September 18, 2023

B·R·A·H·M·S GmbH, part of Thermo Fisher Scientific
Anne Kummerow
Regulatory Affairs Specialist
Neuendorfstrasse 25
Hennigsdorf, Brandenburg 16761
Germany

Re: K222251
Trade/Device Name: 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
Regulation Number: 21 CFR 866.6010
Regulation Name: Tumor-associated antigen immunological test system
Regulatory Class: Class II
Product Code: QXS
Dated: May 11, 2023
Received: May 11, 2023

Dear Anne Kummerow:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ying Mao -S

Ying Mao, Ph.D.
Branch Chief
Division of Immunology and Hematology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K222251

Device Name
B·R·A·H·M·S™ CgA II KRYPTOR™

Indications for Use *(Describe)*

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).

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

807.92(a)(1), (2), (3)

The assigned 510(k) Number: K222251

1. Applicant

BRAHMS GmbH, part of Thermo Fisher Scientific
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16761 Hennigsdorf, Germany

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Date prepared: September 14th, 2023

2. Purpose for Submission

New Device

3. Device Trade Name

B·R·A·H·M·S™ CgA II KRYPTOR™

4. Regulatory Information

A Regulation section:
21 CFR 866.6010 Tumor-associated antigen immunological test system
21 CFR 862.1660 Quality Control material (assayed and unassayed)
21 CFR 862.1150 Calibrator
21 CFR 866.4520 Immunofluorometer equipment

B Classification:
Assay: Class II

C Product code:

QXS, Chromogranin A (CgA) [Class II]

D Panel:
Immunology (82) (Assay)
Clinical Chemistry (75) (Calibrator and Controls)

5. Intended Use *807.92(a)(5)*

A. Intended use(s):

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).

B. 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 diagnostic 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) [1-6]. CgA values may rise during treatment with proton pump inhibitors.

C. Limitations
The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the Interference section below could lead to erroneous results.

Accurate results are dependent on following the proper sample collection, storage, and handling procedures.

D. Special Conditions for use statement(s):
Prescription use only

E. Special Instruments requirements:
B·R·A·H·M·S KRYPTOR compact PLUS analyzer

6. Device Description *807.92(a)(4)*

The B·R·A·H·M·S CgA II KRYPTOR assay is based on the formation of a complex comprised of a Chromogranin A (CgA) analyte “sandwiched” between two monoclonal mouse anti-CgA antibodies. 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 measurement principle is based on a non-radiative energy transfer from a donor (cryptate) to an acceptor (Alexa Fluor™647) when they are part of an immunocomplex (TRACE technology (Time-Resolved Amplified Cryptate Emission)).

The fluorescent signal is proportional to the concentration of the analyte to be measured.

With this principle B·R·A·H·M·S CgA II KRYPTOR is a homogenous one-step immunoassay for the quantification of CgA II in human serum. 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. Results can be retrieved after a 29 min incubation time.

7. Substantial Equivalence Information

a. Predicate Device Name(s)/510(k) number(s)/product code(s):
ARCHITECT CEA / K990774 / DHX

b. Reference Device Name(s) 510(k) number(s)/product code(s):
B·R·A·H·M·S™ PCT sensitive KRYPTOR™ / B·R·A·H·M·S™ KRYPTOR™ compact PLUS / K171338 / PMT, PRI, NMT, JZT

6.1 Technological Characteristics 807.92(a)(6):

c. **Comparison with predicate**:

The intended use and fundamental scientific technology between the predicate device and the new device are substantially equivalent. Comparison of the B·R·A·H·M·S CgA II KRYPTOR to a well-established clinical comparator method based on tumor imaging and gold standard evaluation by RECIST 1.1 criteria met the predefined clinical performance criteria for sensitivity and specificity in detecting tumor progressions.

Item	**Subject Device:** **B·R·A·H·M·S CgA II KRYPTOR**	**Predicate Device:** **K990774** **ARCHITECT CEA**
Intended Use and 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.

