.** For other kit configurations, refer to table at the end of the instructions.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.010ml (10µl), 0.050ml (50µl) and 0.100ml (100µl) 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 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 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; a red top gel separator tube (also known as a "Tiger" top tube) can be used. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml of the specimen is required.

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.

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 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.

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

9.0 TEST PROCEDURE

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

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.010ml (10µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the PAPP-A Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 30 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 (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 PAPP-A Enzyme Reagent to all wells.
9. Cover and incubate for 30 minutes at room temperature
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate.
12. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's inst**
13. **ruction 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 four (4) additional times.**
14. Add 0.100ml (100µl) of substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
15. Incubate at room temperature for 15-20 minutes.
16. 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.**
17. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 32 µg/ml with PAPP-A diluent and multiply result by dilution factor. For a 1:5 dilution add 40µl of diluent to 10µl of high concentration sample.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PAPP-A in unknown specimens.

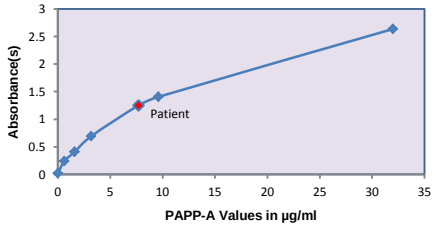
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PAPP-A concentration in µg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of PAPP-A 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/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 in the patient/ sample (1.251) intersects the dose response curve at (7.7 µg/ml) PAPP-A 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 (µg/ml)
Cal A	A1	0.019	0.021	0
	A2	0.022		
Cal B	B1	0.208	0.243	0.64
	B2	0.277		
Cal C	A6	0.421	0.410	1.6
	B6	0.399		
Cal D	C6	0.696	0.695	3.2
	D1	0.693		
Cal E	E1	1.420	1.406	9.6
	E2	1.391		
Cal F	F1	2.625	2.635	32.0
	F2	2.644		
Ctrl # 1	C3	0.880	0.895	4.6
	D3	0.910		
Ctrl #2	E3	1.675	1.787	11.6
	F3	1.898		
Patient	G1	1.306	1.251	7.7
	H1	1.196		

FIGURE 1



*The above data and table below is for example only. Do not use it for calculating your results.

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 (32 µg/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 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 PAPP-A AccuBind® ELISA 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 RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the PAPP-A AccuBind® ELISA Test System are detailed in Table 1.

It is recommended to compare values based on the Multiple of Median (MoM) established for the laboratory when assessing patient samples. By dividing the value of the patient sample by the MoM will give a percent value that is used frequently for evaluation.

TABLE 1
Expected Values for the PAPP-A Test System

Term of Gestation (full weeks)	PAPP-A Concentration (µg/ml)
9	3.86
10	7.1
11	10.1
12	16.68
13	23.2

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 total precision of the PAPP-A AccuBind® Microplate EIA Test System was determined by analyses on six different levels of pool control and patient sera. The mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2.

TABLE 2
Precision data for the PAPP-A Test System

Sample	Mean Value (pg/hl)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Control 1	0.69	0.03	3.65	0.05	7.89
Control 2	2.17	0.14	6.29	0.20	9.33
Control 3	8.25	0.42	5.11	0.60	7.29
Patient 1	1.35	0.05	3.68	0.13	9.51
Patient 2	5.12	0.22	4.24	0.43	8.49
Patient 3	28.02	1.51	5.39	2.60	9.29

*As measured in forty experiments in duplicate over a 20 day period.

14.2 Sensitivity

The LoB/LoD/LoQ of the PAPP-A AccuBind® ELISA Test System were calculated in conformance with CLSI EP17-A2 Protocols for Determination of Limits of Detection of Limits of Quantitation. The LoB is 0.019 µg/ml and the LoD=LoQ=0.074 µg/ml.

14.3 Accuracy

14.3.1 Linearity

The linearity of the PAPP-A Accubind® ELISA test system was tested by diluting human serum samples containing high levels of PAPP-A (10 to 37 µg/ml) with low PAPP-A diluent. The system produces excellent linearity through the range of the test up to 37 µg/ml.

14.3.2 Recovery

The recovery of the PAPP-A AccuBind® Microplate ELISA Test System was calculated for five patient samples spiked with 0.5, 2.0, 4.0, 10.0, and 30.0 µg/ml PAPP-A. Recoveries were determined to be within 15% of the expected values for all samples.

14.3.3 Method Comparison

The PAPP-A AccuBind® ELISA Test System was compared with a reference immunoassay method. Biological specimens from low, normal and relatively high PAPP-A level populations were used; the values ranged from 0.1 µg/ml – 36 µg/ml. The total number of such specimens was 50. The least square regression equation and the correlation coefficient were computed for this PAPP-A ELISA in comparison with the reference method.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	6.52	y = 0.34 + 0.97(x)	0.992
Reference (x)	6.68		

14.4 Specificity

The % cross reactivity of the PAPP-A 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 PAPP-A needed to displace the same amount of labeled analog.

