EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN220027

B Applicant

BRAHMS GmbH, Part of Thermo Fisher Scientific

C Proprietary and Established Names

B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System.

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QWH	Class II with special controls	21 CFR 862.1602 – Prognostic test for development or progression of preeclampsia	Clinical Chemistry and Toxicology Devices

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System.

B Measurand:

Placental growth factor (PlGF)
Soluble fms-like tyrosine kinase-1 (sFlt-1)

C Type of Test:

Immunofluorescent quantitative assay

III Indications for Use:

A Indication(s) for Use:

The B·R·A·H·M·S™ sFlt-1/ PlGF KRYPTOR™ Test System is comprised of the B·R·A·H·M·S PlGF plus KRYPTOR assay and the B·R·A·H·M·S sFlt-1 KRYPTOR assay.

The B·R·A·H·M·S PlGF plus KRYPTOR is an automated immunofluorescent assay using Time-Resolved Amplified Cryptate Emission (TRACE™) technology for the quantitative determination of the concentration of Placental Growth Factor (PlGF) in human serum and plasma (K2 EDTA) on the B·R·A·H·M·S KRYPTOR analyzer.

The B·R·A·H·M·S sFlt-1 KRYPTOR is an automated immunofluorescent assay using Time-Resolved Amplified Cryptate Emission (TRACE) technology for the quantitative determination of the concentration of soluble fms-like tyrosine kinase-1 (sFlt-1), also known as VEGF receptor-1, in human serum and plasma (K2 EDTA) on the B·R·A·H·M·S KRYPTOR analyzer.

The B·R·A·H·M·S PlGF plus KRYPTOR is to be used in conjunction with the B·R·A·H·M·S sFlt-1 KRYPTOR along with other laboratory tests and clinical assessments to aid in the risk assessment of pregnant women (singleton pregnancies between gestational age 23+0 to 34+6/7weeks) hospitalized for hypertensive disorders of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension) for progression to preeclampsia with severe features (as defined by American College of Obstetricians and Gynecologists (ACOG) guidelines) within 2 weeks of presentation.

B Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

B·R·A·H·M·S sFlt-1 KRYPTOR is not indicated to be used as a stand-alone diagnostic assay.

B·R·A·H·M·S PlGF plus KRYPTOR is not indicated to be used as a stand-alone diagnostic assay.

B·R·A·H·M·S sFlt-1 KRYPTOR must be run in conjunction with B·R·A·H·M·S PIGF plus KRYPTOR and the same patient sample must be used to run both assays.

For in vitro diagnostic use only.

Patient samples should not be diluted.

C Special Instrument Requirements:

B·R·A·H·M·S KRYPTOR compact Plus

IV Device/System Characteristics:

A Device Description:

The B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System is comprised of the B·R·A·H·M·S PlGF plus KRYPTOR assay and the B·R·A·H·M·S sFlt-1 KRYPTOR assay.

B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S™ sFlt-1 KRYPTOR assays are supplied as two reagent kits that are run on the B·R·A·H·M·S KRYPTOR compact PLUS. Each kit is sufficient for 75 determinations, and is comprised of two bottled reagents:

PlGF

Cryptate conjugate: Lumi4®-Tb labeled, anti-human PlGF antibody, buffer, bovine albumin, rat immunoglobulins, bovine immunoglobulins, goat immunoglobulins, trehalose, mannitol.
XL conjugate: Cyanin 5.5 labeled, anti-human PlGF antibody, buffer, bovine albumin, rat immunoglobulins, bovine immunoglobulins, goat immunoglobulins.

sFlt-1

Cryptate conjugate: Lumi4®-Tb labeled, anti-human sFlt-1 antibody, buffer, bovine albumin, bovine immunoglobulins, mouse immunoglobulins, dextran, preservative.
XL conjugate: Cyanin 5.5 labeled, anti-human sFlt-1 antibody, buffer, bovine albumin, bovine immunoglobulins, mouse immunoglobulins, dextran, preservative.

The B·R·A·H·M·S KRYPTOR compact PLUS is an automated test platform. The analyzer consumables are:

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
B·R·A·H·M·S KRYPTOR BUFFER	Phosphate Buffer Saline
B·R·A·H·M·S KRYPTOR compact REACT	60 plates

Also supplied and required are calibrators - single level B·R·A·H·M·S PlGF plus KRYPTOR CAL and two levels B·R·A·H·M·S sFlt-1 KRYPTOR CAL. The instrument is calibrated on a 15 day cycle, and re-calibrated with each new lot of reagents.

Also supplied and required are three levels of controls - B·R·A·H·M·S PlGF plus KRYPTOR QC Controls and B·R·A·H·M·S sFlt-1 KRYPTOR QC.

B Principle of Operation

B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S™ sFlt-1 KRYPTOR assays are immunofluorescent quantitative assays. The assays are formatted as homogenous immunoassays not requiring a free-bound separation step because of the detection method; referred to as Time-Resolved Amplified Cryptate Emission (TRACE) technology. For each assay, when antigen present in the patient sample is bound between the two antibodies forming an immunocomplex, and the sample subjected to a fluorescence excitation, a long lived fluorescent emission proportional to the analyte concentration is measured. After measurement of the fluorescence,

the intensity is compared against the standard calibration curve to derive the concentrations of sFlt-1 and PlGF in the sample. The concentration ratio for sFlt-1/PlGF is calculated and reported by the test system.

V Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Third Edition.

CLSI EP06, Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition.

CLSI EP07, Interference Testing in Clinical Chemistry, 3rd 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 EP37, Supplemental Tables for Interference Testing in Clinical Chemistry, 1st edition.

