510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K222251

B Applicant

B.R.A.H.M.S GmbH, part of Thermo Fisher Scientific

C Proprietary and Established Names

B·R·A·H·M·S CgA II KRYPTOR
B·R·A·H·M·S CgA II KRYPTOR CAL
B·R·A·H·M·S CgA II KRYPTOR QC

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QXS	Class II	21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Chromogranin A (CgA)

C Type of Test:

Quantitative, Automated immunofluorescent assay using Time-Resolved Amplified Cryptate Emission (TRACE) technology

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

B·R·A·H·M·S CgA II KRYPTOR is an automated immunofluorescent assay using Time-Resolved Amplified Cryptate Emission (TRACE) technology for quantitative determination of Chromogranin A concentration in human serum.

B·R·A·H·M·S CgA II KRYPTOR is to be used in conjunction with other clinical methods as an aid in monitoring of disease progression during the course of disease and treatment in patients with gastroentero-pancreatic neuroendocrine tumors (GEP-NETs, grade 1 and grade 2).

C Special Conditions for Use Statement(s):

Rx – For Prescription Use Only

The Instruction for use of the device contains the following warnings:

- B·R·A·H·M·S CgA II KRYPTOR should not be used for cancer screening or cancer diagnosis.
- B·R·A·H·M·S CgA II KRYPTOR is not indicated to be used as a stand-alone monitoring assay and should be used in conjunction with clinical signs and symptoms and other diagnostic evidence. In cases where the laboratory results do not agree with the clinical picture or history, additional tests should be performed.
- The results reported by the laboratory to the physician must include the identity of the Chromogranin A 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 Chromogranin A levels is changed, additional tests should be carried out to determine the baseline values.
- High levels of Chromogranin A (CgA) could also be found in cases of benign diseases (such as gastro-intestinal disorders, kidney failure and cardiovascular disorders) and in cancers other than NETs (such as adenocarcinoma of the breast, lung, or colon). CgA values may rise during treatment with proton pump inhibitors.

D Special Instrument Requirements:

B·R·A·H·M·S KRYPTOR compact PLUS analyzer (DEN150009, K171338)

IV Device/System Characteristics:

A Device Description:

The B·R·A·H·M·S CgA II KRYPTOR assay contains sufficient reagents for 50 determinations. Reagents are stored at 2 to 8°C in their original shipping containers until use.

Materials Provided:

- Cryptate Conjugate: One vial lyophilized, contains anti-CgA monoclonal antibody conjugated with cryptate, buffer, bovine albumin, bovine immunoglobulins, murine immunoglobulins, trehalose, mannitol. Volume of 4.1 mL after automated reconstitution with B·R·A·H·M·S KRYPTOR compact Solution 1 and 2 within the instrument.
- XL Conjugate: One vial, anti-CgA monoclonal antibody conjugated with Alexa Fluor 647, buffer, bovine albumin, bovine immunoglobulins, murine immunoglobulins, potassium fluoride.
- Diluent: One vial, Human serum, preservative, EDTA

Materials Required but Provided Separately:

- B·R·A·H·M·S CgA II KRYPTOR Calibrator: Lyophilized recombinant CgA in horse serum, reconstitute with 0.65 mL de-ionized water with conductivity of less than 50 µS/cm [range: 990 - 1,210 ng/mL]
- B·R·A·H·M·S CgA II KRYPTOR QC Controls:
  - Control 1: Lyophilized recombinant CgA in horse serum, reconstitute with 2 mL de-ionized water with conductivity of less than 50 µS/cm (range: 64 - 96 ng/mL)
  - Control 2: Lyophilized recombinant CgA in horse serum, reconstitute with 2 mL de-ionized water with conductivity of less than 50 µS/cm (range: 400 - 600 ng/mL)
- B·R·A·H·M·S KRYPTOR Analyzer Consumables:
  - Reaction plates B·R·A·H·M·S KRYPTOR compact REACT: 60 reaction plates, containing 96 wells each. Additionally, 60 adhesive cover films are included.
  - Dilution plates B·R·A·H·M·S KRYPTOR compact DILCUP: 30 dilution plates, containing 24 wells each. Additionally, 30 adhesive cover films are included.
  - B·R·A·H·M·S KRYPTOR compact Solution 1: ProClin 150 solution
  - B·R·A·H·M·S KRYPTOR compact Solution 2: Potassium fluoride solution
  - B·R·A·H·M·S KRYPTOR compact Solution 3: Active chlorine and sodium hydroxide solution
  - B·R·A·H·M·S KRYPTOR compact Solution 4: Sodium hydroxide solution
  - KRYPTOR BUFFER: Phosphate Buffer Saline (PBS) buffer, not reconstituted, five liters after reconstitution

B Principle of Operation:

B·R·A·H·M·S CgA II KRYPTOR is a homogenous one-step immunoassay for the quantification of CgA II in human serum using TRACE technology.