TABLE 5

Substance	Cross Reactivity
Free βhCG	ND
AFP	ND
hPRL	ND
FSH	ND

15.0 REFERENCES

1. Sorensen S. *Clinical Chemistry*. 57, 1023-1031 (2011).
2. Qin QP. *Clinical Chemistry*. 43, 2323-2332 (1997).
3. Fialova L., Malbohan IM. *Bratisl Lek Listy*. 103, 194-205 (2002).
4. Kagan KO. *Ultrasound Obste Gynecol.* 31, 493-502 (2008).
5. Spencer K. *Ultrasound Obste Gynecol.* 13, 231-237 (1999).
6. Baluja-Conde IB. *Rev Biomed.* 259-271 (2005).
7. Smith GCS. *The Journal of Clinical Endocrinology and Metabolism.*
8. Ong YTC. *British Journal of Obstetrics and Gynaecology.* 107, 1265-1270 (2000).
9. Bischof P. *Archives of Gynecology.* 227, 315-326 (1979).

Revision: 3 **Date: 2022-11-23** **DCO: 1581**
MP12625 **Product Code: 12625-300**

Size		96(A)	192(B)
Reagent (fill)	A)	0.5 ml set	0.5 ml set
	B)	1 (12ml)	2 (12 ml)
	C)	1 (12ml)	2 (12 ml)
	D)	0.5 ml set	0.5 ml set
	E)	1 (5.0 ml)	2 (5.0 ml)
	F)	1 plate	2 plates
	G)	1 (20ml)	1 (20ml)
	H)	1 (12ml)	2 (12ml)
	I)	1 (8ml)	2 (8ml)

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IVD 2°-8°

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

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Testosterone AccuBind® ELISA Test System

Product Code: 5325-300

Rx Only

1.0 INTRODUCTION

Indications for Use: The device is an Enzyme Immunoassay (EIA) for the quantitative measurement of free testosterone in human serum. Measurement of free testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in males and in females; hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries and adrogenital syndromes.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Testosterone, (17β-Hydroxy-4-androstene-3-one), a C_{19} steroid, is the most potent naturally secreted androgen.[1] In normal post pubertal males, testosterone is secreted primarily by the testes, with only a small amount derived from peripheral conversion of 4-Androstene-3, 17-dione (ASD).[2] In adult women, it has been estimated that over 50% of serum testosterone is derived from peripheral conversion of ASD secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

In the male, testosterone is mainly synthesized in the interstitial Leydig cells and the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary (the female equivalent of ICSH).[3] Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and auxiliary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, orchidectomy, estrogen therapy, Klinefelter's syndrome, hypopituitarism, and hepatic cirrhosis.[2-4]

In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women, 50–60% of the daily testosterone production arises from peripheral metabolism of prohormone, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein-Leventhal syndrome), ovarian tumors, adrenal tumors and adrenal hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

The majority of testosterone is bound to transport proteins: weakly bound to albumin and cortisol binding protein (25-65% females; 45-85% males) and tightly bound to sex hormone-binding globulin (SHBG) (35-75% females; 14-50% males).[8] A small fraction exist as unbound or free testosterone; however, this form is biologically active. Therefore, the free hormone concentration is a better indicator of biological activity than total testosterone.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5)
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the equation in the following below.

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

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

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

4.0 REAGENTS

Materials Provided:

A. Free Testosterone Calibrators – 1ml/vial – Icons A-G
Seven (7) vials of serum reference for Free Testosterone at concentrations of 0 (**A**), 0.2 (**B**), 1.0 (**C**), 2.5 (**D**), 7.5 (**E**), 20 (**F**) and 60 (**G**) in pg/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 3.47.
For example: 1pg/ml x 3.47 = 3.47 pM/L

B. Free Testosterone Controls – 1ml/vial – Icons L, M, N
Three (3) vials of serum reference for Free Testosterone at low, middle, and high established concentrations (range values listed on labels). A preservative has been added. Store at 2-8 °C.

C. Free Testosterone Enzyme Reagent – 13ml/vial – Icon
One (1) vial of Testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with dye. Store at 2-8°C.

D. Free Testosterone Coated Plate – 96 wells – Icon
One 96-well microplate coated with testosterone antibody 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 contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

G. 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."

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

I. Product Instructions

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

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.020 & 0.050ml (20µl & 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. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
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 approved 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 in type, and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.040ml (40µl) 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 accordance with local, state, and federal quality control regulations. 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 buffer 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 - 8°C.

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

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 0.020ml (20µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the Free Testosterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 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 (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 gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

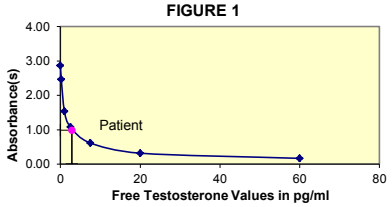
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Free Testosterone in unknown specimens within the analytical measuring range of 0.11-60 pg/ml.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free Testosterone concentration in pg/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of Free Testosterone 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). In the following example, the average absorbance 0.989 intersects the dose response curve at 2.87pg/ml Free Testosterone concentration.

