510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K220972

B Applicant

Beckman Coulter, Inc

C Proprietary and Established Names

Access Thyroglobulin

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MSW	Class II	21 CFR 866.6010 - Tumor-Associated Antigen 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:

Thyroglobulin

C Type of Test:

Quantitative, Chemiluminescent Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Access Thyroglobulin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin levels in human serum using the Access Immunoassay Systems. This device is intended to aid in monitoring for the presence of persistent or recurrent/metastatic disease in patients who have differentiated thyroid cancer (DTC) and have had thyroid surgery (with or without ablative therapy), and who lack serum thyroglobulin antibodies.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For In Vitro Diagnostic Use Only

The Instructions for Use of the device contains the following warning statement:

"The presence of serum autoantibodies to thyroglobulin (TgAb) can interfere with assays for thyroglobulin (Tg). Therefore, sera which contain TgAb, even at very low levels, should not be tested for Tg.

The concentration of thyroglobulin in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Tg assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining Tg levels serially is changed, additional sequential testing should be carried out to confirm baseline values."

D Special Instrument Requirements:

Access Immunoassay Systems (Access Immunoassay System and Access 2 Immunoassay System)

IV Device/System Characteristics:

A Device Description:

Access Thyroglobulin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin levels in human serum using the Access Immunoassay Systems.

Materials included in the Access Thyroglobulin assay

Reagent Pack (2 packs, 50 tests/pack) contains:

- R1a (3.25 mL): Dynabeads paramagnetic particles coated with streptavidin and coupled to biotinylated mouse monoclonal antithyroglobulin antibodies in TRIS buffer with protein (bovine) and preservatives
- R1b (3.10 mL): Mouse monoclonal anti-thyroglobulin-alkaline phosphatase (bovine) conjugate in a TRIS buffer with protein (bovine, murine) and preservatives
- R1c (3.10 mL): HEPES buffer with protein (bovine, murine) and preservatives

Materials needed but not supplied

- Access Thyroglobulin Calibrators: Six levels – 0, 1.0, 10, 100, 250, and 500 ng/mL
- Quality control materials: commercial control material
- Access Thyroglobulin Sample Diluent
- Access Substrate
- Access Wash Buffer II / Unicel DxI Wash Buffer II

To mitigate the risk of biotin interference, the Access Thyroglobulin Reagent Pack has been modified from the previously cleared assay by pre-coupling the biotinylated mouse monoclonal anti-thyroglobulin antibodies to paramagnetic particles coated with streptavidin.

B Principle of Operation:

The Access Thyroglobulin assay is a simultaneous one-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with four biotinylated anti-thyroglobulin antibodies coated on streptavidin paramagnetic particles, and monoclonal anti-thyroglobulin antibody alkaline phosphatase conjugate. The thyroglobulin in the sample binds to the biotinylated antibodies on the solid phase, while the conjugate antibody reacts with a different antigenic site on the thyroglobulin molecule. After incubation in a reaction vessel, 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 thyroglobulin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Access Thyroglobulin Reagents on the Access Immunoassay Systems

B Predicate 510(k) Number(s):

K002905

C Comparison with Predicate(s):

Device & Predicate Device(s):	K220972	K002905
Device Trade Name	Access Thyroglobulin	Access Thyroglobulin
General Device Characteristic Similarities		
Intended Use/ Indications for Use	Access Thyroglobulin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin levels in human serum using the Access Immunoassay Systems. This device is intended to aid in monitoring for the presence of persistent or recurrent/metastatic disease in patients who have differentiated thyroid cancer (DTC) and have had thyroid surgery (with or without ablative therapy), and who lack serum thyroglobulin antibodies.	The Access Thyroglobulin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin levels in human serum and plasma using the Access Immunoassay Systems. This device is intended to aid in monitoring for the presence of local and metastatic thyroid tissue in patients who have had thyroid gland ablation (using thyroid surgery with or without radioactivity) and who lack serum thyroglobulin antibodies.
Technology	Chemiluminescent immunoassay	Same
Analyte	Thyroglobulin	Same
Antibodies	Mouse monoclonal antibodies	Same
Method	Automated	Same
Sample Volume	40 µL	Same
Assay Throughput	~42 minutes	Same
Measuring Range	0.1 – 500 ng/mL	Same
General Device Characteristic Differences		
Sample Matrix	Serum	Serum and Plasma
Assay Architecture	Biotinylated mouse monoclonal anti-thyroglobulin antibodies pre-coupled to paramagnetic particles coated with streptavidin.	No biotinylated mouse monoclonal anti-thyroglobulin antibodies pre-coupled to paramagnetic particles coated with streptavidin.
Biotin Interference	3,510 ng/mL	10 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 Sample
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-laboratory imprecision

