Product information

Users Manual

Progesterone free in saliva ELISA

REF   **IB79304**

Σ **96 wells**

IVD   **For *in-vitro* diagnostic use.**

Contents

1   INTRODUCTION

1.1   Intended Use

Enzyme immunoassay for the *in vitro diagnostic* quantitative measurement of active free progesterone (a female hormone) in saliva.

Measurements obtained by this device may be used in the diagnosis and treatment of disorders of the ovaries or placenta and can be used as an aid for prediction of ovulation.

1.2   Summary and Explanation

Progesterone (4-pregnene-3, 20-dione) is a C21 steroid hormone containing a keto-group (at C-3) and a double bond between C-4 and C-5. Like other steroids, it is synthesized from cholesterol via a series of enzyme-mediated steps *(1)*

The steroid hormone Progesterone is a female sex hormone which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle and it is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy *(2)*

In non-pregnant women progesterone is mainly secreted by the corpus luteum whereas in pregnancy the placenta becomes the major source *(3,4)*. Minor sources for progesterone are the adrenal cortex for both sexes and the testes for males.

The Progesterone level in saliva represents the concentration of the active free Progesterone.

2   PRINCIPLE OF THE TEST

The IBL-AMERICA Salivary Progesterone ELISA kit is based on the competition principle and the microplate separation.

An unknown amount of Progesterone present in the sample and a fixed amount of Progesterone conjugated with horseradish peroxidase compete for the binding sites of a rabbit polyclonal Progesterone -antiserum coated onto the wells. After one hour incubation the microplate is washed to stop the competition reaction. After adding the substrate solution, the concentration of Progesterone is inversely proportional to the optical density measured.

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

3

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 the package insert 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 (21-26°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 microtiterplate 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 1 N acidic solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin, BND and 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 Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from IBL-AMERICA.

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

4

4   REAGENTS

4.1   Reagents provided

1. *Microtiterwells*, 12x8 (break apart) strips, 96 wells;
    Wells coated with a anti-Progesterone antibody (polyclonal).
2. *Standard (Standard 0-6),* 7 vials, 1 mL each, ready to use;
    Concentrations: 0; 10; 50; 100; 500; 1000; 5000 pg/mL
    Conversion: 1000 pg/mL x 3.18 = nmol/L.
     * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
3. *Control*, 2 vials, 1.0 mL each, ready to use;
    Control values and ranges please refer to vial label or QC-Datasheet.
    * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
4. *Enzyme Conjugate*, 1 vial, 26 mL, ready to use;
    Progesterone conjugated to horseradish peroxidase;
    * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
5. *Substrate Solution*, 1 vial, 25 mL, ready to use;
    Tetramethylbenzidine (TMB).
6. *Stop Solution*, 1 vial, 14 mL, ready to use;
    contains 1 n acidic solution.
    Avoid contact with the stop solution. It may cause skin irritations and burns.
7. *Wash Solution*, 1 vial, 30 mL (40X concentrated);
    see „Preparation of Reagents".

    *   BND   = 5-bromo-5-nitro-1,3-dioxane
         MIT   = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available upon request.

4.2   Materials required but not provided

1. Calibrated EIA reader adjusted to read at 450 nm
2. Precision pipettes (100 and 200 µl)
3. Distilled or Deionized water
4. Timer (60 min. range)
5. Reservoirs (disposable)
6. Test tube or microtube rack in a microplate configuration
7. semi logarithmic graph paper or software for data reduction

4.3   Storage Conditions

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

4.4   Reagent Preparation

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

Wash Solution
Add deionized water to the 40X concentrated *Wash Solution*. Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL. *The diluted Wash Solution is stable for 2 weeks at room temperature.*

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 Material Safety Data Sheet.

4.6   Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-AMERICA 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.

