

JUN 1 0 2003

K031348



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## Summary of Safety and Effectiveness

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is K011323.

### I. General Information

**Submitter:**

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Salimetrics, LCC  
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**Date of 510(k) preparation:**

**Device Trade Name:**

HS(high sensitivity) Salivary Cortisol Enzyme  
Immunoassay

**Generic Name:**

Immunoassay for the in vitro quantitative determination  
of cortisol in saliva

**Predicate Device:**

The HS Salivary Cortisol EIA is substantially  
equivalent to the Diagnostic Systems Laboratory Active  
Cortisol EIA, which was approved by FDA (K850141)  
for IVD use.

## VI. Intended Use

Salimetrics HS-Cortisol kit is a competitive immunoassay specifically designed for the quantitative *in vitro* diagnostic measurement of salivary cortisol. Saliva cortisol accurately reflects the amount of serum cortisol in the circulation. Saliva values may be converted into serum values using the equation  $y(\text{serum } \mu\text{g/dL}) = 5.177 + 15.132x(\text{saliva } \mu\text{g/dL})$ . This kit is not intended for use with serum or plasma samples.

## III. Device Description

Test Principle- A microtitre plate is coated with rabbit antibodies to cortisol. Cortisol in standards and unknowns compete with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is inversely proportional to the amount of cortisol present. The optical density readings of the calibrators are used to form a standard curve to which the optical densities of the controls and samples are compared.

Kit Description- The kit consists of an antibody coated 96 well plate, six calibrators at a concentration of 1.800, 0.600, 0.200, 0.067, 0.022, and 0.007  $\mu\text{g/dL}$  of NIST(National Institute of Standards and Technology) cortisol, two controls representing a high (1.00  $\mu\text{g/dL}$ ) and low (0.100  $\mu\text{g/dL}$ ) level of salivary cortisol, the enzyme conjugate (1600X concentrate), assay diluent containing a pH indicator, wash buffer(10X concentrate), tetramethylbenzidine substrate solution, stop solution, and kit insert.

## IV. Prior Marketing History

The HS Salivary Cortisol EIA has been sold as a research tool since November of 1998. The device was cleared for diagnostic use in December of 2001 and afterwards marketed as such. There have been no withdrawals of this device from any country related to safety and effectiveness.

## VII. Contraindications, Warnings and Precautions

- This kit is designed to measure cortisol levels in saliva and should not be used to measure serum or plasma levels.
- A pH value should be obtained on samples that appear yellow or purple after assay diluent is added and the plate is mixed. Samples with pH values  $>$  than 9 or  $<$  3.5 should be recollected.
- Particulate matter may affect antibody binding. Pipette only clear saliva into wells. Saliva may be filtered through cotton to eliminate particulate matter.

- Saliva samples contaminated with blood may give false results.
- Saliva collection and handling recommendations are presented in the kit insert.

## VIII. Comparison to Predicate Device

### Correlation with Serum and Relationship to Predicate Device

The correlation between saliva and serum is highly significant,  $r(64) = .89$ ,  $p < .0001$ . The relationship between the HS Cortisol Salivary EIA and the serum cortisol predicate device, determined by linear regression is  $y(\text{serum } \mu\text{g/dL}) = 5.177 + 15.132x(\text{saliva } \mu\text{g/dL})$ .

### List of similarities and differences between the HS salivary Cortisol EIA and the predicate device

**Table 1** List of assay Characteristics

Device Characteristic	HS Salivary Cortisol EIA	Predicate Device
Basic Principal	Competitive Solid Phase Immunoassay	Competitive Solid Phase Immunoassay
Calibrator matrix	Saliva-like matrix	Serum matrix
Tracer	Horseradish Peroxidase linked to cortisol	Horseradish Peroxidase linked to cortisol
Substrate	Tetramethylbenzidine(TMB)	Tetramethylbenzidine(TMB)
Primary Antibody	goat anti rabbit	goat anti rabbit
Calibrator Range	0.007 - 1.800 $\mu\text{g/dL}$	0.5 - 60 $\mu\text{g/dL}$
Sample Type	saliva	serum or plasma
Instrumentation	standard plate reader	standard plate reader
Intra-assay Precision	Coefficient of variation < 6%	Coefficient of variation < 11%
Inter-assay Precision	Coefficient of variation < 11%	Coefficient of variation < 12%
Sensitivity	<0.007 $\mu\text{g/dL}$	0.1 $\mu\text{g/dL}$
Linearity	84 -105 % recovery	81-119% recovery
Recovery	84-115%	93-124
Control Levels	1.000 $\mu\text{g/dL}$ and 0.100 $\mu\text{g/dL}$	20 $\mu\text{g/dL}$ and 4 $\mu\text{g/dL}$

## VI. Conclusion

The data presented in the 510(k) demonstrates that cortisol can be measured in the HS Salivary Cortisol EIA with comparable accuracy in saliva when compared to the serum predicate device. Saliva cortisol values measured with the HS Salivary Cortisol EIA correlate well with serum cortisol values. There is also a large amount of supporting literature that indicates cortisol can be measured accurately in saliva when samples are collected and stored in an appropriate manner.