The 20-day precision study was performed for the modified Access Thyroglobulin. The native serum samples covering the analytical measuring range of the assay were tested in duplicate per run, two runs per day over 20 days, resulting a total of 80 replicates per sample per reagent lot. Mean, standard deviation (SD) and % coefficient of variation (%CV) were analyzed for each sample. The results are summarized in the table below:

Mean (ng/mL)	N	Within-Run		Between-Run		Between-Day		Within Laboratory	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV
0.11	80	0.01	8.4	0.01	4.7	0.00	0.0	0.01	9.6
0.17	80	0.01	6.2	0.02	14.0	0.01	4.3	0.03	15.9
4.5*	80	0.09	2.1	0.06	1.3	0.05	1.0	0.12	2.6
21*	80	0.4	1.9	0.0	0.0	0.2	0.9	0.5	2.2
133	80	2.2	1.6	1.7	1.2	0.0	0.0	2.7	2.1
431	80	7.2	1.7	8.5	2.0	18.4	4.3	21.5	5.0

* The sample was evaluated for three lots with N=240 datapoints, data from one representative lot was presented.

Lot-to-lot imprecision

The between-lot imprecision of the modified Access Thyroglobulin assay was evaluated by testing samples in two replicates per run, two runs per day, for 20 days, for a total of 80 measurements for each reagent lot. The %CV for the lot-to-lot variation is <5.0% in this study.

Instrument-to-instrument precision

The instrument-to-instrument precision using the modified Access Thyroglobulin was evaluated. The results are summarized in the table below:

Mean (ng/mL)	N	Within-Run		Between-Day		Between-Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
0.48*	75	0.01	2.9	0.01	2.2	0.01	2.0	0.02	4.2
4.3^	90	0.07	1.7	0.04	1.0	0.10	2.3	0.15	3.6
20^	90	0.27	1.3	0.00	0.0	0.57	2.8	0.74	3.6
136*	75	2.44	1.8	1.43	1.1	3.01	2.2	4.13	3.0
446*	75	10.74	2.4	8.15	1.8	0.00	0.0	13.48	3.0

* Sample was tested in three replicates per run, two runs per day for five days on each of three instruments

^ Sample was tested in five replicates per run, one run per day for five days on each of three instruments

2. Linearity:

A linearity study was performed for the modified Access Thyroglobulin assay according to CLSI EP06-Ed2. Two series were prepared: the first series included seven dilution samples prepared by mixing a single native serum sample with thyroglobulin concentration at 0.06 ng/mL with a native serum sample pool with thyroglobulin concentration above 500 ng/mL. The second series included seven dilution samples prepared by mixing a native serum sample with thyroglobulin concentration at 0.05 ng/mL with the serum sample with thyroglobulin concentration at 76.77 ng/mL. For both dilution 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 sets from the two series were combined for a single linearity analysis. For each level, the mean value of the measured values, predicted value and the deviation from linearity were calculated. Predicted values were calculated using a best fitted straight line by a weighted least squares linear regression analysis. Percent deviations from linearity were calculated as differences between the observed values (mean values) and the predicted values divided by the predicted values. The results are summarized in the tables below.

Dilution Range (ng/mL)	Slope	Intercept	R2	Deviation from Linearity (%)
0.05 - 563.72	1.04	-0.002	0.999	-4.1 – 8.0 %

The data support the linearity interval from 0.05 to 563.72 ng/mL with the deviations from linearity within ±10%. The study results support the linearity of the claimed analytical measuring interval (AMI): 0.1 – 500 ng/mL.

