510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K213517

B Applicant

Beckman Coulter, Inc.

C Proprietary and Established Names

Access Thyroglobulin Antibody II

D Regulatory Information

Product Code(s)	Classification	RegulationSection	Panel
JZO	Class II	21 CFR 866.5870 - Thyroid Autoantibody Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Modification of the previously cleared device to mitigate biotin interference

B Measurand:

Anti-Thyroglobulin autoantibody

C Type of Test:

Quantitative, Chemiluminescent Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

Same as Indication for Use below

B Indication(s) for Use:

The Access Thyroglobulin Antibody II assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin antibody levels in human serum and plasma using the Access Immunoassay Systems. The measurement of thyroid autoantibodies may aid in the diagnosis of Hashimoto's disease, nontoxic goiter, and Graves' disease.

C Special Conditions for Use Statement(s):

Rx - For prescription use only
For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

Access 2 Immunoassay System (K121214)
UniCel DxI 600 Access Immunoassay System (K023764)
UniCel DxI 800 Access Immunoassay System (K121790)

IV Device/System Characteristics:

A Device Description:

Materials included in the Access Thyroglobulin Antibody II:

Access Thyroglobulin Antibody II Reagent Pack (2 packs, 50 tests/pack) contains:

- R1a (3.25 mL): Paramagnetic particles coated with streptavidin, coupled to biotinylated human thyroglobulin in TRIS buffer with bovine protein, preservative
- R1b (13.25 mL): Human thyroglobulin bovine alkaline phosphatase conjugate in TRIS buffer with bovine protein, preservative
- R1c (4.5 mL): TRIS buffer with bovine protein, free biotin, alkaline phosphatase blocking reagent, preservative
- R1d (3.1 mL): TRIS buffer with blocking polymer, preservative

Materials needed but not supplied:

- Access Thyroglobulin Antibody II Calibrators: at 0, 50, 250, 1,000, and 2,500 IU/mL
- Quality control materials: commercial control material
- Access Substrate
- Access Wash Buffer II

To mitigate the risk of biotin interference and non-TgAb specific binding of an interferent to alkaline phosphatase (ALP), the Access Thyroglobulin Antibody II reagent pack has been modified from the previously cleared assay by adding free biotin and alkaline phosphatase blocking reagent to existing Well 2 (R1c) of the reagent pack.

B Principle of Operation:

The Access Thyroglobulin Antibody II assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with paramagnetic particles coated with the thyroglobulin protein. The thyroglobulin autoantibodies (TgAb) in the sample bind to the thyroglobulin. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The thyroglobulin-alkaline phosphatase conjugate is added and binds to the TgAb. After the second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of TgAb in the sample. The amount of TgAb in the sample is determined from a stored, multi-point calibration curve.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Access Thyroglobulin Anitbody II

B Predicate 510(k) Number(s):

K112933

C Comparison with Predicate(s):

Device & Predicate Device(s):	K213517 (Candidate Device)	K112933 (Predicate)
Device Trade Name	Access Thyroglobulin Antibody II	Access Thyroglobulin Antibody II
General Device Characteristic Similarities		
Intended Use/ Indications for Use	The Access Thyroglobulin Antibody II assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin antibody levels in human serum and plasma using the Access Immunoassay Systems. The measurement of thyroid autoantibodies may aid in the diagnosis of Hashimoto's disease, nontoxic goiter, and Graves' disease.	Same
Analyte	Thyroglobulin antibody (TgAb)	Same
Methodology	Chemiluminescent immunoassay	Same

Sample types	Human serum and plasma (EDTA or lithium heparin)	Same
Sample volume	10 µL	Same
Calibrator scheme	6-level multipoint calibration curve	Same
General Device Characteristic Differences		
Analytical measuring interval	1.5−2500 IU/mL	0.9−2500 IU/mL
Detection Capability	LoB: 0.0 IU/mL LoD: 0.4 IU/mL LoQ: 1.5 IU/mL	LoB: 0.9 IU/mL LoD: 0.9 IU/mL LoQ: 0.9 IU/mL
Biotin interference	No interference up to 3510 ng/mL	No interference up to 100 ng/mL

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second 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 – Third Edition
- CLSI EP35, Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures – First Edition
- CLSI EP37, Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results met the manufacturer's pre-determined acceptance criteria**.**

1. Precision/Reproducibility:

   Precision and reproducibility measurements were conducted in accordance with the CLSI guideline EP05-A3 as detailed below.

