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

A. 510(k) Number:

k182012

B. Purpose for Submission:

New device

C. Measurand:

Calcitonin

D. Type of Test:

Quantitative, Chemiluminescent Microparticle Immunoassay (CMIA)

E. Applicant:

Axis-Shield Diagnostics Limited

F. Proprietary and Established Names:

ADVIA Centaur® Calcitonin (CALCT) assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1140, Calcitonin test system

2. Classification:

   Class II

3. Product code:

   JKR

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   The ADVIA Centaur® Calcitonin (CALCT) assay is for *in vitro* diagnostic use in the quantitative measurement of calcitonin in human serum using the ADVIA Centaur XP system. Calcitonin measurement is used as an aid in the diagnosis and treatment of diseases involving the thyroid and parathyroid glands, including carcinoma and hyperparathyroidism.

2. Indication(s) for use:

   See Intended use.

3. Special conditions for use statement(s):

   For prescription use only.
   Not for point of care use.

4. Special instrument requirements:

   ADVIA Centaur XP system

I. Device Description:

The ADVIA Centaur® CALCT assay is a fully automated, 2-step immunoassay using direct chemiluminescent technology. The assay utilizes an acridinium-ester-labeled recombinant antibody as the Lite Reagent. The Solid Phase consists of biotinylated anti-calcitonin mouse monoclonal antibody-coated paramagnetic microparticles.

The ADVIA Centaur CALCT Assay Kit contains the following:

- 1 ReadyPack primary reagent pack containing ADVIA Centaur CALCT Lite Reagent and Solid Phase Reagent
- 1 vial ADVIA Centaur CALCT low calibrator
- 1 vial ADVIA Centaur CALCT high calibrator
- ADVIA Centaur systems CALCT Master Curve card
- ADVIA Centaur systems CALCT Calibrator Assigned Value card

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Elecsys Calcitonin Immunoassay

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

k132828

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device ADVIA Centaur® Calcitonin (CALCT) assay (k182012)	Predicate Device Elecsys Calcitonin Immunoassay (k132828)
Intended Use	For in vitro diagnostic use in the quantitative measurement of calcitonin in human serum .	Same
Traceability	Standardized against the WHO 2nd IRP 89/620	Same
Assay principle	Chemiluminescent Microparticle Immunoassay (CMIA)	Same
Test principle	Sandwich principle	Same
Sample type	Human serum and serum separator tubes.	Human serum and plasma (K2-EDTA, K3-EDTA, Lithium Heparin with and without gel)
Sample volume	100 µL	50 µL
Measuring interval	1.75 - 2000.00 pg/mL	1.0 - 2000.0 pg/mL
Calibration interval	14 days Lot-specific	7 days Lot-specific
Reagent stability	Unopened: 2-8°C - up to the stated expiration date On analyzers – 4 weeks	Unopened: 2-8°C - up to the stated expiration date Opened 2-8°C - 12 weeks On analyzers – 4 weeks
Dilution capabilities	1:100 (automated only)	1:100 (automated and manual)

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

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, 3rd Edition

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

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline, 2nd Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, 2nd Edition

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

CLSI EP28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 3rd Edition

L. Test Principle:

The ADVIA Centaur CALCT assay is a fully automated, two-step immunoassay using direct chemiluminescent technology. Calcitonin in the sample is captured by the biotinylated anti-calcitonin mouse monoclonal antibody-coated paramagnetic microparticles present in the Solid Phase reagent. Unbound reagent is removed and the immunocomplexes on the paramagnetic particles are resuspended using the ADVIA Centaur Wash 1. The acridinium-ester-labeled antibody present in the Lite Reagent binds to a different epitope of calcitonin. After a wash step, the Acid and Base Reagents are added to initiate the chemiluminescent reaction. The amount of calcitonin present in the sample is directly proportional to the relative light units (RLUs) detected by the system.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Within-run and total precision were evaluated according to the CLSI EP05-A3 guideline. Precision studies were conducted using 3 serum pools and 2 spiked serum pools tested using 3 reagent lots. Samples were tested in replicates of 2, with 2 runs per day over 20 days, totaling 80 measurements per sample. All 3 lots yielded similar results. The results of one representative lot are summarized in the table below:

Sample	n	Mean (pg/mL)	**Repeatability**		**Total Precision**	
			SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
Sample 1	80	5.51	0.25	4.5	0.47	8.5
Sample 2	80	10.50	0.38	3.6	0.61	5.8
Sample 3	80	36.97	1.33	3.6	1.57	4.3
Sample 4	80	388.46	10.60	2.7	14.91	3.8
Sample 5	80	1615.59	34.56	2.1	53.09	3.3

b. Linearity/assay reportable range:

The linearity of the ADVIA Centaur® CALCT assay was evaluated using 7 diluted samples with calcitonin concentrations evenly distributed throughout the measuring range, prepared from high and low serum pools with concentrations that encompassed the claimed measuring range. Each of the 7 diluted samples, along with the high and low serum pools (a total of 9 concentrations), was tested in replicates of 5, using 3 reagent lots.

