February 7, 2025

Roche Diagnostics
Jane Phillips
Senior Regulatory Manager
9115 Hague Road
Indianapolis, Indiana 46250

Re: K241453
Trade/Device Name: Elecsys sFlt-1 and Elecsys PlGF
Regulation Number: 21 CFR 862.1602
Regulation Name: Prognostic Test for Development or Progression of Preeclampsia
Regulatory Class: Class II
Product Code: QWH
Dated: January 2, 2025
Received: January 6, 2025

Dear Jane Phillips:

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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,

Joseph A. Kotarek -S

Digitally signed by Joseph A. Kotarek -S
Date: 2025.02.07 14:26:55 -05'00'

Joseph Kotarek
Branch Chief
Division of Chemistry and Toxicology 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: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
k241453

Device Name
Elecsys sFlt-1 and Elecsys PlGF

Indications for Use *(Describe)*
Immunoassays for the in vitro quantitative determination of the soluble fms-like tyrosine kinase-1/placental growth factor (sFlt-1/PlGF) ratio in human serum.
The sFlt-1/PlGF ratio is indicated as an aid in the risk assessment of pregnant women, with a singleton pregnancy (23+0 to 34+6/7 weeks' gestation) hospitalized for hypertensive disorders of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension), to develop preeclampsia with severe features within two weeks from testing. The sFlt-1/PlGF ratio should be used in conjunction with clinical assessment and routine laboratory testing.
The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.

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)

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K241453 Elecsys sFlt-1 and Elecsys PlGF
510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Diagnostics
Address	9115 Hague Road P.O. Box 50416 Indianapolis, IN 46250-0457
Contact	Dr. Jane Phillips Phone: (317) 521-3338 Email: jane.phillips@roche.com
Date Prepared	February 2nd, 2025
Proprietary Name	Elecsys sFlt-1 and Elecsys PlGF
Common Name	Elecsys PE Assays
Classification Name	Prognostic test for development or progression of preeclampsia
Product Codes, Regulation Numbers	QWH, 21CFR862.1602
Predicate Devices	B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System (DEN220027)
Establishment Registration	Roche Diagnostics GmbH Mannheim, Germany: 9610126 Roche Diagnostics GmbH Penzberg, Germany: 9610529 Roche Diagnostics Indianapolis, IN United States: 1823260

1. DEVICE DESCRIPTION

1.1. Elecsys sFlt-1

The Elecsys sFlt-1 employs a sandwich principle. The total duration of the assay is 18 minutes.

1st incubation: 20 µL of sample, a biotinylated monoclonal sFlt-1 specific antibody, and a monoclonal sFlt-1 specific antibody labeled with a ruthenium complex (Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$) react to form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

The Elecsys sFlt-1 reagents “M”, “R1” and “R2” are combined in the so-called “rackpack”, a bundle of the three reagent bottles, which is placed on the instrument as a single unit. The reagent rackpack is labeled as SFLT-1.

- M: Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1: Anti-sFlt-1-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated monoclonal anti sFlt-1 antibody (mouse) 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.
- R2: Anti-sFlt-1-Ab~Ru(bpy) (black cap), 1 bottle, 9 mL: Monoclonal anti sFlt-1 antibody (mouse) labeled with ruthenium complex 1.0 mg/L, biotin scavenger antibody 1.0 mg/mL; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Elecsys sFlt-1 will be marketed with the following devices:

- CalSet sFlt-1; secondary, calibrator; JIT, 862.1150 – Class II, exempt
- Elecsys PreciControl Multimarker (cleared in K102157); multi-analyte controls, all kinds (assayed); JJY, 862.1660 – Class I, exempt

1.2. Elecsys PlGF

The Elecsys PlGF employs a sandwich principle. The total duration of the assay is 18 minutes.

1st incubation: 50 µL of sample, a biotinylated monoclonal PlGF specific antibody and a monoclonal PlGF specific antibody labeled with a ruthenium complex (Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)) react to form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

The Elecsys PlGF reagents “M”, “R1” and “R2” are combined in the so-called “rackpack”, a bundle of the three reagent bottles, which is placed on the instrument as a single unit. The reagent rackpack is labeled as PLGF.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-PlGF-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti PlGF antibody (mouse) 0.6 mg/L; phosphate buffer 50 mmol/L, pH 6.0; preservative.

