

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

A. 510(k) Number:

k150528

B. Purpose for Submission:

New Device

C. Measurand:

Cortisol, Salivary

D. Type of Test:

Enzyme Immunoassay

E. Applicant:

IBL International GMBH

F. Proprietary and Established Names:

Cortisol Saliva Luminescence Immunoassay

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1205

2. Classification:

Class II

3. Product code:

NHG

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for Use below

2. Indication(s) for use:

The IBL International Cortisol Saliva Luminescence Immunoassay is intended for the in-vitro diagnostic quantitative determination of Cortisol in human saliva and for use as an aid in the diagnosis and treatment of adrenal disorders.

The device is not intended for point-of-care settings.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Microplate reader, pipette to dispense very accurately 20 μ L of saliva, multichannel pipettors, microplate or orbital shaker, and a plate sealer.

I. Device Description:

The kit comes in two sizes. The kit consists of one size contains 96 tests and the other contains enough for 960 tests. The microtiter plate is white colored and coated with anti-cortisol antibodies (rabbit) coated microplate (1 x 12 x 8 or 10 x 12 x 8).

Each kit contains the following components:

Seven standard (CAL A-G: 0, 0.015, 0.03, 0.10, 0.40, 1.00 and 3.20 μ g/dL), ready to use. Contains commercially available (non-human source) cortisol, buffer, 0.1% BSA and 0.1% ProClin.

Two controls (low and high cortisol), ready to use. Contains commercially available (non-human source) cortisol, buffer, 0.1 BSA and 0.1 % ProClin.

Enzyme conjugate is yellow colored, ready to use. Contains commercially available (non-human source) cortisol (chromatographically purified), conjugate to HRP, stabilizers.

Chemiluminescence reagent 1&2, ready to use. Reagent 1 contains luminol enhancer and reagent 2 contains peroxide solution and stabilizers.

Wash buffer concentrate concentrate (10X). Contains phosphate buffer, tween, and stabilizers.

Adhesive Foil.

Oral fluid collection device:

The saliva collection device is not included in the kit. The saliva collection device is stated in the labeling under the specimen collection and storage section. The recommended saliva collection device for this assay is the Sarstedt Cortisol Salivette (Cat#51.1534.500), which has been registered and listed under D053653

J. Substantial Equivalence Information:

1. Predicate device name(s):

Pantex AM/PM Salivary Cortisol Enzyme Immunoassay

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

k102841

3. Comparison with predicate:

Similarities		
Item	Candidate Device: Cortisol Saliva Luminescence Immunoassay	Predicate Device: Pantex AM/PM Salivary Cortisol EIA Kit (k102841)
Indication for Use	For the <i>in vitro</i> diagnostic quantitative determination of cortisol in human saliva and for use as an aid in the diagnosis and treatment of adrenal disorders.	Same
Sample Type	Saliva	Same
Calculations	Quantitative determination with a standard curve	Same
Quality Control	Cortisol low and high, ready to use	Same
Storage	2 – 8 °C	Same
Test Method	Enzyme Immunoassay	Same
Test Principle	Cortisol in the sample and	Same

Similarities		
Item	Candidate Device: Cortisol Saliva Luminescence Immunoassay	Predicate Device: Pantex AM/PM Salivary Cortisol EIA Kit (k102841)
	enzyme labelled antigen compete for binding sites of the antibodies coated onto the microwells. After adding the substrate solution, the intensity of the luminescence is inversely proportional to the amount of antigen in the sample.	

Differences		
Item	Candidate Device: Cortisol Saliva Luminescence Immunoassay	Predicate Device: Pantex AM/PM Salivary Cortisol EIA Kit (k102841)
Limits of Detection	LoB: 0.004 µg/dL LoD: 0.012 µg/dL LoQ: 0.012 µg/dL	LoB: 0.003 µg/dL LoD: 0.006 µg/dL LoQ: 0.006µg/dL
Measuring Range	0.012 – 3.00 µg/dL	0.01 – 3.0 µg/dL
Reference Intervals	Age: 21-70 AM: 0.079 – 1.290 µg/dL PM: 0.042 – 0.436 µg/dL	Age: 23-68 AM: 0.258 – 1.269 µg/dL PM: 0.025– 0.296 µg/dL

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

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedure

CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures

CLSI EP09-A2 Method Comparison and Bias Estimation Using Patient Samples

L. Test Principle:

Cortisol in the sample and enzyme labelled antigen compete for binding sites of the antibodies coated onto the microwells. After addition of the luminescence substrate solution, the intensity of the luminescence measured is inversely proportional to the amount of antigen in the sample.

