Ultrasensitive Estradiol ELISA

For the quantitative determination of estradiol in serum and plasma

For *In Vitro* Diagnostic Use within the United States of America.
This product is for Research Use Only outside of the United States of America.

Catalog Number: 20-EDLHUU-E01
Size: 96 wells
Version: 7.0 2023-04-12-vk - ALPCO 1.0

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1 INTRODUCTION

1.1 Intended Use

The Ultrasensitive Estradiol ELISA is an enzyme immunoassay for the quantitative measurement of estradiol in serum or plasma (EDTA, heparin, or citrate plasma). For *In Vitro* Diagnostic Use.

The device is **intended to be used** as an aid to diagnosis for individuals where information of the following is required:

- Cycle tempo disorders: oligomenorrhea, amenorrhea and polymenorrhea
- Follow-up of hormonal infertility therapy
- Assessment of ovarian function
- Disorders in pubertal development
- Therapy control in hormonal replacement therapy

The device is **not intended** for tumor diagnostics.

1.2 Summary and Explanation

Estradiol (1,3,5(10)-estratriene-3,17β-diol; 17β-estradiol; E21) is a C18 steroid hormone with a molecular weight of 272.4 Dalton. It is the most potent natural estrogen, produced mainly by granulosa cells of the female ovary and the placenta by the aromatization of androstenedione to estrone, followed by conversion of estrone to estradiol by 17β-HSD. Estradiol is also synthesized in other tissues including testicles, adrenal gland, fat tissue, liver, breast, and brain (1-5, 16). In plasma, estradiol is largely bound to SHBG and albumin. Only a fraction of 2.21 % is free and biologically active, the percentage remaining constant throughout the menstrual cycle (6-8). Estradiol acts primarily as an agonist of the estrogen receptor (ER) subtypes ERα and ERβ, nuclear steroid hormone receptors which trigger the appropriate response at the nuclear level in the target sites. These sites include follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent liver and skin. In the female, estradiol acts as a growth hormone for tissue of the reproductive organs. During the menstrual cycle, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. This estradiol peak stimulates the hypothalamic-pituitary axis to secrete the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are essential for follicular maturation and ovulation. In the luteal phase, estradiol, in conjunction with progesterone, prepares the endometrium for implantation (9-15). During pregnancy, estradiol concentration increases due to placental production and high levels are sustained throughout pregnancy (15.)

Estradiol values should always be interpreted with knowledge of the cycle phase. Elevated values are observed in the periovulatory phase, drug-induced poly-ovulation, over-substitution, slowed metabolism, estrogen-producing tumors, and granulosa cell tumor.

Decreased levels are observed in primary ovarian insufficiency (e.g. postmenopausal), secondary ovarian insufficiency (e.g. pituitary insufficiency, use of ovulation inhibitors), anovulatory cycles, or Corpus luteum insufficiency.

In men, elevated levels of estradiol are found, for example, in obesity and liver cirrhosis, as well as in estrogen-producing tumors (rare).

2 PRINCIPLE OF THE TEST

The Ultrasensitive Estradiol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody (rabbit) directed towards antigenic sites of the estradiol molecule. During the first incubation, the sample is incubated together with Assay Buffer in the coated well. Thereafter, enzyme conjugate (estradiol conjugated to horseradish peroxidase) is added. Estradiol in the sample competes with the added enzyme conjugate for

binding to the coated antibody. 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 inversely 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 WARNING AND PRECAUTIONS

- This kit is for *in vitro* diagnostic use only. For professional use only.
- 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.
- 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 be 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 ALPCO.
- 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 absorbance 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 colored. Do not pour reagents back into vials as reagent contamination may occur.
- Follow laboratory quality assurance 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. All reagents, however, should be treated as potential biohazards in use and for disposal.
- 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 should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation. Waste must be discarded according to local rules and regulations.

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. Remove contaminated clothing and wash it before reuse. If inhaled, take the person to open air. Wash contaminated objects before reusing them.

- Avoid contact with Stop Solution containing < 5 % H2SO4. 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.
- Safety Data Sheets for this product are available upon request directly from ALPCO.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiter wells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with an anti-estradiol antibody (polyclonal). Ready to use.
2. **** Zero Standard,*** 1 vial, 3 mL, concentration: 0 pg/mL, Ready to use.
3. **** Standard (Standard 1 - 6*)**, 6 vials, 1 mL, ready to use;
   Concentrations: 10 – 25 – 50 – 100 – 200 - 400 pg/mL
   Conversion: 1 pg/mL = 3.671 pmol/L
   Calibrated against the following reference material: *17β Estradiol (E-060-1ML, Cerilliant)*
4. **** Control Low & Control High***, 2 vials, 1 mL, ready to use.
   For control values and ranges please refer to vial label or Certificate of Analysis.
5. **** Assay Buffer***, 1 vial, 7 mL, ready to use
6. **** Enzyme Conjugate***, 1 vial, 14 mL, ready to use;
   Estradiol conjugated to horseradish peroxidase
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Contains 3,3‘,5,5‘-tetramethylbenzidine (TMB).
   Keep away from direct sunlight.
8. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains < 5 % H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **** Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see “Preparation of Reagents”.