- R2 Anti-PlGF-Ab~Ru(bpy) (black cap), 1 bottle, 8 mL: Monoclonal anti PlGF antibody (mouse) labeled with ruthenium complex 4.0 mg/L, biotin scavenger antibody 1.0 mg/mL; phosphate buffer 50 mmol/L, pH 6.0; preservative.

Elecsys PlGF will be marketed with the following devices:

- CalSet PlGF; secondary, calibrator; JIT, 862.1150 – Class II, exempt
- PreciControl Multimarker (cleared in K102157); multi-analyte controls, all kinds (assayed); JJY, 862.1660 – Class I, exempt

Both assays contain a blocking agent for exogenous biotin from supplements.

2. INDICATIONS FOR USE

Immunoassays for the in vitro quantitative determination of the soluble fms like tyrosine kinase-1/placental growth factor (sFlt-1/PlGF) ratio in human serum.

The sFlt-1/PlGF ratio is indicated as an aid in the risk assessment of pregnant women, with a singleton pregnancy (23+0 to 34+6/7 weeks' gestation) hospitalized for hypertensive disorders of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension), to develop preeclampsia with severe features within two weeks from testing. The sFlt-1/PlGF ratio should be used in conjunction with clinical assessment and routine laboratory testing.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

3. IMMUNOASSAY ANALYZERS.TECHNOLOGICAL CHARACTERISTICS

The following tables compare the Elecsys sFlt-1 and PlGF with its predicate device, B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System (DEN220027).

Table 1: Technical Characteristics Comparison Table between Elecsys sFlt-1 and PlGF and B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System

Feature	Candidate Device Elecsys sFlt-1 and PlGF	Predicate Device B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System DEN220027
Intended Use	Immunoassays for the in vitro quantitative determination of the soluble fms like tyrosine kinase-1/placental growth factor (sFlt-1/PlGF) ratio in human serum. The sFlt-1/PlGF ratio is indicated as an aid in the risk assessment of pregnant women, with a singleton pregnancy (23+0 to 34+6/7 weeks' gestation) hospitalized for hypertensive disorders of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension), to develop preeclampsia with severe features within two weeks from testing. The sFlt-1/PlGF ratio should be used in conjunction with clinical assessment and routine laboratory testing. The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.	The B·R·A·H·M·STM 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+6/7 weeks) hospitalized for hypertensive disorders of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension) for progression to pre-eclampsia with severe features (as defined by American College of Obstetricians and Gynecologists guidelines) within 2 weeks of presentation.
Assay Method	Sandwich immunoassay	Same
Detection Method	Electrochemiluminescence "ECLIA"	Time-Resolved Amplified Cryptate Emission (TRACETM) technology
Applications/Test Time	18 minute	9 minute
Sample Type/Matrix	Human serum	Human serum/plasma
Sample Anticoagulants	N/A	K_2-EDTA
Handling of R1 and R2	Liquid, ready to use	Same
Buffer composition R1	Phosphate buffer	Bovine albumin
Calibrator	sFlt-1 CalSet and PlGF CalSet	B·R·A·H·M·S sFlt-1 KRYPTOR CAL and B·R·A·H·M·S PlGF KRYPTOR CAL
Calibration Method	2-point calibration	Same
Calibration Interval	Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Calibration interval may be extended based on acceptable verification of calibration by the laboratory. Renewed calibration is recommended as follows: • After 28 days using the same reagent lot • After 7 days (when using the same reagent kit on the analytzer) • As required: e.g. quality control findings	Calibration must be carried out before the first use of each new B·R·A·H·M·S sFlt-1 and PlGF KRYPTOR lot, then repeated on a regular basis automatically managed by the B·R·A·H·M·S KRYPTOR analyzer in order to readjust the standard curve. Controls must be run after each calibration.

	outside the defined limits	
Controls	PreciControl Multimarker	B·R·A·H·M·S sFlt-1 KRYPTOR QC kit and B·R·A·H·M·S PlGF KRYPTOR QC kit
Traceability/Standardization	Elecsys sFlt-1 assay: This method has been standardized against the Quantikine ELISA Human VEGFR1/Flt-1 Immunoassay. ▪ Elecsys PlGF assay: This method has been standardized against the Quantikine ELISA Human PlGF Immunoassay.	An international PlGF reference preparation is not available. Therefore, the assay is calibrated using a reference preparation of recombinant human PlGF as the "Highest Available Standard". This calibration is verified on a regular basis by regression analysis and comparison to theoretically expected results. The PlGF assay results are given in pg/mL.
Reagent Stability	Reagent stability for on analyzer is now 28 days.	• unopened at 2-8 °C: up to the stated expiration date • after opening at 2-8 °C: 12 weeks • on analyzers: 8 weeks
Measuring Range	sFlt-1 80-85000 pg/mL PlGF 10-5400 pg/mL	sFlt-1 315-90,000 pg/mL PlGF 7.6 – 4,000 pg/mL
Biotin Tolerance	Up to 1200 ng/mL	N/A