**Comparison Between the Performance Data of the HS Salivary  
Cortisol EIA and the Predicate Device**

**Table 2** Intra-assay Precision

The **intra-assay** precision was determined from the mean of 12 replicates.

SAMPLE	N	MEAN (ug/dL)	STANDARD DEVIATION (ug/dL)	COEFFICIENT OF VARIATION (%)
HS cortisol Saliva EIA				
Level 1	12	1.591	0.068	4.3
Level 2	12	0.702	0.035	5.0
Level 3	12	0.188	0.011	5.9
Level 4	12	0.115	0.006	5.2
Predicate (Serum EIA)				
Level 1	12	29.2	3.0	10.3
Level 2	12	15.9	0.8	5.0
Level 3	12	8.4	0.2	2.4

**Table 3** Inter-assay Precision

The **inter-assay** precision was determined from the mean of average duplicates for 12 separate runs.

SAMPLE	N	MEAN (µg/dL)	STANDARD DEVIATION (µg/dL)	COEFFICIENT OF VARIATION (µg/dL)
HS cortisol Saliva EIA				
Level 1	12	1.436	0.098	6.9
Level 2	12	1.000	0.086	8.6
Level 3	12	.194	0.013	6.7
Level 4	12	.023	0.002	10.6
Predicate (Serum EIA)				
H	12	15.0	1.2	8.0
M	12	11.4	0.7	6.1
L	12	5.0	0.6	12.0

## Sensitivity

HS Cortisol Saliva EIA: The lower limit of sensitivity was determined by interpolating the mean minus 2 standard deviations for 10 sets of duplicates at 0 µg/dL standard. The minimal concentration of cortisol that can be distinguished from 0 is <.007 µg/dL.

Predicate (Serum EIA): The theoretical sensitivity or minimum detection limit, calculated by the interpolation of the mean minus two standard deviations of ten replicates of the 0 µg/dL Cortisol standard, is 0.1 µg/dL.

**Table 4** Linearity of Dilution

Two saliva samples were diluted with assay diluent and assayed.

SAMPLE	DILUTION FACTOR	EXPECTED (µg/dL)	OBSERVED (µg/dL)	RECOVERY (%)
HS cortisol Saliva EIA		IX.	X.	XI.
Sample 1		---	0.161	---
	1:2	0.081	0.079	97.5
	1:4	0.040	0.042	105.0
	1:8	0.020	0.019	95.0
	1:16	0.010	0.010	100.0
Sample 2		---	0.523	---
	1:2	0.262	0.263	100.4
	1:4	0.131	0.110	84.0
	1:8	0.065	0.056	86.2
	1:16	0.033	0.033	100.0
Sample 3		---	1.708	---
	1:2	0.854	0.782	91.6
	1:4	0.427	0.389	91.1
	1:8	0.214	0.210	98.1
	1:16	0.107	0.106	99.1
Predicate (Serum EIA)				
Sample 1		---	13.0	---
	1:2	6.5	6.9	106
	1:4	3.2	3.8	119
	1:8	1.6	1.8	113
	1:16	---	---	---
Sample 2		---	27.2	---
	1:2	13.6	12.4	91
	1:4	6.8	5.5	81
	1:8	3.4	3.4	100
	1:16	1.7	2.0	118

**Table 5** Recovery

Seven saliva samples containing different levels of endogenous cortisol were spiked with known quantities of cortisol and assayed. Compare to serum results listed below.

SAMPLE	ENDOGENOUS (µg/dL)	ADDED (µg/dL)	EXPECTED (µg/dL)	OBSERVED (µg/dL)	RECOVERY (%)
HS Cortisol Saliva EIA					
1	0.632	0.180	0.812	0.816	100.5
2	0.093	0.180	0.273	0.308	112.8
		0.020	0.113	0.095	84.1
3	0.700	0.500	1.200	1.290	107.5
		0.180	0.88	0.891	101.3
4	0.696	0.020	0.716	0.745	104.1
5	0.039	1.00	1.039	1.060	102.0
6	0.137	1.00	1.137	1.310	115.2
7	0.289	0.020	0.309	0.307	99.4
Predicate (serum EIA)					
1	5.1	5.5	10.6	9.8	93
2	13.3	1.8	15.1	18.7	124
3	20.4	3.6	24.0	24.9	104

**Table 6** Specificity

Percent cross-reactivity in the predicate device is expressed as the ratio of cortisol concentration to the concentration of the reacting compound at 50% binding of the 0 µg/dL cortisol standard.

In the HS Salivary Cortisol EIA, the following compounds were tested at concentrations up to 66,000 ng/mL for cross-reactivity:

Compound	% Cross-reactivity in predicate	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Cortisol EIA
Prednisolone	58.3	100	9.530
Prednisone	10.9	1000	0.421
Cortisone	7.0	1000	0.31
11-Deoxycortisol	5.7	500	3.116
21-Deoxycortisol	1.9	1000	0.745
17-α Hydroxy- progesterone	0.9	1000	0.611
Dexamethasone	0.9	1000	1.277
Tiamcinolone	0.4	1000	0.430
Corticosterone	ND	10,000	0.093
Progesterone	ND	1000	0.060
17-β estradiol	No data	10	ND
DHEA	ND	10,000	ND
Testosterone	No data	10,000	0.007
Transferrin	No data	66,000	ND
Aldosterone	No data	10,000	ND

ND = non-detectable (<0.004)

## Salivary Cortisol Expected Ranges

Each laboratory should establish its own range of expected values. The following values have been reported for salivary cortisol.

