

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k062626

B. Purpose for Submission:

New device

C. Measurand:

Cortisol

D. Type of Test:

Quantitative ELISA

E. Applicant:

IBL-Hamburg

F. Proprietary and Established Names:

Cortisol ELISA

G. Regulatory Information:

1. Regulation section:

21 CFR § 862.1205 Cortisol (hydrocortisone and hydroxycorticosterone) test system

2. Classification:

Class II

3. Product code:

CGR – Radioimmunoassay, cortisol

4. Panel:

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The IBL Cortisol enzyme linked immunosorbent assay is for the in-vitro-diagnostic quantitative determination of cortisol in human serum and saliva. The Cortisol ELISA kit is useful as an aid in the differential diagnosis of Cushing syndrome and Addison's disease.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Calibrated EIA reader adjusted to read at 450nm.

I. Device Description:

The kit contains the components below.

Microtiter Plate – Break-apart strips coated with anti-cortisol antibodies.

Enzyme Conjugate - Ready to use. Contains: Cortisol (chromatographically purified) conjugated to HRP and stabilizers.

Standard A-G – previously cleared (k052359)

Controls - previously cleared (k052359)

TMB Substrate Solution - Ready to use, TMB, Buffer, stabilizers.

TMB Stop Solution - Ready to use, 1 M H₂SO₄.

Wash Buffer Concentrate (10x) - phosphate buffer, Tween, stabilizers.

Adhesive Foil

J. Substantial Equivalence Information:

1. Predicate device name(s):

IBL Cortisol LIA TEST KIT

2. Predicate 510(k) number(s):

k052359

3. Comparison with predicate:

Similarities		
Item	Cortisol ELISA (IBL) (device)	Cortisol LIA (IBL) Predicate
Sample	Serum and saliva	Serum and saliva
Test principle	Competitive immunoassay. Competition is between a labeled and non-labeled antigen for a fixed number of antibody binding sites. The amount of labeled analyte bound to the antibody is inversely proportional to the concentration of the analyte present in the sample.	Competitive immunoassay. Competition is between a labeled and non-labeled antigen for a fixed number of antibody binding sites. The amount of labeled analyte bound to the antibody is inversely proportional to the concentration of the analyte present in the sample
Calibration	Quantitative from standard curve with 7 calibrators	Quantitative from standard curve with 7 calibrators
Quality control	2 Controls at different levels	2 Controls at different levels
Assay range	Saliva: 0.015 – 4.0 µg/dL Serum: 0.75 – 200 µg/dL	Saliva: 0.015 – 4.0 µg/dL Serum: 0.75 – 200 µg/dL
Microtiter plate	Break apart strips coated with anti-cortisol antibodies	Break apart strips coated with anti-cortisol antibodies

Differences		
Item	Cortisol ELISA (IBL) (device)	Cortisol LIA (IBL) Predicate
Method	Enzyme linked immunosorbent assay	Luminescence immunoassay
Detection	Colorimetric detection	Luminescence detection

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labeled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed color is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra assay precision for saliva was determined by repeated measurements of control saliva samples within the same run. The mean CV was 5.7% (range 3.2 – 7.6%). Inter assay precision for saliva was determined by repeated double measurements of control saliva samples in 10 different runs on consecutive days. The mean CV was 7.4% (range 6.2 – 9.1%).

Intra assay variation for serum was determined by repeated measurements of samples after 1:51 dilution with standard A. The mean CV was 8.8% (range 3.8 – 11.8%). Inter assay variation for serum was determined by repeated double measurements of control sera samples after 1:51 dilution in 10 different runs. The mean CV was 11.2% (range 10.8 – 12.0%).

b. Linearity/assay reportable range:

Reportable range:

Saliva: 0.015 – 4 µg/dL Cortisol (anal.Sens. to highest standard- higher samples must be diluted to give results within the standard curve)

Serum: 0.75 – 200 µg/dL Cortisol (anal.Sens. to highest standard multiplied with dilution factor of 50)

Linearity of saliva samples

Saliva samples having different cortisol levels were serially diluted with zero standard. Dilutions covering the full measuring range of the assay were performed for each sample. Each dilution was measured in duplicate in one assay run. No relevant deviation of the expected linearity was observed. The

mean linearity recovery for saliva was 97% (range was 83 to 114% recovery).