4. NON-CLINICAL PERFORMANCE EVALUATION

Non-clinical performance evaluation for Elecsys sFlt-1 and Elecsys PlGF assay executed with the study briefly summarized.

4.1. Precision

Precision measurements were conducted to evaluate repeatability (within-run precision) and intermediate precision (within-laboratory precision) according the CLSI guideline EP05-A3. Precision of the Elecsys sFlt-1 and PlGF assays was evaluated on a single **cobas e** 411 immunoassay analyzer according to CLSI EP5-A3 guideline. One reagent lot was evaluated. The precision study was conducted using the study design of testing 2 aliquots of each control

(PC (=PreciControl) Multimarker) and human sera (HS) in single determinations per run, 2 runs per day for 21 days. Assay calibration was performed as specified in the package insert. The samples were run in randomized order on the **cobas e** 411 analyzer.

Table 2: Summary of 21-Day Precision for Elecsys PlGF on the cobas e 411 analyzer

Sample	Mean	Repeatability		Intermediate precision		n
	pg/mL	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	
Human serum 1	46.3	1.03	2.2	1.51	3.3	84
Human serum 2	89.9	1.18	1.3	2.03	2.3	84
Human serum 3	441	4.46	1.0	7.27	1.7	84
Human serum 4	11.8	0.670	5.7	1.17	9.9	83
Human serum 5	74.0	1.87	2.5	4.11	5.6	84
Human serum 6	39.4	1.06	2.7	2.09	5.3	84
Human serum 7	96.0	1.58	1.6	2.38	2.5	84
Human serum 8	74.7	1.34	1.8	2.28	3.1	84
Human serum 9	4923	78.4	1.6	116	2.4	84
Human serum 10	8894	141	1.6	178	2.0	84
PC[a)] Multimarker 1	96.9	1.47	1.5	2.26	2.3	84
PC Multimarker 2	950	8.68	0,9	17.1	1.8	84

a) PC = PreciControl

Table 2: Summary of 21-Day Precision for Elecsys sFlt-1 on the cobas e 411 analyzer

Sample	Mean	Repeatability		Intermediate precision		n
	pg/mL	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	
Human serum 1	1690	21.1	1.2	28.2	1.7	84
Human serum 2	632	6.45	1.0	11.1	1.8	84
Human serum 3	3867	43.0	1.1	74.1	1.9	84
Human serum 4	89.3	2.02	2.3	3.15	3.5	83
Human serum 5	36992	682	1.8	1287	3.5	84
Human serum 6	76873	1870	2.4	2842	3.7	84
Human serum 7	18037	246	1.4	405	2.2	84
Human serum 8	2522	28.6	1.1	46.0	1.8	84
Human serum 9	78.6	1.85	2.3	2.77	3.5	84
Human serum 10	90.7	2.02	2.2	3.32	3.7	84
PC[a)] Multimarker 1	106	2.13	2.0	2.98	2.8	84
PC Multimarker 2	1044	9.46	0.9	17.3	1.7	84

Table 3: 21-Day Precision Results Ratio Summary

Sample	Mean	Repeatability		Intermediate precision		n
	Ratio	SD	CV (%)	SD	CV (%)	
Human serum 1	37	0.736	2.0	0.985	2.7	84
Human serum 2	7	0.118	1.7	0.133	1.9	84
Human serum 3	9	0.094	1.1	0.123	1.4	84
Human serum 4	8	0.369	4.9	0.528	7.0	83
Human serum 5	501	17.9	3.6	27.4	5.5	84
Human serum 6	1957	71.8	3.7	101	5.1	84
Human serum 7	188	2.95	1.6	3.92	2.1	84
Human serum 8	34	0.497	1.5	0.798	2.4	84
Human serum 9	0.016	0.0005	2.9	0.0005	3.4	84
Human serum 10	0.01	0.0003	2.5	0.0004	3.7	84
PC Multimarker 1	1	0.025	2.3	0.026	2.3	84
PC Multimarker 2	1	0.015	1.4	0.017	1.5	84