EN ISO 17511:2020, In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples.

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:
   The precision performance of the B·R·A·H·M·S PlGF plus KRYPTOR assay, B·R·A·H·M·S sFlt-1 KRYPTOR assay, and the sFlt-1/PlGF ratio was evaluated following the guidelines of CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Third Edition.

   PlGF

   Study #1 - Repeatability and Within-Laboratory Precision: Single-Site Study
   The purpose of this study was to determine the repeatability and within laboratory precision of the B·R·A·H·M·S PlGF plus KRYPTOR assay using the B·R·A·H·M·S KRYPTOR compact PLUS instrument. In the study, 17 serum samples (12 native and five spiked) and three controls were assayed with PlGF concentrations ranging from 8.7 to 6,040 pg/mL. Each sample was assayed in duplicates per run, two runs per day across 20 days for a total of 80 replicates; using three lots of PlGF reagent kits, three lots of calibrators, and one lot of controls. The testing was conducted by two operators using four B·R·A·H·M·S KRYPTOR compact PLUS instruments. Repeatability and within laboratory precision were calculated by

a two-way nested ANOVA with factors day and instrument as variance components. The following is a summary of the results:

Sample	PlGF Mean (pg/mL)	Repeatability %CV	Between Day %CV	Between Instrument %CV	Within Laboratory %CV
Serum 1	8.7	14.6	0.0	4.3	15.2
Serum 2	10.6	12.3	0.0	6.0	13.7
Serum 3	10.6	12.3	0.0	4.6	13.1
Serum 4	11.2	11.8	0.0	4.0	12.5
Serum 5	17.2	7.6	0.0	4.0	8.6
Serum 6	20.7	6.0	0.0	3.6	7.0
Control 1	35.1	4.1	1.3	2.8	5.1
Serum 7	42.8	3.1	0.0	3.9	5.0
Serum 8	59.4	2.8	0.0	3.5	4.5
Serum 9	79.8	2.3	2.8	2.3	4.3
Control 2	103	2.1	1.2	0.7	2.5
Serum 10	147	1.7	2.6	2.5	4.0
Serum 11	364	1.1	2.5	2.2	3.5
Control 3	426	1.0	0.6	1.5	1.9
Serum 12	817	0.9	3.3	2.3	4.1
Serum 13	973	0.8	0.0	2.0	2.2
Serum 14	1,906	0.8	0.0	2.2	2.4
Serum 15	3,382	1.1	0.0	2.1	2.3
Serum 16	5,379	1.0	0.7	2.5	2.8
Serum 17	6,040	0.9	0.0	2.8	2.9

The lot-to-lot precision was calculated by a two-way nested ANOVA with factors of lot and instruments as variance components. The following is a summary of the results:

Sample	PlGF Mean (pg/mL)	Between lot %CV
Serum 1	8.7	3.3
Serum 2	10.6	7.1
Serum 3	10.6	2.4
Serum 4	11.2	5.1
Serum 5	17.2	2.4
Serum 6	20.7	2.3
Control 1	35.1	0.0
Serum 7	42.8	3.0
Serum 8	59.4	2.5
Serum 9	79.8	3.9
Control 2	103	0.8
Serum 10	147	3.6
Serum 11	364	3.0

Control 3	426	0.0
Serum 12	817	3.8
Serum 13	973	0.0
Serum 14	1,906	0.0
Serum 15	3,382	0.0
Serum 16	5,379	0.0
Serum 17	6,040	0.4

Study #2 - Reproducibility: Multisite Study

A three site reproducibility study was conducted testing five samples (four native and one spiked), and using one lot of reagent kit, one lot calibrator, and one lot of controls. In the study, at each site, using one instrument, each sample was assayed in replicates of five per run, one run per day over five days, for a total of 25 replicates. Reproducibility was analyzed by two-way nested ANOVA with factors days and sites as variance components. The results are summarized below:

Sample	PlGF Mean (pg/mL)	Repeatability	Within-Laboratory	Reproducibility
		% CV	% CV	% CV
Serum 1	15.2	9.5	10.9	11.1
Serum 2	19.7	7.2	7.2	7.4
Serum 3	238	1.4	1.7	2.6
Serum 4	894	1.0	2.0	4.6
Serum 5	5,939	1.5	2.2	3.6

Study #3 Reproducibility with intended use samples

A three instrument reproducibility study was conducted with samples representative of the intended use patient population. In the study, four samples of pooled serum obtained from pregnant women were assayed in replicates of five in one run per day over five days using three instruments for a total of 75 measurements per sample. The testing used one lot of B·R·A·H·M·S PlGF plus KRYPTOR. The results were analyzed for %CV for repeatability, within-laboratory, and reproducibility via two-way nested ANOVA with factors instrument and days as variance components. The results are summarized below:

Sample	PlGF Mean (pg/mL)	Repeatability	Within-Laboratory	Reproducibility
		% CV	% CV	% CV
Serum 1	15	9.8	9.9	9.9
Serum 2	55	2.9	3.7	4.5
Serum 3	141	1.7	1.8	2.8
Serum 4	235	1.4	2.1	2.9

sFlt-1

Study #1 - Repeatability and Within-Laboratory Precision: Single-Site Study

The purpose of this study was to determine the repeatability and within laboratory precision of the B·R·A·H·M·S sFlt-1 KRYPTOR assay using the B·R·A·H·M·S KRYPTOR compact