A sample volume of 14 µL is needed for each test. The sample probe of the analyzer pipettes and dispenses the conjugates from the reagent kit and the sample into the wells. The probe is heated to incubate the reagent-sample mixture, so it is at reaction temperature (37°C) prior to dispensing and mixing in the reaction well. Incubation lasts for 29 minutes. During this time, a complex comprised of a Chromogranin A (CgA) analyte "sandwiched" between two monoclonal mouse anti-CgA antibodies is formed. One of the antibodies (537/H2) is directed at the epitope AA124–144 and labelled with DiSMP cryptate, the other antibody (541/E2) binds to AA280-301 and is labelled with Alexa Fluor 647. The signal generated by the cryptate at 620 nm serves as an internal reference and is measured simultaneously with the long-lived acceptor signal at 665 nm

which is the specific signal. The fluorescent signal is proportional to the concentration of the analyte to be measured. The linear direct measuring range of the assay is from 20−3,000 ng/mL, going up to 1,000,000 ng/mL with automated dilution.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Abbott Architect CEA

B Predicate 510(k) Number(s):

K990774

C Comparison with Predicate(s):

Device & Predicate	K222251	K990774
Device Trade Name	B·R·A·H·M·S CgA II KRYPTOR	ARCHITECT CEA
General Device Characteristic Similarities		
Intended Use/ Indications For Use	B·R·A·H·M·S CgA II KRYPTOR is an automated immunofluorescent assay using Time-Resolved Amplified Cryptate Emission (TRACE) technology for quantitative determination of Chromogranin A concentration in human serum. B·R·A·H·M·S CgA II KRYPTOR is to be used in conjunction with other clinical methods as an aid in monitoring of disease progression during the course of disease and treatment in patients with gastroentero-pancreatic neuroendocrine tumors (GEP-NETs, grade 1 and grade 2).	The ARCHITECT CEA assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of Carcinoembryonic Antigen (CEA) in human serum and plasma. The ARCHITECT CEA assay is to be used as an aid in the prognosis and management of cancer patients in whom changing concentrations of CEA are observed.
Classification	Class II	Same
Regulation	21 CFR § 866.6010 Tumor-associated antigen immunological test system	Same
Measurement	Quantitative	Same
Traceability	Traceable to in-house reference	Same
Units	ng/mL	Same
General Device Characteristic Differences		
Analyte	Chromogranin A	Carcinoembryonic Antigen
Sample Type	Serum	Serum and plasma

Principles of operation	Automated fluorescent immunoassay using TRACE (Time-resolved amplified cryptate emission) technology.	Automated chemiluminescent microparticle immunoassay (CMIA)
Instrument	B·R·A·H·M·S KRYPTOR compact PLUS analyzer	ARCHITECT analyzer system
Reportable Range	20–3,000 ng/mL, up to 1,000,000 ng/mL with automated dilution	0–500 ng/mL, extended to 1500 ng/mL

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) and International Organization for Standardization (ISO) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP06, 2nd Edition, Evaluation of Linearity of Quantitative Measurement Procedures
- CLSI EP07, 3rd Edition, Interference Testing in Clinical Chemistry
- CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition
- 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 EP34, 1st Edition, Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking
- CLSI EP37, 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry
- ISO 14971: 2019, Medical devices, Application of risk management to medical devices – Third edition
- ISO 15223-1: 2016 Medical Devices – Symbols to be used with Medical Device Labels, Labelling and Information to be Supplied Part 1: General Requirements
- ISO 17511: 2020, In vitro diagnostic medical devices, Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples – Second edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

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

1. Precision/Reproducibility:

Precision testing was performed in accordance with CLSI guideline EP05-A3.

a) Within-Laboratory Precision:

The studies were performed at a single site on B·R·A·H·M·S KRYPTOR compact PLUS analyzer. Twelve levels of native human serum sample were run in two replicates per run, two runs daily over the course of 20 days (2 x 2 x 20, n=80 for each sample). The data were analyzed for repeatability (within-run), between-run, between-day, and within-laboratory precision. The mean ng/mL and percent coefficient of variation (%CV) are summarized in table below.

Sample	Mean (ng/mL)	N	Within-Run (Repeatability)		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	23.0	80	1.2	5.2%	0.6	2.5%	1.9	8.2%	2.3	10.0%
2	26.0	80	1.3	5.1%	0.8	3.1%	2.1	7.9%	2.6	9.9%
3	34.6	80	1.3	3.7%	0.6	1.8%	2.7	7.8%	3.1	8.8%
4	56.3	80	1.6	2.8%	1.4	2.4%	4.3	7.6%	4.8	8.4%
5	86.3	80	1.5	1.7%	3.0	3.5%	6.4	7.4%	7.2	8.3%
6	132	80	1.7	1.3%	3.4	2.6%	8.5	6.4%	9.3	7.1%
7	228	80	3.8	1.7%	5.2	2.3%	14.4	6.3%	15.7	6.9%
8	463	80	5.2	1.1%	10.4	2.2%	16.7	3.6%	20.4	4.4%
9	744	80	12.0	1.6%	11.5	1.6%	12.9	1.7%	21.1	2.8%
10	1177	80	13.2	1.1%	29.5	2.5%	33.6	2.9%	46.6	4.0%
11	1825	80	24.5	1.3%	38.8	2.1%	71.9	3.9%	85.3	4.7%
12	2687	80	42.7	1.6%	72.1	2.7%	181.0	6.7%	199.0	7.4%

b) Lot-to-Lot Precision:

The study was performed at a single site using three different lots of B·R·A·H·M·S CgA II KRYPTOR reagents on one instrument. Ten levels of native human serum sample were run in five replicates per run, one run per day for five days (3 x 5 x 1 x 5, n=75 for each sample). The data were analyzed for the repeatability (within-run), between-lot and total precision. The results are summarized in table below.