4.2. Linearity/Assay Reportable Range

The linearity study was conducted with the Elecsys sFlt-1 and PlGF assays on the **cobas e** 411 analyzer to demonstrate that measurements across the claimed measuring range for each parameter are linear. This was done according to CLSI EP06-Ed2, Study Design B. The linearity study was performed with more than two sets of panel members with each set partially covering the proposed linearity interval. The main reason for this study design is the large measuring range and limitations in the number of dilutions of each high sample. The concentration ranges of the diluted samples cover the entire measuring range of the assays. Samples were assayed in 4-fold determination within a single run, on one instrument. SD and CVs were calculated for each 4-fold determination.

Reportable ranges were established as:

- Elecsys sFlt-1 is 80-85000 pg/mL.
- Elecsys PlGF is 10-5400 pg/mL.

Attention: For the Elecsys sFlt-1 assay, please use the PreciControl Multimarker controls with the lowest control level for sFlt-1 being approximately 100 pg/mL.

4.3. Analytical Sensitivity

4.3.1. Limit of Blank (LoB)

LoB of the Elecsys sFlt-1 and PlGF assays on the **cobas e** 411 analyzer was determined according to CLSI EP17-A2. Limit of Blank determines the highest observed measurement values for samples free of analyte. The Limit of Blank was determined as the 95th percentile of measurements of blank samples. The zero-level (blank) sample used was a human serum sample from pregnant female diluted with horse serum.

In total 60 determinations for analyte free samples have been obtained. Limit of Blank determines the highest observed measurement values for samples free of analyte. The Limit of Blank was determined as the 95^{th} percentile of measurements of blank samples.

Table 4. LoB for PlGF

Reagent lot	LoB (pg/mL)
1	0.732
2	0.946
3	0.482

All lots met the predetermined acceptance criterion. The LoB claim in the labeling will be set to ≤ 2 pg/mL.

Table 5. LoB for sFLT-1

Reagent lot	LoB (pg/mL)
1	4.00
2	3.51
3	2.92

All lots met the predetermined acceptance criterion. The LoB claim in the labeling will be set to ≤ 6 pg/mL.

4.3.2. Limit of Detection (LoD)

LoD of the Elecsys sFlt-1 and PlGF assays on the **cobas e** 411 analyzer was determined according to CLSI EP17-A2. The LoD determines the lower limit for samples with analyte. The LoD was determined as the lowest amount of analyte in a sample that can be detected with a 95% probability.

Experimental Design:

- Three reagent lots
- One **cobas e** 411 analyzer
- Six runs on ≥ three days
- Two replicates per sample per run
- Five samples
- 60 replicates per sample per reagent lot

Five low-analyte serum samples (non-pregnant female from single donor) were measured in two-fold determination, six runs over at least three days. In total, 60 determinations per reagent lot were performed. A pooled estimate of the precision (SD total) for the 5 low level samples was calculated. The LoD was established according to the following EP17-A2 calculation:

LoD = LoB + 1.653 x SD total (of low analyte samples)

Table 6. LoD for PlGF

Reagent Lot	LoD (pg/mL)
1	2.19
2	2.19
3	1.65

All lots met the predetermined acceptance criterion. The LoD claim in the labeling will be set to ≤ 3 pg/mL.

Table 7. LoD for sFLT-1

Reagent Lot	LoD (pg/mL)
1	6.03
2	4.95
3	4.44

All lots met the predetermined acceptance criterion. The LoD claim in the labeling will be set to ≤ 10 pg/mL.

4.3.3. Limit of Quantitation (LoQ)

The LoQ of the Elecsys sFlt-1 and PlGF assays was determined on the **cobas e** 411 analyzer according to CLSI Guideline EP17-A2. LoQ determines the lowest amount of analyte that can be quantitatively determined with stated accuracy and stated experimental conditions. The LoQ was determined as the lowest concentration of analyte which can be quantified with an intermediate precision CV of no more than 20%.

Experimental Design:

- Three reagent lots
- One **cobas e** 411 analyzer
- Five days
- One run per day
- Five replicates per sample per run
- ≥ four low level serum samples
- 20 replicates per sample per reagent lot

A set of four human serum samples (non-pregnant female or pregnant female from single donor diluted with horse serum) with concentrations in the specified LoQ-area were evaluated. The mean value and the intermediate precision (CV) and standard deviation (SD) for each LoQ sample were calculated.