PLUS instrument. In the study, 16 samples (13 native and three spiked) were assayed with sFlt-1 concentration ranging from 48.1 to 68,944 pg/mL. Each sample was assayed in duplicates per run, two runs per day across 20 days for a total of 80 replicates; using three lots of PlGF reagent kits, calibrators, and one lot of controls. The testing was conducted by two operators using four B·R·A·H·M·S KRYPTOR compact PLUS instruments. Repeatability and within laboratory precision were calculated by a two-way nested ANOVA with factors day and instrument as variance components. The following is a summary of the results:

Sample	sFlt-1 Mean (pg/mL)	Repeatability %CV	Between Day %CV	Between Instrument %CV	Within Laboratory %CV
Serum 1	48.1	9.4	0.0	6.9	11.6
Serum 2	75.9	5.9	0.7	2.3	6.3
Serum 3	102	4.7	1.7	2.2	5.5
Serum 4	247	1.8	1.4	3.3	4.0
Serum 5	518	0.9	1.4	2.5	3.0
Serum 6	1,040	0.5	1.4	2.3	2.7
Control 1	1,559	0.7	2.3	3.5	4.2
Serum 7	1,605	0.6	1.4	2.2	2.7
Serum 8	2,932	0.5	1.4	1.9	2.4
Control 2	3,055	0.4	1.8	3.3	3.7
Serum 9	4,648	0.6	1.3	1.9	2.4
Control 3	9,772	0.6	2.3	2.6	3.5
Serum 10	10,453	0.6	1.2	2.4	2.8
Serum 11	22,832	0.7	2.4	1.6	3.0
Serum 12	50,452	0.9	2.5	2.3	3.5
Serum 13	68,944	0.7	2.3	3.0	3.8

The lot-to-lot precision was calculated by a two-way nested ANOVA with factors lot and instruments as variance components. The following is a summary of the results:

Sample	sFlt-1 Mean (pg/mL)	Between lot %CV
Serum 1	48.1	0.0
Serum 2	75.9	1.6
Serum 3	102	2.4
Serum 4	247	3.3
Serum 5	518	2.7
Serum 6	1,040	2.3
Control 1	1,559	4.4
Serum 7	1,605	2.1
Serum 8	2,932	1.6
Control 2	3,055	3.7

Serum 9	4,648	0.5
Control 3	9,772	3.2
Serum 10	10,453	0.0
Serum 11	22,832	0.0
Serum 12	50,452	0.0
Serum 13	68,944	0.0

Study #2 - Reproducibility: Multisite Study

A three site reproducibility study was conducted testing five serum samples, and using one lot of reagent kit, one lot calibrator, and one lot of controls. In the study, at each site, using one instrument, each sample was assayed in replicates of five per run, one run per day over five days, for a total of 25 replicates. Reproducibility was analyzed by two-way nested ANOVA with factors days and sites as variance components. The results are summarized below:

Sample	sFlt-1 Mean (pg/mL)	Repeatability	Within-Laboratory	Reproducibility
		% CV	% CV	% CV
Serum 1	20.9	28.3	28.5	38.4
Serum 2	263	2.3	3.4	3.5
Serum 3	1,697	0.8	2.3	3.0
Serum 4	11,401	1.7	3.2	3.3
Serum 5	40,067	0.7	3.8	5.1

Study #3 Reproducibility with intended use samples

A three instrument reproducibility study was conducted with samples representative of the intended use patient population. In the study, five samples of pooled serum obtained from pregnant women were assayed in replicates of five in one run per day over five days using three instruments for a total of 75 measurements per sample. The testing used one lot of B·R·A·H·M·S sFlt-1 KRYPTOR. The results were analyzed for %CV for repeatability, within-laboratory, and reproducibility via two-way nested ANOVA with factors instrument and days as variance components. The results are summarized below:

Sample	sFlt-1 Mean (pg/mL)	Repeatability	Within-Laboratory	Reproducibility
		% CV	% CV	% CV
Serum 1	1,244	0.6	1.2	2.6
Serum 2	5,225	0.6	1.0	2.0
Serum 3	6,848	0.5	1.0	2.1
Serum 4	32,305	0.9	2.4	2.6
Serum 5	28,625	0.8	1.8	1.8

sFlt-1/PlGF ratio

Study #1 - Repeatability and Within-Laboratory Precision: Single-Site Study

The purpose of this study was to determine the precision and reproducibility performance of the sFlt-1/PlGF ratio. In the study, 14 samples were selected from the individual 20-day precision data of the sFlt-1 and PlGF assays (see above) with concentrations such that the

range of ratios reasonably encompassed that expected in the intended use population; which based on the clinical study ranged from 1.5 to 1146. Repeatability and within laboratory precision were calculated via a two-way nested ANOVA software with factors day and instruments as variance components. The following is a summary of the analysis:

Mean sFlt-1 conc., pg/mL	Mean PlGF conc., pg/mL	Mean Ratio	Repeatability %CV	Within-Laboratory %CV
1605	817	2.0	1.2%	5.7%
4648	1906	2.4	1.1%	3.7%
518	147	3.5	1.7%	5.4%
4648	817	5.7	1.0%	5.4%
1605	147	11	1.9%	5.4%
22832	817	28	1.1%	5.4%
4648	147	32	1.8%	5.1%
1605	42.8	38	3.4%	6.3%
68944	817	85	1.2%	6.0%
4648	42.8	109	3.3%	6.1%
22832	147	156	1.9%	5.1%
68944	147	471	1.8%	6.0%
22832	42.8	534	3.2%	5.7%
68944	42.8	1613	3.2%	6.3%

Study #2 - Reproducibility: Multisite Study

The reproducibility of the sFlt-1/PlGF ratio was evaluated by selecting 6 samples from the individual multisite data of the sFlt-1 and PlGF assays (see above). Reproducibility was analyzed via two-way nested ANOVA software with factors days and sites as variance components. The results are summarized below:

Mean sFlt-1 conc., pg/mL	Mean PlGF conc., pg/mL	Mean Ratio	Repeatability %CV	Within-Lab %CV	Reproducibility %CV
1697	238	7.1	1.3%	2.4%	3.4%
11401	894	12.7	1.9%	3.5%	4.5%
40067	894	44.6	1.3%	4.1%	4.1%
11401	238	47.9	1.7%	3.0%	3.3%
1697	19.7	86.6	7.5%	7.5%	8.4%
40067	238	168	1.5%	3.4%	3.5%

2. Linearity:

The linearity performance of the B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S™ sFlt-1 KRYPTOR run on the B·R·A·H·M·S KRYPTOR compact PLUS was established following the guidelines of CLSI EP06 Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition.