Group	Number	AM range in µg/dL	PM range in µg/dL
Children ages 2.5-5.5	112	0.060-0.700	0.08-0.660
Children ages 8-11	285	0.112-0.904	ND-0.249
Adolescents ages 12-18	403	0.046-0.950	ND-0.296
Males ages 21-30	26	0.112-0.743	ND-0.308
Females ages 21-30	20	0.272-1.348	ND-0.105
Males ages 31-50	67	0.122-1.551	ND-0.359
Females ages 31-50	31	0.094-1.515	ND-0.181
Males ages 51-70	28	0.112-0.812	ND-0.228
Females ages 51-70	23	0.149-0.739	0.02-0.254
<u>All adults</u>	<u>192</u>	<u>0.094-1.551</u>	<u>ND-0.359</u>

ND= none detected

Group	Number	Overall range
Children, neonatal	275	0.010-3.606
Children, age 6 months	165	0.010-2.890

## CORRELATION WITH SERUM:

The correlation between serum and saliva cortisol was determined by assaying 68 matched samples from presumed normal adults using the predicate device for serum and the Salimetrics HS salivary cortisol EIA. The correlation between saliva and serum was highly significant,  $r(64) = .89$ ,  $p < .0001$ . The relationship between the HS Cortisol Salivary EIA and the serum cortisol predicate device, determined by linear regression is  $y(\text{serum } \mu\text{g/dL}) = 5.177 + 15.132x(\text{saliva } \mu\text{g/dL})$ .

Saliva explained 79.2% of the variance in serum,  $F(1,63) = 240.528$ ,  $p < 0.0001$ . The intercept is 5.177 (SE = 0.450, and is significantly above zero,  $t = 11.512$ ,  $p < 0.0001$ ). The slope of saliva is 15.132 (SE = 0.976), and is also significant,  $t = 15.509$ ,  $p < 0.0001$ . Scores with residuals  $> 3$  and  $dfbeta > 1$  (N = 3) were removed to minimize influential observations.

Since both serum (skew = 1.325) and saliva (skew = 1.793) are skewed, after taking a log transformation, the R<sup>2</sup> was only slightly reduced, with logged saliva explaining 74.7% of the variance in logged serum,  $F(1,63) = 185.530$ ,  $p < 0.0001$ . The new constant is 2.947 (SE = 0.054), and is significant,  $t = 54.544$ ,  $p < 0.0001$ , and the slope of the saliva is also significant,  $t = 13.621$ ,  $p < 0.001$  (B = 0.538, SE = 0.040).



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration  
2098 Gaither Road  
Rockville MD 20850

JUN 1 0 2003

Ms. Eve Schwartz  
VP of Operations  
Salimetrics, LLC  
P.O. Box 395  
State College, PA 16804-0395

Re: k031348  
Trade/Device Name: HS Salivary Cortisol Enzyme Immunoassay Kit  
Regulation Number: 21 CFR 862.1205  
Regulation Name: Cortisol (hydrocortisone and hydroxycorticosterone) test system  
Regulatory Class: Class II  
Product Code: NHG  
Dated: May 28, 2003  
Received: May 30, 2003

Dear Ms. Schwartz:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

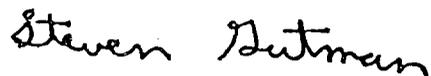
Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

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This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address <http://www.fda.gov/cdrh/dsma/dsmamain.html>.

Sincerely yours,

A handwritten signature in black ink that reads "Steven Gutman". The signature is written in a cursive style.

Steven I. Gutman, M.D., M.B.A.  
Director  
Office of *In Vitro* Diagnostic Device  
Evaluation and Safety  
Center for Devices and  
Radiological Health

Enclosure



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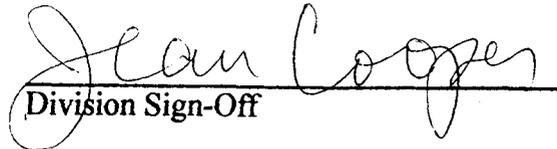
### INDICATIONS FOR USE STATEMENT

510(k) number: K011323

Device Name: HS Salivary Cortisol Enzyme Immunoassay Kit

#### Indications for Use:

Salimetrics HS-Cortisol kit is a competitive immunoassay specifically designed for the quantitative *in vitro* diagnostic measurement of salivary cortisol. This kit may be used to measure adrenal cortical function and as a screen for Cushing's and Addison's disease. Saliva cortisol accurately reflects the amount of serum cortisol in the circulation. This kit is not intended for use with serum or plasma samples.

  
Division Sign-Off

Office of In Vitro Diagnostic Device  
Evaluation and Safety

510(k) K031348

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Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use

OR

Over-the-Counter Use