LoQ samples were sorted according to the concentration of their measured mean value. LoQ is defined as the mean value of that sample which is the first that fulfills the specification for the intermediate precision and for which no sample with higher concentration exists that exceeds this specification. Data are summarized in a table and a graphic is generated plotting the measured mean values versus the intermediate precision (CV) of the LoQ samples.

Table 8. LoQ for PlGF

Reagent Lot	LoQ (pg/mL)
1	8.97
2	7.77
3	7.48

All lots met the predetermined acceptance criterion. The LoQ claim in the labeling will be set to ≤ 10 pg/mL.

Table 9. LoQ for sFLT-1

Reagent Lot	LoQ (pg/mL)
1	10.2
2	12.6
3	11.8

All lots met the predetermined acceptance criterion. The LoQ claim in the labeling will be set to ≤ 15 pg/mL.

4.4. High-Dose Hook Effect

The high-dose hook effect (HDHE) of the Elecsys sFlt-1 and PlGF assays was assessed on one **cobas e** 411 analyzer using a dilution series.

There is no high-dose hook effect at sFlt-1 concentrations up to 200000 pg/mL.
There is no high-dose hook effect at PlGF concentrations up to 100000 pg/mL.

4.5. Human Anti-Mouse Antibodies (HAMA)

The effect of the presence of human anti-mouse antibodies (HAMA) on the Elecsys sFlt-1 and Elecsys PlGF assays was assessed on one **cobas e** 411 analyzer. The differentiation between HAMA- negative and HAMA-positive samples was assessed.

Heterophilic human anti-mouse antibodies (HAMA) interference was tested up to a HAMA concentration of 81 μg/L. No HAMA interference for Elecsys PlGF was found. For samples with sFlt-1 concentrations ⩽ 2190 pg/mL, HAMA was shown to interfere, resulting in sFlt-1/PlGF ratios with ⩾10% positive bias”

4.6. Interferences

For each interfering substance four human serum samples with four different concentrations of PlGF and sFLT-1 were tested.

Biotin:

One aliquot of each serum sample was spiked with the interfering substance; another aliquot was spiked with the same volume of isotonic NaCl solution (dilution pool). The interfering pool was then diluted into the dilution pool in 11 increments. The recovery for each sample was calculated by comparison to the reference (unspiked) sample.

Lipemia (Intralipid):

One aliquot of each serum sample was spiked with the interfering substance; another aliquot was spiked with the same volume of isotonic NaCl solution (dilution pool). The interfering pool was then diluted into the dilution pool in 10% increments. The recovery for each sample was calculated by comparison to the reference (unspiked) sample.

Hemoglobin:

Fresh hemolysate was prepared from fresh EDTA plasma. One aliquot of each serum sample was spiked with the hemolysate (interfering pool), another aliquot was spiked with the same volume of isotonic NaCl solution (dilution pool). The interfering pool was then diluted into the dilution pool in 10% increments. The recovery for each sample was calculated by comparison to the reference (unspiked) sample.

Bilirubin (90 % unconjugated / 10 % conjugated bilirubin):

One aliquot of each serum sample was spiked with the interfering substance, and another aliquot was spiked with the same volume of the solvent (0.1 mol/L NaOH) of the interfering substance

(dilution pool). The interfering pool was then diluted into the dilution pool in 10% increments. The recovery for each sample was calculated by comparison to the reference (unspiked) sample.

Rheumatoid Factor (RF):

One aliquot of each serum sample was spiked with the interfering substance, and another aliquot with the same volume of the solvent (buffer matrix) of the interfering substance (dilution pool). The interfering pool was then diluted into the dilution pool in 6 increments 6 dilution steps were prepared respectively 0, 10, 30, 50, 60 and 100%. The recovery for each sample was calculated by comparison to the reference sample.

Table 10: Interferences

Compound	Highest concentration tested
Bilirubin	451.4 µmol/L µmol/L or 26.4 mg/dL
Hemoglobin	0.621 mmol/L or 1000 mg/dL
Intralipid	2000 mg/dL
Rheumatoid factors	1200 IU/mL
Biotin	1200 ng/mL

Bilirubin concentrations at >26.4 mg/dL in the samples can result in an up to 10 % decrease of the sFlt-1/PlGF ratio around the cutoff of 38 as shown in a spiking experiment.

