510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K253490

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Glucose HK Gen.3; ONLINE DAT Methadone II; cobas pro integrated solutions

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CFR	II	21 CFR 862.2160 - Glucose Test System	CH - Clinical Chemistry
DJR	II	21 CFR 862. 3620- Methadone Test System	CH - Clinical Chemistry
JJE	I	21 CFR 862. 2160 - Discrete photometric chemistry analyzer for clinical use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Addition of previously cleared assays to a modified instrument platform

B Measurand:

Glucose and Methadone

C Type of Test:

Glucose: Quantitative photometric
Methadone: Kinetic interaction of microparticles in a solution

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
cobas pro integrated solutions is an automated analyzer, intended for running qualitative, semiquantitative, and quantitative clinical chemistry and immunochemistry assays as well as ion-selective measurements.

Glucose HK Gen.3 is an in vitro test for the quantitative determination of glucose in human serum, plasma, urine and CSF on cobas c systems. Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia, and pancreatic islet cell tumors.

ONLINE DAT Methadone II is an in vitro diagnostic test for the qualitative and semiquantitative detection of methadone in human urine on cobas c systems at a cutoff concentration of 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program. Semiquantitative assays are intended to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas chromatography/mass spectrometry (GC-MS).

Methadone II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. GC-MS is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

cobas pro integrated solutions

IV Device/System Characteristics:

A Device Description:

cobas pro integrated solutions
The cobas pro integrated solutions is a fully automated, random-access, software-controlled system intended for in vitro quantitative and qualitative analysis of analytes in body fluids. The quantitative analysis also includes semi-quantitative. The cobas c 703 is being added to the cobas pro integrated solutions.

Glucose HK Gen. 3
The reagent working solutions include:

- R1: MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}, 24 mmol/L; ATP, ≥ 4.5 mmol/L; NADP, ≥ 7.0 mmol/L; preservative
- R3: HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}, 4 mmol/L; HK (yeast), ≥ 300 µkat/L; G-6-PDH (E. coli), ≥ 300 µkat/L; preservative

ONLINE DAT Methadone II
The reagent working solutions include:
R1: Conjugated methadone derivative; buffer; bovine serum albumin; 0.09 % sodium azide
R2: Microparticles attached to methadone antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

B Principle of Operation:

Glucose HK Gen. 3:
Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) specifically oxidizes G-6-P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide reduced (NADH). One micromole of NADH is produced for each micromole of glucose consumed. The NADH produced absorbs light at 340 nm and can be detected spectrophotometrically as an increased absorbance. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration.

ONLINE DAT Methadone II:
The Methadone assay is based on the kinetic interaction of microparticles in a solution (KIMS) as measured by changes in light transmission. In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases. When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

C Instrument Description Information:

1. Instrument Name:

   cobas pro integrated solutions

2. Specimen Identification:

The specimen is in a tube with a barcode label. The system identifies the specimen by scanning the barcode. This is the primary use case for sample identification. The system also supports manual entry of sample IDs to support the needs of the customer.

3. Specimen Sampling and Handling:

   Specimen sampling and handling procedures are analyte specific and documented in the respective reagent method sheets.

4. Calibration:

   The software of the cobas pure integrated solution automatically recommends calibration for all tests requiring calibration. Calibration methods and procedures are analyte specific and documented in the respective reagent method sheets.

5. Quality Control:

   Quality control procedures are analyte specific and documented in the respective reagent method sheets.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Glucose HK Gen. 3, cobas pro integrated solutions
ONLINE DAT Methadone II

B Predicate 510(k) Number(s):

K191899, K021505

C Comparison with Predicate(s):

Comparison with Glucose HK Gen. 3

Device & Predicate Device(s):	K253490	K191899
Device Trade Name	Glucose HK Gen.3	Glucose HK Gen.3
General Device Characteristic Similarities		
Intended Use/Indications for Use	In vitro test for the quantitative determination of glucose in human serum, plasma, urine, and CSF.	Same
General Device Characteristic Differences		
Instrument Platform	cobas c 703 in cobas pro integrated solutions	cobas c 503 in cobas pro integrated solutions

Comparison with ONLINE DAT Methadone II

Device & Predicate Device(s):	K253490	K021505
Device Trade Name	ONLINE DAT Methadone II	ONLINE DAT Methadone II
General Device Characteristic Similarities		
Intended Use/Indications for Use	In vitro test for the qualitative and semiquantitative detection of methadone in human urine at a cutoff concentration of 300 ng/mL.	Same
General Device Characteristic Differences		
Instrument Platform	cobas c 703 in cobas pro integrated solutions	cobas c 503 in cobas pro integrated solutions

