510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K203270

B Applicant

Siemens Healthcare Diagnostics Products Ltd.

C Proprietary and Established Names

IMMULITE/IMMULITE® 1000 Cortisol

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CGR	Class II	21 CFR 862.1205 - Cortisol (Hydrocortisone and Hydroxycorticosterone) Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modified device. Change in polyclonal capture antibody supplier.

B Measurand:

Cortisol

C Type of Test:

Chemiluminescence Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

For in vitro diagnostic use with the IMMULITE® and IMMULITE 1000 Analyzers - for the quantitative measurement of cortisol (hydrocortisone, Compound F) in serum, as an aid in the clinical assessment of adrenal status.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with IMMULITE® and IMMULITE 1000 analyzers

IV Device/System Characteristics:

A Device Description:

The IMMULITE/IMMULITE® 1000 Cortisol assay is comprised of the following components:

Component	Volume	Ingredients
Cortisol Test Unit (solid phase)	1 bead/Test unit	Polyclonal rabbit anti-cortisol antibody.
Cortisol Reagent Wedge (liquid phase)	7.5 mL	Alkaline phosphatase (bovine calf intestine) conjugated to cortisol in buffer, with preservative.
Cortisol Adjustors (Low and High)	3 mL	Cortisol in processed human serum, with preservative.
IMMULITE/IMMULITE 1000 Cortisol Sample Diluent (sold separately)		
Cortisol Sample Diluent	25ml	Cortisol-free human serum, with preservative

B Principle of Operation:

IMMULITE/IMMULITE® 1000 Cortisol is a solid-phase, enzyme-labeled chemiluminescent competitive immunoassay. The solid phase (bead) is coated with polyclonal rabbit anti-cortisol

antibody. The liquid phase consists of alkaline phosphatase (bovine calf intestine) conjugated to cortisol. The patient sample and the reagent are incubated together with the coated bead for 30 minutes. During this time, cortisol in the sample competes with enzyme-conjugated cortisol in the reagent for a limited number of antibody binding sites on the bead. Unbound patient sample and enzyme conjugate are then removed by centrifugal washes. Finally, chemiluminescent substrate is added to the test unit containing the bead and the signal is generated inversely proportion to the bound enzyme.

V *Substantial Equivalence Information:*

A Predicate Device Name(s):

IMMULITE Cortisol

B Predicate 510(k) Number(s):

K931409

C Comparison with Predicate(s):

Device & Predicate Device(s):	K203270	K931409
Device Trade Name	IMMULITE/IMMULITE® 1000 Cortisol (Modified)	IMMULITE/IMMULITE® 1000 Cortisol (Unmodified)
General Device Characteristic Similarities		
Intended Use/Indications For Use	For in vitro diagnostic use with the IMMULITE® and IMMULITE 1000 Analyzers - for the quantitative measurement of cortisol (hydrocortisone, Compound F) in serum, as an aid in the clinical assessment of adrenal status.	Same
Analyte	Cortisol	Same
Automated	Automated Assay	Same
Measurement	Quantitative	Same
Sample Type	Human serum	Same
Calibration Range	1-50 µg/dL (28 to 1380 nmol/L)	Same
Technology	Chemiluminescent enzyme immunoassay	Same
Sample Volume	10 µL	Same

Device & Predicate Device(s):	K203270	K931409
Device Trade Name	IMMULITE/IMMULITE® 1000 Cortisol (Modified)	IMMULITE/IMMULITE® 1000 Cortisol (Unmodified)
General Device Characteristic Similarities		
Detection Enzyme conjugate	Alkaline phosphatase (bovine calf intestine) conjugated to cortisol	Same
Capture Antibody	Polyclonal rabbit anti-cortisol	Same
General Device Characteristic Differences		
Detection Limit	LoB: 0.008 µg/dL (0.22 nmol/L)	Analytical Sensitivity: 0.20 µg/dL (5.5 nmol/L)
	LoD: 0.053 µg/dL (1.46 nmol/L)	Not Applicable
	LoQ: 0.2 µg/dL (5.52 nmol/L)	Not Applicable

VI Standards/Guidance Documents Referenced:

CLSI EP06-A2: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

CLSI EP07: Interference Testing in Clinical Chemistry, 3rd Edition

CLSI EP-09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd Edition.