Heterophilic human anti-mouse antibodies (HAMA) in human specimens can react with reagent antibodies, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products for diagnosis or therapies can be prone to this interference and anomalous values may be observed. Specimens from patients who have received mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies and may interfere in assays that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design. sFlt-1 and PlGF values determined on patient samples using other manufacturers' assays

may result in significantly different ratios, which could lead to wrong diagnostic conclusions. Therefore, only the Elecsys sFlt-1 and Elecsys PlGF assays should be used to calculate the ratio.

4.7. Analytical Specificity/Cross-Reactivity

A cross-reactivity study was conducted with Elecsys sFlt-1 and PlGF assays on the **cobas e** 411 analyzer to evaluate the potential cross-reacting compounds using human serum samples. Samples were measured in the presence and absence of the potential cross-reactants and cross-reactivity was calculated with one lot of reagent.

Elecsys sFlt-1 assay
The monoclonal antibodies used are highly specific against human sFlt-1. The following cross-reactivities were found in samples with sFlt-1 levels between 1300 and 9000 pg/mL.

Table 11. sFlt-1 Cross Reactivity

Substance	Concentration tested (pg/mL)	Cross-reactivity (%)
VEGFR2	11000000	< 0.01 %
VEGFR3	1430000	< 0.01 %
VEGF-B	10000	< 2.1 %

Elecsys PIGF assay
The monoclonal antibodies used are highly specific against human PIGF. The following cross-reactivities were found in samples with PIGF levels between 65 and 180 pg/mL.

Table 12. PlGF Cross Reactivity

Substance	Concentration tested (pg/mL)	Cross-reactivity (%)
VEGF-B	10000	< 0.1 %
VEGF 165	10000	< 0.2 %
VEGF/PIGF-1	10000	< 0.9 %

Recombinant PlGF-2 protein shows up to 42 % cross-reactivity and interferes with the Elecsys PlGF assay (please see section on Precautions and warnings).

Limitations - interference

4.8. Pharmaceutical substances Exogenous Interferences – Drugs

Elecsys sFlt-1 and Elecsys PIGF assays

In vitro tests were performed on 15 commonly used pharmaceuticals. No interference with the Elecsys sFlt-1 and Elecsys PIGF assays was found.

Table 13. Common Drugs Tested

Compound	Concentration (mg/L)
Acetaminophen	260
Acetylcysteine	150
Acetylsalicylic acid	1000
Ampicillin-Na	1000
Ascorbic acid	300
Cefoxitin	2500
Cyclosporine	5
Doxycycline	50
Heparin	5000 U/L (sFlt-1) 500 U/L (PIGF)*
Ibuprofen	500
Levodopa	20
Metronidazole	200
Phenylbutazone	400
Rifampicin	60
Theophylline	100

*Heparin interference with Elecsys PIGF was found for heparin concentrations > 500 U/L (see section Precautions and warnings).

13 additional pharmaceuticals and substances were tested with the Elecsys sFlt-1 and Elecsys PlGF assays. No interference with the Elecsys sFlt-1 and Elecsys PIGF assays was found.

Table 14. Additional Substances Tested

Compound	**Concentration (mg/L)**
Albumin	60000
Caffeine	59
Calcium	200
Dihydralazin	200
Ethanol	5 % (v/v)
Folic Acid	2.4
Gentamicin	10
Iron	72
Labetalol	1.5
Magnesium Sulfate	252
Methyldopa	7.2
Metroprolol	5
Nifedipine	0.6

5. CLINICAL PERFORMANCE EVALUATION

Praecis "Preeclampsia Risk Assessment: Evaluation of Cut-offs to Improve Stratification"

PRAECIS is a validation of the Roche sFlt-1/PlGF ratio cut-off of 38 as an aid in the risk assessment of pregnant women with a singleton pregnancy (23+0 to 34+6/7 weeks' gestation) with a hypertensive disorder of pregnancy to develop preeclampsia with severe features within two weeks from testing.

Study population included pregnant women (≥18 years of age) and gestational age 23+0 to 34+6/7 weeks who are admitted to the hospital with (or develop while hospitalized) a hypertensive disorder of pregnancy (preeclampsia, chronic hypertension with or without superimposed preeclampsia, or gestational hypertension).

Clinical Performance criteria of NPV and PPV were met for sFlt-1/PlGF ratio at a cutoff of 38 for the Intended Use and the Intended Use (Study Target) population. The primary study objective was fulfilled.