Sample	Mean (ng/mL)	N	Within-Run (Repeatability)		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV
1	26.3	75	1.7	6.3%	0.3	1.2%	1.7	6.4%
2	48.9	75	1.5	3.1%	0.0	0.0%	2.2	4.4%
3	96.8	75	2.2	2.3%	0.1	0.1%	2.7	2.8%
4	513	75	8.3	1.6%	8.3	1.6%	13.0	2.5%
5	757	75	13.6	1.8%	14.0	1.9%	21.3	2.8%
6	963	75	13.0	1.4%	23.6	2.4%	29.8	3.1%
7	1,441	75	26.3	1.8%	39.1	2.7%	53.2	3.7%
8	2,030	75	41.3	2.0%	88.9	4.4%	107	5.3%
9	2,540	75	48.2	1.9%	19.2	0.8%	68.2	2.7%
10	2,895	75	68.5	2.4%	0.0	0.0%	94.6	3.3%

c) *Site-to-Site Reproducibility:*

The study was performed at three external sites using a single lot of B·R·A·H·M·S CgA II KRYPTOR reagents. Five levels of native serum sample were run in three replicates per run, two runs per day over the course of five days (3 x 3 x 2 x 5, n=90 for each sample). The combined results with the factors of site and run used to calculate the repeatability (within-run), between-run, between-day, between-site and reproducibility are summarized in table below:

Sample	N	Mean (ng/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	90	25.7	1.7	6.5%	0.0	0.0%	0.6	2.2%	1.5	5.8%	2.3	9.0%
2	90	53.6	1.9	3.6%	1.5	2.8%	2.9	5.5%	0.0	0.0%	3.8	7.1%
3	90	104	2.1	2.0%	1.5	1.5%	2.0	2.0%	2.9	2.8%	4.4	4.2%
4	90	515	9.5	1.8%	5.9	1.1%	9.6	1.9%	12.	2.5%	19.4	3.8%
5	90	1,089	16.7	1.5%	23.9	2.2%	41.0	3.8%	38.	3.6%	63.6	5.8%
6	90	1,723	29.2	1.7%	63.4	3.7%	61.8	3.6%	0.0	0.0%	93.2	5.4%
7	90	2,923	62.3	2.1%	66.8	2.3%	64.5	2.2%	178	6.1%	210.0	7.2%

2. Linearity:

a) *Linearity studies:*

Linearity studies were conducted in accordance with CLSI guideline EP06, 2nd Edition. Three series of samples were prepared by mixing three High samples with Low sample to span the analytical measuring interval of the B·R·A·H·M·S CgA II KRYPTOR. Low sample used two individual normal human serum samples. Three High samples were pooled human serum with CgA concentrations of 3,401 ng/mL, 301 ng/mL and 52.3 ng/mL. Samples in three series were measured in five replicates and the 'measured value' (mean CgA ng/mL) was compared to its predicted value for deviation percentage using a weighted least squares regression analysis. This deviation was then compared to the allowable deviation from linearity (ADL). The results are summarized in table below.

Sample	Range (ng/mL)	Slope (95%CI)	Intercept (95%CI)	R^2	% Bias**
1	296–3401	1.02 (1.01; 1.03)	-66.11 (-73.07; -59.15)	0.998	-2.6% − 4.9%
2	39–301	1.08 (1.07; 1.10)	-10.96 (-12.77; -9.15)	0.997	-4.2% − 7.8%
3	15–52	1.04 (1.01; 1.06)	-2.05 (-2.85; -1.24)	0.994	-6.8% − 3.3%
1-2-3*	15–3401	0.98 (0.96; 0.99)	-0.45 (-1.41; 0.51)	0.992	-14.1% − 12.7%

*Combined from Sample 1, 2 and 3

**Minimum and maximum deviation from linearity

The results support the linearity of the claimed B·R·A·H·M·S CgA II KRYPTOR 'analytical measuring interval' (AMI) of 20 to 3,000 ng/mL.

b) *Dilutional Recovery Studies:*

Dilutional recovery studies were conducted using 10 pools of human serum samples with different CgA concentrations. The samples were diluted on the instrument with the diluent of the assay, with the following user specified dilution factors 1:2, 1:3, 1:5, 1:10, 1:20 and 1:25 (for samples with concentration below 1,000 ng/mL) and 1:2, 1:3, 1:5,

1:10, 1:20, 1:25 and 1:50 (for samples with concentration above 1,000 ng/mL). The samples were measured by two operators over six runs, in duplicates with three lots of reagents on three instruments. The sample testing was divided between the reagent lots and instruments (all samples were not tested by all lots and instruments). The mean recoveries were between 97.6 % and 109.6 %. The results show that the dilutions performed by the instrument are within specifications.

c) *Spiking Recovery Studies:*

Spiking recovery studies were conducted with 13 individual human serum samples with different CgA concentrations ranging from 43.1 ng/mL to 2,875 ng/mL, covering the AMI of the B·R·A·H·M·S CgA II KRYPTOR. Seven samples (Group A) were spiked (1:1) with a sample at 88.8 ng/mL prepared with recombinant CgA antigen diluted in normal human serum and six samples (Group B) were spiked (1:1) with a sample at 938 ng/mL prepared with recombinant CgA antigen diluted in normal human serum. The samples were measured in duplicate by one operator in a single run on one instrument with one lot of B·R·A·H·M·S CgA II KRYPTOR, before and after spiking and the recoveries between the 'measured values' (mean CgA ng/mL) and the expected concentrations were calculated for the 13 spiked samples. The measured CgA ng/mL in spiked samples and expected CgA ng/mL calculated for each sample and the % recovery of measured CgA ng/mL is presented in the table below.