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.

FIGURE 1



EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	2.867	2.867	0.0
	B1	2.867		
Cal B	C1	2.489	2.470	0.2
	D1	2.451		
Cal C	E1	1.509	1.533	1.0
	F1	1.556		
Cal D	G1	1.071	1.084	2.5
	H1	1.097		
Cal E	A2	0.620	0.614	7.5
	B2	0.608		
Cal F	C2	0.333	0.318	20
	D2	0.303		
Cal G	E2	0.171	0.168	60
	F2	0.165		
Ctrl L	G2	1.333	1.384	1.339
	H2	1.434		
Ctrl M	A3	0.734	0.737	5.284
	B3	0.739		
Ctrl N	C3	0.192	0.187	47.107
	D3	0.182		
Patient	C4	0.997	0.989	2.870
	D4	0.980		

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

11.0 Q.C. PARAMETERS

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

1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. 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 IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be 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 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 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 RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the Free Testosterone AccuBind® ELISA Test System are detailed in Table 1.

TABLE I

Population	Range (in pg/ml)
Male / 20-39	9.2-34.6
Male / 40-59	6.1-30.3
Male / ≥60	6.1-27.9
Female / 20-39	0.2-6.1
Female / 40-59	0.3-4.4
Female / ≥60	0.5-3.4

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 Accuracy

The Free Testosterone AccuBind® ELISA Test System was compared with a reference ELISA method. Biological specimens from from low, normal, and elevated concentrations were assayed. The total number of such specimens was 137. The least square regression equation and the correlation coefficient were computed.

TABLE 2

	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	y=1.017x-0.24	0.997
Reference (x)		

14.2 Precision

This study was conducted during 20 days of testing. The human serum and control sample were tested in duplicate, two times per day for a total of 80 measurements per sample. Three (3) different reagent lots, three (3) serum pools, and three (3) controls were used for the study (low, medium, and high concentration). The results of a representative lot are shown below:

TABLE 3

Lot 1 N=32	Mean (pg/ml)	Within-Run		Total	
		SD	CV%	SD	CV%
Ctrl 1	2.51	0.09	3.7%	0.20	7.8%
Ctrl 2	10.98	0.40	3.6%	0.96	8.7%
Ctrl 3	22.72	0.83	3.6%	2.18	9.6%
Serum 1	0.98	0.06	5.9%	0.12	12.4%
Serum 2	4.53	0.26	5.7%	0.36	8.0%
Serum 3	53.62	4.24	7.9%	4.32	8.1%

TABLE 4

	Mean (pg/ml)	Within-Run Precision		Within-Kit Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%	SD	CV%
Ctrl 1	2.48	0.11	4.57	0.20	8.20	0.21	8.51
Ctrl 2	11.04	0.47	4.23	0.84	2.60	0.87	8.00
Ctrl 3	23.24	1.00	4.31	1.80	7.73	1.83	7.95
Pnt 1	0.97	0.05	4.88	0.09	9.14	0.08	9.43
Pnt 2	4.62	0.23	4.88	0.32	6.89	0.33	7.20
Pnt 3	54.66	3.25	5.95	3.92	7.17	4.13	7.55

14.3 Detection Limits

The LOB (limit of the blank), the LOD (limit of detection) and the LOQ (limit of quantitation) were determined in accordance with CLSI EP 17-A A guideline, Protocols for Determination of Limits of Detection).

TABLE 5

LoB	LoD	LoQ
0.0295 pg/ml	0.0519 pg/ml	0.0519 pg/ml

14.4 Cross Reactivity

Cross reactivity was determined by testing those compounds most likely to interfere with the Monobind Free Testosterone ELISA Test System. The specificity of the assay was determined in accordance with CLSI EP07-A2. The results of the cross-reactivity study are as follows.