CLSI EP15-A3: User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edition.

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.

CLSI EP34: Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking First Edition.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   The precision/reproducibility information provided was reviewed and found to be acceptable to support that the modified device performs as described in k931409.

2. Linearity:

   The linearity information provide was reviewed and found to be acceptable to support the linear range of 1-50 µg/dL (28 to 1380 nmol/L) described in k931409.

3. Analytical Specificity/Interference:

   Cross-reactivity:
   A cross-reactivity study was conducted to evaluate the potential cross-reactivity of the assay. Potential cross-reactants were spiked at a ratio of 1:19 into an aliquot of a blank sample (charcoal- adsorbed human serum) and blank samples were also prepared which consisted of charcoal-adsorbed human serum spiked 1:19 with the solvents used to prepare the cross-reactants. The testing was performed using 1 reagent lot with cross reactant and blank samples assayed in duplicate on 1 instrument. Cross-reactivity was calculated as follows:

$$\%\ \text{Cross-reactivity} = \frac{\text{Observed cross-reactivity mean dose}}{\text{Cross-reactant added concentration (µg/dL)}} \times 100\%$$

Result Summary

Compound	Cross-Reactant added concentration (µg/dL)	% Cross-Reactivity
Aldosterone	1000	ND
Androstenedione	10000	ND
Betamethasone	1000	ND
Corticosterone	400	0.92%
Cortisone	400	1.77%
11- Deoxycorticosterone	400	ND
11-Deoxycortisol	100	4.05%
21-Deoxycortisone	500	ND
Dexamethasone	400	ND
DHEA-SO4	10,000	ND
Estriol	100	ND

Compound	Cross-Reactant added concentration (µg/dL)	% Cross-Reactivity
Estrone	500	ND
Fludrocortisone	1,000	ND
Fluticasone	22	ND
17α-hydroxyprogesterone	400	ND
Methotrexate	100	ND
Methylprednisolone	200	1.12%
Prednisolone	8	16.01%
Prednisone	16	ND
Pregnanediol	2,000	ND
Progesterone	400	ND
Spironolactone	1,000	ND
Tetrahydrocortisol	1,000	ND
Tetrahydrocortisone	400	ND
Triamcinolone	5,000	ND
Allotetrahydrocortisol	100	2.06%
α-Cortolone	1,000	ND
a-Cortol	1000	ND
β-Cortol	1,000	ND
β-Cortolone	1,000	ND
11-Dehydrocorticosterone	1000	ND
20α-dihydrocortisol	1,000	0.28%
20β-dihydrocortisol	1000	ND
20α-dihydrocortisone	1,000	ND
Estradiol	1,000	ND

ND = Not Detected

The labeling includes the following limitation statement;

> "Circulating cortisol results may be falsely elevated in samples obtained from patients being treated with prednisolone or prednisone (converted to prednisolone in vivo). Circulating cortisol results may also be falsely elevated due to cross-reactivity with 11-Deoxycortisol during metyrapone stimulation testing and therapy, potentially masking a hypoadrenalism. Caution must therefore be exercised with cortisol determinations for patients undergoing therapy with these and structurally related synthetic corticosteroids."