Sample	Original Sample (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	%Recovery
A1	43.1	65.7	67.4	103%
A2	220	154	145	94%
A3	962	525	570	109%
A4	1,244	666	622	93%
A5	1,981	1,035	994	96%
A6	2,515	1,302	1,252	96%
A7	2,875	1,482	1,352	91%
B1	77.0	508	495	97%
B2	474	706	714	101%
B3	1,097	1,018	1,040	102%
B4	1,789	1,364	1,396	102%
B5	1,994	1,466	1,450	99%
B6	2,594	1,766	1,727	98%

The % recovery ranged from 91% to 109%, supporting the spike recovery of the B·R·A·H·M·S CgA II KRYPTOR across the claimed AMI (20 to 3,000 ng/mL).

d) *High Dose Hook Effect:*

Hook effect of the B·R·A·H·M·S CgA II KRYPTOR was determined by spiking recombinant human CgA in normal human serum. Twenty-nine dilutions with CgA concentrations ranging from 200,000 ng/mL down to 12 ng/mL were measured in duplicate with one lot of reagents on one instrument. The measurement of samples above 3,000 ng/mL was done without the auto-dilution process. No high dose hook effect is expected with the B·R·A·H·M·S CgA II KRYPTOR assay for CgA concentrations up to 23,000 ng/mL.

Note: High concentration samples (> 3,000 ng/mL) are detected in the first few seconds of incubation and may be diluted by the appropriate dilution factor, then re-assayed automatically. Potential Hook Effect is detected by kinetics analysis of the samples by B·R·A·H·M·S KRYPTOR analyzer family.

e) Extended Measuring Interval:

Extended measuring range study was conducted in accordance with CLSI guideline EP34, 1st Edition. A total of 10 individual human serum samples with concentrations between 25,000 ng/mL and 50,000 ng/mL were diluted with dilution factor at 1:25. Samples with concentrations between 75,000 ng/mL and 1,000,000 ng/mL were diluted with dilution factor at 1:500. Manual dilutions and dilutions performed by the B·R·A·H·M·S KRYPTOR compact PLUS instrument were done with one B·R·A·H·M·S CgA II KRYPTOR reagent lot. The measurements were done by one operator with three runs on two instruments. Recovery results for dilution factor at 1:25 for samples with concentrations between 25,280 ng/mL up to 47,137 ng/mL are between 94% and 104%. Recovery results for dilution factor at 1:500 for samples with concentrations between 75,051 ng/mL up to 1,067,721 ng/mL are between 96% and 102%. These results showed that the dilution at 1:25 and 1:500 performed by the B·R·A·H·M·S KRYPTOR compact PLUS instrument are consistent with manual dilutions.

3. Analytical Specificity/Interference:

Interference study was performed according to CLSI guidelines EP07 (3rd Edition) and EP37 (1st Edition) guidelines to determine the effect of various endogenous and exogenous substances on the B·R·A·H·M·S CgA II KRYPTOR assay. Two or three native human serum pools with a target concentration of 93.7, 619, 86.3, 132 and 1,177 ng/mL were split between three lots of B·R·A·H·M·S CgA II KRYPTOR reagents, two lots of B·R·A·H·M·S CgA II KRYPTOR CAL and QC. Testing was conducted by four operators using four instruments. The samples were supplemented with potentially interfering or cross-reactive compounds and measured in three replicates. The percent bias was determined by comparing the result of sample with interferent or cross-reactant to a control sample without the interferent or cross-reactant. An interference or cross-reactivity within ± 10% bias between the mean spiked sample value and the mean control value was considered non-significant.

a) Endogenous Substance Interference:

The following endogenous substances were tested using B·R·A·H·M·S CgA II KRYPTOR assay. No significant interference was found for each substance at the concentrations listed below.

Endogenous Substance	Concentration
Albumin	50 g/L
Bilirubin (Unconjugated)	500 mg/L
Hemoglobin	10 g/L
Triglycerides	5 g/L
HAMAs	300 µg/L
Rheumatoid factors	1,000 kIU/L

b) *Exogenous Substance Interference:*

The potential interference of 29 commonly used drugs (including those used for cancer treatment) and dietary supplements, was evaluated using B·R·A·H·M·S CgA II KRYPTOR assay. No significant interference was found for each substance at the concentrations listed below.