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

5

5   SPECIMEN COLLECTION AND PREPARATION

Samples containing sodium azide should not be used in the assay. The saliva samples should be completely colorless. Even the slightest red color shows blood contamination. Such blood contamination will give falsely elevated concentration values. In case of visible blood contamination the patient should discard the sample, rinse the sampling device with tap water, also rinse the mouth with (preferably) cold water, wait for 10 minutes and take a new sample. Do not chew anything during the sampling period. Any pressure on the teeth may result in falsely elevated measurements due to an elevated content of gingival liquid in the saliva sample.

5.1   Specimen Collection

For the correct collection of saliva we are recommending to only use appropriate devices made from ultra-pure polypropylene. Do not use any PE devices or Salivettes for sampling; this in most cases will result in significant interferences. Glass tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper. Please contact IBL-America for more details.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem at least any food of animal origin (meat or dairy products) should be avoided prior to finalizing the collection. In the morning breakfast should be done only after finalizing the collection procedure. During the day the collection period should be timed just before an anticipated meal. As the steroid hormone secretion in saliva as well in serum shows an obvious dynamic secretion pattern throughout the day it is important to always collect 5 samples during a 2 hour period; this means every 30 minutes one sample. If possible the volume of each single sample should be a minimum of 0.5 ml (better 1 ml). Saliva flow may be stimulated by drinking water. This is allowed and even recommended before and during the collection period. Drinking of water is not allowed during the last 5 minutes before taking the single samples. The typical timing for a morning collection period would be as follows. Wake-up at 6:00 AM, drinking water and brushing teeth, 1st sample at 6:15 AM, followed by samples at 6:45 AM, 7:15 AM, 7:45 AM, and 8:15 AM, followed by breakfast at 8:25 AM. The typical timing for an afternoon collection period would be like: 1st sample at 5:00 PM, followed by samples at 5:30 PM, 6:00 PM, 6:30 PM, 7:00 PM, followed by dinner at 7:10 PM. Modest variation in the collection timing will not be critical, and the collection time-frame can be extended up to 3 hours.

5.2   Specimen Storage and Preparation

Saliva samples in general are stable at ambient temperature for several days. Therefore mailing of such samples by ordinary mail without cooling will not create a problem. Storage at 4°C can be done for a period of up to one week. Whenever possible samples preferable should be kept at a temperature of -20°C. Even repeated thawing and freezing is no problem. Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation. Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully. Then the samples have to be centrifuged for 5 to 10 minutes. Now the clear colorless supernatant is easy to pipette. If the sample should show even a slight reddish tinge it should be discarded. Otherwise the concentration value most probably will be falsely elevated. Due to the episodic variations of the steroid secretion we highly recommend the strategy of multiple sampling. If such a set of multiple samples has to be tested the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single samples in a separate sampling device and perform the testing from this mixture..

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 *Standard 0* solution and re-assayed 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 saliva + 90 µl *Standard 0* (mix thoroughly)
b) Dilution 1:100:   10 µl of dilution a) + 90 µl *Standard 0* (mix thoroughly).

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

6

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 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of coated strips in the frame holder.
2. Dispense **100 µl** of each Progesterone Standards and Controls into appropriate wells.
3. Dispense **100 µl** of each sample into selected wells.
4. Dispense **200 µl** of Enzyme Conjugate into each sample and standard well and mix the plate for thoroughly for 10 seconds.
5. Incubate for **60 minutes** at room temperature.
6. Briskly shake out the contents of the wells and rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the inverted wells sharply on absorbent paper towel to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **200 µl** of Substrate Solution to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the reaction by adding **100 µl** of Stop Solution to each well.
10. Determine the absorbance of each well at 450 ± ☐10 nm.
    It is recommended to read the wells within 10 minutes.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. 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 > 5000 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Conversion:
Progesterone Conversion: 1000 pg/mL x 3.18 = nmol/L.

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Version 09-01/12
JS
Updated 140801
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com
7

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and <u>cannot</u> be used in place of data generation at the time of assay.