TABLE 6

Sample	Conc. (ng/ml)	Cross Reactivity	
		Spiked Serum	Blank Serum
11-Deoxycortisol	1000	0.000%	ND
11-KetoTestosterone	10	0.647%	0.519%
11β-Hydroxytestosterone	100	0.065%	0.054%
17α-ethynyl estradiol	1000	0.000%	ND
17α-Estradiol	1000	0.000%	0.000%
17β-Estradiol	100	0.000%	ND
17-Hydroxypregnenolone	1000	0.000%	ND
17-Hydroxprogesterone	10	0.000%	0.000%
3-EstriolGluc	1000	0.000%	ND
3-EstriolSul	1000	0.000%	ND
3β-Androstanediol	500	0.000%	ND
5α-Dihydrotestosterone	100	0.054%	0.042%
Aldosterone	8000	0.000%	0.000%
Amitriptyl HCl	1000	0.000%	ND
Androsterone	1000	0.000%	ND
Androstenedione	1000	0.004%	0.002%
Clomiphene Citrate	1000	0.000%	ND
Corsticosterone	1000	0.000%	0.000%
Corstisone	1000	0.000%	0.000%
Cortisol	1000	0.000%	0.000%
Cyproterone acetate	1000	0.000%	ND
D-5-Androstene-3β,17β-diol	1000	0.000%	ND
Danazol	1000	0.000%	ND
DHEA	100000	0.000%	0.000%
DHEA-S	1000	0.000%	0.000%
Desogestrel	100	0.000%	ND
Dexamethasone	1000	0.000%	ND
Epistestosterone	1000	0.001%	0.001%
Estriol	1000	0.000%	0.000%
Estrone	1000	0.000%	0.000%
Ethisterone	1000	0.000%	0.000%
Ethynodiol	1000	0.000%	0.000%
Ethynodiol diacetate	50	0.000%	ND
Flunisolide	1000	0.000%	ND
Fluoxymesterone	1000	0.000%	ND
Lynestrol	1000	0.000%	ND
Medoxyprogesterone acetate	1000	0.000%	ND
Methyl Testosterone	100	0.000%	ND
Mestranol	1000	0.000%	ND
Norethindrone	50	0.000%	ND
Norethinodrone acetate	50	0.000%	ND
Norgestimate	1000	0.000%	ND
Norgestrel (Levonorgestrel)	50	0.000%	ND
Norethynodrel	50	0.000%	ND
Oxymetholone	100	0.000%	ND
Prednisolone	1000	0.000%	ND
Prednisone	800	0.000%	0.000%
Progesterone	1000	0.000%	0.000%
Salbutamol	1000	0.000%	ND
Spironolactone	1000	0.000%	0.000%
Stanozolol	1000	0.000%	0.000%
Testosterone Cypionate	12	0.002%	0.000%
Testosterone enanthate	100	0.000%	0.000%
Testosterone SO4	1000	0.004%	0.003%
Testosterone Propionate	1000	0.000%	0.000%
Testosterone Undecanoate	12	0.011%	0.053%
Triamcinolone	50	0.000%	0.000%

14.5 Interference

Using CLSI-A2 Interference Testing in Clinical Chemistry as a guide, potential interferents were tested utilizing charcoal-stripped human serum spiked with known concentrations of interferent. The following results of % binding values even at higher than normal interferent levels indicate that there is no significant binding on the free testosterone-HRP conjugate.

TABLE 7

Substance	Highest concentration at which no significant interference was observed
Acetaminophen	20 mg/dl
Acetylcysteine	150 mg/dl
Ascorbic Acid	6 mg/dl
Bilirubin Conjugated	15 mg/dl
Bilirubin Unconjugated	20 mg/dl
Biotin	100 ng/ml
Caffeine	6 mg/dl
Cholesterol	503 mg/dl
Creatine	30 mg/dl
Dextran	5000 mg/dl
Digoxin	6.1 ng/ml
Doxycycline	50 mg/L
Erythromycin	6 mg/dl
Gentamicin	1 mg/dl
HAMA	440 ng/ml
Heparin	3 U/ml
Hemoglobin	500 mg/dl
Human Serum Albumin	2.5 g/dl
Ibuprofen	50 mg/dl
Immunoglobulin G	4 g/dl
Levodopa	20 mg/L
Lidocaine	1.2 mg/dl
Lipemia (glycerides)	1000 mg/dl
Methyldopa	20 mg/L
Nicotine	0.1 mg/dl
Phenobarbital	15 mg/dl
Protein: Total	10.5 g/dl
Rheumatoid Factor	1110 IU/ml
Salicylic Acid	60 mg/dl
SHBG	200 µg/ml
Triglycerides	900 mg/dl
Urea	500 mg/dl

15.0 REFERENCES

1. Dorfman, RI and Shipley, RA, ED: Androgens, New York,: John Wiley and Sons, 1956.
2. Horton R, Tait JF: Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of conversion to testosterone. J.Clin Invest 45: 301-303, 1966.

3. Faiman C and Winter. JSD, Reyes, FI, Clin *Obstet Gynaecol,* 3, 467 (1976).
4. Sizonenka, PC, *Pediatrician,* 14, 191 (1987).
5. Cummings DC, Wall SR: Non sex hormone binding globulin bound testosterone as a marker for hyperandrogenism. J. Clin Endocrinol Metab. 61:873-876, 1985.
6. Lashansky, G, et. al., *J Clin Endocrinol Metab,*58, 674 (1991)
7. Tietz, NW, ED: Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, WA Saunders Co, 1995.
8. Smith SW: Free Testosterone, *AACC Endo*; 11(3), 59-62 (1993).

Revision: 5 **Date: 2018-Oct-01** **DCO: 1315**
MP5325 **Product Code: 5325-*300***

Size		96(A)	192(B)
Reagent (fill)	A	1ml set	1ml set
	B	1ml set	1ml set
	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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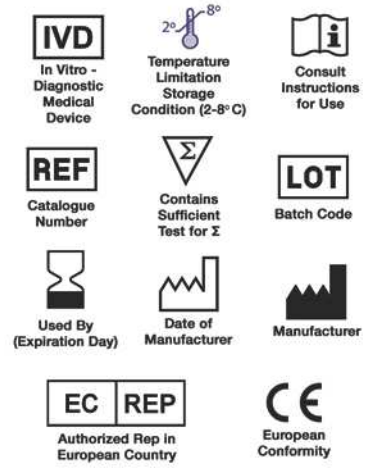
Glossary of Symbols
(EN 980/ISO 15223)



PERFORMANCE CHARACTERISTICS – DETECTION LIMIT STUDY

PURPOSE Define the low end of detection of the Free Testosterone AccuBind® ELISA Test System.