Item	Subject Device: B·R·A·H·M·S CgA II KRYPTOR	Predicate Device: K990774 ARCHITECT CEA
Operating Principle (Technology)	Automated fluorescent immunoassay using TRACE (Time-resolved amplified cryptate emission) technology.	Automated chemiluminescent microparticle immunoassay (CMIA)
Measured Analyte	Chromogranin A (CgA)	Carcinoembryonic Antigen (CEA)
Test Matrix	Serum	Serum or plasma
Methodology	Automated, quantitative	Same
Sample volume	14 µL	10 µL
Antibody	2 antibodies labelled with a) cryptate (the donor, to be excited by laser light) and b) fluorophore (the acceptor; fluorescence emission)	2 antibodies labelled with a) paramagnetic microparticles (for immune complex formation) b) acridinium (chemiluminescent dye, signal emission)
Assay Principle	Sandwich Immunoassay	same
Principal Operator	Professional user	Same
Calibrator (CAL)	1 calibrator	2 calibrators
Instrument / Analyzer	B·R·A·H·M·S KRYPTOR compact PLUS analyzer	ARCHITECT analyzer series

6.2 Summary of Non-Clinical Test - Performance and Safety Testing *807.92(b)(1)*:

Standards Body	Standard Name
Clinical Laboratory Standards Institute (CLSI)	CLSI EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
CLSI	CLSI EP06-Ed2 – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
CLSI	CLSI EP07-A3 - Interference testing in clinical chemistry; approved guideline - Third Edition
CLSI	CLSI EP09-A3 - Measurement Procedure Comparison And Bias Estimation Using Patient Samples - 3rd Edition
CLSI	CLSI EP17 A2: - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition
CLSI	CLSI EP25 A:2009 - Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
CLSI	CLSI EP28-A3c - Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
CLSI	CLSI EP34 1st Edition - Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking, 1st Edition

Standards Body	Standard Name
International Organization for Standardization (ISO)	ISO 14971:2019 Medical Devices – Application of Risk Management to Medical Devices
ISO	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	ISO 17511:2020 In Vitro diagnostic medical devices – Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials

8. Test Principle

The B·R·A·H·M·S CgA II KRYPTOR assay is a one-step immunoassay to determine the presence of CgA in human serum using TRACE™ technology. One reagent antibody is labelled with Alexa Fluor™ 647 (the acceptor). The second antibody is labelled with cryptate (the donor). After excitation with laser light, spatial proximity of the two labelled antibodies in the antigen/antibody complex allows for energy transfer from the donor to the acceptor molecule and subsequent fluorescent signal emission as relative light units (RLUs) which can be detected by the B·R·A·H·M·S KRYPTOR instrument. A direct relationship exists between the amount of CgA in the sample and the RLUs detected by the B·R·A·H·M·S KRYPTOR instrument's optical system.

9. Performance Characteristics

a. Analytical Performance

i. Repeatability and Within-Laboratory Precision

Precision (repeatability, within-laboratory, reproducibility and lot-to-lot precision) was determined following CLSI Guideline EP05-A3.
Repeatability and Within-Laboratory coefficient of variation (CV) were calculated from the measurement of samples over 20 days, with 2 runs per day in 2 replicates:

Sample ID N = 12	Mean Value (ng/mL)	Repeatability CV	Within-Laboratory CV
P03	23.0	5.2 %	10.0 %
P04	26.0	5.1 %	9.9 %
P05	34.6	3.7 %	8.8 %
P06	56.3	2.8 %	8.4 %
P07	86.3	1.7 %	8.3 %
P08	132	1.3 %	7.1 %
P09	228	1.7 %	6.9 %
P10	463	1.1 %	4.4 %
39PRE05	744	1.6%	2.8 %
P11	1,177	1.1 %	4.0 %
P12	1,825	1.3 %	4.7 %
P13	2,687	1.6 %	7.4 %

Lot-to-lot CVs were calculated from the measurement of samples over 5 days, with 5 replicates per day using 3 reagent lots and 1 instrument:

Sample	Mean Value (ng/mL)	Lot-to-Lot	
		SD	CV
39LOT_01	26,3	0.3	1.2%
39LOT_02	48,9	0.0	0.0%
39LOT_03	96,8	0.1	0.1%
39LOT_04	513	8.3	1.6%
39LOT_05	757	14.0	1.9%
39LOT_06	963	23.6	2.4%
39LOT_07	1,441	39.1	2.7%
39LOT_08	2,030	88.9	4.4%
39LOT_09	2,540	19.2	0.8%
39LOT_10	2,895	0.0	0.0%

ii. Reproducibility

For reproducibility, samples have been measured on 5 days, with 2 runs per day in 3 replicates using 1 reagent lot at 3 different sites (different instruments).