PlGF

In the study, three separate panels that together cover the full measuring range were evaluated. A high concentration for each panel was prepared with recombinant PlGF antigen. The high concentration samples had expected concentrations at 4,424 pg/mL, 943 pg/mL, and 101 pg/mL for panels 1, 2, and 3, respectively. Known volumes of each respective high concentration sample in the panel were mixed with a low concentration sample (expected concentration of zero) to prepare different levels for linearity testing in each panel.
With each linearity panel, the samples were measured in four replicates with one lot of reagent kit using one instrument. The results for each linearity panel were combined and analyzed by weighted linear regression to find the best fit straight line to the data with zero intercept; with expected concentrations based on the dilutions on the x-axis and average observed concentration on the y-axis. At each concentration, the deviation from linearity was calculated based on the difference between the observed concentration and the predicted concentration of the best fit straight line. The deviation from linearity at each level supports the claimed PlGF measuring range of 7.6 to 4,000 pg/mL.

sFlt-1
In the study, five separate panels that together covered the full measuring range were evaluated. A high concentration for each panel was prepared with recombinant sFlt-1 antigen. The high concentration samples had expected concentrations at 100,000 pg/mL, 50,000 pg/mL, 10,000 pg/mL, 1,000 pg/mL, and 200 pg/mL for panels 1, 2, 3, 4 and 5 respectively. Known volumes of each respective high concentration sample in the panel were mixed with a non-zero sample with an expected concentration of 19.2 pg/mL. With each linearity panel, the samples were measured in five replicates with one lot of reagent kit using one instrument. The results for each linearity panel were combined and analyzed by weighted linear regression to find the best fit straight line to the data with an intercept; with expected concentrations based on the dilutions on the x-axis and average observed concentration on the y-axis. At each concentration, the deviation from linearity was calculated based on the difference between the observed concentration and the predicted concentration of the best fit straight line. The deviation from linearity at each level supports the claimed sFlt-1 measuring range of 315 to 90,000 pg/mL.

sFlt-1/PlGF Ratio
Not applicable

High dose hook effect
A study was conducted to assess whether PlGF and sFlt-1 concentrations above the upper limit of the measuring range are reported with a value within the measuring range because of a hook effect when the immunoassay reagents are under conditions of antigen excess.

PlGF
In the study, 16 samples were prepared with recombinant PlGF antigen at target PlGF concentrations ranging from 40 to 1,600,000 pg/mL. Each sample was measured in duplicate with one reagent lot and one instrument. The results of the study support the sponsor's labeling claim that the B·R·A·H·M·S PlGF plus KRYPTOR exhibited no high dose hook effect at PlGF concentrations up to 800,000 pg/mL.

sFlt-1
In the study, 17 samples were prepared with recombinant human sFlt-1 at target sFlt-1 concentrations ranging from 10 to 400,000 pg/mL. Each sample was measured in duplicate with one reagent lot and one instrument. The results of the study support the sponsor's labeling claim that the B·R·A·H·M·S sFlt-1 KRYPTOR exhibited no high dose hook effect at sFlt-1 concentrations up to 400,000 pg/mL.

sFlt-1/PlGF Ratio
Not applicable

3. Analytical Specificity/Interference:
The analytical specificity performance of the B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S sFlt-1 KRYPTOR run on the B·R·A·H·M·S KRYPTOR compact PLUS was evaluated by testing for interference from endogenous and exogenous substances, and cross-reactivity.

Interference from endogenous and exogenous substances

PlGF
In the study, two samples of either: (1) pooled human serum from healthy pregnant women spiked with PlGF at 40 and 500 pg/mL (50 and 60 pg/mL PlGF for folic acid testing, 50 and 130 pg/mL PlGF for iron testing) or (2) recombinant human PlGF diluted in defibrinated normal human plasma with PlGF at 30 and 400 pg/mL were prepared and divided into two aliquots; i.e. test sample (with added interferent) and control sample (with no added interferent). Sample testing was conducted with two lots of the reagent kit using three instruments (except for folic acid testing which used one lot and one instrument). Each test and control sample was assayed in replicates of three (except folic acid and iron testing which included five replicates). The data showed that the % interference for endogenous substances was between -9.3% and +10.8%, and between -6.1% and 15.4% for exogenous substances with the B·R·A·H·M·S PlGF plus KRYPTOR assay.

sFlt-1
In the study, two samples of pooled of human serum from healthy pregnant women spiked with sFlt-1 at 1500 and 10,000 pg/mL (2400 and 11,000 pg/mL sFlt-1 for folic acid testing, 50 and 130 pg/mL sFlt-1 for iron testing) were prepared, and divided into two aliquots, i.e., test sample (with added interferent) and control sample (with no added interferent). Sample testing was done with one lot of reagent kit and using one instrument. Each test and control sample was assayed in replicates of three (except folic acid and iron testing which included five replicates). The data showed that the % interference for endogenous substances was between -10.8% and -0.6%, and between -1.8% and +2.2% for exogenous substances with the B·R·A·H·M·S PlGF plus KRYPTOR assay.

