March 30, 2023

Roche Diagnostics
Dr. Leslie Patterson
Regulatory Affairs Program Manager
9115 Hague Road
Indianapolis, IN 46250

Re: K230161
Trade/Device Name: ONLINE TDM Phenytoin - Free Phenytoin application
Regulation Number: 21 CFR 862.3350
Regulation Name: Diphenylhydantoin test system
Regulatory Class: Class II
Product Code: MOJ
Dated: January 19, 2023
Received: January 20, 2023

Dear Dr. Leslie Patterson:

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,

Paula V. Caposino -S
Digitally signed by Paula V. Caposino -S
Date: 2023.03.30 18:04:32 -04'00'

Paula Caposino, Ph.D.
Acting Deputy Division Director
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: 06/30/2023
See PRA Statement below.

Submission Number *(if known)*

K230161

Device Name

ONLINE TDM Phenytoin - Free Phenytoin application

Indications for Use *(Describe)*

ONLINE TDM Phenytoin - Free Phenytoin application is an in vitro test for the quantitative determination of free phenytoin in human serum and plasma on cobas c systems. The determination of free phenytoin is used in monitoring levels of free phenytoin to ensure appropriate therapy.

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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ONLINE TDM Phenytoin - Free Phenytoin application
K230161 - 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	Leslie Patterson Phone: (317) 225-8563 Email: leslie.patterson@roche.com
Date Prepared	March 21, 2023
Proprietary Name	ONLINE TDM Phenytoin - Free Phenytoin application
Common Name	Free Phenytoin application
Classification Name	Diphenylhydantoin test system
Product Codes, Regulation Numbers	MOJ, 862.3350
Predicate Devices	Reagent Application for Free Phenytoin (K952555)
Establishment Registration	Roche Diagnostics GmbH Mannheim, Germany: 9610126 Roche Diagnostics GmBH Penzberg, Germany: 9610529 Roche Diagnostics Indianapolis, IN United States: 1823260

1. DEVICE DESCRIPTION

The ONLINE TDM Phenytoin - Free Phenytoin application is an in vitro test for the quantitative determination of free phenytoin in human serum and plasma on **cobas c** systems. The determination of free phenytoin is used in monitoring levels of free phenytoin to ensure appropriate therapy.

Prior to measurement using the ONLINE TDM Phenytoin - Free Phenytoin application, the sample is processed by ultrafiltration to remove the bound phenytoin generating a result for free phenytoin.

The ONLINE TDM Phenytoin - Free Phenytoin application is based on the kinetic interaction of microparticles in a solution (KIMS). Phenytoin antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions, photometrically detected by turbidity measurements is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of phenytoin in the sample. A competitive reaction takes place between the drug conjugate and phenytoin in the serum sample for binding to the phenytoin antibody on the microparticles. The resulting turbidity is indirectly proportional to the amount of drug present in the sample.

1.1. Reagents - working solutions

R1: Phenytoin conjugate, 1.0 µg/mL; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer, pH 7.3; stabilizer; preservative

R2: Anti-phenytoin antibody (mouse monoclonal); latex microparticle, 0.003 % (w/w); 3-(N-morpholino) propane sulfonic acid (MOPS) buffer, pH 7.4; stabilizer; preservative

2. INDICATIONS FOR USE

The ONLINE TDM Phenytoin - Free Phenytoin application is an in vitro test for the quantitative determination of free phenytoin in human serum and plasma on **cobas c** systems. The determination of free phenytoin is used in monitoring levels of free phenytoin to ensure appropriate therapy.

3. TECHNOLOGICAL CHARACTERISTICS

The following table compares the ONLINE TDM Phenytoin - Free Phenytoin application on **cobas c** 503 with its predicate device, COBAS-FP Reagents for Free Phenytoin on COBAS FARA II chemistry system (K952555).