Table 15: Performance of the sFLT-1 and PlGF Assays in the Intended Use Population

Assay	N of sPE a)	N of non-sPE	Total N	Prevalence (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV b) (%) (95% CI)
ratio > 38	170	84	254	33.45	91.40 (86.41, 95.00)	77.30 (72.68, 81.47)	94.70 (91.54, 96.94)
ratio ≤ 38	16	286	302				
Total	186	370	556				

Assay	N of sPE	N of non-sPE	Total N	PPV c) (%) (95% CI)	Accuracy (%) (95% CI)	LR- d) (95% CI)	LR+ e) (95% CI)
ratio > 38	170	84	254	66.93 (60.77, 72.68)	82.01 (78.56, 85.12)	0.11 (0.07, 0.18)	4.03 (3.32, 4.88)
ratio ≤ 38	16	286	302				
Total	186	370	556				

a) sPE = preeclampsia with severe features

b) NPV = negative predictive value

c) PPV = positive predictive value

d) LR- = negative likelihood ratio

e) LR+ = positive likihood ratio

Table 16: Impact of hypertensive disorder at presentation on the prognostic performance of Roche Elecsys sFlt-1/PlGF ratio at cutoff of 38

Hypertensive disorders	**n**	**Sensitivity (%) (95% CI)**	**Specificity (%) (95% CI)**	**PPV (%) (95% CI)**	**NPV (%) (95% CI)**
Preeclampsia	142	95.06 (87.84, 98.64)	72.13 (59.17, 82.85)	81.91 (72.63, 89.10)	91.67 (80.02, 97.68)
Superimposed Preeclampsia	68	93.75 (79.19, 99.23)	55.56 (38.10, 72.06)	65.22 (49.75, 78.65)	90.91 (70.84, 98.88)

Hypertensive disorders	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Chronic Hypertension	232	87.76 (75.23, 95.37)	82.51 (76.22, 87.72)	57.33 (45.38, 68.69)	96.18 (91.87, 98.58)
Gestational Hypertension	114	83.33 (62.62, 95.26)	78.89 (69.01, 86.79)	51.28 (34.78, 67.58)	94.67 (86.90, 98.53)

Table 17: Impact of maternal age at presentation on the prognostic performance of Roche Elecsys sFlt-1/PlGF ratio at cutoff of 38

Maternal age	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
< 35 years	370	90.91 (84.66, 95.21)	81.51 (75.99, 86.23)	73.17 (65.70, 79.78)	94.17 (90.05, 96.95)
≥ 35 years	186	92.59 (82.11, 97.94)	69.70 (61.10, 77.39)	55.56 (44.70, 66.04)	95.83 (89.67, 98.85)

Table 18: Impact of gestational age at presentation on the prognostic performance of of Roche Elecsys sFlt-1/PlGF ratio at cutoff of 38

Gestational age	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
< 30 weeks	188	100.00 (95.14, 100.00)	81.58 (73.23, 88.22)	77.89 (68.22, 85.77)	100.00 (96.11, 100.00)
≥ 30 weeks	368	85.71 (77.84, 91.61)	75.39 (69.64, 80.54)	60.38 (52.33, 68.04)	92.34 (87.87, 95.56)

6. REFERENCE RANGE STUDY

Expected values

Roche completed a study to collect samples from apparently healthy pregnant women in the US, to test them for sFlt-1 and PlGF to calculate normal reference ranges. Subjects were enrolled at 9 sites across the US. Women enrolled were representative of the US population in terms of race and ethnicity and were currently within gestational weeks 23 weeks + 0 days - 34 weeks + 6 days. Within the gestational time ranges there were 380 evaluable subjects with 78 % Caucasian, 14 % African American, 6 % Asian and 2 % other.[21] In a population of 380 healthy pregnant women between week 23+0 up to week 34+6, the sFlt-1/PlGF ratio median was established at 3.21, the 2.5th percentile at 0.857 and the 97.5th percentile at 13.5.

Expected values in the healthy population are for information only, the clinical application of the sFlt-1/PlGF ratio in the intended use population is outlined in the section titled “Interpretation of the sFlt-1/PlGF ratio” above.

7. CONCLUSIONS

The information provided in this 510(k) Premarket Notification supports the determination that the Elecsys sFlt-1 and PlGF assay is substantially equivalent to the predicate device, B·R·A·H·M·S sFlt-1/ PlGF KRYPTOR Test System (DEN220027).