Sample No.	[CgA] Mean (ng/mL)	Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
		SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
2.1	25.7	1.66	6.5%	0.0	0.0%	0.55	2.2%	1.50	5.8%	2.30	9.0%
2.2	53.6	1.93	3.6%	1.53	2.8%	2.94	5.5%	0.00	0.0%	3.83	7.1%
2.3	104	2.07	2.0%	1.51	1.5%	2.04	2.0%	2.88	2.8%	4.37	4.2%
2.4	515	9.52	1.8%	5.87	1.1%	9.55	1.9%	12.6	2.5%	19.4	3.8%
2.5	1,089	16.7	1.5%	23.9	2.2%	41.0	3.8%	38.9	3.6%	63.6	5.8%
2.6	1,723	29.2	1.7%	63.4	3.7%	61.8	3.6%	0.0	0.0%	93.2	5.4%
2.7	2,923	62.3	2.1%	66.8	2.3%	64.5	2.2%	178	6.1%	210	7.2%
2.8	92,561	2,669	2.9%	2,993	3.2%	1,584	1.7%	2,803	3.0%	5,143	5.6%

iii. Detection Capability

The LoB (Limit of Blank), LoD (Limit of Detection) and LoQ (Limit of Quantitation) of the assay were determined based on the guidelines from CLSI EP17-A02.

a. LoB Test Protocol

LoB was determined by testing a total of 30 replicates of 4 different blank samples (120 total replicates) over 8 days with 2 reagents lots on 2 B·R·A·H·M·S KRYPTOR compact PLUS instruments. The LoB was determined to be 11.3 ng/mL, using the parametric option.

b. LoD Test Protocol

LoD was determined by testing a total of 30 replicates of 4 different low CgA concentration samples (120 total replicates) over 7 days with 2 reagents lots on 2 B·R·A·H·M·S KRYPTOR compact PLUS instruments. The LoD was determined to be 14.0 ng/mL, using the parametric option.

c. LoQ Test Protocol

LoQ was determined by testing a total of 60 replicates of 7different pools of human serum samples collected from healthy individuals (420 total replicates) over 5 days with 3 reagents lots on 3 B·R·A·H·M·S KRYPTOR compact PLUS instruments. LoQ was defined as the lowest concentration that can be reproducibly measured with an intermediate within-laboratory precision CV of ≤ 20%. The LoQ was determined to be 20.0 ng/mL.

iv. Linearity

Linearity was determined following CLSI Guideline EP06-A on study design and revised CLSI EP06-A 2nd Edition guidance for analysis.
The B·R·A·H·M·S CgA II KRYPTOR assay is linear from 20.0 ng/mL (LoQ) up to 1,000,000 ng/mL. Samples above 3,000 ng/mL will be diluted automatically.

Sample	Range (ng/mL)	Slope (95%CI)	Intercept (95%CI)	R^2
Sample 01	296-3401	1.021 (1.009;1.033)	-66.11 (-73.07;-59.15)	0.998
Sample 02	39-301	1.081 (1.065;1.096)	-10.96 (-12.77;-9.150)	0.997
Sample 03	15-52	1.036 (1.012;1.061)	-2.048 (-2.853;-1.243)	0.994
Sample 01-02-03 (combined data)	15-3401	0.975 (0.961;0.990)	-0.447 (-1.407;0.514)	0.992

v. Dilution Recovery

Dilution recovery was determined by diluting 10 pools of human serum samples. Dilutions were done by B·R·A·H·M·S KRYPTOR compact PLUS instruments using the kit diluent. The recoveries between the pure values and each dilution level values and mean recoveries were calculated. The mean recovery values are ranging from 97.6% - 109.6%.

vi. Spike Recovery

A total of 13 individual serum samples with different CgA concentrations were used to assess spike recovery of the B·R·A·H·M·S CgA II KRYPTOR. 7 of the samples were spiked (1:1) with a low concentration recombinant CgA antigen sample and the 6 other samples were spiked with a second recombinant CgA antigen sample of higher concentration. The samples were measured (in duplicate) before and after spiking and the recoveries between the measured mean values and the expected concentrations were calculated for the 13 spiked pools. The spiked pools had individual recovery values ranging from 91% - 109%.

vii. High Dose Hook Effect

B·R·A·H·M·S CgA II KRYPTOR is a homogenous immunoassay and does not require separation or washing steps. It is thus possible to obtain data without interrupting the immunological reaction. 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.