The linear regression equation of a representative lot is shown below:

$y = 0.988\ x + 0.012 \qquad R^2 = 1.00$

The linearity study supports the sponsor's claimed measuring rang: 1.75 – 2000 pg/mL.

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

Traceability:

The ADVIA Centaur CALCT assay is traceable to the World Health Organization (WHO) 2nd International Reference Preparation for Calcitonin (Human); NIBSC code: 89/620. Assigned values for calibrators are traceable to this standard.

d. Detection limit

The analytical limits at low levels were determined in accordance with CLSI EP17-A2 guideline.

Limit of blank (LoB) was determined using 3 reagent lots on 1 analyzer, measuring 4 zero-level serum pools over 3 days, 1 run per day, 5 replicates per run, for a total of 60 blank measurements per reagent lot. LoB was calculated as follows: sample measurements were ranked from lowest to highest, then the rank position of the 95th percentile was calculated using the equation: Rank position = 0.5+n*0.95.

Limit of detection (LoD) was determined using 3 reagent lots on 1 analyzer, measuring sample pools with low concentration of calcitonin (0.50-6.00 pg/mL). The samples were analyzed over 4 days (reagent lot 1) and 3 days (reagent lot 2 and 3), with 1 run per day, 7 replicates per run ensuring a minimum of 60 replicates per reagent lot. The LoD was determined as the lowest concentration of calcitonin that can be detected with 95% probability.

Limit of quantitation (LoQ) was determined using the data used to generate LoD, by comparing the Total Error (=Bias + 2*SD) at each pool level. The LoQ was determined as the lowest concentration of calcitonin that can be detected at a total error of 20%.

The detection limits are summarized in the table below:

LoB	LoD	LoQ
1.29 pg/mL	1.65 pg/mL	1.75 pg/mL

e. *Analytical specificity:*

Endogenous Interference was evaluated using human serum pools with calcitonin concentrations of 8.88 -14.12 pg/mL (serum 1) and 380.21 – 515.68 pg/mL (serum 2). Each interferent was spiked at 2 concentrations, and an equivalent control was prepared for each spike using the diluent without the interferent. Samples were tested in replicates of 5, using 3 reagent lots. The sponsor defined significant interference as a difference greater than ± 10% of the control value. The endogenous interference effects on calcitonin quantitation are summarized in the table below:

Substance	Highest concentration tested at which no significant interference was observed
Conjugated bilirubin	40 mg/dL
Unconjugated bilirubin	60 mg/dL
Hemoglobin	500 mg/dL
Cholesterol	500 mg/dL
Protein albumin (human)	6.0 g/dL
Total protein	12.0 g/dL
Protein gamma globulin (human)	6.0 g/dL
Rheumatoid factor	200 IU/mL
Intralipid	3000 mg/dL

Exogenous Interference was evaluated using human serum pools with calcitonin concentrations of 5.33 - 12.94 pg/mL (serum 1) and 196.79 - 520.50 pg/mL (serum 2). Each interferent was spiked at 2 concentrations, and an equivalent control was prepared for each spike using the diluent without the interferent. Samples were tested in replicates of 5, using 3 reagent lots. The sponsor defined significant interference as a difference greater than ± 10% of the control value. The exogenous interference effects on calcitonin quantitation are summarized in the table below:

Substance	Highest concentration tested at which no significant interference was observed
Acetaminophen	1000 mg/L
Amiodarone	6.08 ug/mL
Ascorbic Acid	300 mg/L
Carbimazol	30ug/mL
Cobozantinib	0.6 µg/mL
Fluocortolon	270 ng/mL
Heparin Na	300 U/dL
Hydrocortisone	984 ng/mL

Substance	Highest concentration tested at which no significant interference was observed
Ibuprofen	500 mg/dL
Iodide	0.2 µg/mL
Octreotid Acetate (Sandostatin)	5.2 ng/mL
Perchlorate	200 µg/mL
Prednisolone	3 µg/mL
Propranolol	2 µg/mL
Propylthiouracil	7.2 µg/mL
Silwet L720	0.2 mg/dL
Thiamazol	80 µg/mL
Vandetanib	1 µg/mL

In addition, a study was conducted to determine biotin interference. Biotin was added to serum samples containing different levels of calcitonin. The samples were tested against appropriate controls and the observed bias is presented in the table below. Interference was defined as a difference >±10% of the control sample values.