Dilution Recovery

Verification studies were performed to determine the sample manual dilution recovery of the modified Access Thyroglobulin assay on the Access 2 Immunoassay system. For "over range" manual sample dilution, three serum samples with thyroglobulin concentration at 1202.05, 2003.56, and 2998.53 ng/mL were diluted 1:5 (1 part sample and 4 parts diluent) or 1:10 (1 part sample and 9 parts diluent) in Access Thyroglobulin Sample Diluent according to the Instructions for Use of the assay. Each dilution preparation was measured for a total of

eight datapoints on each of three reagent pack lots on one Access 2 Immunoassay System. The percent of recovery was calculated by comparing the observed value to the expected value for each sample. The results support manual sample dilution suggested for the Access Thyroglobulin assay.

High Dose Hook Effect:

High dose hook effect of the modified Access Thyroglobulin assay was evaluated by testing five samples with analyte concentration above the analytical measuring interval: 600, 10450, 20300, 30150 and 41000 ng/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 40,000 ng/mL.

3. Analytical Specificity/Interference:

Potential interfering and cross-reacting substances were evaluated for their ability to cross react or interfere with the performance of the modified Access Thyroglobulin. The studies were performed following the CLSI EP07-Ed3.

Interference:

Each of potential (endogenous and exogenous) interfering substances was tested at two thyroglobulin concentrations: 25 ng/mL and 100 ng/mL. Test serum samples were prepared by spiking the potential interfering substances at one concentration. Results were compared to matched control samples which were prepared by spiking an equal volume of solvent (blank) where appropriate. The test samples and control samples were measured in eight replicates using three reagent lots on the Access 2 Immunoassay System. The recovery was calculated by comparing measurements of the test and control samples. No interference (≤±10% difference of test from control) for all three lots of the modified Access Thyroglobulin reagents up to the concentrations of the potential interfering substances tested as shown in the table below:

Interfering Substance	Concentration
Biotin	3510 ng/mL
Bilirubin	10 mg/dL
Triolein	1800 mg/dL Triglyceride
Hemoglobin	1000 mg/dL
Protein	5 g/dL
Aspirin	50 mg/dL
Acetaminophen	20 mg/dL
Ibuprofen	40 mg/dL
Thyroxine (T4)	218.5 µg/dL
Cabozantinib-S-Malate	15.3 mg/dL
Lenvatinib Mesylate	2.62 mg/dL

Cross reactivity:

Each potential cross-reacting substance was tested in serum samples at two thyroglobulin concentrations: 20 ng/mL and 100 ng/mL. Test samples were prepared by spiking with the potential cross-reacting substances at one concentration. Results were compared to matched control samples which were spiked with an equal volume of solvent (blank) where appropriate. The test samples and control samples were measured in eight replicates using each of three modified reagent lots on the Access 2 Immunoassay System. The recovery was calculated by comparing measurements of the test and control samples. No cross reactivity (≤±10% difference of test from control) for all three lots of the modified Access Thyroglobulin reagents up to the concentrations of the potential cross-reacting substances tested as shown in the table below:

Cross-Reacting Substance	Concentration
3,3',5-Triiodo-L-thyronine (T3)	100 ng/mL
L-Thyroxine (T4)	10 µg/dL
Thyroxine Binding Globulin (TBG)	50 µg/mL
Thyroid Stimulating Hormone (TSH)	235 mIU/mL)

Blocking Effect of HAMA/Heterophilic Ab:

Eighty heterophilic serum samples were tested in replicates of six to evaluate blocker effectiveness in the modified Access Thyroglobulin assay using three reagent lots (27 samples tested with Lot 1, 27 samples tested with Lot 2, and 26 samples tested with Lot 3) on one Access 2 Immunoassay System. Out of 80 samples, HAMA interference was observed in a total of 19 samples.

The following cautionary note of interference has been included in the "Limitations" section of the product insert: "Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g., HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies."

4. Assay Reportable Range:

The assay reportable range for the modified Access Thyroglobulin is the same as the claimed analytical measuring interval (AMI): 0.1–500 ng/mL.

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

Traceability:

The calibration traceability of the modified Access Thyroglobulin assay has not changed. Refer to K002905.

Stability:

The stability of the modified Access Thyroglobulin assay is evaluated according to the recommendation of CLSI EP25-A.