Exogenous Substance	Concentration	Exogenous Substance	Concentration
Acetaminophen	238.3 mg/L	Etoposide	114 mg/L
Alprazolam	6.0 mg/L	Everolimus	6 mg/L
Amlodipine	100.2 g/L	Fluorouracil	684 mg/L
Aspirin	546.6 mg/L	Interferon (IFN-α- 2b)	3,000 kU/L
Biotin	3,510 µg/L	Lanreotide	72 mg/L
Fish oil	2.4 g/L	Octreotide	12 mg/L
Hydrochlorothiazide	6.0 mg/L	Oxaliplatin	96.9 mg/L
Ibuprofen	499.6 mg/L	Sunitinib	22.5 mg/L
Lisinopril	300.4 µg/L	Temozolomide	228 mg/L
Lorazepam	998.3 µg/L	Temsirolimus	15 mg/L
Metoprolol	5.0 mg/L	Pancrealipase	400 kU/L
Bevacizumab	720 mg/L	Hydrocodone	200.3 µg/L
Capecitabine	2.85 g/L	Oxycodone	500.9 µg/L
Carboplatin	1 g/L	Multivitamin*	
Cisplatin	2 g/L		

*Vitamin A (16.7 kIU/L), Vitamin C (1,000 mg/L), Vitamin D (5.33 kIU/L), Vitamin E (100 IU/mL), Thiamin (B1) (200 mg/L), Riboflavin (B2) (250 mg/L), Niacin (170 mg/L), Vitamin B6 (170 mg/L) and Vitamin B12 (3,333 µg/L)

c) *Cross-Reactivity:*

The potential cross-reactivity of the following substances was evaluated using B·R·A·H·M·S CgA II KRYPTOR assay. No significant interference was found for each substance at the concentrations listed below.

Cross-Reactive Substance	Concentration
Parastatin	100 nmol/L
Catestatin	452 nmol/L
Pancreastatin	182 nmol/L
Vasostatin I	9 nmol/L
Vasostatin II Cterm	15 nmol/L
Vasostatin II	5 nmol/L
Chromostatin	10 nmol/L
Chromogranin A protein fragment	217 nmol/L
Chromogranin B (Secretogranin 1)	72 nmol/L
Chromogranin C (Secretogranin 2)	148 nmol/L
WE14	606 nmol/L

4. Assay Reportable Range:

The claimed B·R·A·H·M·S CgA II KRYPTOR assay Analytical Measuring Interval (AMI) is 20−3,000 ng/mL. The device allows auto-dilution of samples—the Extended Measuring Interval (EMI) 'upper limit of quantitation' (ULOQ to the maximum dilution concentration)

is 1,000,000 ng/mL. The Reportable Range (the lower limit of the AMI to the maximum dilution concentration) is 20 to 1,000,000 ng/mL.

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

a) Traceability:

There is no recognized international standard for Chromogranin A (CgA). Calibration of the CgA is traceable to in-house reference calibrators (first lot produced during development of the assay), whose CgA values have been assigned in accordance with ISO 17511:2020 (2nd Edition).

b) Kit Stability:

The stability of the B·R·A·H·M·S CgA II KRYPTOR reagent kit was determined according to CLSI guideline EP25-A.

i) Reagent kit stability (shelf-life):

A real-time stability study was performed using unopened B·R·A·H·M·S CgA II KRYPTOR reagent stored at 2°C to 8°C. Seven samples with concentrations from 25 ng/mL to 2,781 ng/mL were tested in five replicates per sample at manufacturing time point (Time 0) and at 2, 3 and 4 months of storage time points. Results support the 4-month shelf-life stability claim for the B·R·A·H·M·S CgA II KRYPTOR reagent kit at 2°C to 8°C.

An accelerated stability study was conducted B·R·A·H·M·S CgA II KRYPTOR reagent at three elevated temperatures of 45°C, 37°C and 30°C. Four serum samples were measured in duplicates at 11 different times points [including day zero (0) and between day 3 and 52 days depending on the temperature] with each reagent stored at the three elevated temperatures. The results support a temporary shelf-life claim for the B·R·A·H·M·S CgA II KRYPTOR reagents at 2°C to 8°C for 9 months.

An additional real-time stability study conducted on three B·R·A·H·M·S CgA II KRYPTOR reagent lots using human serum samples for > 9 months storage claims is ongoing.

ii) Onboard stability:

The B·R·A·H·M·S CgA II KRYPTOR reagent unit onboard stability study was performed by testing seven serum samples for up to 30 days after a reagent unit was reconstituted and placed on-board a B·R·A·H·M·S KRYPTOR compact PLUS instrument (on-board storage temperature, 2°C to 8°C). The results support on-board stability claim for the B·R·A·H·M·S CgA II KRYPTOR reagents at 2°C to 8°C for 29 days.

c) Sample Stability:

i) Sample stability at room temperature (RT):

Twelve fresh serum samples were stored at RT (18−25°C) and tested at 5 hours, 24 hours, 48 hours and 72 hours by one operator with two lots of B·R·A·H·M·S CgA II KRYPTOR reagents using two instruments. The CgA concentration data obtained at the different time points were plotted against T0 measurements and analyzed by

Passing-Bablok regression analysis. The slopes between 1.02 and 1.08 and correlation coefficients between 0.97 and 0.99 support 48 hours sample stability claim at room temperature (18−25°C).

ii) Sample stability at -20°C:
Four serum samples across AMI were divided each into 10 aliquot samples. Aliquot samples were stored at -20°C and tested in duplicate at day 0 (fresh measurement), day 15, day 31 and day 32 by one operator with one lot of B·R·A·H·M·S CgA II KRYPTOR reagents using one instrument. Percent concentration change was computed based on linear regression analysis of aliquot-specific mean concentrations vs. time. The results support sample stability claims of one month at -20°C.

iii) Freeze-Thaw stability:
Four serum samples across AMI were aliquoted. Four of those aliquots were tested at time point T0 (fresh measurement) and the other four aliquots after claimed number of freeze/thaw cycles (each cycle consisted of freezing and storage at -20°C for at least 12 hours and thawing for about 30 min at room temperature before re-freezing or measurement). The samples were tested by one operator with one lot of B·R·A·H·M·S CgA II KRYPTOR reagent using one instrument. The results support serum CgA concentrations stability up to four freeze and thaw cycles for samples stored at -20°C.