Linearity of serum samples

Serum samples (already diluted 1:50) having different cortisol levels were serially diluted with zero standard. The measured concentrations were multiplied with 50 to get the serum concentration. Dilutions covering the full measuring range of the assay were performed for each sample. Each dilution was measured in duplicate in one assay run. No relevant deviation of the expected linearity was observed. The mean linearity recovery for serum was 105% (range was 90 to 120% recovery).

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The complete Kit will have a shelf life of 9 months at 2 – 8°C. Traceability, stability, and expected values of calibrators and controls were previously established (k052359).

d. Detection limit:

The analytical sensitivity was calculated from the mean of the OD of the zero calibrator minus 2 SD of 26 replicate analyses. The lowest detectable level that can be distinguished from the zero standard is 0.015 µg/mL using 4 parameter logistics for curve fit.

e. Analytical specificity:

The cross-reactivity of the cortisol antiserum has been measured against various compounds. The percent of cross-reactivity is expressed as the ratios of cortisol concentration to the concentration of the reacting compound at 50% binding of the zero standard.

	Cortisol LIA	Cortisol ELISA
Compound	Cross-reactivity (%)	
Cortisol, Hydrocortisone	100	100
Prednisolone	57	29
11-Deoxycortisol	12	16
Corticosterone	2.5	2.4
Cortisone	2.0	3.3
6 β -Hydroxycortisol	1.6	1.4
Prednisone	1.0	2.2
17 -Hydroxyprogesterone	0.5	1.2
Deoxycorticosterone	0.3	0.5
6 -Methyl-17 -Hydroxyprogesterone	0.1	0.3
Progesterone	<0.05	<0.01
Dexamethasone	<0.05	0.06
17 -Hydroxypregnenolone	<0.01	0.02
Dehydroisoandrosterone	<0.01	<0.01
Androstenedione	<0.01	<0.01
Estriol	<0.01	<0.01
6 -Methyl-17 -Hydroxyprogesterone-acetate	<0.01	<0.01
Pregnenolone	<0.01	<0.01
Estrone	<0.01	0.03
Testosterone	<0.01	0.02
17 -Hydroxyprogesterone-17 sulphate	<0.01	<0.01
Androsterone sulphate	<0.01	0.02
Testosterone sulphate	<0.01	<0.01
Cholesteryl sulphate	<0.01	<0.01
17 β -Estradiol-17 sulphate	<0.01	0.02
DHEA-S	<0.01	<0.01

The following substances do not have a significant effect (+/-20 % of expected) on the test results up to the below stated concentrations:

	Serum	
	Conc.	Cortisol (μ g/dL)
Hemoglobin	4.0 mg/mL	0.06; 0.33; 0.62
Bilirubin	0.5 mg/mL	0.07; 0.35; 0.63
Triglyceride	30 mg/mL	0.07; 0.40; 0.75
	Saliva	
	Conc.	Cortisol (μ g/dL)
Thimerosal	0.50 %	0.19; 0.25; 0.34
Blood	0.125 %	0.09; 0.26
NaN3	0.60 %	0.23; 0.31

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

The device was compared to the predicate Cortisol LIA from IBL with 129 serum samples (117 patient serum samples supplemented with 12 spiked samples to cover measuring range). Another comparison was performed with the same predicate device using 130 saliva samples (16 patient saliva samples supplemented with 14 spiked samples to cover the measuring range).

Sample type	Regression equation	Correlation (R ²)
Serum (n=129)	y=1.174x-2.191	0.995
Saliva (n=130)	y=0.915x+0.056	0.990

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

	n	µg/dL		nmol/L	
		AM	PM	AM	PM
Saliva	725	0.5 – 1.5	0.03 – 0.3	13.8 – 41.4	0.83 – 8.3
Serum	125	5 – 25	2 – 12	138 - 690	55.2 – 331.2

Reference

(Westermann J, Demir A, Herbst V. Determination of Cortisol in Saliva and Serum by a Luminescence-Enhanced Enzyme Immunoassay. Clin Lab 2004;50:11-24)

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.