Interference:
The sponsor stated that a study was conducted in accordance with CLSI EP07 to evaluate interference for conjugated and unconjugated bilirubin, hemoglobin, intralipid and biotin. Working stock solutions of the interfering substances were prepared and spiked into 5 patient samples containing cortisol concentrations ranging from 3.3 – 23.1 µg/dL. Blank control samples were prepared by spiking an aliquot of the same patient sample with an equivalent volume of the solvent used to prepare the test substance. The testing was performed using 1 reagent lot with interferent and blank samples assayed in triplicate on one instrument. Mean percent recovery was calculated as:

$$\% \text{ recovery} = \frac{\text{mean observed dose (interferent spike)}}{\text{mean control dose (blank spike)}} \times 100$$

The interference study results support the following labeling limitations:

Hemolysis: Presence of packed red blood cells in concentrations up to 30 µL/mL has no effect on results, within the precision of the assay.
Lipemia: Presence of triglycerides in concentrations up to 3000 mg/dL has no effect on results, within the precision of the assay.
Bilirubin: Samples spiked with 100 and 200 mg/L of conjugated and unconjugated bilirubin were analyzed. Bilirubin may interfere with the assay, causing elevation of values.
Biotin: Specimens that contain biotin at a concentration of 3500 ng/mL demonstrate a less than or equal to 10% change in results. Biotin concentrations greater than this may lead to incorrect results for patient samples.

4. Assay Reportable Range:

   Cortisol: 1-50 µg/dL (28 to 1380 nmol/L).

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The assay is traceable to an internal standard manufactured using qualified materials and measurement procedures.

6. Detection Limit:

   The sponsor conducted Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies is to evaluate the detection capability of the modified IMMULITE/IMMULITE® 1000 Cortisol assay and stated these studies were conducted in accordance with CLSI EP17-A2.

   Limit of Blank:
   Four blank samples were prepared using charcoal-adsorbed human serum matrix and tested on 2 reagent lots, 1 instrument, 1 run per day for 3 days with 6 replicates per day resulting in a total of 72 replicates for each reagent lot. The limit of blank was estimated by the 95th percentile of 72 values for each lot. LoB was taken as the highest LoB from both reagent lots.

Limit of Detection:
Four low analyte samples (native serum samples diluted with charcoal-adsorbed human serum) were tested using 2 reagent lots, 1 instrument, 1 run per day for 3 days with 6 replicates per day resulting in a total of 72 replicates for each reagent lot. For the low level samples, parametric LoD equations were applied using the LoB value. LoD was determined for both reagent lots, with the maximum of the two values used to set LoD.

Limit of Quantitation:
The limit of quantitation was determined using 8 human samples (native serum samples diluted with charcoal-adsorbed human serum) spanning the low end range of the assay (from 0.16-3.38 µg/dL) and tested with 2 reagent lots for 5 test days, 2 runs per day and 5 replicates per run, for a total of 50 measurements per sample, per reagent lot. For each lot, regression analysis was performed based on within-laboratory precision plotted against the mean concentration obtained for each sample. The LoQ was defined as the largest cortisol concentration having a predicated within-laboratory CV of 20%.

LoB/LoD/LoQ estimates are summarized below:

Limit of Blank (LoB)	0.008 µg/dL
Limit of Detection (LoD)	0.053 µg/dL
Limit of Quantitation (LoQ)	0.20 µg/dL

The reportable range of the modified IMMULITE/IMMULITE® 1000 Cortisol assay is 1 to 50 µg/dL.

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed by comparing the modified device to the predicate device (unmodified IMMULITE/IMMULITE® 1000 Cortisol Assay). The sponsor states the study was performed in accordance with CLSI EP09c. A total of 152 native patient samples were assayed in duplicate split across 4 runs, using 3 instruments (41 patients on runs 1-3 using instruments 1-3, and 30 patients on run 4 using instrument 1). The first replicate for the modified assay was compared to the mean of the two replicates for the predicate (unmodified) device.

Sample type	N	Sample Range Tested (µg/dL)	Regression equation
Serum	152	2.01 – 48.3	y = 0.951x - 0.155 µg/dL r = 0.991

2. Matrix Comparison:

Not applicable. Only serum samples are recommended for use with this assay.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

References ranges are based on literature and were reviewed in k931409.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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