Table 1: ONLINE TDM Phenytoin - Free Phenytoin application Technical Characteristics

	Candidate Device: ONLINE TDM Phenytoin - Free Phenytoin application	**Predicate Device: COBAS-FP Free Phenytoin**
Intended Use / Indications for Use	The ONLINE TDM Phenytoin - Free Phenytoin application is an in vitro test for the quantitative determination of free phenytoin in human serum and plasma on **cobas c** systems. The determination of free phenytoin is used in monitoring levels of free phenytoin to ensure appropriate therapy.	The COBAS-FP Reagents for Free Phenytoin are an in vitro test for the quantitative determination of free phenytoin in serum and heparinized plasma on the COBAS FARA II chemistry system.
Assay Method	Kinetic Interaction of Microparticles in a Solution (KIMS)	Fluorescence Polarization Immunoassay (FPIA)
Sample Type/Matrix	Serum and Lithium heparin, K2-EDTA, K3-EDTA plasma	Serum and heparinized plasma
Calibrator	Preciset TDM I calibrators (diluted)	COBAS FP Free Phenytoin Calibrators
Controls	TDM Control Set (Level I and II, ultrafiltrated)	COBAS FP Free Phenytoin Controls
Measuring Range	0.400-4.00 µg/mL	0.09-4 µg/mL
Lower Limits of Measurement	LoB (Limit of Blank) = 0.100 µg/mL (0.396 µmol/L) LoD (Limit of Detection) = 0.200 µg/mL (0.792 µmol/L) LoQ (Limit of Quantitation) = 0.400 µg/mL (1.58 µmol/L)	Sensitivity (Analytical): 0.09 µg/mL

4. NON-CLINICAL PERFORMANCE EVALUATION

Performance characteristics were evaluated with ONLINE TDM Phenytoin - Free Phenytoin application on **cobas c** 503 and are briefly summarized below.

All acceptance criteria were met.

4.1. Precision

4.1.1. Repeatability and Intermediate Precision

Precision was determined in accordance with the CLSI EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer. The results are summarized below. All acceptance criteria were met.

Repeatability	**Mean**		**SD**		**CV**
	µg/mL	µmol/L	µg/mL	µmol/L	%
Control 1[a)]	1.28	5.07	0.0277	0.110	2.2
Control 2[b)]	2.80	11.1	0.0670	0.265	2.4
Human Serum 1	0.739	2.93	0.0198	0.0784	2.7
Human Serum 2	1.19	4.71	0.0348	0.138	2.9
Human Serum 3	1.74	6.89	0.0408	0.162	2.4
Human Serum 4	2.50	9.90	0.0596	0.236	2.4
Human Serum 5	3.52	13.9	0.0723	0.286	2.1

Intermediate precision	**Mean**		**SD**		**CV**
	µg/mL	µmol/L	µg/mL	µmol/L	%
Control 1[a)]	1.28	5.07	0.0365	0.145	2.9
Control 2[b)]	2.80	11.1	0.0795	0.315	2.8
Human Serum 1	0.739	2.93	0.0241	0.0954	3.3
Human Serum 2	1.19	4.71	0.0379	0.150	3.2
Human Serum 3	1.74	6.89	0.0600	0.238	3.5
Human Serum 4	2.50	9.90	0.0797	0.316	3.2
Human Serum 5	3.52	13.9	0.100	0.396	2.8

a) TDM Control Set Level I, ultrafiltrated, b) TDM Control Set Level II, ultrafiltrated

4.2. Analytical Sensitivity

4.2.1. Limit of Blank (LoB)

For determination of LoB, one analyte-free sample (ultrafiltrate of human phenytoin-free serum) was measured with three reagent lots in 6 runs, each run with 10-fold determination, distributed over 6 days, on one **cobas c** 503 analyzer. The LoB was determined according to CLSI EP17-A2. The LoB claim in the labeling will be set to LoB = 0.100 µg/mL (0.396 µmol/L).

