

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

A. 510(k) Number:

k060480

B. Purpose for Submission:

Modifications to reagent pack

C. Measurand:

Cortisol

D. Type of Test:

Enzyme-linked Immunosorbent Assay (ELISA)

E. Applicant:

Ortho-Clinical Diagnostics

F. Proprietary and Established Names:

VITROS Immunodiagnostic Product Cortisol Reagent Pack

VITROS Immunodiagnostic Products Cortisol Calibrator

VITROS Immunodiagnostic Products Metabolism Controls

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CGR	II	862.1205	75
JIS	II	862.1150	75
JJX	I (reserved)	862.1660	75

H. Intended Use:

1. Intended use(s):

See Indications For Use below.

2. Indication(s) for use:

Device Name:

VITROS Immunodiagnostic Products Cortisol Reagent Pack

VITROS Immunodiagnostic Products Cortisol Calibrator

VITROS Immunodiagnostic Products Metabolism Controls

Indications for Use:

The measurement of cortisol in human serum, plasma (heparin or EDTA) or urine aids in the assessment of adrenal status.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

VITROS ECI/ECiQ Immunodiagnostic System

I. Device Description:

The VITROS Immunodiagnostic Cortisol Reagent Pack uses luminescence as the signal in the determination of cortisol concentration in serum, plasma, or urine. Coated microwells are used as the solid phase separation system. The system is comprised of three main elements:

- The VITROS Immunodiagnostic Products Cortisol Reagent Pack, VITROS Immunodiagnostic Products Cortisol Calibrators (cleared under k983990) and VITROS Immunodiagnostic Products Metabolism Controls (cleared under k983990), which are combined by the VITROS Immunodiagnostic System to perform the VITROS Cortisol assay.
- The VITROS Immunodiagnostic System – instrumentation, which provides automated use of the immunoassay kits. The VITROS Immunodiagnostic System was cleared for market by a separate 510(k) pre- market notification (k962919).
- Common reagents used by the VITROS System in each assay. The VITROS Immunodiagnostic Products Signal Reagent and VITROS Immunodiagnostic Products Universal Wash Reagent were cleared as part of the VITROS Immunodiagnostic Products Total T3 Reagent Pack and VITROS Immunodiagnostic Products Total T3 Calibrators 510(k) premarket notification (k964310).

Note: High Sample Diluent B was cleared as part of the VITROS Immunodiagnostic Products Total β -hCG Reagent Pack and VITROS Immunodiagnostic Products Total β -hCG Calibrators 510(k) premarket notification (k970894). The VITROS System and common reagents are dedicated specifically for use with the VITROS Immunodiagnostic Products range of immunoassay products.

J. Substantial Equivalence Information:

Predicate		k983990 VITROS Cortisol Assay
Describe the item being compared		
<p>The modified VITROS Immunodiagnostic Products Cortisol assay is substantially equivalent to VITROS Immunodiagnostic Products Cortisol assay previously cleared under k983990. The modifications to the assay only affect the VITROS Immunodiagnostic Products Cortisol Reagent Pack. There are no modifications to the current VITROS Immunodiagnostic Products Cortisol Calibrators or the VITROS Immunodiagnostic Products Metabolism Controls previously cleared under k983990.</p>		
Similarities		
Device Characteristics	Predicate Device	New Device
Sample type	serum, plasma (EDTA or heparin) and urine	serum, plasma (EDTA or heparin) and urine
Basic principle	Solid phase immunoassay	Solid phase immunoassay
Tracer	Enzyme labeled	Enzyme labeled
Instrumentation	VITROS Immunodiagnostic System	VITROS Immunodiagnostic System
Sample volume	25 uL	25 uL
Incubation time and temperature	30 minutes at 37 Celsius	30 minutes at 37 Celsius
Differences		
Device Characteristic	Predicate Device	New Device
Biotinylated Antibody Reagent HRP Conjugate Reagent	<u>Antibody</u> Sheep polyclonal anti-cortisol antibody biotinylated antibody reagent (pool of two bleeds from a single sheep immunized in-house at Pollards Wood) <u>Concentration</u> 1.5 mg/Kg Contains Bovine Alpha Globulin	<u>Antibody</u> Sheep polyclonal anti-cortisol antibody biotinylated antibody reagent (pool of eight bleeds from two sheep immunized in-house at Pollards Wood); <u>Concentration</u> 0.5 mg/Kg Remove Bovine Alpha Globulin; Add ANS (8-anilino-1-naphthalenesulfonic acid)
Reportable Range	0 to 1700 nmol/L	4.39 to 1700 nmol/L