Within-Laboratory Precision

To evaluate within-laboratory precision, a panel of five samples was prepared by pooling individual native serum samples to cover the AMI of the modified Access Thyroglobulin Antibody II assay. Each sample was assayed in two replicates per run, two runs per day for 20 days, using one reagent pack lot on one Access 2 Immunoassay System to obtain a total of 80 replicates per sample. Three quality controls were run in duplicate on each day to qualify each test run. Mean, standard deviation (SD) and % coefficient of variation (%CV) were analyzed for each sample. The results are summarized in the table below:

Sample	N	Mean (IU/mL)	Within-Run		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	3.1	0.3	10.3	0.2	5.9	0.0	0.0	0.4	11.8
Sample 2	80	5.3	0.4	7.5	0.2	4.2	0.0	0.0	0.5	8.6
Sample 3	80	95	4.1	4.3	3.2	3.4	0.0	0.0	5.2	5.4
Sample 4	80	506	19.0	3.8	18.6	3.7	0.1	0.0	26.6	5.3
Sample 5	80	1485	77.7	5.2	75.4	5.1	0.4	0.0	108.3	7.3

Instrument-to-instrument precision

To evaluate between-instrument precision, a panel of five serum samples was prepared by pooling individual native serum samples to cover the AMI of the modified Access Thyroglobulin Antibody II assay. Each sample was assayed in five replicates per run, one run per day over five days, using one reagent pack lot on three Access 2 Immunoassay Systems to obtain a total of 75 replicates per sample. Three quality controls were run in duplicate on each day to qualify each test run. The results are summarized in the table below:

Sample	N	Mean (IU/mL)	Within-Run		Between-Day		Between-Instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	75	3.4	0.3	8.1	0.1	2.4	0.2	6.2	0.4	10.4
Sample 2	75	5.3	0.5	8.7	0.3	6.2	0.4	6.9	0.7	12.8
Sample 3	75	94	5.2	5.5	2.1	2.2	1.8	2.0	5.9	6.2
Sample 4	75	508	26.9	5.3	9.2	1.8	19.5	3.8	34.5	6.8
Sample 5	75	1611	207.0	12.8	127.6	7.9	0.0	0.0	243.1	15.1

Lot-to-lot imprecision:

Lot-to-lot imprecision was evaluated by testing samples using three lots of the modified Access Thyroglobulin Antibody II assay. The %CV for lot-to-lot imprecision is less than 10% for all samples tested.

2. Linearity:

The linearity of the modified Access Thyroglobulin Antibody II assay was assessed in accordance with the CLSI guideline EP06-Ed2. Low and high pooled native samples were used to prepare six overlapping linearity series for the AMI. For each series, the lowest sample was tested in replicates of eight, while all other samples were tested in replicates of four on one Access 2 Immunoassay System using one reagent lot. The data were analyzed for weighted linear regression by comparing observed results to expected concentrations for each sample level. Deviation from linearity was calculated by determining the difference between observed and linear fit values. The results are summarized in the table below:

Dilution Range (ng/mL)	Slope	Intercept	R2	% Deviation from Linearity
0.39 – 20.38	0.93	0.009	0.99	< 15 IU/mL: -0.5 to 0.2 IU/mL ≥ 15 IU/mL: 1.8% to 7.0%
0.85 – 69.40	1.08	-0.05	0.98	< 15 IU/mL: -0.02 to 0.69 IU/mL ≥ 15 IU/mL: -7.7% to 6.5%
50.70 – 302.02	0.91	-0.92	0.97	-8.7% to 11.5%
211.24 – 850.53	1.26	-47.18	0.90	-16.8% to 17.4%*
459.43 – 1418.88	1.10	-24.97	0.96	-7.7% to 11.0%
961.35 – 2774.13	1.01	-43.97	0.93	-13.0% to 16.1% ^

* one sample with concentration of 607.1 IU/mL showed 16.8% of deviation from the linearity, and one sample with concentration of 728.2 IU/mL showed 17.4% of deviation from the linearity. The rest of the samples had %deviation from the linearity within ±10%.

^ one sample with concentration of 1807.8 IU/mL showed -13.0% of deviation from the linearity, and one sample with concentration of 2943.1 IU/mL showed 16.1% of deviation from the linearity. The rest of the samples had %deviation from the linearity within ±10%.

The data support the linearity of the claimed analytical measuring interval (AMI) of 1.5 IU/mL to 2,500 IU/mL for the modified Access Thyroglobulin Antibody II assay.

