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

A. 510(k) Number:

k102841

B. Purpose for Submission:

New Device

C. Measurand:

Cortisol, Salivary

D. Type of Test:

Quantitative Enzyme Immunoassay

E. Applicant:

Pantex, Division of Bioanalysis, Inc.

F. Proprietary and Established Names:

Pantex AM/PM Salivary Cortisol Enzyme Immunoassay

G. Regulatory Information:

1. Regulation section:

   21 CFR §862.1205, Cortisol test system

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:

   For the *in vitro* diagnostic quantitative determination of free and protein bound salivary cortisol in human saliva as an aid in the assessment of Cushing Syndrome and Addison's Disease. Measurements of Cortisol in saliva are used in the diagnosis and treatment of disorders of the adrenal gland.

3. Special conditions for use statement(s):

   For prescription use only

   Not for use with serum or plasma samples

4. Special instrument requirements:

   Microplate reader capable of reading 450 nm with 4 parameter data reduction or comparable software, device to dispense very accurately 50 µL of saliva, multichannel pipettors, microplate or orbital shaker, and a plate sealer.

I. Device Description:

The kit consists of a goat anti-rabbit gamma globulin coated microplate (12x8, breakable strip wells), 7 ready-to-use cortisol calibrators (gravimetrically prepared at 0.1 – 30 ng/mL), low and high controls, rabbit anti-cortisol antibody, analog 10X concentrated cortisol-peroxidase, substrate solution, stop reaction solution, and 10X concentrate wash solution.

The collection device that was used for all performance characteristic studies is not included in the kit. The collection device is stated in the labeling both under the performance characteristics and materials needed but not supplied. The recommended collection device for this assay is the VWR Sample Mailing Tube, Cat #16465-260 (VWR International).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Salimetrics Salivary Cortisol HS EIA Kit

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

   k011323

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	For the *in vitro* diagnostic quantitative determination of free and protein bound salivary Cortisol in human saliva as an aid in the assessment of Cushing Syndrome and Addison's Disease. Measurements of Cortisol in saliva are used in the diagnosis and treatment of disorders of the adrenal gland.	Same
Analyte	Free and protein-bound cortisol	Same
Sample Type	Saliva	Same
Calculations	Quantitative determination with standard curve	Same
Quality Control	Use of reference controls recommended	Same
Storage	2 to 8ºC	Same
Test Method	Enzyme Immunoassay	Same
Sample Type	Saliva	Same
Test Principle	Cortisol in the sample competes with cortisol-enzyme conjugate for binding sites to antibody bound to a microwell. Unbound components are washed away and enzyme is measured by a colored reaction with the TMB substrate.	Same

Differences		
Item	Device	Predicate
Interferences	Substances tested that showed no significant interference: • Coffee • Food • Cigarette Smoking • Chewing Gum • Alcoholic Beverage	Not Available
Collection Device	VWR Sample Mailing Tube Cat # 16465-260	Polypropylene Vials and Salimetrics Oral Swab (SOS), Item # 5001.02

Differences		
Item	Device	Predicate
Expected Values	2.58 – 12.69 ng/mL (AM) 0.25 – 2.96 ng/mL (PM)	0.94 – 15.51 ng/mL (AM) ND – 3.59 ng/mL (PM)
Limits of Detection	LoB: 0.03 ng/mL LoD: 0.06 ng/mL LoQ: 0.06 ng/mL	LoD: <0.03 ng/mL
Calibrator Ranges	0.1 – 30 ng/mL	0.12 – 30 ng/mL
Measuring Range	0.1 – 30 ng/mL	0.12 – 30 ng/mL

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

None referenced

L. Test Principle:

Cortisol in the sample competes with cortisol-enzyme conjugate for binding sites to antibody bound to a microwell. Unbound components are washed away and enzyme is measured by a colored reaction with the tetramethybenzemidine (TMB) substrate. Concentration of the sample is interpolated from the calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Study Protocol:

A precision study was performed by testing 3 levels (approximately 0.6, 4, and 25 ng/mL) of pooled clinical saliva samples, assayed in duplicate with 2 runs per day for 20 days (n=80) using 3 different reagent kit lots for each study.