Standard	Absorbance Units (450 nm)
Standard 0 (0 pg/mL)	1.89
Standard 1 (10 pg/mL)	1.71
Standard 2 (50 pg/mL)	1.54
Standard 3 (100 pg/mL)	1.39
Standard 4 (500 pg/mL)	0.95
Standard 5 (1000 pg/mL)	0.71
Standard 6 (5000 pg/mL)	0.44

7 EXPECTED NORMAL VALUES

In order to determine the normal range of SLV Progesterone, saliva samples from 80 adult male and 120 female apparently healthy subjects, ages 21 to 75 years, were collected in the morning and analyzed using the IBL-AMERICA SLV Progesterone ELISA kit.
The following ranges were calculated from this study.

	Age group		Salivary progesterone pg/mL
Women	21 - 50 yrs.	Follicular phase n = 40	19.6 – 86.5 pg/mL
	21 - 50 yrs.	Luteal phase n = 40	99.1 – 332.6 pg/mL
	51 - 75 yrs.	Postmenopausal n = 40	6.0 – 56.4 pg/mL
Men	21 - 50 yrs.	n = 40	12.7 – 57.4 pg/mL
	51 - 75 yrs.	n = 40	15.2 – 65.1 pg/mL

Therapy should not be decided based on results alone. The results should be correlated to other clinical observations and diagnostic tests.
Furthermore, we recommend that each laboratory establish its own range for the population tested, because the values differ between age, new born, children, adolescents and adults.

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 IBL-AMERICA directly.

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

8

9   PERFORMANCE CHARACTERISTICS

9.1   Assay Dynamic Range

The range of the assay is between 3.8 – 5000 pg/mL.

9.2   Specificity

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Progesterone.

Steroid	% cross reactivity
Progesterone	100.0
Desoxycorticosterone	1.1
Pregnenolone	0.35
17α-Hydroxyprogesterone	0.3
Corticosterone	0.2
11-Desoxycortisol	0.1
Estriol	<0.1
Estradiol 17β	<0.1
Testosterone	<0.1
Cortisone	<0.1
DHEA-S	<0.02
Cortisol	<0.02

9.3   Sensitivity

The lowest detectable level of progesterone that can be distinguished from the Zero Standard is 3.8 pg/mL at the 95 % confidence limit.

9.4   Reproducibility

Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 5 saliva samples within one run. The within-assay variability is shown below:

Mean (pg/mL)	1328.7	650.1	293.8	186.9	23.3
SD (pg/mL)	75.9	30.4	15.8	10.6	1.8
CV (%)	5.7	4.7	5.4	5.7	7.6
n =	20	20	20	20	20

Inter-Assay

The inter-assay (between-day) variation was determined by duplicate measurements of 5 saliva samples over 10 days.

Mean (pg/mL)	55.5	565.4	1338.0	567.9	630.6
SD (pg/mL)	4.3	41.5	70.9	40.5	45.1
CV (%)	7.7	7.3	5.3	7.1	7.2
n =	20	20	20	20	20

Inter-Lot

The Inter-Lot (between-lot) variation was determined by triplicate measurements of five saliva samples in three different kit lots. The between lot variability is shown below:

Mean (pg/mL)	60.4	630.3	1574.8	360.3	635.1
SD(pg/mL)	4.3	37.6	64.1	28.7	33.4
CV%	7.1	6.0	4.1	8.0	5.3
n=	9	9	9	9	9

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

9

9.5   Recovery

Recovery of the IBL-AMERICA Progesterone ELISA was determined by adding increasing amounts of the analyte to five different saliva samples containing different amounts of endogenous analyte. Each sample (native and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Concentration pg/mL	13.9	87.9	147.8	283.0	386.8
Average % recovery	96.4	103.2	100.3	97.3	102.2
Range of from	91.1	101.1	94.9	92.7	97.8
% recovery to	101.6	104.8	103.8	106.6	105.7

9.6   Linearity

In total six saliva samples containing different amounts of analyte were serially diluted with Standard 0 and assayed with the IBL-AMERICA ELISA.