*Contains non-mercury preservative.

4.1.1 Equipment and material required but not provided

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

4.2 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 microplate must always be stored in the resealable aluminum pouch containing a desiccant. Do not open the pouch until it has reached room temperature. The microtiter plate consists of 12 individual strips. Each strip can be divided into 8 individual wells.
Unused wells must be immediately returned to the aluminum pouch with the desiccant and stored again tightly resealed at 2 °C to 8 °C.
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 if within expiration date printed on the label.

4.3 Preparation of Reagents

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

4.4 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.

4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, ALPCO must 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 must be stored until a final solution has been found. After this, they should be disposed of according to the official regulations.

5 SAMPLE COLLECTION, STORAGE, AND PREPARATION

Human serum or plasma (EDTA, heparin or citrate plasma) can be used in this assay.
Do not use hemolytic, icteric or lipemic specimens. Refer to Interfering Substances for more information.
Please note: Samples containing sodium azide should not be used in the assay.

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.

Plasma:
Whole blood should be collected into centrifuge tubes containing anticoagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Note: Whole blood should not be frozen before centrifugation.

5.2 Sample 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	7 days
	at -20 °C (in aliquots)	up to 12 months

5.3 Sample Preparation and Dilution

Samples can be assayed without additional preparation.

Very high estradiol concentrations are expected during pregnancy. Therefore, dilution of such samples will be required.

Example:
Dilution 1:100: 10 µL sample + 990 µL *Zero Standard*; Mix thoroughly.

6. ASSAY PROCEDURE

6.1 General Remarks

- 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 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.
- Absorbance is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in “Assay 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.

− 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 Assay 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 correct pipetting volumes.

1. Secure the desired number of microtiter wells in the frame holder.
2. Pipette **50 µL** of each ***Standard, Control,*** and **sample** with new disposable tips into appropriate wells.
3. Add **50 µL *Assay Buffer*** into each well.

Thoroughly mix for 10 seconds. It is important to completely mix in this step.

4. Incubate for **60 minutes** at room temperature.

5. Add **100 µL *Enzyme Conjugate*** into each well.

Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

6. Incubate for **60 minutes** at room temperature.

7. Wash the wells as follows:

If the wash step is performed manually:

Briskly shake out the contents of the wells.

Rinse the wells **4 times** with **300 µL** 1X working *Wash Solution* per well.

If an automated plate washer is used:

Rinse the wells **4 times** with **400 µL** 1X working *Wash Solution* per well.

At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!

8. Pipette **100 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 (measurement wavelength) and at 620 nm or 630 nm (reference wavelength for recommended background subtraction)** 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.

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, it is recommended to retest the samples.

3. Samples with concentrations exceeding the highest standard can be further diluted with *Zero Standard* and re-assayed as described in “Assay Procedure,” or must be reported as > 400 pg/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 semi-logarithmic 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)
Standard 0 (0 pg/mL)	1.956
Standard 1 (10 pg/mL)	1.687
Standard 2 (25 pg/mL)	1.399
Standard 3 (50 pg/mL)	1.046
Standard 4 (100 pg/mL)	0.765
Standard 5 (200 pg/mL)	0.462
Standard 6 (400 pg/mL)	0.309

7. REFERENCE VALUES

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

In a study conducted with apparently healthy subjects, using the Ultrasensitive Estradiol ELISA the following data were observed:

Population		n	Mean (pg/mL)	Median (pg/mL)	2.5^{th} - 97.5^{th} Percentile (pg/mL)	Range (min. - max.) (pg/mL)
Males		69	34.55	27.09	14.33 – 119.52	12.85 – 156.50
Females	Follicular Phase	16	65.03	38.60	27.60 – 235.66	26.05 – 297.53
	Ovulation Phase	16	102.09	96.80	27.93 – 239.29	23.82 – 263.53
	Luteal Phase	16	93.52	76.42	44.62 – 282.27	42.31 – 352.98
	Post-Menopause	19	52.51	33.27	14.41 – 126.30	12.70 – 129.00
Pregnancy	2nd trimester	42	6 576	5 247	1 112 – 22 997	689 – 27 160
	3rd trimester	41	11 857	10 955	3 551 – 25 039	2 859 – 28 837

Values above or below the reference range should be considered as suspicious and require additional testing.

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 quality assurance in the laboratory 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 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 ALPCO.

9. ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 5.6 pg/mL – 400 pg/mL pg/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

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

Substance	Concentration Range of Spiked Substance (pg/mL)	Mean Cross-Reactivity (%)
11-Desoxycortisol	10 – 1000	0
17-OH Progesterone	1.2 – 120	0.1
21-OH Progesterone	35 – 3500	0
Aldosterone	0.075 – 7.5	0
Androstendione	0.22 – 22	0
Androsterone	10 – 1000	0
Corticosterone	0.5 – 50	0
Cortisol	16 – 1600	0
Cortisone	16 – 1600	0
Creatinine	500 – 50000	0

Substance	Concentration Range of Spiked Substance (pg/mL)	Mean Cross-Reactivity (%)
DHEA	0.5 – 50	0
DHEA-S	300 – 30000	0
Estriol	1.5 – 150	0
Estrone	0.03 – 0.3	2.2
Glucose	100 – 10000 **µg/mL**	0
Prednisolone	35 – 3500	0
Prednisone	35 – 3500	0
Pregnenolone	35 – 3500	0
Progesterone	42.2 – 4220	0.1
Testosterone	1 – 100	0

9.3 Sensitivity

Limit of Blank (LoB)	3.146 pg/mL
Limit of Detection (LoD)	5.583 pg/mL
Limit of Quantification (LoQ)	7.445 pg/mL
Measuring range	5.583 pg/mL – 400 pg/mL
Linear range	13.0 g/mL – 400 pg/mL

9.4 Reproducibility

9.4.1 Within-run Precision

The within-run precision was determined with 4 samples covering the complete measuring range in 5 independent runs within 5 days in 5 replicates per run. CV was calculated as mean CV of 5 runs.

Sample	n	Mean (pg/mL)	CV (%)
1	5	10.15	9.3
2	5	21.41	6.9
3	5	42.00	2.6
4	5	138.93	2.9

9.4.2 Between-run Precision

The between-run variation was determined with 4 samples. The 4 samples are measured in 5 days with 5 replicates per run. 25 data points are generated per sample (5 replicates x 5 runs = 25 data points).

Sample	n	Mean (pg/mL)	CV (%)
1	25	10.15	11.0
2	25	21.41	10.6

3	25	42.00	6.0
4	25	138.93	5.3

9.4.3 Between-lot Precision

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

Sample	n	Mean (pg/mL)	CV (%)
1	18	9.92	6.9
2	18	35.04	4.5
3	18	79.40	6.9
4	18	134.72	3.0

9.5 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 (pg/mL)		6.33	37.98	77.51	124.02
Average Recovery (%)		94.9	101.5	105.4	100.5
Range of Recovery (%)	**from**	90.3	96.8	100.8	93.6
	to	98.4	106.8	110.5	107.4

9.6 Linearity

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

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (pg/mL)		196.88	283.46	379.00	446.00
Average Recovery (%)		96.4	96.8	91.6	95.2
Range of Recovery (%)	**from**	86.4	88.7	85.5	86.1
	to	105.6	103.4	95.5	100.4

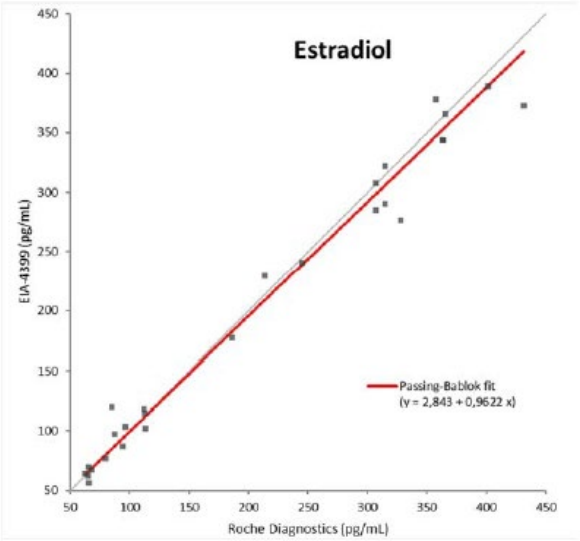
9.7 Method Comparison

A comparison of Ultrasensitive Estradiol Sensitive ELISA (y) and the Roche Diagnostics, ECLIA (x) gave the following correlation:

n = 27

r = 0.990

y = 0.9622x + 2.843



10. LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use and in compliance with laboratory quality assurance guidelines. 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 0.063 mg/mL) and triglyceride (up to 7.5 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 on the measurement of estradiol in a sample.

10.3 High-Dose-Hook Effect

"High-Dose Hook Effect" is not detected up to 8,000 pg/mL of estradiol.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. 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 ALPCO.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results agree 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.

12 LITERATURE

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