SCOPE Determining the limit of the blank (LoB), limit of detection (LoD), and the limit of quantitation (LoD) for the Free Testosterone ELISA in conformance with CLSI EP17-A2 Protocols for Determination of Limits of Detection of Limits of Quantitation (2012).

The limit of blank (LoB) is defined as the highest value expected to be seen in a series of observed test results on a sample that contains no analyte.

The limit of detection (LoD) is defined as the actual concentration at which an observed test result is likely to exceed the limit of blank and may therefore be declared as a detectable value.

The limit of quantitation (LoQ) is defined as the lowest actual concentration which the analyte is reliably detected to be acceptable for clinical use.

RESPONSIBILITY The Research and Development Officer and Quality Control technician have the responsibility to prepare samples, monitor laboratory site, record and report testing data per this procedure.

The project coordinator is assigned by the R&D Manager to coordinate, monitor, and record data during the duration of the study.

STUDY SITE Tests are performed at Monobind, Inc., by personnel trained and familiar with performing the Free Testosterone ELISA test System

MATERIALS

1. Monobind Free Testosterone AccuBind® ELISA Test System
2. Low or non-detectable testosterone serum samples
3. Low serum testosterone samples at or below the minimum assay measuring range

TEST PROCEDURE

A. Test Sample Preparation

1. Process samples according to accepted laboratory practices and procedures for handling, storing, and processing biological samples.
2. Obtain sufficient Free Testosterone charcoal-stripped human serum samples.
3. Screen the sample to determine its Free Testosterone value utilizing the Free Testosterone AccuBind® ELISA Test System.

B. Detection Studies

1. Limit of Blank (LoB)
    a. Obtain a Free Testosterone stripped sample(s) that is considered the “lowest or analyte free” sample.
    b. Utilizing 3 samples over a 3 day period, 24 per run, 2 runs a day, for a total of 6 assays run and 144 measurements.
    c. Calculate the LoB per CLSI, EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation for the full 144 measurements over the three samples.

    $LoB = Mean_{BLANK} + (1.645/(1-(1/(4f)))SD_{BLANK}$

    where f = Ns-K, and Ns = The number of low level sample results, and K = The number of low level samples.
2. Limit of Detection (LoD)

a. Spike testosterone free serum with high grade testosterone to obtain desired high and low concentrations.

b. Create ten (10) low level Free Testosterone samples that range from zero to four (4) times the LoB value according to the dilution ratios shown below:

Assigned Value for low level specimens

	1st Step Dilution		Assigned Value
	Serum (pg/L)	CalcVal after X times dil (pg/L)	Calculated Value (pg/L)
Serum-1	0.771	0.9000	0.0200
Serum-2	0.812	0.8000	0.0400
Serum-3	0.926	0.7000	0.0600
Serum-4	1.022	0.6000	0.0800
Serum-5	1.145	0.5000	0.1000
Serum-6	0.771	0.4000	0.1200
Serum-7	0.812	0.3000	0.1400
Serum-8	0.926	0.2000	0.1600
Serum-9	1.022	0.1000	0.1800
Serum-10	1.145	0.0000	0.2000

c. Generate 4 replicates for 400 total measurements over a ten (10) day period.

d. Calculate the LoD per the guidelines as described above in 1.d. Do not average the replicates when calculating the LoD.

$LoD = LoB + (1.645/(1-(1/(4f)))SD_{Detection} \times 1.645$

3. Limit of Quantitation (LoQ)

a. If total error (TE) is < than goal for total error, then LoD = LoQ.

b. If total error fails, assay forty (40) samples with a Free Testosterone value greater than the LoD value with the Free Testosterone ELIS Test System.

C. Data Collection and Statistical Analysis

1. Document the observed results.

2. Utilize the Analyse-It software to perform the statistical analysis.

ACCEPTANCE CRITERIA

1. The quality control values shall fall within the established mean and ± 2 SD for the assay to be accepted.

2. Total Error ≤ 0.05 pg/ml

3. LoB ≤ 0.1 pg/ml

4. LoD ≤ 0.3 pg/ml

5. LoQ ≥ LoD

SUMMARY

Total Error	*Limit of the Blank (LoB)*	*Limit of Detection (LoD)*	*Limit of Quantitation (LoQ)*
0.027 pg/ml	0.0295 pg/ml	0.0519 pg/ml	0.0519 pg/ml

CONCLUSION

Based on the data shown, the highest value expected to be seen in a series of observed test results on a sample that contains no analyte is 0.0295 pg/ml. The limit of detection (LoD) or the actual concentration at which an observed test result is likely to exceed the limit of blank and may therefore be declared as a detectable value is 0.0519 pg/ml.