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

STANDARDS
Title and Reference Number
Interference Testing in Clinical Chemistry; Approved Guideline (CLSI EP 7-A)
Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (CLSI EP09-A2)
Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (CLSI EP5-A2)
How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition (CLSI C28-A2)
Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)
Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A)

L. Test Principle:

A competitive immunoassay technique is used. This depends on competition between Cortisol present in the sample with a horseradish peroxidase (HRP)-labeled Cortisol conjugate for a limited number of binding sites on a biotinylated sheep anti-cortisol polyclonal antibody. The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent (a substituted acetanilide) is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent increases the level and duration of the light produced. The light signals are read by the VITROS Immunodiagnostic System. The amount of HRP conjugate bound is inversely proportional to the concentration of Cortisol present in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

Precision was evaluated following CLSI (formerly NCCLS) EP5-A2. Two replicates of each of 3 freeze-dried control sera were assayed on 2 separate occasions per day on at least 20 different days. An identical experiment was performed for urine (including the urine extraction pretreatment steps) to assess precision for urine samples. The experiments were performed using 2

reagent lots on 2 different VITROS Immunodiagnostic Systems. Results are summarized below:

Conversion: $\mu\text{g/dL} = \text{nmol/L} \times 0.03625$

Serum (nmol/L)								
	Mean Cortisol Concentration	Within-run*		Within-calibration**		Within-lab***		# Observed
		SD	CV (%)	SD	CV (%)	SD	CV (%)	
System 1	93.5	2.07	2.2	4.43	4.7	4.42	4.9	80
	259	4.41	1.7	10.9	4.2	11.3	4.5	80
	771	16.6	2.2	36.0	4.7	38.2	5.1	80
System 2	84.1	1.86	2.2	2.81	3.3	3.33	3.8	80
	233	5.99	2.6	7.32	3.1	9.08	3.7	80
	668	11.9	1.8	19.9	3.0	28.2	4.0	80

Urine (nmol/L)								
	Mean Cortisol Concentration	Within-run*		Within-calibration**		Within-lab***		# Observed
		SD	CV (%)	SD	CV (%)	SD	CV (%)	
System 1	106	19.3	18.2	25.7	24.2	24.3	22.9	92
	307	21.0	6.8	34.6	11.3	37.1	12.1	92
	767	137	17.9	159	20.7	156	20.3	92
System 2	119	27.9	23.4	35.1	29.5	34.3	28.8	96
	363	42.0	11.6	48.4	13.3	51.4	14.2	96
	874	150	17.2	174	19.9	181	20.7	96

* Within-run (Repeatability): Within-run precision was determined using duplicate determinations.

** Within-calibration: Total within-calibration precision was determined using a single lot of reagent over a single calibration interval.

*** Within-lab: Total within-lab precision was estimated using a single reagent lot calibrated weekly.

b. Linearity/assay reportable range:

The measuring range of the VITROS Cortisol assay is 4.39 nmol/L to 1700 nmol/L. Linearity of the assay was evaluated using protocols based on CLSI (formerly NCCLS) EP6-A. Two plasma sample pools were prepared with Cortisol titers near zero and at the high end of the range. A Cortisol stock solution was prepared in methanol. The high pool (pool 11) was prepared by mixing donor samples with an additional spike of Cortisol. The low pool (pool 1) was prepared from charcoal stripped plasma. The low and high

concentration pools were mixed to give 9 additional pools of intermediate concentrations. A minimum of six singleton determinations of each of the individual pools were made together with singleton determinations of the QC In-house Controls with each of the two Master Lots on two different VITROS Immunodiagnostic Systems. Results are summarized below.