Results Summary:

Results were calculated and are listed in the table below:

Sample	Concentration (ng/mL)	Within Run		Between Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV
Level 1	0.6	0.02	3.79	0.05	7.80	0.05	9.09
Level 2	4.00	0.15	3.60	0.051	1.26	0.19	4.56
Level 3	25.00	0.44	1.76	0.29	1.15	0.82	3.24

b. Linearity/assay reportable range:

Study Protocol:

Linearity was evaluated following CLSI guideline EP6-A. A high spiked cortisol sample of human saliva (33.788 ng/mL) and low cortisol sample of human saliva (0.093 ng/mL) were used in this study. Dilutions were prepared from these two samples to provide various concentrations of cortisol. A total of ten levels of cortisol concentrations were tested. The cortisol concentrations ranged from 0.093 – 33.788 ng/mL.

Results Summary:

Statistical evaluations using linear regression showed that the assay is linear from 0.093 – 33.788 ng/mL, yielding a linear regression result of $y = 0.973\ x – 0.0540$, with a correlation coefficient of $R^2 = 0.9976$.

The data provided support the sponsor's claim that the measuring range of this assay is 0.1 to 30 ng/mL.

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

Traceability

Controls and calibrators are included in the test kit. The assigned cortisol values of the calibrators are traceable to the National Institute of Standards and Technology (NIST) cortisol reference material (code 921). Cortisol standard material is purchased from a commercial vendor and prepared stock solution used to make the standards, calibrators and controls is directly compared to NIST material.

Value Assignment of Calibrators and Controls

The initial lot of calibrator materials (stock solution) is value assigned through a correlation study using NIST cortisol reference material. Based on the correlation study with the NIST material, the cortisol values for the stock solution are established. All subsequent standards, calibrators and controls are value assigned against the stock solution. Verification is conducted by direct comparison with each kit lot to the NIST reference material.

7 calibrators are provided at 0, 0.1, 0.3, 1.0, 3.0, 10, and 30 ng/mL.

2 controls are provided at 1.0 and 20 ng/mL.

Stability

The shelf-life and open-vial stability testing protocols for the controls and calibrator materials and the acceptance criteria were described and found to be acceptable. Calibrator materials are stable until expiration date (9 months) when stored at 2-8ºC.

Reagent stability protocol and acceptance criteria was also evaluated and found to be acceptable. Reagents are stable in storage at 2-8ºC for 9 months.

Open vial stability protocol and acceptance criteria was evaluated and found to be acceptable. Open vial reagents are stable at 2-8 ºC for 31 days.

Sample stability was investigated and the protocol and acceptance criteria were found to be acceptable. Specimens are stated to be stable when stored for no greater than 7 days at 37 °C, 7 days at 2 – 8 °C, and 7 days at -15 °C (freeze up to 7 times).

d. Detection limit:

The limit of the blank (LoB), The limit of detection (LoD), and Limit of Quantitation (LoQ) for salivary cortisol was determined from 120 measurements (40 measurements per day over 3 days) on one cortisol deficient sample and one low level cortisol sample (< 0.1 ng/mL). The LoB was determined to be 0.0392 ng/mL, and the LoD was determined to be 0.0519 ng/mL. The calculated LoQ based on a stated bias of 13.6% CV was 0.0519 ng/mL.

This assay has a measuring range of 0.1 to 30 ng/mL.

e. Analytical specificity:

Interference Study Protocol:

An in-vitro interference study was conducted by spiking three levels of Cortisol (low, medium and high) with high concentrations of five potentially interfering substances. The in-vivo experiment was conducted by simulating the intake of five potentially interfering substances by spiking control saliva with either the potential interferent itself or an extract of the potential interferent (food was homogenized and centrifuged, and the supernatant was spiked into control saliva).

Result Summary:

Based on the sponsor's definition of non-significant interference (within 10% recovery of the control sample), the following claims were made:

> The below compounds at the indicated concentration do not cause significant interference with the assay.

Potential Interferent	% Recovery of Low Pool	% Recovery of Medium Pool	% Recovery of High Pool
Caffeine 800 µg/mL	101.7	104.3	91.9
Food 426 mg/mL	99.6	99.8	104.0
Nicotine 800 µg/mL	101.7	107.6	106.0
Chewing Gum 270 mg/mL	104.4	102.4	103.7
Ethanol 0.1%	96.8	97.3	109.4

The Package insert contains the following limitation: "Avoid food consumption, drinking coffee or alcohol, smoking or chewing gum one (1) hour prior to sample collection. Rinse mouth thoroughly with water 15 minutes prior to collection."