High Dose Hook Effect:

High dose hook effect of the modified Access Thyroglobulin Antibody II assay was evaluated by testing five serum samples with analyte concentration above the analytical measuring interval: 3,000, 15,000, 27,000, 39,000 and 51,000 IU/mL. Each sample was tested in replicates of five using three lots of reagents on one Access 2 Immunoassay System. The results showed no high dose hook effect up to an analyte concentration of 50,000 IU/mL.

3. Analytical Specificity/Interference:

Interference

Interference studies were performed in accordance with the CLSI guidelines EP07 3rd Ed and EP37.

Patient sera representing two clinically relevant concentrations (4 IU/mL and 100 IU/mL) of TgAb were spiked with endogenous and exogenous interfering substances including biotin. Paired spiked and unspiked (control) samples were assayed in six replicates with three

reagent pack lots of the modified Access Thyroglobulin Antibody II on one Access 2 Immunoassay System, and the mean values were used to evaluate the observed bias by comparing measurements of the spiked and control samples. No significant interference (≤ ±10% of difference) was demonstrated at the final test concentrations listed in the table below.

Interfering Substance	Test concentration
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	65 mg/dL
Bilirubin	40 mg/dL
Biotin	3510 ng/mL
Hemoglobin	500 mg/dL
Heparin (Sodium)	8000 U/dL
Total protein (human serum albumin)	6 g/dL
Ibuprofen	50 mg/dL
Multi-vitamin*	1:20
Triglycerides (intralipid)	500 mg/dL

* Includes Vitamin C, 60 mg; Vitamin D3, 10 mcg (400 IU); Vitamin E, 13.5 mg; Thiamin, 1.1 mg, Riboflavin, 1.7 mg; Niacin, 20 mg; Vitamin B_6, 2 mg; Vitamin B_{12}, 6 mcg; Biotin, 300 mcg; Pantothenic Acid, 10 mg; Iron, 9 mg; Iodine, 150 mcg; Zinc, 3 mg; Manganese, 2 mg; Chromium, 25 mcg; Molybdenum 25 mcg.

Cross-reactivity

The cross-reactivity of the modified Access Thyroglobulin Antibody II assay was evaluated with 10 serum samples from patients with rheumatoid arthritis and systemic lupus erythematosus and 20 serum samples tested positive for autoantibodies to cyclic citrullinated peptide, nuclear antigen, double stranded DNA, and rheumatoid factor. Each sample was tested in six replicates using three reagent pack lots on one Access 2 Immunoassay System. The results are summarized in the following table:

	N	N of Positive
Autoimmune diseases and conditions		
Rheumatoid arthritis (RA)	5	0
Systemic lupus erythematosus (SLE)	5	1*
Autoantibodies		
Anti-cyclic citrullinated peptide (anti-CCP)	5	1^
Anti-nuclear antigen (anti-ANA)	5	0
Anti-double stranded DNA (anti-dsDNA)	5	0
Rheumatoid factor (RF)	5	0

* SLE sample with TgAb concentration of 906 IU/mL

^ one positive for anti-CCP with TgAb concentration of 6.23 IU/mL

4. Assay Reportable Range:

The assay reportable range is 1.5 − 2500 IU/mL.

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

i) Traceability:

Refer to K112933. No change to the traceability of the modified Access Thyroglobulin Antibody II assay.

ii) Kit stability:

Shelf-life stability − A real-time stability study was performed in accordance with the CLSI guideline EP25 Ed2 to determine the shelf-life of the modified Access Thyroglobulin Antibody II kit stored at 2–10°C. One of the reagent pack lots was pre-stressed to environmental test conditions that simulated potential winter and summer transport. Five serum samples with TgAb concentrations spanning the AMI in five replicates was tested using three reagent pack lots on five Access 2 Immunoassay Systems at multiple time points throughout the claimed stability period and at least one month past the expiration date. Three quality controls were run in duplicate on each day to qualify each test run. Results support the 12-month shelf-life stability at 2–10°C for the modified Access Thyroglobulin Antibody II assay.

In-use reagent stability − In-use stability of the modified Access Thyroglobulin Antibody II assay was evaluated by testing samples using three lots of reagent packs which were opened and stored at 2–10°C. The commercial control samples and patient samples, representing low, medium, and high TgAb concentrations, were tested in duplicate at 10 time points over 58 days. The data supports the open vial stability of the modified Access Thyroglobulin Antibody II up to 56 days stored at 2–10°C after initial use.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted in accordance with the CLSI guideline EP17-A2. The studies evaluated two reagent pack lots of the modified Access Thyroglobulin Antibody II assay on two Access 2 Immunoassay Systems.