Biotin test concentration (ng/mL)	Calcitonin concentration (pg/mL)	Bias (%)
500,000	268.92	3.7
1,500	8.23	-19.6
750	8.66	-15.4
375	8.91	-13
187.5	8.36	-18.3
93.75	8.45	-17.4
46.875	9.01	-12
23.439	9.30	-9.2

The following statement was included within the Limitations section of the labeling:

Patient samples with low calcitonin levels may demonstrate falsely depressed results in presence of biotin greater than 40 ng/mL. Do not test samples from patients who take high doses of biotin. If biotin interference is suspected, follow your established internal procedures to investigate the interference or test with an alternative assay that is not affected by biotin interference.

Cross Reactivity was evaluated using human serum pools with calcitonin concentrations of 7.57-11.65 pg/mL, according to CLSI EP07-A2 guideline. Each of these pools was spiked with cross-reactants as detailed in the table below, and an equivalent control was prepared for each spike using the diluent without the interferent. Samples were tested in replicates of 5, using 3 reagent lots. Cross-reactivity was estimated using the following formula:

% cross-reactivity = 100 x [(dose of cross-reactivity sample (pg/mL) - dose of control sample (pg/mL)) ÷ concentration of cross-reactivity compound spike (pg/mL)]

The sponsor defined significant cross-reactivity as greater than ± 0.1% of the control value.

Cross reactant	Concentration tested (ng/mL)	% Cross-Reactivity
Adrenocortitcotropic hormone (ATCH)	200	0.00
C-peptide	80000	0.00
Insulin	67000	0.00
Prolactin	2000	0.00
PTH	300	0.00
TSH	2000 µIU/mL	0.00
Salmon calcitonin	200	0.00
Porcine calcitonin	1000	0.00
Chicken calcitonin	1000	0.00
Gastrin	4000	0.00
Procalcitonin	100	0.00
Calcitonin gene-related peptide	2000	0.00
Elcatonin	200000	0.00
Katacalcin	80000	0.00
Pentagastrin	7.5	-0.03

High Dose Hook Effect

To evaluate the hook effect, a serum pool depleted of calcitonin was spiked with synthetic calcitonin at a target concentration of 1,500,000 pg/mL and diluted serially. Samples were tested in replicates of 5 using 1 reagent lot. The results demonstrated that there is no observable high dose hook effect in the ADVIA Centaur CALCT assay for samples with calcitonin concentrations up to approximately 1,280,000 pg/mL.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device

A method comparison study was performed to compare the ADVIA Centaur® CALCT assay (candidate device) run on the ADVIA Centaur XP system to the Roche Elecsys Calcitonin assay (predicate device) run on the cobas e411 analyzer. A total of 139

serum specimens of US origin were evaluated. Specimens were tested in singlicate across 4 reagent lots and 6 days of testing. Specimens that did not report a result within the measurement range of the predicate method were not included, resulting in a 97 specimen method comparison, with calcitonin concentrations between 1.01 and 1813.00 pg/mL, as reported by the predicate device. Results of the Passing-Bablok regression analysis are presented below:

$y = 0.97 x + 1.09$ $r = 0.98$

b. Matrix comparison:

Not applicable. Serum is the only claimed sample type.

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:

A reference range study was performed according to CLSI EP28-A3c guideline. A total of 240 serum samples were obtained from apparently healthy adult US donors (n=120 males and n=120 females). The age range of the participants was 22-79 years. Reference ranges were determined using the 97.5th percentiles as upper limit of normal. The study was conducted at 1 site using 1 reagent lot over 3 days of testing. The reference range study result summary is presented below:

Cohort	N	97.5th percentile	Lower limit of 92% CI	Upper limit of 92% CI
Apparently healthy females	120	9.53 pg/mL	5.77 pg/mL	18.14 pg/mL
Apparently healthy males	120	13.38 pg/mL	12.02 pg/mL	14.86 pg/mL

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 801 and 809, as applicable.

O. Conclusion:

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