All kit standards, control, and patient saliva samples must be run in duplicates.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All performance data were obtained using Sarstedt Cortisol Salivette (Cat#51.1534.500) saliva collection tube.

a. *Precision/Reproducibility:*

Study Protocol:

A precision study based on CLSI guideline EP5-A3 was performed by testing six assays daily using 3 different reagent lots and two operators. Within each assay, the high, and low kit controls and 7 saliva pools were used. Each pool consists of the following: 2 low concentrated cortisol samples (up to 0.3 µg/dL), 2 medium concentrated cortisol samples (0.3 to 0.8 µg/dL), 2 high concentrated cortisol samples (0.8 to 1.0 µg/dL) and 1 very high concentrated cortisol samples (1.0 to 3.2 µg/dL) run in triplicate for 21 days. Total N is 63. The precision results of the statistical analysis are summarized in the tables below:

Level	Mean conc. (µg/dL)	within run		between run		between day		Total Precision = Within Laboratory Precision	
		SD (µg/dL)	% CV	SD (µg/dL)	% CV	SD (µg/dL)	% CV	SD (µg/dL)	% CV
1	0.060	0.003	5.0%	0.003	5.0%	0.002	3.3%	0.003	5.0%
2	0.230	0.008	3.5%	0.007	3.0%	0.006	2.6%	0.009	3.9%
3	0.623	0.020	3.2%	0.024	3.9%	0.021	3.4%	0.028	4.5%
4	0.587	0.017	2.9%	0.017	2.9%	0.013	2.2%	0.021	3.6%
5	0.988	0.030	3.0%	0.033	3.3%	0.028	2.8%	0.037	3.7%
6	0.924	0.028	3.0%	0.033	3.6%	0.029	3.1%	0.038	4.1%
7	1.781	0.054	3.0%	0.063	3.5%	0.054	3.0%	0.059	3.3%

Spiking Recovery:

Three human saliva samples ranging from 0.242-2.528 µg/dL containing different

levels of endogenous cortisol were spiked with known quantities of cortisol and assayed. The percent recovery ranged from 93.7% to 109.6%.

b. Linearity/assay reportable range:

Study Protocol:

Linearity was evaluated based on the CLSI EP6-A guideline. A high spiked cortisol saliva sample (3.486 µg/dL) and a low cortisol concentration saliva sample (0.009 µg/dL) were used in the study. Dilutions were prepared from these two saliva samples to produce intermediate concentrations. A total of 12 levels of cortisol concentrations (0.009, 0.322, 0.660, 0.949, 1.263, 1.666, 1.886, 2.430, 2.585, 3.017, 3.267, 3.486 µg/dL) were tested with each sample tested in duplicates. Statistical evaluation using linear regression with 11 levels tested (sponsor excluded the highest level) showed that the assay is linear from 0.012 – 3.134 µg/dL, yielding a linear regression result of $y = 1.03x - 0.05$ with $r^2 = 0.99$

The sponsor's claim measuring range of this assay is 0.012 to 3.0 µg/dL.

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

Traceability:

The assigned cortisol values of the calibrators are traceable to the National Institute of Standards and Technology (NIST) cortisol reference material (code 921).

Value Assignment of Calibrators and Controls

A stock solution was prepared by weighing in a fixed amount of Cortisol. The kit calibrators are produced from this stock solution. Value assignment of the kit calibrators is performed with multiple replicates and verification is conducted by direct comparison with each kit lot calibrators to the NIST reference material.

Target values of the calibrators are: CAL A-G: 0, 0.015, 0.03, 0.10, 0.40, 1.00 and 3.20 µg/dL.

The kit controls are produced similarly to the kit calibrators. The controls are measured in 5 different runs and in quadruple measurements (reaching 20 values). The mean is calculated and the controls ranges are defined as $\pm 18\%$ from mean value for Control 1 and as $\pm 15\%$ for Control 2.

Target ranges of controls are:

Control 1 is 0.049 to 0.102 µg/dL

Control 2 is 0.47 to 0.88 µg/dL

Stability of the kit, including calibrators and controls:

Sponsor claimed that the kit is stable unopened for 18 months when stored at 2-8°C. Once open, the kit is stable for 19 days when stored at 2-8°C. Real-time stability and open kit stability has been performed. Study protocol and acceptance criteria has been provided and found to be acceptable.

d. Detection limit:

The limit of blank (LoB), the limit of detection (LoD), and the limit of quantitation (LoQ) for salivary cortisol was conducted according to CLSI EP17-A guidance document.

LoB was determined from five contrived samples utilizing 5 different lots of Standard A, on one analyzer. LoB study was performed using 2 reagent lots, over 3 days, run in duplicate, one run per day, for a total number of 150 determinations.

LoD was determined from five low salivary cortisol samples (0.007 – 0.023 µg/dL), on one analyzer, with 2 reagent lots, run in duplicate, one run per day for 3 days, for a total number of 150 determinations. Each sample generated a total of 30 values. LoQ was determined from five salivary cortisol samples (0.006-0.108 µg/dL) on one analyzer, with 2 reagent lots, run in duplicate, one run per day for 3 days, for a total number of 150 determinations. Each sample generated a total of 30 values. LoQ is defined as an inter-assay precision of less than 20% CV.

The LoB was determined to be 0.004µg/dL; the LoD was determined to be 0.012 µg/dL and the LoQ was determined to be 0.012 µg/dL.

This assay has a measuring range of 0.012 to 3.0 µg/dL.

e. Analytical specificity:

Interference Study Protocol:

The interference study was conducted by spiking three levels of saliva samples (low, medium and high) with high concentrations of potentially interfering substances. The study was conducted by spiking the saliva samples with commercially available interfering substances.