The LoB was determined with analyte-free samples including four serum, four lithium heparin (LiHep) plasma and four EDTA samples. Each blank sample was tested over three days with one run per day and five replicates per run to obtain a total of 60 replicates per sample type per reagent lot. The LoB was estimated as the 95th percentile of the measurements and determined to be 0 IU/mL for all sample types across two lots of reagent pack. The claimed LoB is 0 IU/mL.

The LoD were determined using 11 serum or LiHep plasma samples with low TgAb levels. Each sample was tested over five days with one run per day and nine replicates per run to obtain a total of 45 replicates per sample type per lot. The LoD was calculated as the LoB + 1.645 x SD of the replicates for the low-level samples and determined as 0.4 IU/mL for both serum and LiHep plasma. The claimed LoD is 0.4 IU/mL.

LoQ used the same samples and testing protocol as described for LoD study. The LoQ, defined as the mean TgAb value of the sample which fulfills the specification of ≤ 1.5 IU/mL

that corresponds to the TgAb concentration that can be quantitatively determined with ≤ 20% CV, was estimated as 1.5 IU/mL for serum, and 0.9 IU/mL for LiHep plasma. The claimed LoQ is 1.5 IU/mL which is the lower limit of the AMI for the modified Access Thyroglobulin Antibody II.

7. Assay Cut-Off:

   Refer to K112933

B Comparison Studies:

1. Method Comparison with Predicate Device:

   A method comparison study was performed in accordance with the CLSI guideline EP09c. The study evaluated a total of 123 native serum samples and native sample pools spanning the AMI of the modified Access Thyroglobulin Antibody II assay. Each sample was assayed in one replicate on a single Access 2 Immunoassay System using three lots of the candidate device and one lot of the predicate. Three commercial quality controls were run in duplicate on each day to qualify each test run. Results based on Passing-Bablok regression analysis are summarized in table below:

Lot	N	Concentration Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R
1	120	1.79 − 2216.25	0.99 (0.98 − 1.02)	-0.24 (-0.68 − 0.22)	0.99
2	123	1.79 − 2216.25	1.03 (1.00 − 1.06)	-0.13 (-0.68 − 0.30)	0.99
3	84	1.79 − 2216.25	1.01 (0.97 − 1.05)	-0.16 (-1.77 − 0.37)	0.99

2. Matrix Comparison:

   To demonstrate that lithium (Li)-Heparin and EDTA plasma samples yield results comparable with serum samples tested by the modified Access Thyroglobulin Antibody II assay, 38 matched samples with TgAb concentration across the claimed AMI of the assay were tested in singlicate with one reagent lot on one Access 2 Immunoassay System. Passing-Bablok regression analysis was performed, and the results are summarized in the following table:

	N	Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R
Li-Heparin Plasma vs Serum	38	1.5 − 2284.6	1.00 (0.99 − 1.06)	0.27 (-0.18 − 0.98)	0.99
EDTA Plasma vs Serum	38	1.6 − 2181.9	0.95 (0.91 − 0.99)	0.54 (-0.06 − 1.41)	0.99

C Clinical Studies:

1. Clinical Sensitivity:

   Please refer to K112933

2. Clinical Specificity:

   Please refer to K112933

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

   Not applicable

D Clinical Cut-Off:

Please refer to K112933

E Expected Values/Reference Range:

The reference range for unmodified Access Thyroglobulin Antibody assay (predicate) was previously established based on a study in which 137 samples from males < 30 years of age were screened for their serum TSH, and absence of personal or family history of thyroid disease and non-thyroid autoimmune disease. The 95% non-parametric upper reference limit was determined to be < 4 IU/mL. Additionally, 519 normal samples collected in the U.S. from both males and females ranging in age from 18–74 years old were tested. The results indicated that 96% of these samples < 4 IU/mL.

To verify this reference range, a total of 30 serum samples collected from apparently healthy donors were tested with modified Access Thyroglobulin Antibody on Access 2 Immunoassay System. The results showed that 28 of 30 (93.3%) have value < 4 IU/mL with the established 95% non-parametric upper reference limit was < 4 IU/mL. Similar results were also observed for the predicate using this sample cohort.

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.