Pool Number	Calculated Concentration (nmol/L)	Mean Measured Concentration (nmol/L)	Calculated % Recovery
11	pooled	2090*	-
10	1880*	1870*	99.5
9	1670	1710	102.4
8	1460	1470	100.7
7	1250	1260	100.8
6	1050	1040	99.0
5	837	837	100.0
4	629	614	97.6
3	420	410	97.6
2	212	195	92.0
1	pooled	4.02	-
Measured as a % of Calculated: Overall Mean (%) =			98.8

* Extrapolated values – concentration greater than highest calibrator. The reference calibrators consist of seven levels (0, 14, 82, 659, 1267 and 1725 nmol/L) so a Master Curve is calibrated for each Master Lot up to 1725 nmol/L. The customer calibrators consist of three levels (0, 125 and 750 nmol/L) and recreate the lot specific Master Curve on the customer’s instrument when a calibration is run.

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

Both the Calibrators and Metabolism Controls are value-assigned by performing multiple determinations using several VITROS Immunodiagnostic Systems. The Calibrators are traceable to in-house reference calibrators, which have been value-assigned to correlate to samples measured by gas chromatography/mass spectrometry. The calibration and curve-shape for the VITROS Cortisol assay is defined by a set of Reference Calibrators in the Reference Calibration Curve (RCC).

There are two types of magnetic cards provided to the customer with every calibrator pack. These are the lot calibration card and the protocol card. The lot calibration card contains encoded data which is specific to a lot of reagents, for example the master calibration data. The protocol card contains encoded information that allows the System to process the assay associated with the calibrators, for example assay incubation times and sample volumes.

The Reagent Packs and Calibrators were subjected to simulated transport conditions. The data in this report supported the stability of the VITROS Cortisol reagent pack and calibrators up to the Week 22 timepoint when stored at 2-8°C. Therefore a shelf life of 16 Weeks is claimed for the reagent pack and calibrator when stored at 2-8°C.

VITROS Cortisol Calibrators consist of three levels with nominal values of 0, 125 and 750 nmol/L cortisol. VITROS Metabolism controls consist of three levels with nominal values of 90, 250 and 750 nmol cortisol/L.

d. Detection limit:

The sponsor defined analytical sensitivity as the concentration calculated from the mean light unit minus two standard deviations of a sample containing no analyte, where 20 replicates were determined. The analytical sensitivity of this assay is 3 nmol/L.

The Limit of Quantitation (LoQ) was evaluated using five plasma pools spiked with cortisol resulting in concentrations of 4.39, 10.5, 17.2, 17.3 and 23.1 nmol/L. Ten consecutive replicates of the samples were performed twice. Three Master Lots and three VITROS Immunodiagnostic Systems were used during the study for at least three days using three different calibrations. Each run included singleton determination of the Calibrators and QC in-house controls. The lowest sample from the the concentrations tested giving accurate precision (defined as CV < 15%) was 4.39 nmol/L. The LoQ for this assay is 4.39 nmol/L (the LoQ is the low end of the reportable range of the assay).

e. Analytical specificity:

Specificity of the VITROS Cortisol assay was evaluated by testing the following substances as recommended by CLSI (formerly NCCLS) Protocol EP7-A. They were found not to interfere (defined as bias <10%) with the assay at a cortisol concentration of 241 to 288 nmol/L (8.74 to 10.4 mg/dL). Interferent testing was conducted by preparing stock solutions of the interferent. The stock solution was added to the VITROS Metabolism Control, Level 2 with a nominal value of 250 nmol/L. The corresponding control pool was prepared by reconstituting the VITROS Metabolism Control, Level 2 with water or with water/solvent (at the same ratio as the test substance pool, but without the test substance). The analyte levels present in the control sample were established using the VITROS Cortisol assay on the VITROS Immunodiagnostic System.

Test Substance	Concentration Tested	Interference Less Than: (%)
Azide	1.5 mmol/L	9.9
Bilirubin	1.71 mmol/L	4.7
Triolein	33.9 mmol/L	2.1
Dipyron	3.00 mmol/L	6.3
Biotin (serum)	0.040 μ mol/L	-2.1
Biotin (extracted urine)	0.040 mmol/L	0.7

To evaluate the cross reactivity of prednisolone, 11-deoxycortisol, cortisone, and corticosterone in the VITROS Cortisol assay, three determinations at each dilution were made and analyzed as a percent cross-reactivity at 50% of zero standard binding of cortisol compared with the concentration at 50% displacement for the cross-reactant. The cross-reactivity at 50% displacement is defined as the point where the reduction in signal corresponds to 50% of the signal achieved in the absence of analyte, shown as a percentage of the analyte concentration giving the same fall in signal. For the remaining cross-reactants, the mean value of three determinations was compared with the concentration of the cross-reactant used for the test:

Cross-reactivity at 50% displacement	
Test Substance	% Cross Reactivity
Prednisolone	26.8%
11-Deoxycortisol	0.95%
Corticosterone	1.03%
Cortisone	0.13%

Test Substance	Concentration Tested (nmol/L)	Mean sample value of cross reactant pool (nmol/L) (measurement of sample containing no cortisol)	Mean % Cross-reactivity
Estriol	96685	4.84	0.006
Fludrocortisone	26281	1011	3.85
Prednisone	139509	106	0.076
Testosterone	346741	23.0	0.007
Progesterone	317965	1460	0.459
17- α -Hydroxyprogesterone	151286	836	0.553
β -Cortol	135685	7.32	0.006
β -Cortolone	81855	<3.00	ND
Dexamethasone	127389	310	0.244

Test Substance	Concentration Tested (nmol/L)	Mean sample value of cross reactant pool (nmol/L) (measurement of sample containing no cortisol)	Mean % Cross-reactivity
11- β -Hydroxyprogesterone	30257	761	2.52
6- α -Methylprednisolone	26702	1585	5.94
11-Deoxycorticosterone	86580	30.6	0.035
Tetrahydrocortisol	81855	131	0.159
Topiramte	2946000	<3.00	ND

*ND = Not Detectable. Concentrations were below the analytical sensitivity of the assay, <3.00 nmol/L.

Hemoglobin caused the bias shown at the concentrations indicated in the table below:

	Hemoglobin Concentration	Units = nmol/L	
		Analyte Conc.*	Bias**
Hemoglobin	0.062 mmol/L	310	25
Hemoglobin	0.310 mmol/L	310	136

* Average test concentration of replicate determinations using two different lots of reagent determined from twelve samples containing approximately 300 nmol cortisol/L

** Estimate of the average difference observed.

Azide at concentrations greater than or equal to 0.05% have also been shown to interfere with this assay.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 214 banked serum samples and 100 urine samples were collected. There were no inclusion or exclusion criteria required for this testing. Each of the 214 serum samples and 100 urine samples were assayed in singleton on the VITROS Immunodiagnostic System. One VITROS Immunodiagnostic System and one lot of reagents were used to test the predicate. Two VITROS

Immunodiagnostic Systems and two Master Lots were used to test the VITROS Cortisol assay. The claimed working range of the VITROS Cortisol Assay is 4.39 to 1700 nmol/L. The range of serum samples tested was 14.5 to 1370 nmol/L. The range of urine samples tested was 3.33 to 685 nmol/L.

Results were determined using linear regression and are summarized below.

Serum: VITROS Cortisol Assay = 0.986 * (predicate) - 14.2 nmol/L

Urine: VITROS Cortisol Assay = 0.798 * (predicate) - 22.03 nmol/L

b. Matrix comparison:

Twelve fresh blood samples were drawn into heparin plasma, EDTA plasma and serum tubes. They were assayed on the day they were drawn, after 5 and 7 days storage at 2-8°C and after 4 weeks at -20 ° C in order to demonstrate that serum results match plasma results. Results are summarized below.

Study	Sample Range Tested (nmol/L)	Mean % Difference Against Serum		Range of % Differences Against Serum	
		Heparin	EDTA	Heparin	EDTA
Fresh Samples	144 - 529	0.0	-6.7	-8.5 to 4.9	-10.2 to -3.0
Stored at 2-8 °C for 5 Days	147 - 490	0.2	-6.2	-8.9 to 8.1	-11.3 to -0.4
Stored at 2-8 °C for 7 Days	151 - 479	-3.1	-3.1	-8.5 to 4.6	-9.8 to 6.0
Stored at -20 °C for 4 Weeks	157 - 531	-1.5	-7.1	-8.4 to 4.3	-13.9 to -2.0

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 serum expected ranges, 123 to 626 nmol/L (obtained from 100 samples) and 46.2 to 389 nmol/L (obtained from 100 samples), were obtained from the sera of apparently healthy patients sampled in the morning before 10:00 AM and in the evening after 5 PM, respectively. The urine expected range is 35.7 to 699 nmol cortisol/24 hours (obtained from 42 samples) was obtained from 24-hour urine samples collected from apparently healthy patients.

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.