PERFORMANCE CHARACTERISTICS – CROSS REACTIVITY & INTERFERENCE

A. Cross Reactivity

PURPOSE Evaluate whether various analytes or substances cross-react with the quantitation of Free Testosterone when utilizing the Free Testosterone AccuBind® ELISA Test System

SCOPE Determine the cross-reactivity of various analytes or substances with the Free Testosterone ELISA test system according to CLSI EP07-A2, Interference Testing in Clinical Chemistry (2005).

RESPONSIBILITY The Research and Development Officer and Quality Control technician have the responsibility to prepare samples, monitor laboratory site, record and report testing data per this procedure.

The project coordinator is assigned by the R&D Manager to coordinate, monitor, and record data during the duration of the study.

STUDY SITE Tests are performed at Monobind, Inc., by personnel trained and familiar with performing the Free Testosterone AccuBind® ELISA test System

MATERIALS

1. Monobind Free Testosterone AccuBind® ELISA Test System
2. Charcoal stripped human serum sample with no free testosterone
3. Male serum with endogenous free testosterone
4. Purified cross-reactants from commercial sources (e.g. Sigma-Aldrich)

TEST PROCEDURE

A. Sample Preparation

1. Process samples according to accepted laboratory practices and procedures for safe handling, storing, and processing biological samples.
2. The male serum is a pool of five individual samples. Assay to determine the free testosterone value, and record.
3. The blank serum is human serum that has been charcoal-stripped of free testosterone.

B. Test procedure

1. The interferent stocks solutions were prepared by dissolving the interfering compounds in appropriate organic buffer.
2. Spike the male serum and blank serum samples with known concentrations of cross-reactants to commercially accepted levels as specified in Table 1.

Table 1

Sample	Cross Reactant Conc. (ng/ml)
11-Deoxycortisol	1000
11-KetoTestosterone	10
11β-Hydroxytestosterone	100
17α-ethynyl estradiol	1000
17α-Estradiol	1000
17β-Estradiol	100
17-Hydroxypregnenolone	1000
17-Hydroxprogesterone	10
3-EstriolGluc	1000
3-EstriolSul	1000
3β-Androstanediol	500
5α-Dihydrotestosterone	100
Aldosterone	8000

Amitriptyl HCl	1000
Androsterone	1000
Andronstenedione	1000
Clomiphene Citrate	1000
Corsticosterone	1000
Corstisone	1000
Cortisol	1000
Cyproterone acetate	1000
D-5-Androstene-3β,17β-diol	1000
Danazol	1000
Dehydroepiandrosterone	100000
Dehydroepiandrosterone Sulfate	1000
Desogestrel	100
Dexamethasone	1000
Epistestosterone	1000
Estriol	1000
Estrone	1000
Ethisterone	1000
Ethynodiol	1000
Ethynodiol diacetate	50
Flunisolide	1000
Fluoxymesterone	1000
Lynestrol	1000
Medoxyprogesterone acetate	1000
Methyl Testosterone	100
Mestranol	1000
Norethindrone	50
Norethinodrone acetate	50
Norgestimate	1000
Norgestrel (Levonorgestrel)	50
Norethynodrel	50
Oxymetholone	100
Prednisolone	1000
Prednisone	800
Progesterone	1000
Salbutamol	1000
Spironolactone	1000
Stanozolol	1000
Testosterone Cyprionate	12
Testosterone Undecanoate	12
Testosterone enanthate	100
Testosterone SO4	1000
Testosterone Propionate	1000
Triamcinolone	50

3. Assay the samples in duplicate with the Free Testosterone AccuBind® ELISA Test System.
4. Using the average of the duplicates, calculate the cross-reactivity, ensuring the units are consistent:

$$\left(\frac{Observed\ Value - Unspiked\ Value}{Mass\ of\ cross - reactant}\right) \times 100$$

ACCEPTANCE CRITERIA

1. The % variation from specimens tested without interfering substance should be ≤ 10%
2. The quality control values shall fall within the established mean and ±2SD for the assay to be accepted.