For each substance, the results were analyzed for the highest concentration of that substance tested that did not show significant interference. The results are summarized below for both analytes.

Interfering Substance	Highest concentration of interferent tested that did not show significant interference
Endogenous *	
Albumin	6.0 g/dL
Bilirubin	25 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Exogenous	
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	65.2 mg/dL
Ascorbic acid	3.0 mg/dL
Caffeine	5.9 mg/dL
Calcium	20 mg/dL
Dihydralazin (Dihydralazine)	20 mg/dL
Ethanol	5% (v/v)
Folic Acid	2.4 mg/dL
Gentamicin	1.0 mg/dL
Heparin (intravenous)	3000 U/L
Ibuprofen	50 mg/dL
Iron (ferrous)	72 mg/dL
α-Methyldopa	1.5 mg/dL
Metoprolol	0.5 mg/dL

* Concentration based on the amount of substance added to the samples; actual concentrations of endogenous substances in the samples were higher since the native concentrations of these substances in the samples were not known.

sFlt-1/PlGF Ratio

In the study, four samples from healthy pregnant women spiked sFlt-1 were selected from the individual data from sFlt-1, and the same samples spiked with PlGF were selected to analyze for interference from each of albumin, bilirubin, hemoglobin, and triglycerides based on the sFlt-1/PlGF ratio.

Substance	Concentration	Test sample average ratio	Blank sample average ratio	% Interference
Albumin	6.0 g/dL	38.6	44.0	-12.4
		21.1	21.5	-1.7
		3.2	3.2	-0.3
		252	291	-13.7
Bilirubin	25 mg/dL	44.4	43.8	1.3
		21.4	20.8	2.9
		3.2	3.2	0.1
		295	283	4.1
Hemoglobin	500 mg/dL	38.6	4.7	-11.8
		20.5	21.5	-4.8

		3.1	3.3	-5.3
		256	289	-11.2
Triglycerides	3000 mg/dL	36.2	38.5	-5.8
		17.3	18.5	-6.3
		2.6	2.8	-7.9
		242	253	-4.2

Interference from potential cross-reactive substances
In the study, two samples of pooled human serum from pregnant women were prepared and divided into two aliquots, i.e., test sample (with added substance) and control sample (with no added substance). The concentrations tested for each substance were 10,000 pg/mL, 5,000 pg/mL, 1,000 pg/mL and 100 pg/mL. Sample testing was conducted with one lot of reagent kit for each analyte using two instruments. Each test and control sample was assayed in replicates of five replicates. For each substance, the results were analyzed to identify the highest concentration of that substance that did not show significant interference. The results are summarized below.

PlGF

Interfering substance	Highest concentration of interferent tested that did not show significant interference
PlGF-2	100 pg/mL
PlGF-3	100 pg/mL
VEGF-A (VEGF-165)	5,000 pg/mL
VEGF-B	10,000 pg/mL
VEGF/PlGF heterodimer	10,000 pg/mL

sFlt-1

Interfering substance	Highest concentration of interferent tested that did not show significant interference
PlGF-2	10,000 pg/mL
PlGF-3	10,000 pg/mL
VEGF-A (VEGF-165)	5,000 pg/mL
VEGF-B	10,000 pg/mL
VEGF/PlGF heterodimer	10,000 pg/mL

sFlt-1/PlGF Ratio

Interfering substance	Highest concentration of interferent tested that did not show significant interference
PlGF-2	100 pg/mL
PlGF-3	100 pg/mL
VEGF-A (VEGF-165)	5,000 pg/mL
VEGF-B	5,000 pg/mL

VEGF/PlGF heterodimer	10,000 pg/mL

Cross-reactivity

PlGF

A cross-reactivity study was conducted to quantify the level of cross-reactivity of the PlGF assay with certain substances structurally similar to the antigen; endogenous growth factors that share amino acid sequences with PlGF. In the study, two test samples were prepared by spiking individually various cross-reactive substances into native serum samples containing PlGF. These samples were paired with two reference samples of the same serum that were not spiked and with the same PlGF concentration. Each sample was assayed in replicates of three using two reagent kit lots and three instruments. The results from each of the test samples and reference samples were averaged. The difference in results between test and reference samples was calculated for each of the PlGF concentrations, and each analyzed for % cross-reactivity using the below equation. The table summarizes the cross-reactivities from the study.

% Cross-Reactivity = (PlGF result from test sample – PlGF result from reference sample) x 100% / Concentration of cross-reactive substance

Substance	Substance Concentration	Test sample average PlGF, pg/mL	Control sample average PlGF, pg/mL	% Cross-Reactivity
PlGF-2	10,000 pg/mL	1,101	36.1	10.6%
		1,636	498	11.4%
PlGF-3	10,000 pg/mL	373	36.1	3.4%
		801	498	3.0%
VEGF-A (VEGF 165)	10,000 pg/mL	35.4	34.6	0.01%
		480	474	0.06%
VEGF/PlGF Heterodimer	10,000 pg/mL	131	34.6	0.96%
		534	474	0.60%

sFlt-1

Substance	Substance Concentration	Test sample average sFlt-1, pg/mL	Control sample average sFlt-1, pg/mL	% Cross-Reactivity
PlGF-1 (PlGF)	10,000 pg/mL	1,511	1,526	-0.2%
		10,064	10,061	0.0%
PlGF-2	10,000 pg/mL	1,507	1,526	-0.2%
		10,065	10,061	0.0%
PlGF-3	10,000 pg/mL	1,515	1,526	-0.1%
		10,055	10,061	-0.1%
VEGF-A (VEGF-165)	10,000 pg/mL	1,515	1,526	-0.1%
		10,057	10,061	0.0%
VEGF-B	10,000 pg/mL	1,501	1,526	-0.3%