Cross-Reactivity Study Protocol:

The sponsor evaluated cross-reactivity by using a cortisol-free saliva pool. Structurally related compounds were spiked into the saliva pool at concentrations up to 10,000 ng/mL for cross reactivity evaluation. % Cross-reactivity was calculated using the following the equation:

$$\% \text{ Cross-reactivity} = \frac{\text{Mean cortisol of the Cross-Reactant pool}}{\text{Concentration of Cross-Reactant}} \times 100$$

Result Summary:

Based on the calculated % Cross-reactivity, only prednisolone was found to significantly cross-react with the proposed assay. The interference of prednisolone is stated in the package insert of the device.

Cross-reactant Tested	Concentration Tested	% Cross-reactivity
Cortisol	10,000 ng/mL	100
Dihydroisoandrosterone	10,000 ng/mL	0.0076
6-ß-Hydroxycortisol	10,000 ng/mL	1.7177
6-methyl-17-Hydroxyprogesterone	10,000 ng/mL	0.1427
Prednisone	10,000 ng/mL	1.0874
Prednisolone	10,000 ng/mL	25.9001
17-OH-Progesterone	10,000 ng/mL	0.0284
Progesterone	10,000 ng/mL	0.0079
17-OH-Pregnenolone	10,000 ng/mL	0.0066
Pregnenolone	10,000 ng/mL	0.0038

Desoxycorticosterone	10,000 ng/mL	0.0517
11-Desoxycortisol	10,000 ng/mL	1.8133
Dexamethasone	10,000 ng/mL	0.0164
Cortisone	10,000 ng/mL	0.7600
Corticosterone	10,000 ng/mL	1.0847
Aldosterone	10,000 ng/mL	0.0070
Androstenedione	10,000 ng/mL	0.0038
Testosterone	10,000 ng/mL	0.0042
5α DHT	10,000 ng/mL	0.0019
DHEA-SO4	10,000 ng/mL	0.0031
Androstanedione	10,000 ng/mL	0.0028
Estradiol 17β	10,000 ng/mL	0.0024
Estradiol 17α	10,000 ng/mL	0.0003
Estrone	10,000 ng/mL	0.0010
Estriol	10,000 ng/mL	0.0015

f. Assay cut-off:

2. Comparison studies:

a. Method comparison with predicate device:

Study Protocol:

A total of 160 patient saliva samples from a commercial vendor were used in the method comparison study. These samples were all collected in the recommended collection tubes, and together with 6 spiked samples they ranged from 0.1 ng/mL to 29.85 ng/mL. The spiked samples used to cover the upper measuring range of the proposed assay were spiked with different volumes of 1000 ng/mL concentration of stock calibrator solution (prepared with the NIST cortisol reference material, code 921) with target concentrations between 10 ng/ml and 30 ng/ml. Linear regression analysis was performed between the proposed salivary cortisol assay and the predicate device.

Result Summary:

The Result of Linear Regression analysis yielded a regression equation of $y = 1.0269x + 0.0994$ with a correlation coefficient of $r = 0.9898$.

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 listed expected normal values for the assay of 2.58 – 12.69 ng/mL (AM) and 0.25 – 2.96 ng/mL (PM) were determined in saliva specimens from a population of 152 healthy adults (76 male, 76 female, ages 23-68) for AM analysis and 152 healthy adults (76 male, 76 female, ages 23-68) for PM analysis. The inclusion criteria for the healthy adult population was adults of all ages, genders and race with normal TSH and TPO levels, not currently undergoing medical treatment or drug therapy, and free of illness on the day of sample collection. The exclusion criteria included patients on cortisol therapy, on hormone therapy or taking oral contraceptives, patients with implanted contraceptive devices, history of thyroid or other autoimmune disease, history of Cushings syndrome or Addison's disease, pregnant or lactating women, and any patient who had consumed an alcoholic beverage within 24 hours of sample collection. The labeling states that each laboratory should check the validity of this reference range and if necessary establish its own patient population specific reference interval.

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.