Shelf-life (unopened): Real-time stability and accelerated stability studies were performed to verify the shelf-life of the modified Access Thyroglobulin assay. For the real-time stability study, three lots of reagents were subjected to simulated summer shipping and winter shipping conditions and then stored at the recommended storage condition, 2–10ºC as initial timepoint (T0). Five native patient serum samples within the AMR were tested with a minimum of two replicates at T0 and at each test time point. The data support a shelf-life of the modified Access Thyroglobulin assay for 365 days (12 months). The real-time stability study is on-going.

An accelerated stability study was carried out using one modified Access Thyroglobulin stored under elevated temperature storage conditions: 32°C for 49 days and 37°C for 20 days. Five patient serum samples, within the range of the assay, were assayed using three replicates at each time point of six approximately equally spaced time points. The accelerated stability study supports a claim for a 25-month shelf-life for the modified Access Thyroglobulin at 2–10ºC.

Open in-use stability: The modified Access Thyroglobulin was evaluated for in-use stability. Three lots of reagent packs were opened and stored at 10°C. Each lot was tested using commercial control samples and patient samples, representing low, medium, and high thyroglobulin concentrations in duplicate at the following time-points 0, 2, 5, 8, 13, 16, 21, 23, 28, 30 days. Fresh reagent packs were tested at each time point as reference. The data supports the open vial stability of the modified Access Thyroglobulin up to 28 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) for the modified Access Thyroglobulin assay were verified based on the CLSI EP17-A2.

The LoB was determined by testing four distinct serum samples containing no measurable Thyroglobulin using two regent lots. For each Lot, LoB samples were run in five replicates per run, one run per day, for three days on two Access 2 Immunoassay Systems. LoB was defined as the value corresponding to the 95th percentile of the rank position of the distribution of values. The claimed LoB is 0.02 ng/mL which is the highest LoB value observed across two reagent lots.

The LoD was determined by testing eight native serum specimens using two reagent lots. For each Lot, LoD samples were run in nine replicates per run, one run per day, for five days on two Access 2 Immunoassay Systems. The LoD was determined by fitting the precision profile model between within-lab standard deviation (SD) and concentration. The SD was multiplied by the 95th percentile of the standard normal distribution and added to the LoB to

calculate the LoD per CLSI EP17-A2. The claimed LoD is 0.05 ng/mL which is the highest LoD value observed across lots.

The LoQ was determined by testing the same samples used for the LoD study using two reagent lots on two Access 2 Immunoassay Systems over five days. The LoQ was defined as the lowest value with within-lab precision ≤ 20% CV. The claimed LoQ is 0.05 ng/mL.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison study was conducted by testing 102 serum samples using three reagent lots of the modified Access Thyroglobulin assay (candidate device) and one lot of the unmodified Access Thyroglobulin assay (predicate device) on one Access 2 Immunoassay System at one site. Passing-Bablok regression analysis and Pearson's correlation were performed, and the results are summarized in the following table:

Lot#	N	Range of Observations (ng/mL)	Intercept [95% CI]	Slope [95% CI]	Correlation Coefficient (r)
1	102	0.17 – 407.67	-0.013 [-0.03 to 0.01]	0.99 [0.98 to 1.00]	1.00
2	102	0.17 – 407.67	0.0004 [-0.02 to 0.02]	0.97 [0.95 to 0.98]	1.00
3	102	0.17 – 407.67	-0.02 [-0.03 – 0.00]	0.96 [0.96 – 0.97]	1.00

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Refer to K002905

2. Clinical Specificity:

Refer to K002905

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

Not applicable

D Clinical Cut-Off:

Refer to K002905

E Expected Values/Reference Range:

The reference range for unmodified Access Thyroglobulin assay (predicate) was previously established based on a study of testing 152 apparently healthy adults (females and males combined). The results ranged from 1.15 to 130.77 ng/mL, with a median of 9.08 ng/mL, and 2.5th and 97.5th percentiles of 1.59 and 50.03 ng/mL.

To verify this reference range, 28 serum samples from apparently healthy donors were each tested on three lots of modified Access Thyroglobulin assay (candidate device) and one lot of the predicate device. The results showed thyroglobulin levels ranging from 4.14 to 130.46 ng/mL with 97.5th percentiles of 46.44 ng/mL, 44.76 ng/mL, and 46.36 ng/mL for three lots, respectively. Similar results were also observed for the predicate.

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.