- In other words, potential Hook Effect is detected by kinetics analysis of the samples by B·R·A·H·M·S KRYPTOR analyzer family. Measurement is stopped for samples greater than 3,000 ng/mL. If automatic dilution is activated, then the B·R·A·H·M·S KRYPTOR analyzer automatically dilutes the sample at an appropriate dilution. If automatic dilution is not activated, then the B·R·A·H·M·S KRYPTOR analyzer family adds the dilution of the sample to the worklist and the user has to validate the worklist to launch the dilution of the sample. This process allows for sample measurements greater than 3,000 ng/mL up to 1,000,000 ng/mL.

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viii. Interference and Cross-Reactivity

Interference studies were based on CLSI EP07-A3 as a guideline. Cross-reactivity studies were based on CLSI EP07-A2 as a guideline and were verified for compliance with EP07-A3.

Interference was assessed in accordance with CLSI Guideline EP7-A3 and CLSI EP37 1st edition. The substances evaluated with the B·R·A·H·M·S CgA II KRYPTOR were found not to affect the test performance (bias ≤ 10%) at concentrations reasonably and consistently found in clinical situations. The substances included the following:

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

Exogenous Interfering Substance	Concentration
Acetaminophen	238.3 mg/L
Alprazolam	6.0 mg/L
Amlodipine	100.2 µg/L
Aspirin	546.6 mg/L
Biotin	3,510 ng/mL
Fish Oil	2.4 g/L
Hydrochlorothiazide	6.0 mg/L
Ibuprofen	499.6 mg/L
Lisinopril	300.4 µg/L
Lorazepam	998.3 µg/L
Metoprolol	5.0 mg/L
Multivitamin: Vitamin A Vitamin C Vitamin D Vitamin E Thiamin (B1) Riboflavin (B2) Niacin Vitamin B6 Vitamin B12	 16.7 kIU/L 1,000 mg/L 5.33 kIU/L 100.0 IU/L 200 mg/L 250 mg/L 170 mg/L 170 mg/L 3,330 µg/L
Bevacizumab	720 mg/L
Capecitabine	2.85 g/L
Carboplatin	1 g/L
Cisplatin	2 g/L
Etoposide	114 mg/L
Everolimus	6 mg/L

Exogenous Interfering Substance	Concentration
Fluorouracil	684 mg/L
Interferon (IFN-α-2b)	3,000 kU/L
Lanreotide	72 mg/L
Octreotide	12 mg/L
Oxaliplatin	96.9 mg/L
Sunitinib	22.5 mg/L
Temozolomide	228 mg/L
Temsirolimus	15 mg/L
Pancrelipase	480 kU/L
Hydrocodone	200.3 µg/L
Oxycodone	500.9 µg/L

Cross-reactivity effects were tested with substances that are structurally similar to the CgA protein. The percent of cross-reactivity was between -21.6% - 0.03%. The results are shown below.

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

ix. Sample Stability

The following studies were performed to confirm the sample stability claims.

a. Short-Term Sample Stability at Room Temperature

- A study with 12 serum samples stored at room temperature (18-25°C) confirms a stability claim of 48 hours for storage at room temperature.

b. Long-Term Sample Stability at -20°C

- A total of 4 serum samples, each divided into 10 aliquots, were tested after storage at -20°C to demonstrate long-term stability. The study confirms a stability claim of 1 month for storage at -20°C.

c. Freeze Thaw Sample Stability

- A total of 28 serum samples were tested in two studies to demonstrate freeze-thaw stability. The studies confirm a stability claim of 4 freeze-thaw cycles (storage at -20°C).

x. Reagent Stability

The following studies were conducted to support reagent stability claims based on CLSI EP25-A.

a. Real Time Stability for B·R·A·H·M·S CgA II KRYPTOR

Real time stability study with native samples for 1 lot of unopened B·R·A·H·M·S CgA II KRYPTOR reagent stored at 2-8°C has been carried out for 4 months. In combination with an accelerated stability study, stability for reagents at 2-8°C is supported for a duration of 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.