4.2.2. Limit of Detection (LoD)

For determination of LoD, 5 serum samples with low analyte concentrations (spiked with phenytoin and ultrafiltrated) were measured on three reagent lots with 2-fold determination per run on one **cobas c** 503 analyzer. Six runs were distributed over 6 days. The LoD was determined according to CLSI EP17-A2. The LoD claim in the labeling will be set to LoD = 0.200 µg/mL (0.792 µmol/L).

4.2.3. Limit of Quantitation (LoQ)

For determination of LoQ, 6 serum samples (spiked with phenytoin and ultrafiltrated) were measured with three reagent lots on one **cobas c** 503. These samples were tested in 1 run per day over 5 days, 5 replicates per run for each LoQ sample. The Limit of Quantitation (LoQ) was determined according to CLSI EP17-A2. For calculation according to the RMS model, the bias has not been considered. TErel corresponds to the intermediate precision of the LoQ samples. The LoQ claim in the labeling will be set to LoQ = 0.400 µg/mL (1.58 µmol/L).

4.3. Linearity/Assay Reportable Range

The linearity of the ONLINE TDM Phenytoin - Free Phenytoin application was assessed according to CLSI EP06-A-Ed2.

A dilution series was prepared from a spiked human ultrafiltrated serum pool (sample High) and a negative ultrafiltrated serum pool (sample Blank). The dilution series spanning the measuring range was prepared to obtain ≥ 9 levels. Samples were assayed on one **cobas c** 503 analyzer in 1 run using 3 reagent lots and 4 replicates per sample. The process was the same with K3-EDTA plasma. The linearity data is analyzed according to CLSI EP06-Ed2.

Linearity was confirmed for the measuring range of 0.400-4.00 µg/mL (1.58-15.8 µmol/L).

4.4. Dilution

Post Dilution Check experiments were performed for samples above the measuring range and verify dilution of samples via the rerun function is a 1:2 dilution. Three ultrafiltrates were prepared with phenytoin concentrations above the measuring range; the volumes of the ultrafiltrate were adjusted to the weighed amount of phenytoin to achieve final concentrations of 5.00 µg/mL, 6.00 µg/mL and 7.00 µg/mL. The samples were measured with the ONLINE TDM Phenytoin - Free Phenytoin application on the **cobas c** 503 via the automatic rerun function and after manual dilution. The ONLINE TDM Phenytoin - Free Phenytoin application demonstrated % deviation results of -7.3% to -11.6% when measuring samples above the measuring range and using the automatic rerun function.

4.5. Endogenous Interferences

Endogenous substances (hemoglobin, lipemia (Intralipid), conjugated and unconjugated bilirubin, Immunoglobulin G (IgG), albumin, rheumatoid factor, total protein, triglycerides and HAMA) were evaluated for potential interference with the ONLINE TDM Phenytoin - Free Phenytoin application on the **cobas c** 503 analyzer.

Endogenous interferents were tested for analytical interference (endogenous substances added to the ultrafiltrate).

All predefined acceptance criteria were met, and the proposed labeling claims for each endogenous substance can be found below:

Endogenous Substance	Claim No interference up to
Hemolysis	H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L)
Icterus	I index of 60 for conjugated bilirubin (approximate conjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL)
Triglycerides	700 mg/dL (7.98 mmol/L)
Albumin	60 g/L
Total protein	between concentrations of 2-12 g/dL
Rheumatoid factors	1200 IU/mL

Endogenous substances were tested for phenytoin release and phenytoin binding effects (endogenous substances were present in the sample before ultrafiltration).

Endogenous Substance	Maximum Concentration without significant effect
Hemolysis	H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L)
Lipemia	L index of 1000 (1000 mg/dL)
Immunoglobulin G	60 g/L

Conjugated and unconjugated bilirubin, triglycerides and rheumatoid factors result in higher free phenytoin concentrations. No significant phenytoin release was observed for conjugated bilirubin up to 18 mg/dL, for unconjugated bilirubin up to 9 mg/dL, for triglycerides up to 183 mg/dL and for rheumatoid factors up to 284 IU/mL.