Result Summary:

Sponsor's definition of non-significant interference is <10% difference between spiked and unspiked sample. Results are summarized in the table below.

Potential Interferent	Tested concentration range of potential interfering Substance	Highest concentration tested showing non-significant interference
Blood	0.10 – 106 mg/mL	Blood will interfere at all levels
Ethanol	0.079 – 7.9 mg/mL	0.079 mg/mL
Food	0.11 – 11.1 mg/mL	1.11 mg/mL
Chewing gum	0.11 – 11.1 mg/mL	11.1 mg/mL
Nicotine	0.1 – 10 mg/mL	1.0 mg/dL
Caffeine	0.1 – 1000 µg/mL	1000 µg/mL

The package insert has the following limitations: “The patient should not eat, drink, chew gum, or brush teeth for 30 min. before sampling. Otherwise, rinse mouth thoroughly with cold water 5 minutes prior to sample collection. Do not collect samples when oral disease, inflammation, or lesions exist (blood contamination).”

Cross-Reactivity Study Protocol:

The sponsor evaluated cross-reactivity by using analyte free zero standards.

Cortisol and the cross reactants were spiked into the standards to obtain a suitable range of concentrations. Two replicates of each contrived sample were tested. Each of the potential cross-reactive substances were tested in at least 6 different concentrations between 1-10, 000 µg/dL concentration range.

The % cross-reactivity will be calculated as:

$$\% \text{ cross -reactivity} = \frac{\text{concentration of cortisol at } 50\% \text{ B/B}_0}{\text{concentration of cross-reactant giving } 50\% \text{ B/B}_0}$$

Cross-reactant tested	Highest concentration Tested (µg/dL)	Calculated cross reactivity (%)
Cortisol	10000	102 %
Androstenedione	10000	<0.1 %
11-Deoxycortisol	10000	5.35 %
Estrone	10000	<0.01 %
Estriol	10000	<0.1 %
Medroxyprogesterone 17-acetate	10000	<0.01 %
17 α-Hydroxyprogesterone	10000	0.74 %
Dehydroisandrosterone	10000	<0.01 %

Pregnenolone	10000	<0.01 %
11-Deoxycorticosterone	10000	0.10 %
Medroxyprogesterone	10000	0.03 %
4,5 α -Dihydrotestosterone	10000	<0.01 %
Progesterone	10000	0.03%
Prednisolone	10000	15.82 %
Corticosterone	10000	0.63 %
Prednisone	10000	0.95 %
Cortisone	10000	1.73 %
Dexamethason	10000	<0.01 %
Testosterone	10000	<0.01 %
Androsterone	10000	<0.01 %
Dihydrotestosterone	10000	<0.01 %
Methyltestosterone	10000	<0.01 %
19-Norethindrone	10000	<0.01 %
Ethinylestradiol	10000	<0.01 %
Epiestriol	10000	<0.01 %
6 β -Hydroxycortisol	10000	0.73 %
β -Estradiol	10000	<0.01 %

Sponsor has the following limitation based on their cross reactivity results in their package insert: “The use of topical creams or medication containing prednisolone and 11-Deoxycortisol should be avoided as they can cause preanalytical contamination of the saliva sample.”

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Study Protocol:

A total of 180 human saliva specimens collected using a Cortisol Salivette (Sarstedt Inc. Cat. 51.1534.500) were collected from one clinical site and used in the method comparison study against the predicate device (Pantex Cortisol Salivary EIA). Samples were aliquoted into four 0.2 mL aliquot tubes, frozen and shipped to the sponsor’s facility. A total of 25 contrived samples were included in the method comparison to cover the measuring range. Eleven samples were excluded from the analysis due to the cortisol concentration being outside the measurement range of the predicate k102841. All testing was performed in-house over 5 days and the samples were run in duplicate on both candidate device and the predicate device. Samples range tested from 0.012 to 2.942 $\mu\text{g/dL}$ and results are summarized in the table below.

Result Summary:

N	r ²	r	Weighted Deming regression
169	0.986	0.993	$y = 0.902x - 0.017$

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:

The expected normal values for the assay are 0.079 – 1.290 µg/dL (AM) and 0.041 – 0.436 µg/dL (PM). The reference intervals were established using saliva samples from healthy adults 155 saliva samples were collected in AM and 168 saliva samples were collected in PM at two time points (earlier than 10:00 am and between 2:00 pm and 4:00 pm) at one collection site. Saliva was collected using Cortisol Salivette form Sarstedt Int. The inclusion criteria for the healthy adult population was adults between 21-70 years old of age, representing different races with normal TSH and TPO levels, were not currently undergoing medical treatment or drug therapy, and were free of illness on the day of the sample collection. The exclusion criteria included patients on cortisol therapy, on hormone therapy or taking oral contraceptives, patients with implanted contraceptive device, history of thyroid or other autoimmune disease, history of Cushing's syndrome or Addison's disease, pregnant or lactating women.

The label states that it is recommended that each laboratory establish its own range of normal values.

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.