SUMMARY

		Male Serum Spiked Samples		Blank Serum Spiked Samples	
Sample	Conc. (ng/ml)	Conc (pg/ml)	Cross Reactivity	Conc. (pg/ml)	Cross Reactivity
Base Pool	0	7.408	-	0	
11-Deoxycortisol	1000	7.269	0.000%	0.000	ND
11-KetoTestosterone	10	72.139	0.647%	51.855	0.519%
11β-Hydroxytestosterone	100	72.815	0.065%	54.000	0.054%
17α-ethynyl estradiol	1000	7.351	0.000%	0.000	ND
17α-Estradiol	1000	7.282	0.000%	0.153	0.000%
17β-Estradiol	100	7.363	0.000%	0.000	ND
17-Hydroxypregnenolone	1000	7.35	0.000%	0.000	ND
17-Hydroxprogesterone	10	7.969	0.000%	1.012	0.000%
3-EstriolGluc	1000	7.825	0.000%	0.000	ND
3-EstriolSul	1000	11.729	0.000%	0.000	ND
3β-Androstanediol	500	7.917	0.000%	0.000	ND
5α-Dihydrotestosterone	100	60.975	0.054%	42.382	0.042%
Aldosterone	8000	12.80	0.000%	4.919	0.000%
Amitriptyl HCl	1000	7.301	0.000%	0.000	ND
Androsterone	1000	8.061	0.000%	0.000	ND
Andronstenedione	1000	50.552	0.004%	19.248	0.002%
Clomiphene Citrate	1000	7.263	0.000%	0.000	ND
Corsticosterone	1000	10.099	0.000%	0.777	0.000%
Corstisone	1000	9.90	0.000%	0.478	0.000%
Cortisol	1000	7.316	0.000%	0.298	0.000%
Cyproterone acetate	1000	10.70	0.000%	0.000	ND
D-5-Androstene-3β,17β-diol	1000	7.782	0.000%	0.000	ND
Danazol	1000	12.30	0.000%	0.000	ND
Dehydroepiandrosterone	100000	7.311	0.000%	7.457	0.000%
Dehydroepiandrosterone Sulfate	1000	7.45	0.000%	0.238	0.000%
Desogestrel	100	7.436	0.000%	0.000	ND
Dexamethasone	1000	7.381	0.000%	0.000	ND
Epistestosterone	1000	21.612	0.001%	8.215	0.001%
Estriol	1000	7.368	0.000%	0.122	0.000%
Estrone	1000	7.679	0.000%	0.036	0.000%
Ethisterone	1000	8.597	0.000%	0.003	0.000%
Ethynodiol	1000	8.042	0.000%	0.114	0.000%
Ethynodiol diacetate	50	7.563	0.000%	0.000	ND

Flunisolide	1000	7.456	0.000%	0.000	ND
Fluoxymesterone	1000	7.450	0.000%	0.000	ND
Lynestrol	1000	7.395	0.000%	0.000	ND
Medoxyprogesterone acetate	1000	7.426	0.000%	0.000	ND
Methyl Testosterone	100	7.163	0.000%	0.000	ND
Mestranol	1000	7.338	0.000%	0.000	ND
Norethindrone	50	7.541	0.000%	0.000	ND
Norethinodrone acetate	50	7.428	0.000%	0.000	ND
Norgestimate	1000	7.478	0.000%	0.000	ND
Norgestrel (Levonorgestrel)	50	7.463	0.000%	0.000	ND
Norethynodrel	50	7.544	0.000%	0.000	ND
Oxymetholone	100	7.401	0.000%	0.000	ND
Prednisolone	1000	7.828	0.000%	0.000	ND
Prednisone	800	10.442	0.000%	0.966	0.000%
Progesterone	1000	11.025	0.000%	0.248	0.000%
Salbutamol	1000	8.026	0.000%	0.000	ND
Spironolactone	1000	7.310	0.000%	0.112	0.000%
Stanozolol	1000	7.442	0.000%	0.081	0.000%
Testosterone enanthate	100	7.304	0.000%	0.044	0.000%
Testosterone SO4	1000	75.970	0.004%	28.182	0.003%
Testosterone Propionate	1000	9.589	0.000%	1.008	0.000%
Triamcinolone	50	7.544	0.000%	0.043	0.000%
Base Pool for below analytes		38.436	-	0.077	-
Testosterone Cypionate*	12	38.685	0.002%	0.021	0.000%
Testosterone Undecanoate*	12	38.262	-0.001%	0.015	-0.001%

*C_{MAX} for the two compounds is 11 ng/ml or 11000 pg/ml

CONCLUSION

1. No cross reactivity above 0.647% was detected with the performance of Free Testosterone AccuBind® ELISA Test System upon addition of massive amounts of selected substances to the human serum pool.

B. Interference

PURPOSE Conduct interference testing to identify potential interferents when utilizing the Free Testosterone ELISA Test System

SCOPE Determine potential intereferents with the Free Testosterone ELISA test system according to CLSI EP07-A2, Interference Testing in Clinical Chemistry (2005)

RESPONSIBILITY The Research and Development Officer and Quality Control technician have the responsibility to prepare samples, monitor laboratory site, record and report testing data per this procedure.

The project coordinator is assigned by the R&D Manager to coordinate, monitor, and record data during the duration of the study.

STUDY SITE Tests are performed at Monobind, Inc., by personnel trained and familiar with performing the Free Testosterone AccuBind® ELISA test System

MATERIALS

1. Monobind Free Testosterone AccuBind® ELISA Test System
2. Charcoal-stripped human serum pool
3. Commercially available interferents for spiking

TEST PROCEDURE

A. Sample Preparation

1. Process samples according to accepted laboratory practices and procedures for safe handling, storing, and processing biological samples.
2. The male serum is a pool of five individual samples. Assay to determine the free testosterone value, and record.
3. The blank serum is human serum that has been charcoal-stripped of free testosterone.