b. Real Time Stability for B·R·A·H·M·S CgA II KRYPTOR CAL

- Real time stability study for 3 lots of unopened B·R·A·H·M·S CgA II KRYPTOR calibrators stored at 2-8°C has been carried out for up to 112 weeks. Real time stability for calibrators at 2-8°C is claimed at 104 weeks (24 months).

c. Real Time Stability for B·R·A·H·M·S CgA II KRYPTOR QC

- Real time stability study for 3 lots of unopened B·R·A·H·M·S CgA II KRYPTOR controls stored at 2-8°C has been carried out for up to 109 weeks at least. Real time stability for controls at 2-8°C is claimed at 104 weeks (24 months).

d. On-board Stability for B·R·A·H·M·S CgA II KRYPTOR Assay

- In-use stability study for 1 lot of opened and reconstituted B·R·A·H·M·S CgA II KRYPTOR reagents stored at 2-8°C on board a B·R·A·H·M·S KRYPTOR compact PLUS instrument has been carried out for up to 30 days. In-use stability for B·R·A·H·M·S CgA II KRYPTOR reagents stored at 2-8°C is claimed at 29 days, with a required calibration after 15 days.

e. On-board Stability for B·R·A·H·M·S CgA II KRYPTOR CAL

- In-use stability study for 1 lot of opened and reconstituted B·R·A·H·M·S CgA II KRYPTOR calibrator stored at room temperature (18-25°C) on board a B·R·A·H·M·S KRYPTOR compact PLUS instrument has been carried out for up to 6 hours. In-use stability for B·R·A·H·M·S CgA II KRYPTOR calibrators stored at room temperature is claimed at 5 hours.

f. On-board Stability for B·R·A·H·M·S CgA II KRYPTOR QC

- In-use stability study for 1 lot of opened and reconstituted B·R·A·H·M·S CgA II KRYPTOR controls stored at room temperature (18-25°C) on board a B·R·A·H·M·S KRYPTOR compact PLUS instrument has been carried out for up to 6 hours. In-use stability for B·R·A·H·M·S CgA II KRYPTOR controls stored at room temperature is claimed at 5 hours.

- In-use stability study for 1 lot of opened and reconstituted B·R·A·H·M·S CgA II KRYPTOR controls stored at 2-8°C has been carried out for up to 29 hours. In-use stability for B·R·A·H·M·S CgA II KRYPTOR controls stored at 2-8°C is claimed at 24 hours.
- In-use stability study for 1 lot of opened and reconstituted B·R·A·H·M·S CgA II KRYPTOR controls stored at ≤ -20°C has been carried out for up to 36 days. In-use stability for B·R·A·H·M·S CgA II KRYPTOR controls stored at ≤ -20°C is claimed at 31 days.

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b) Clinical Studies

i. 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 US. 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).

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

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

ii. Clinical Sensitivity and Specificity:

The prospective study validated a statistically significant association of a 50% increase of CgA serum concentrations to a value of greater than 100 ng/ml between consecutive monitoring visits in 153 adult GEP-NET patients (grade 1 and 2) with tumor progression as classified by RECIST 1.1 criteria. The study yielded (estimate and 95% confidence interval):

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

*Cut-off 50% CgA increase and CgA > 100 ng/mL

Performance Measurement	Value	Lower CI*	Upper CI*
Sensitivity	34.4% (33/96)	23.2%	45.5%
Specificity	93.4% (339/363)	90.2%	96.0%
PPV	57.9% (33/57)	40.5%	73.6%
NPV	84.3% (339/402)	79.3%	89.1%

Performance Measurement	Value	Lower CI*	Upper CI*
PLR	5.20	2.90	9.66
NLR	0.70	0.58	0.83
Prevalence	20.9% (96/459)		

*CI = 95% Confidence interval
PPV = positive predictive value; NPV = negative predictive value
PLR = positive likelihood ratio; NLR = negative likelihood ratio

iii. Expected Values/Reference Ranges:

The B·R·A·H·M·S CgA II KRYPTOR reference limit was determined by testing a total of 206 samples from self-declared healthy individuals in the USA. The 95th quantile was estimated 187.0 ng/mL (90% confidence interval 123.5 to 255.6 ng/mL).

10. Proposed Labeling

The labeling is sufficient, and it satisfies the requirements of 21 CFR Part 809.10.

11. Conclusion

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