6. Detection Capability:

CLSI guideline EP17-A2 was followed to determine the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the B·R·A·H·M·S CgA II KRYPTOR.

LoB:
Four blank samples (CgA depleted serum samples) were tested in five replicates per run, three runs per sample using two B·R·A·H·M·S CgA II KRYPTOR reagent lots (Lot A and Lot B; 4 x 5 x 3 = 60 replicates for each lot) on two instruments. The LoB was calculated using the parametric option as the distribution of the LoB values was considered as normal. This gave the LoB value 11.1 ng/mL for Lot A and 11.3 ng/mL for Lot B. The claimed LoB for the B·R·A·H·M·S CgA II KRYPTOR is 11.3 ng/mL.

LoD:
Four low CgA concentration serum samples were run in five replicates per run, three runs per sample using two B·R·A·H·M·S CgA II KRYPTOR reagent lots (Lot A and Lot B; 4 x 5 x 3 = 60 replicates for each lot) on two instruments. The parametric analysis was used as 120 measurement results from four LoD samples followed normal distribution in each group. This gave the LoD value 14.0 ng/mL for Lot A and 13.9 ng/mL for Lot B. The claimed LoD for the B·R·A·H·M·S CgA II KRYPTOR is 14.0 ng/mL.

LoQ:
A precision profile was created using seven low CgA concentration serum samples using three lots of B·R·A·H·M·S CgA II KRYPTOR reagents (Lot A, Lot B and Lot C). A within-laboratory precision study of 60 measurements (3 lots x 5 days x 2 runs x 2 replicates) was performed. Within-laboratory %CV was calculated for each sample and for the three reagent lots. The estimated LoQ of B·R·A·H·M·S CgA II KRYPTOR determined using CgA

concentration with 20% CV estimates, was 7.9 ng/mL for Lot A, 9.1 ng/mL for Lot B and 8.6 ng/mL for Lot C. The lowest measurand concentration that can be measured with respect to pre-specified % CV acceptance criterion was set at 20 ng/mL. The claimed LoQ for the B·R·A·H·M·S CgA II KRYPTOR is 20 ng/mL.

7. Assay Cut-Off:

   See Clinical Cut-Off below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable. The predicate measures a different analyte. See clinical studies.

2. Matrix Comparison:

   Not applicable. The assay is for serum samples only.

C Clinical Studies:

The effectiveness of the B·R·A·H·M·S CgA II KRYPTOR as an aid in monitoring the course of progressive or non-progressive disease in patients with well-defined Gastroentero-pancreatic neuroendocrine tumors (GEP-NETs) grade 1 and 2, was determined through a prospective, multi-center, observational study in the expected study duration of 32 months. Course of disease was assessed by standard imaging (CT/MRI scans) and tumors were classified by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria for progression (progressive disease) vs. no progression (complete response, partial response, or stable disease). Tumor progression was coded in binary format based on RECIST tumor classification. The two classes of tumor progression were defined as: (1) "progression" (or "positive") if the target tumor response was categorized as "Progressive Disease (PD)" and (2) "no progression" (or "negative") if the target tumor response was categorized as "Complete Response (CR)", "Partial Response (PR)" or "Stable Disease (SD)".

Inclusion criteria

- Primary well-differentiated G1 and G2 neuroendocrine tumor located in jejunum, ileum, colon, rectum, duodenum, appendix, stomach, or pancreas
- Measurable disease according to RECIST criteria (version 1.1)
- CT or MRI order obtained and within 4 weeks of CgA measurement
- B·R·A·H·M·S CgA II KRYPTOR baseline measurement available
- Patient has discontinued the following treatments for a least 3 weeks before study start
  - Proton pump inhibitors (PPI)
  - Corticoids
  - H2-receptor antagonists
- Baseline ECOG PS <2

Exclusion criteria

- Other active malignancy with the exclusion of melanoma or other cancers that occurred more than 5 years ago
- Participation in another clinical trial involving an investigational therapeutic (exception: diagnostic studies and studies evaluating known therapies)
- No measurable disease by RECIST criteria (version 1.1)
- Severe renal dysfunction defined as creatinine of 1.5x ULN
- Severe liver dysfunction in the absence of liver metastasis defined by aspartate aminotransferase (AST), serum total bilirubin and/or alanine transaminase (ALT) 1.5x ULN; Severe liver dysfunction in the presence of liver metastasis defined by AST and ALT over 5x ULN and total bilirubin over 1.5x ULN
- Severe gastrointestinal disorders (chronic atrophic gastritis, pancreatitis, inflammatory bowel disease, irritable bowel syndrome)
- Severe cardiovascular disease (severe symptomatic congestive heart failure, pulmonary artery hypertension, acute coronary syndrome)
- Patients receiving active treatment with the following medications and samples were collected less than 3 weeks after discontinuing:
  - Proton pump inhibitors (PPI)
  - Corticoids
  - H2-receptor antagonists
- Chronic alcohol and/or substance abuse
- Known Pregnancy

One hundred seventy-five (175) patients were enrolled from four clinical facilities, including three geographically distributed sites in U.S. and one site in Germany. Twenty-two (21) patients were excluded from analysis due to unavailable baseline RECIST images or unavailable follow-up visits. The remaining 153 patients had an evaluable baseline visit and at least one follow-up visit with a total 612 data points (153 baseline visits and 459 follow-up visits).