		9,975	10,061	-0.9%
VEGF/PlGF heterodimer	10,000 pg/mL	1,515	1,526	-0.1%
		9,968	10,061	-0.9%

4. Assay Reportable Range:
The reportable range for the sFlt-1/PlGF ratio is based on the analytical measuring range for the individual assays for sFlt-1 and PlGF. From the data collected during the clinical study (see section C.12), the range in sFlt-1/PlGF was found to be between 0.4 and 7367. The results of the individual assays for PlGF and sFlt-1 are not used for interpretation of results.

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

Metrological Traceability
The metrological traceability of the B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S™ sFlt-1 KRYPTOR run on the B·R·A·H·M·S KRYPTOR compact PLUS was established following the guidelines in EN ISO 17511:2020 In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples.

PlGF
The metrological traceability of the PlGF assay, through the calibrators was established using a commercially available reference preparation of PlGF that is considered by the sponsor as the highest reference for the assay. The sponsor's detailed metrological traceability was reviewed and found to be acceptable.

sFlt-1
The metrological traceability of the sFlt-1 assay, through the calibrators was established using a commercially available reference preparation of sFlt-1 that is considered by the sponsor as the highest reference for the assay. The sponsor's detailed metrological traceability was reviewed and found to be acceptable

sFlt-1/PlGF Ratio
Not applicable

Stability
In support of specimen storage stability, two studies were conducted.

1. *Specimen stability: One day stability at room temperature and 2-8 °C*

PlGF and sFlt-1
The product labeling describes that samples may be stored up to 1 day at room temperature and at 2 – 8°C. To validate sample specimen stability claims in the labeling, a short-term stability study was conducted with both serum and K2EDTA plasma. In the study, 25 fresh serum and 25 K2EDTA plasma samples collected from pregnant women

were stored under two conditions of 18-25°C (room temperature) and 2-8°C. For each storge condition, the samples were tested in singlicate at the following time points:

Fresh (within 3 hours of blood collection)
24 hours (± 4.5 hours from sample collection)
48 hours (± 3.5 hours from sample collection)

The data at time points 24 and 48 hours was analyzed for stability by comparison to the reference time point of fresh. The stability results showed that PlGF and sFlt-1 are stable in serum and K2EDTA plasma up to 48 hours at 2-8°C and 48 hours at room temperature, which supports the labeling claim of one day stability at room temperature and 2-8 °C.

2. *Specimen stability: Six month stability at -70°C*

PlGF and sFlt-1

To support the testing of clinical samples that were stored for up to six months in the PRAECIS clinical study, a separate stability study was conducted to establish that the storage conditions (-70°C) yield stable samples, and therefore the results of the clinical study are representative of intended use samples. In the study, 20 fresh serum and 20 K2EDTA plasma samples collected from pregnant women (representing the intended use population) were assayed for reference (time zero), and then stored at -70°C. The stored samples were tested in singlicate at the following time points: 6 months and 15 to 19 months (depending on the sample).

At each time point, each sample was tested in singlicate using one instrument and one reagent lot. The results for each sample were analyzed by linear regression across all three time points to derive an estimate percentage of change in concentration at month 6 versus time zero. A second analysis was conducted by directly calculating the percentage change in concentration between time zero and the different time points. The stability data showed that PlGF and sFlt-1 are stable in serum and EDTA plasma up to six months at -70°C which supports the use of the stored samples in the clinical study.

6. Detection Limit:

The detection capability of the B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S™ sFlt-1 KRYPTOR run on the B·R·A·H·M·S KRYPTOR compact PLUS was evaluated for limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) following the recommendations in CLSI EP17-A2.

PlGF

LoB

The LoB was evaluated using four zero-analyte samples. In the study, for each of three reagent lots, the four samples were each assayed in replicates of five per run over four runs with four different instruments per run for a total of 80 replicates per lot. The LoB was

analyzed for each lot by the non-parametric method of the CLSI guidance. The claimed LoB of the assay, selected from the highest value obtained across the three lots, is 2.5 pg/mL.

LoD
The LoD was evaluated using four pooled serum samples obtained from pregnant women representative of the intended use population with PlGF concentrations near the estimated detection limit. In the study, for each of three reagent lots, the four samples were each assayed in replicates of five per run over four runs with four different instruments per run for a total of 80 replicates per lot. The LoD was analyzed for each lot by the method of the CLSI guidance, whereby LoD is equal to LoB + Cp (SDL) where Cp is a multiplier to give the 95th percentile of the normal distribution and SDL is the pooled standard deviation of all low-level samples. The claimed LoD of the assay, selected from the highest value obtained across the three lots, is 4.9 pg/mL.

LoQ
The LoQ was evaluated using 9 pooled serum samples with PlGF concentrations ranging from 5.3 to 18 pg/mL. In the study, for each of two instruments with two separate reagent lots, the 9 samples were each assayed in replicates of 2 per run, one run per day over 9 days for a total of 18 measurements per sample per instrument/lot. Across each of the two instruments/lots, for each sample the within-laboratory CV% was calculated. The LoQ was analyzed per instrument/lot to be the lowest analyte concentration with a within-laboratory precision no greater than 20%. The claimed LoQ for PlGF was based on the highest value from the two instruments/lots, which as 7.6 pg/mL.

sFlt-1

LoB
The LoB was evaluated using four zero-analyte samples. In the study, for each of three reagent lots, the four samples were each assayed in replicates of five per run over three runs with three instruments over six days for a total of 60 replicates per lot. The LoB was analyzed for each lot by the non-parametric method of the CLSI guidance. The claimed LoB of the assay, selected from the highest value obtained across the three lots, is 17.8 pg/mL.