Three of these samples were serially diluted directly, and the other 3 samples at first were spiked with progesterone and then serially diluted up to 1:128.

The percentage recovery was calculated by comparing the expected and measured values for progesterone. A linearity of 3.8 – 4600 pg/mL has been identified as the usable range for this assay. Samples above this range must be diluted and re-run.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (pg/mL)	58.3	98.7	1073	2802	6000	5000
Average % Recovery	104.1	97.5	95.79	98.96	102.6	103.5
Range of from	99.9	89.9	91.2	93.6	99.1	94.4
Recovery % to	106.7	100.8	105.5	107.3	106.5	107.7

Comparison Studies

A study was performed that evaluated 306 saliva samples collected from adult men and women ages 20 – 75. The samples were run on the IBL-AMERICA test and a commercially available LIA method to determine the concentration of free progesterone in the saliva samples. A correlation of 0.9373 and regression formula of $y = 0.912x + 6.066$ was obtained versus this method.

An additional study was performed using 101 saliva samples from adult men and women ages 20 - 75. These samples were compared to the LIA method, and yielded a correlation of $r = 0.9923$ with the following regression formula.

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

10
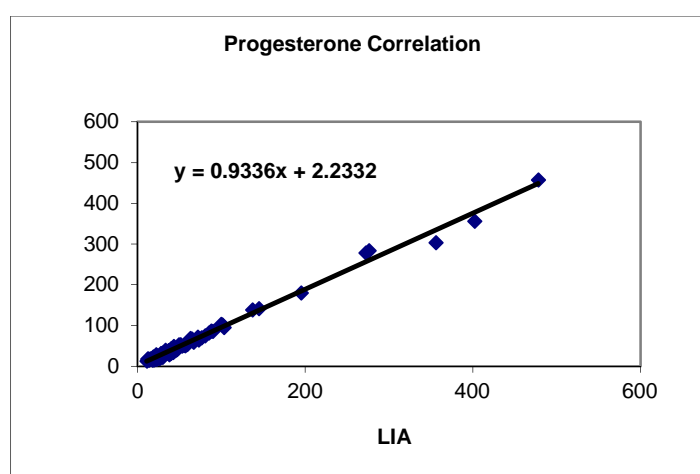
10 LIMITATIONS OF USE

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.

The patient should not eat, drink, chew gum or brush teeth for 30 minutes before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

10.1 Interfering Substances

Blood contamination of more than 0.16% in saliva samples will affect results, and usually can be seen by eye.

Concentrations of Sodium Azide ≥ 0.02% interferes in this assay and may lead to false results.

10.2 High-Dose-Hook Effect

No hook effect was observed in this test.

11 LEGAL ASPECTS

Only for countries where the declaration of European Conformity (CE mark) is applicable.

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 IBL-AMERICA.

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. Miller W.L. (1988): Molecular biology of steroid hormone synthesis, *Endocrin.* Rev. 9, 295-318
2. Filicori M., Butler J.P., Crowley W.F. Jr. (1984): Neuroendocrine regulation of the corpus luteum in the human*, J. Clin. Invest.* 73, 1638
3. Csapol Al., Pulkkinen M. O., Wiest W.G. (1973): Effects of lutectomy and progesterone replacement therapy in early pregnancy patients. *Am J. Obstet. Gynecol*. 115, 759
4. Henson M.C. (1998): Pregnancy maintenance and the regulation of placental progesterone biosynthesis in the baboon. *Human Reproduction Update*, 4, 389-405

Manufactured for :
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

11

SYMBOLS USED WITH IBL-AMERICA ELISAS

Symbol	English	Deutsch	Français	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
📖i	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
∑	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
🌡	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
⧗	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
🏭	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore

Version 09-01/12
JS
Updated 140801

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955   Fax.: +1 (763) - 780-2988
Email: ibl@ibl-america.com   Web: www.ibl-america.com

12