Phenytoin binds to albumin and total protein (the albumin fraction within total protein). Increased protein concentrations result in decreased free phenytoin concentrations.

4.6. Analytical Specificity/Cross-Reactivity

A cross-reactivity study was conducted with the ONLINE TDM Phenytoin - Free Phenytoin application on the **cobas c** 503 analyzer to evaluate the potential cross-reacting compounds. For each potential cross-reacting compound two ultrafiltrated human serum pools were prepared by spiked with phenytoin to obtain approximately 1.00 and 2.50 µg/mL phenytoin. The phenytoin concentration was determined at least in 5-fold determination and compared to the reference aliquot. All acceptance criteria for cross reactivity were met.

Compound	Concentration tested (µg/mL)	% Cross Reactivity
Fosphenytoin	387	≤ 50.0 %
m-HPPH	500	≤ 10.0 %
p-HPPH	220	≤ 5.0 %
5(*p*-methylphenyl)-5-phenylhydantoin	500	≤ 5.0 %

4.7. Exogenous Interferences – Drugs

An exogenous interference study was conducted to evaluate commonly used pharmaceuticals and in addition, special pharmaceuticals were tested with the ONLINE TDM Phenytoin - Free Phenytoin application on the **cobas c** 503 analyzer. No increase in free phenytoin concentrations was observed at the concentrations tested.

Drug	Concentration tested mg/L (µg/mL)
Acetaminophen	156
N-Acetyl-L-cysteine	150
N-Acetylsalicylic acid	30.0
Amitriptyline	0.48
Amlodipine	0.075
Amobarbital	36.0
Amoxicillin	54.0
Ampicillin	75.0
L-Ascorbic acid	52.5
Atorvastatin	0.750
Budesonide	0.00630
Carbamazepine-10,11-epoxide	140
Chlordiazepoxide	6.90
Chlorpromazine	3.30
Citalopram	5.43
Cyclosporin A	1.80
Diazepam	30.0
Diphenhydramine	50.0
Doxycyclin	18.0
Ethosuximide	1000
Furosemide	15.9
Gabapentin	26.7
Gentamicin	30.0
Glutethimide	36.0
Heparin, sodium	3300 IU/L
Hydantoin	500
10-Hydroxycarbamazepine	105
Hydroxychloroquine	0.624
Imipramine	0.315
Levodopa	7.50
Levothyroxin	0.429
Lisinopril	0.246
Losartan	1.164
Mephobarbital (Methylphenobarbital)	10.5
Metformin	12.0
Methsuximide	260
Methyldopa	22.5

Metoprolol	1.50
Metronidazol	123
Oxcarbazepine	100
Pentobarbital	126
2-Phenyl-2-ethyl-malonamide (PEMA)	100
Phenylsuximide	500
Prednisone	0.0990
Primidone	120
Promethazine	0.297
Rifampicin	48.0
Secobarbital	15.9
Sertraline	0.927
Simvastatin	1.68
Theophylline	60.0
Vancomycin	120

Increased concentrations of free phenytoin were correctly observed in the presence of butabarbital (secbutabarbital), carbamazepine, cefoxitin, ethotoin, p-hydroxyphenobarbital, ibuprofen, oxaprozine, phenylbutazone, d-propoxyphene and valproic acid; this effect may occur at therapeutic concentrations. These drugs bind to human albumin in serum and release phenytoin, thus increasing the concentration of free phenytoin. When these drugs were spiked into serum, significant effects were observed at concentrations above those indicated in the following table:

Drug	**No significant effect up to mg/L (µg/mL)**
Butabarbital (Secbutabarbital)	100
Carbamazepine	56.0
Cefoxitin	188
Ethotoin	15.0
p-Hydroxyphenobarbital	240
Ibuprofen	54.8
Oxaprozine	141
Phenylbutazone	32.1
d-Propoxyphene	40.0
Valproic acid	25.7

The above drugs, which increase the free phenytoin concentration when present in serum, were also tested for analytical interference by spiking the drugs into the ultrafiltrate. No analytical interference from these drugs was observed when present in ultrafiltrate, confirming that the increase of free phenytoin concentration observed when present in serum is a result from competition for albumin binding.