LoD
The LoD was evaluated using four samples comprised of pooled human serum or diluted human serum, and sFlt-1 concentrations near the estimated detection limit. In the study, for each of three reagent lots, the four samples were each assayed in replicates of five per run over three runs with three instruments over six days for a total of 60 replicates per lot. The LoD was analyzed for each lot by the method of the CLSI guidance, whereby equal to LoB + Cp (SDL) where Cp is a multiplier to give the 95th percentile of the normal distribution and SDL is the pooled standard deviation of all low-level samples. The claimed LoD of the assay, selected from the highest value obtained across the three lots, is 28.5 pg/mL.

LoQ
The LoQ was evaluated using five pooled normal human serum samples with sFlt-1 concentrations ranging from 12.6 to 47.2 pg/mL. In the study, for each of two instruments

with two separate reagent lots, the five samples were each assayed in replicates of 2 per run, one run per day over 7 days for a total of 14 measurements per sample per instrument/lot. Across each of the two instruments/lots, for each sample the within-laboratory CV% was calculated. The LoQ was analyzed per instrument/lot to be the lowest analyte concentration with a within-laboratory precision no greater than 20%. The claimed LoQ for PlGF was based on the highest value from the two instruments/lots, which as 29.4 pg/mL pg/mL.

The summary results for LoB, LoD and LoQ are shown below.

Analyte	LoB	LoD	LoQ	Claimed Measuring Range
PlGF (pg/mL)	2.5	4.9	7.6	7.6 to 7,000 pg/mL
sFlt-1 (pg/mL)	17.8	28.5	29.4	75.3 to 90,000 pg/mL

sFlt-1/PlGF Ratio
Not applicable

7. Assay Cut-Off:

   See Other Clinical Supportive Data in section C.12.

B Comparison Studies:

8. Method Comparison:

   Not applicable.

9. Matrix Comparison:
   A matrix equivalency study to serum was conducted to support use of the B·R·A·H·M·S PlGF plus KRYPTOR and B·R·A·H·M·S™ sFlt-1 KRYPTOR assays with K2EDTA plasma, as claimed in the product labeling.

   PlGF and sFlt-1
   In the study, the equivalence between serum and K2EDTA plasma samples was established by assaying 46 matched samples collected from pregnant women. Each sample was assayed in singlicate using one lot of reagent kit and one instrument on two runs over two days. The PlGF and sFlt-1 concentration data obtained from the serum and K2EDTA plasma samples were plotted and analyzed by Passing-Bablok regression analysis. The slope and intercept of the regression line were calculated, and summarized as follows:

Analyte	Range (serum)	Slope	Intercept	Correlation coefficient (Spearman)
PlGF	88 – 1757 pg/mL	0.943	7.0	0.976
sFlt-1	166 – 3400 pg/mL	0.971	31.0	0.974

The study results support the sponsor's claim that human serum and K2EDTA plasma are acceptable sample types to be used with the PlGF and sFlt-1 assays.

sFlt-1/PlGF Ratio
Not applicable.

C Clinical Studies:

10. Clinical Sensitivity:

See section C.12.

11. Clinical Specificity:

See section C.12.

12. Other Clinical Supportive Data:
The clinical performance of the B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System was evaluated in a clinical study titled *PRAECIS: Preeclampsia Risk Assessment: Evaluation of Cut-offs to Improve Stratification. Identification and Validation of a Cut-off for the Ratio of Soluble Fms-like Tyrosine Kinase-1 to Placental Growth Factor (sFlt-1/PlGF) to Stratify Risk in Pregnant Women with Hypertensive Disorders of Pregnancy.*

The study was a prospective, multicenter, blinded, non-interventional study conducted across 18 sites in the US. The purpose of the two-part study was the identification (part 1) and validation (part 2) of the cut-off for the ratio of sFlt-1 to PlGF used to prognose the risk of developing preeclampsia with severe features (sPE) in women hospitalized for a hypertensive disorder of pregnancy.

In the study (part 2), 520 women with singleton pregnancies between 23+0 and 34+6/7 weeks gestation hospitalized (or being hospitalized) for a hypertensive disorder of pregnancy were enrolled. Hypertensive disorders included: preeclampsia (PE), chronic hypertension (HTN) with or without superimposed PE or gestational hypertension, as defined by American College of Obstetricians and Gynecologists (ACOG) 2013 guidelines (Hypertension in Pregnancy. Obstet Gynecol. 2013 Nov;122(5):1122-31.) and the 2019 update (Chronic Hypertension in Pregnancy, OBSTETRICS & GYNECOLOGY, VOL. 133, NO. 1, JANUARY 2019). Women who met the criteria for sPE at the time of enrollment were excluded. Women that met the following criteria were also not eligible for the study: withdrew patient consent, multiple fetus pregnancy, outside the gestational age range, or delivered before specimens could be collected. Patients with multiple hospitalizations were not excluded; 34 patients were enrolled twice, one patient was enrolled three times. The study population comprised 556 enrollments of 520 patients, and with the following races:

Race	**% (n)**
American Indian or Alaskan Native	0.38 (2)
Asian	5.96 (31)

Black/African American	29.62 (154)
Hawaii or Other Pacific Islander	0.58 (3)
Other	4.23 (22)
Unknown or Undeclared	5.19 (27)
White/Caucasian	54.04 (281)

For each patient enrolled into the study, samples (serum and K2EDTA plasma) were collected for determination of sFlt-1 and PlGF levels at baseline, and derivation of the sFlt-1/PlGF ratio. The samples were stored frozen (-70°C) and sent to a central laboratory for later testing as a batch.