Serum samples were collected and transported to the laboratory at room temperature and tested using B R·A·H·M·S CgA II KRYPTOR reagent kits and B·R·A·H·M·S KRYPTOR instrument available at each study site. If fresh serum could not be analyzed within ≤ 24 hours, samples were aliquoted and stored frozen at ≤ -80°C and tested later. The 153 patients included in the study showed median of 63 years of age. The baseline demographic distribution and clinical assessment is summarized in table below.

Variable	Class	n = 153	
		#	%
(Baseline) Demographic data			
Sex	Male	91	59.S
	Female	62	40.S
Race	White or Caucasian	143	93.S
	Other	5	3.3
	African American	4	2.6
	Asian	1	0.7
Ethnicity	Not Hispanic or Latino	141	92.2
	Hispanic or Latino	8	5.2
	Unknown	3	2.0
	Not Reported	1	0.7

(Baseline) Clinical assessment			
ECOG PS*	0	103	67.3
	1	50	32.7
Physical/ clinical assessment	None of the above	86	56.2
	Diarrhea	43	28.1
	Abdominal pain	21	13.7
	Flushing syndrome	20	13.1
	Weight loss	8	5.2
	Palpitations	7	4.6
	Shortness of breath	7	4.6
	Decreased appetite	5	3.3

*Eastern Cooperative Oncology Group Performance Scale

Disease status and imaging at baseline is summarized in the table below.

Variable	Class	n = 153	
		#	%
(Baseline) Disease status			
Primary tumor site	Pancreas	55	35.9
	Other	46	30.1
	Ileum	46	30.1
	Jejunum	3	2.0
	Colon	1	0.7
	Duodenum	1	0.7
	Stomach	1	0.7
Primary tumor stage	0	10	6.5
	I	1	0.7
	II	1	0.7
	III	2	1.3
	IV	139	90.8
Tumor grade	G1	61	39.9
	G2	92	60.1
Site of metastases	Liver	134	87.6
	Lymph node	62	40.S
	Other	46	30.1
	None	7	4.6
	Lung	2	1.3
Autonomous hormone secretion syndrome	Non-functional tumor	96	62.7
	Other	54	35.3
	Multiple endocrine neoplasia type 1	2	1.3
	Insulinoma	1	0.7
	Verner-Morrison syndrome	1	0.7
(Baseline) Imaging			
Method of assessment	CT	86	56.2
	MRI	67	43.8
Number of lymph nodes measuring ≥ 15mm in short axis	0	98	82.4
	1	15	12.6
	2	4	3.4
	4	2	1.7
	5	0	0.0

The analysis for the time to follow-up visits shows that 92% (421/459) follow-up visits occurred within the first 24 months of the study and 8% (38/459) follow-up visits occurred between 24 and 36 months after study initiation. The analysis for the time between visits shows that 96% (439/459) follow-up visits occurred within 2 and 13 months after the previous visit, i.e., within one of the recommended time windows in the study protocol. Seven follow-up visits occurred earlier than 2 months after the previous visit and 13 visits later than 13 months after the previous visit. The overall number of progressions ranged from one (13.7%) over two (7.8%) over three (3.3%) to more than three (5.2%). 69.9% of the patients did not have any tumor progression. The overall number of no progressions ranged from zero (5.9%) over one (32.7%) over two (22.2%) over three (15.0%) to more than three (24.2%).

Variable	Class	n = 153	
		#	%
Tumor progression			
Number of progressions	0	107	69.9
	1	21	13.7
	2	12	7.8
	3	5	3.3
	>3	8	5.2
Number of no-progressions	0	9	5.9
	1	50	32.7
	2	34	22.2
	3	23	15.0
	>3	37	24.2
Visits			
Number of visits (baseline and follow-up)	2	41	26.8
	3	30	19.6
	4	26	17.0
	5	27	17.6
	6	10	6.5
	7	12	7.8
	8	6	3.9
	9	1	0.7

A total of 612 visits/observations (153 baseline visits, 459 follow-up visits) was undertaken with an average number of four observations/visits per patient. The B·R·A·H·M·S CgA II KRYPTOR result is considered as positive when 50% increase of CgA serum concentrations (ΔCgA>50%) to a value of greater than 100 ng/mL between consecutive monitoring visits. The test result is considered as negative when a change of CgA serum concentrations is less than or equal to 50% increase or to a value of CgA concentration below or equal to 100 ng/mL between monitoring visits. The results of the B·R·A·H·M·S CgA II KRYPTOR from 153 adult GEP-NET patients (grade 1 and 2) were analyzed and comparing with clinical assessment based on tumor progression as classified by RECIST 1.1 criteria. The clinical performance characteristics of the B·R·A·H·M·S CgA II KRYPTOR relative to progression were evaluated, with two-sided 95% confidence interval (CI), for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). The results are summarized in tables below.