For phenobarbital and mephenytoin, when spiked into serum before ultrafiltration, significantly increased values for free phenytoin were observed above concentrations of 34.5 µg/mL and 40.0 µg/mL, respectively. When spiked into the ultrafiltrate, increased values for free phenytoin were found at concentrations above 90 µg/mL (356 µmol/L) for phenobarbital and above 60 µg/mL (238 µmol/L) for mephenytoin, indicating analytical interference of these drugs with the assay in addition to the competition for albumin binding. For these drugs, analytical interference starts at higher drug concentrations compared to the competitive effect.

4.8. Sample Matrix Comparison

The effect on quantitation of free phenytoin values in the presence of anticoagulants with the ONLINE TDM Phenytoin - Free Phenytoin application was determined on the **cobas c** 503 analyzer by comparing values obtained from samples collected in serum, Li-Heparin, K2-EDTA and K3-EDTA plasma tubes; after centrifugation the samples were spiked and ultrafiltrated. The study was performed using ≥50 samples, 1 lot of reagent and measured on 1 **cobas c** 503 analyzer. All predefined acceptance criteria were met, supporting the labeling claim that serum, Li-Heparin, K2-EDTA and K3-EDTA plasma are acceptable sample types.

Anticoagulant	Slope	Intercept (µg/mL)	Correlation Coefficient (Pearson r)	Concentration of Samples (µg/mL, serum)
Serum vs. Li-Heparin plasma	1.018	-0.0149	0.988	0.592 – 3.84
Serum vs. K2-EDTA plasma	0.923	-0.0328	0.992	0.592 – 3.83
Serum vs. K3-EDTA plasma	0.950	-0.0282	0.992	0.592 – 3.84

4.9. Method Comparison to Phenytoin - Free Phenytoin Application on COBAS INTEGRA 400 plus

A method comparison was performed with the Phenytoin - Free Phenytoin Application on the COBAS INTEGRA 400 plus and the ONLINE TDM Phenytoin - Free Phenytoin application on the **cobas c** 503 analyzer (candidate device), using a total of 138 native human ultrafiltrated serum samples (≤10% spiked), 1 lot of reagent, 1 **cobas c** 503 analyzer, in 3 runs, in singlicate. The sample concentrations were between 0.400 µg/mL (1.58 µmol/L) and 3.78 µg/mL (15.0 µmol/L). The results can be found below:

Passing/Bablok	Deming Regression
y = 1.035x - 0.0165 µg/mL	y = 1.019x + 0.0107 µg/mL
τ = 0.972	r = 0.998

4.10. Stability

The stability data supports Roche Diagnostic's claims as reported in the package labeling.

5. ADDITIONAL INFORMATION

The ONLINE TDM Phenytoin - Free Phenytoin application is intended to be used with the following calibrators and controls:

- Preciset TDM I calibrators (diluted)
- TDM Control Set (Level I and II, ultrafilatrated)

Preciset TDM I, product code DKB, is a Class II 510(k) Exempt device and therefore, is not included with this submission.

TDM Control Set, product code JJY, is a Class I 510(k) Exempt device and therefore, is not included with this submission.

Other devices required but not provided:

- Serum Filters

6. CONCLUSIONS

The analytical performance data for ONLINE TDM Phenytoin - Free Phenytoin application met the acceptance criteria and support the substantial equivalence of ONLINE TDM Phenytoin - Free Phenytoin application on **cobas c** 503 analyzer to the predicate.