All women enrolled in the study were followed for two weeks or until delivery within two weeks. The development of sPE within a two-week window of enrollment was the study endpoint/clinical outcome. A central adjudication committee (panel of 3 independent obstetricians) was assembled to review data and arrive at consensus regarding each patient's diagnoses for the development of sPE following the 2013 ACOG clinical practice guidelines.

The interpretation of results of the subject device based on the sFlt-1/PlGF ratio determined in part 1 were as follows:

High risk if sFlt-1/PlGF ratio ≥ 40
Low risk if sFlt-1/PlGF ratio < 40

The severe preeclampsia status per adjudication panel and subject device serum results were analyzed based on the number of enrollments (n=556). The following is a summary of serum results.

		Severe preeclampsia status		
		Yes	No	Total
sFlt-1/PlGF	High risk	174 True positive	93 False positive	267
	Low risk	12 False negative	277 True negative	289
	Sum	186	370	556

Prevalence for developing sPE within two weeks in the clinical study = 33% (186/556).

Of the 556, results, there were 93 false positives and 12 false negatives.

The positive predictive value (PPV) and negative predictive value (NPV) are summarized below:

PPV (95% CI)	NPV (95% CI)
65% (59.3, 70.6)	96% (92.9, 97.6)

The positive likelihood ratio (PLR) and negative likelihood ratio (NLR) are summarized below:

PLR (95% CI)	NLR (95% CI)
3.72 (3.11, 4.46)	0.09 (0.05, 0.15)

The sensitivity and specificity are summarized below:

Sensitivity (95% CI)	Specificity (95% CI)
94% (89.1, 96.3)	75% (70.2, 79.0)

The clinical results were stratified into subgroups based on the four hypertensive disorders of pregnancy, gestation age (<30 weeks and ≥ 30 weeks), and maternal age (18-34 years and 35-50 years). There was no significant difference in clinical performance across the hypertensive disorders of pregnancy, gestation age, or maternal age.

There was no significant difference in clinical performance when testing K2EDTA plasma samples in the same study.

D Clinical Cut-Off:

See section C.12 above.

E Expected Values/Reference Range:

In a population of 166 healthy pregnant women between week 23+0 up to week 34+6 gestation, the sFlt-1/PlGF ratio median was established at 3.2, the 2.5th percentile at 0.8 and the 97.5% percentile at 31.

F Other Supportive Performance Characteristics Data:

Not applicable.

Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Training:

The sponsor's training program documentation was reviewed and found acceptable as part of risk management activities.

Identified Risks and Mitigations:

Risks to Health	Mitigation Measures
Incorrect performance of the test leading to false positive results.	Certain design verification and validation activities and documentation. Certain labeling information, including certain limiting statements and performance characteristics.
Incorrect interpretation of test results.	Certain design verification and validation activities, including certain licensed practitioner training as part of risk management activities. Certain labeling information, including certain limiting statements.

Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

Clinical benefit may be foreseen for the following reasons:

- A true positive test could help risk stratify a patient who may benefit from increased surveillance and preparedness for the possibility of developing preeclampsia with severe features (sPE).

- Because progression to sPE can occur rapidly, heightened level of suspicion with a positive test could aid in risk stratification that would allow for preparation and subsequent rapid intervention in the event that clinical circumstances dictate such intervention is necessary.

- True positive patients may benefit from increased frequency of monitoring of their condition by their healthcare provider and earlier step-up care (e.g., referral to higher level hospital with level III or IV NICU). Such advanced knowledge of risk could be especially helpful in rural or community settings where anticipation of clinical deterioration could help in planning for transfers to higher levels of care.

- With a higher level of suspicion, a clinician may keep a patient in the hospital for a longer period of observation, as a positive test could indicate a high risk to go on to sPE.

B Summary of the Assessment of Risk:

The probable risks associated with the use of the B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System are from undetected instances of false positive test results and/or misinterpretation.

Based on the reported clinical performance of the device in the clinical validation study, the positive predictive value is 65%, indicating that 35% of the time a patient with a positive result did not go on to develop preeclampsia with severe features, i.e., false positive result. The risk of

harm to the patient caused by a false positive result classifying the patient as high likelihood of progressing to sPE are due to inappropriate clinical decisions. Patients with a positive test result would be more closely monitored and preparation for possibility of progression to sPE could be initiated. However, no active intervention/treatment is to be undertaken based on a positive test result.

When used as intended, the device is a risk predictor for the future progression to sPE, and should not be misinterpreted to be used for the diagnosis of sPE, used for decisions of delivery or hospital discharge, or used for monitoring the patient's condition by repeated testing. The risk of harm to the patient caused by misinterpretation arises from appropriate clinical management interventions, such as health complications to the mother and baby, or lowering vigilant expectant management.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

The risks of false positive results are mitigated by the requirement of certain design verification and validation activities, including certain studies to ensure clinical and analytical performance. Certain labeling information, including limiting statements in the patient test reports and labeling, serve to reduce the risk of false positive results and/or incorrect interpretation of results.

When used as intended, general controls are insufficient, but in light of the special controls, the probable benefits of this device outweigh the probable risks of this device.

VII Conclusion:

The De Novo request is granted, and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: QWH

Device Type: Prognostic test for development or progression of preeclampsia

Device Class: Class II with special controls.

Regulation: 21 CFR 862.1602