B. Test Procedure

1. Create a base pool containing the sample matrix of interest, which can comprise a volume of 4-6 ml.
2. The interferent stocks solutions were prepared by dissolving the interfering compounds in appropriate buffer.
3. It is recommended that screening experiments be done with both low and high analyte pools, as interference may be dependent upon analyte concentration.
4. Spike human serum samples with increasing amounts of interferent. The interferent volume should be no more than 5% of the matrix volume.
5. Document the concentrations of interferent added.
6. Assay according to instructions for Free Testosterone AccuBind® ELISA Test System.
7. Determine if interferent substances cause intereference at specified concentrations by calculating percent interference.

C. Data Collection and Statistical Analysis

1. Document the observed results.
2. Observe for effect of interferent on a sample by comparing the concentration of unspiked to spiked.
3. Using the average of the duplicates, calculate the percent interferance, ensuring the units are consistent:

$$\frac{Observed\ Value - Unspiked\ Value}{Unspiked\ Value}$$

ACCEPTANCE CRITERIA

1. Non-significant interference:
    a. The % variation from specimens tested without interfering substance should be ≤10%
    b. Recovery after the addition of interferents should be ≥90%
2. The quality control values shall fall within the established mean and ±2SD for the assay to be accepted.

SUMMARY

Effect of Human Serum Albumin (HSA)

Sample	Concentration Added (g/dL)			
	0	1.25	2.5	5
1	2.298	1.993	2.220	1.997
2	10.832	10.606	9.254	8.357
3	5.018	4.747	4.513	4.182
4	0.989	1.088	1.087	1.086

Effect of Sex Hormone Binding Globulin (SHBG)

Sample ID	SHBG Added	OD	Percent
	µg/ml	450nm	B/B(0)
1	0	2.487	100.00%
2	6.25	2.473	99.42%
3	12.5	2.483	99.82%
4	25	2.458	98.81%
5	50	2.460	98.91%
6	100	2.452	98.59%
7	200	2.436	97.93%

		Male Serum High Spiked Samples Base Concentration: 38.498 pg/ml	Male Serum Mid-Level Spiked Samples Base Concentration: 7.916 pg/ml	Blank Serum Spiked Samples
Sample	Concentration Added	Interference	Interference	Interference
Acetaminophen	20 mg/dl	0.699%	2.804%	ND
Acetylcysteine	150 mg/dl	1.597%	-0.189%	ND
Ascorbic Acid	6 mg/dl	3.720%	1.832%	ND
Bilirubin Conjugated	15 mg/dl	2.268%	-2.830%	ND
Bilirubin Unconjugated	20 mg/dl	1.626%	-2.893%	ND
Biotin	100 ng/ml	3.704%	-2.590%	ND
Caffeine	6 mg/dl	2.151%	1.983%	ND

Cholesterol	503 mg/dl	2.551%	4.156%	ND
Creatine	30 mg/dl	1.574%	3.550%	ND
Dextran	5000 mg/dl	0.899%	2.665%	ND
Digoxin	6.1 ng/ml	-1.735%	-3.499%	ND
Doxycycline	50 mg/L	-1.995%	-4.775%	ND
Erythromycin	6 mg/dl	0.714%	-0.076%	ND
Gentamicin	1 mg/dl	-1.998%	-4.207%	ND
Hemoglobin*	500 mg/dl	0.774%	-2.700%	ND
Heparin	3 U/ml	-3.008%	-5.584%	ND
Ibuprofen	50 mg/dl	-1.961%	0.227%	ND
Immunoglobulin G	4 g/dl	-1.397%	-1.592%	ND
Levodopa	20 mg/L	3.075%	-1.187%	ND
Lidocaine	1.2 mg/dl	0.158%	1.377%	ND
Lipemia	1000 mg/dl	-0.696%	-3.209%	ND
Methyldopa	20 mg/L	-0.722%	-3.095%	ND
Nicotine	0.1 mg/dl	-6.003%	0.417%	ND
Phenobarbital	15 mg/dl	-7.374%	-0.644%	ND
Protein: Total	10.5 g/dl	2.400%	-2.994%	ND
Rheumatoid Factor	1110 IU/ml	-1.839%	-4.775%	ND
Salicylic Acid	60 mg/dl	-5.117%	4.005%	ND
SHBG	96nM	-4.592%	-2.451%	ND
Triglycerides	900 mg/dl	-3.372%	-4.169%	ND
Urea	500 mg/dl	-1.704%	0.733%	ND

*Hemoglobin interference of male serum mid-spiked samples were tested with a base pool concentration of 9.409pg/ml

Human anti-mouse antibodies (HAMA) interference was evaluated with human serum samples with low and high endogenous HAMA. Less than 10% interference was observed for HAMA at 440 ng/ml and 185 ng/ml.

CONCLUSION

1. The results showed % binding values between 97.93% – 99.42% even at higher than normal interferent levels for HSA and SHBG. Results showed no interference above 10% for interferents tested on high, mid-level, and blank Free Testosterone concentration. In conclusion, the results showed that there was no significant binding by interferents on the free testosterone-HRP conjugate