		Tumor Progression		Total
		Progression	No Progression	
Binary CgA increase*	Positive	33	24	57
	Negative	63	339	402
Total		96	363	459

*Cut-off: 50% ΔCgA, Upper Limit of Normal (ULN) of CgA = 100 ng/mL

	Value	Lower 95% CI	Upper 95% CI
Sensitivity (n/N)	34.4% (33/96)	23.2%	45.5%
Specificity (n/N)	93.4% (339/363)	90.2%	96.0%
PPV (n/N)	57.9% (33/57)	40.5%	73.6%
NPV (n/N)	84.3% (339/402)	79.3%	89.1%
PLR	5.20	2.90	9.66
NLR	0.70	0.58	0.83
Prevalence	20.9% (96/459)		

To account for the correlations of the multiple CgA II measurements within each patient, confidence intervals were calculated using bootstrap method and the Boot package (R version 4.0.5). The test meets minimum requirements of the statistical validity including the requirement that PPV (57.9%) is statistically significantly higher than prevalence (20.9%); the lower bound of 95% confidence interval for PPV (40.5%) is above prevalence (20.9%); NPV (84.3%) is statistically higher than 1−prevalence (79.1%); the lower bound of 95% confidence interval for NPV (79.3%) is above 1−prevalence (79.1%); PLR (5.20) is > 1 and NLR (0.70) is < 1.

The performance of binary CgA increase for diagnosis of tumor progression according to RECIST 1.1 for patients with tumor grade G1 (estimates and 95% confidence intervals) are summarized in the tables below.

Patients with tumor grade 1		Tumor Progression		Total
		Progression	No Progression	
Binary CgA increase*	Positive	7	6	13
	Negative	24	117	141
Total		31	123	154

*Cut-off: 50% ΔCgA, CgA = 100 ng/mL

Patients with tumor grade 1	Value	Lower 95% CI	Upper 95% CI
Sensitivity (n/N)	22.6% (7/31)	6.5%	41.7%
Specificity (n/N)	95.1% (117/123)	90.4%	99.1%
PPV (n/N)	53.8% (7/13)	18.2%	85.7%
NPV (n/N)	83.0% (117/141)	73.2%	91.9%
PLR[1]	4.63	1.05	24.38
NLR	0.81	0.61	1.00
Prevalence	20.1% (31/154)		

[1]One (1) out of 10,000 bootstrap samples excluded from computation of CI due to non-evaluable PPV and PLR as no test-positive visit was in the corresponding sample.

The performance of binary CgA increase for diagnosis of tumor progression according to RECIST 1.1 for patients with tumor grade G2 (estimates and 95% confidence intervals) is summarized in the tables below.

Patients with tumor grade 2		Tumor Progression		Total
		Progression	No Progression	
Binary CgA increase*	Positive	26	18	44
	Negative	39	222	261
Total		65	240	305

*Cut-off: 50% ΔCgA, CgA = 100 ng/mL

Patients with tumor grade 2	Value	Lower 95%CI	Upper 95%CI
Sensitivity	40.0% (26/65)	26.1%	53.2%
Specificity	92.5% (222/240)	88.5%	95.9%
PPV	59.1% (26/44)	38.1%	76.0%
NPV	85.1% (222/261)	79.2%	90.3%
PLR	5.33	2.80	10.64
NLR	0.65	0.50	0.81
Prevalence	21.3% (65/305)		

In summary, the clinical study results support the intended use of B·R·A·H·M·S CgA II KRYPTOR as an "aid in monitoring of disease progression during the course of disease and treatment in patients with gastroentero-pancreatic neuroendocrine tumors (GEP-NETs, grade 1 and grade 2)."

D Clinical Cut-Off:

The clinical cut-off was derived from a retrospective, bicentric observational pilot study of 102 patients with diagnosed well-differentiated G1 and G2 GEP-NETs in the U.S. During routine monitoring visits serum CgA concentrations were assessed in comparison to standard imaging (CT/MRI) and tumors were classified by RECIST 1.1 criteria for progression (progressive disease) vs. no progression (complete response, partial response or stable disease).

Positive test result

ΔCgA > 50% and CgA > 100 ng/mL

An increase of CgA serum concentrations of more than 50% to a value of greater than 100 ng/mL between consecutive monitoring visits defines a positive test result representing a higher probability that a tumor progression has occurred.

Negative test result

ΔCgA ≤ 50% or CgA ≤ 100 ng/mL

A change of CgA serum concentrations of equal or less than 50% increase between monitoring visits or to a value of 100 ng/mL or less defines a negative test result representing a lower probability that a tumor progression has occurred.

E Expected Values/Reference Range:

The reference range of the B·R·A·H·M·S CgA II KRYPTOR was established per CLSI EP28-A3c. Serum specimens from 208 samples from healthy subjects (age ≥ 22 and ≤ 87 years, collected in the U.S.) were assessed using the B·R·A·H·M·S CgA II KRYPTOR on the B·R·A·H·M·S KRYPTOR compact PLUS analyzer. The results are presented in the table below:

N = 208*	
	CgA (ng/mL)
Min	18.3
Max	447.7
Mean	75.4
Median	61.8
Standard deviation	59.6
95th percentile	187.0
97.5th percentile	270.1

*Two values identified as outliers by Dixon's outlier test were excluded from the analysis because their deviation from the preceding value was more than 33% of the total range covered by the samples

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.