Comparison of the cobas pro integrated solutions

Device & Predicate Device(s):	K253490	K191899
Device Trade Name	cobas pro integrated solutions	cobas pro integrated solutions
General Device Characteristic Similarities		
Intended Use/Indications for Use	Intended for clinical chemistry and immunochemistry assays as well as ion-selective measurements	Same
General Device Characteristic Differences		
Instrument platforms	cobas c 703 in cobas pro integrated solutions	cobas c 503 in cobas pro integrated solutions

VI Standards/Guidance Documents Referenced:

- Clinical and Laboratory Standards Institute (CLSI) EP05-A3. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
- CLSI EP06 2nd Edition. Evaluation of the Linearity of Quantitative Measurement Procedures.
- CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- IEC 61010-1 Edition 3.1 2017-01 consolidated version. Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General Requirements (including: Corrigendum 1).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Repeatability (within-run precision) and intermediate precision (within-laboratory precision) were evaluated following the recommendations in the CLSI guideline EP05-A3.

Glucose HK Gen.3: Repeatability and Intermediate Precision

The precision of the Glucose HK Gen.3 assay was evaluated on one candidate device using three lots of reagent. The protocol consisted of testing two sample replicates per run, two runs per day for 21 days. Repeatability and within-lab precision were calculated. The samples were randomized in each run separately. Two serum-based controls and five levels of individual serum samples were used (Serum 1 was diluted, Serum 2 was native, and Serum 3-5 were spiked). The protocol was repeated for each application (serum, urine, CSF). For urine, individual samples were also used (Urine 1 was native and Urine 2-5 were spiked) while for CSF, pooled samples were used (CSF 1-2 were diluted, CSF 3 was native, and CSF 4-5 were spiked). The results for a representative reagent lot are summarized in the table below.

Summary of precision results – Glucose HK Gen.3

Sample	N	Mean (mg/dL)	Repeatability		Mean (mg/dL)	Within-Laboratory Precision	
			SD (mg/dL)	CV (%)		SD (mg/dL)	CV (%)
Serum Control 1	84	104	0.400	0.4	104	0.782	0.8
Serum Control 2	84	254	1.112	0.4	254	1.910	0.8
Serum 1	84	4.0	0.095	2.4	4.0	0.104	2.6
Serum 2	84	68	0.371	0.5	68	0.494	0.7
Serum 3	84	112	0.567	0.5	112	1.072	1
Serum 4	84	389	1.484	0.4	389	1.874	0.5
Serum 5	84	740	3.837	0.5	740	5.044	0.7

Sample	N	Mean (mg/dL)	Repeatability		Mean (mg/dL)	Within-Laboratory Precision	
			SD (mg/dL)	CV (%)		SD (mg/dL)	CV (%)
Urine Control 1	84	27	0.267	1	27	0.297	1.1
Urine Control 2	84	290	1.748	0.6	290	2.414	0.8
Urine 1	84	3.8	0.263	6.9	3.8	0.263	6.9
Urine 2	84	14	0.207	1.5	14	0.225	1.6
Urine 3	84	70	0.407	0.6	70	0.567	0.8

Sample	N	Mean (mg/dL)	Repeatability		Mean (mg/dL)	Within-Laboratory Precision	
			SD (mg/dL)	CV (%)		SD (mg/dL)	CV (%)
Urine 4	84	375	1.802	0.5	375	2.216	0.6
Urine 5	84	730	3.315	0.5	730	4.090	0.6

Sample	N	Mean (mg/dL)	Repeatability		Mean (mg/dL)	Within-Laboratory Precision	
			SD (mg/dL)	CV (%)		SD (mg/dL)	CV (%)
CSF Control 1	84	57	0.303	0.5	57	0.368	0.6
CSF Control 2	84	30	0.231	0.8	30	0.254	0.9
CSF 1	84	4.5	0.254	5.7	4.5	0.265	5.9
CSF 2	84	38	0.234	0.6	38	0.335	0.9
CSF 3	84	71	0.335	0.5	71	0.402	0.6
CSF 4	84	375	1.748	0.5	375	2.774	0.7
CSF 5	84	726	3.081	0.4	726	4.234	0.6

ONLINE DAT Methadone II

The precision of the ONLINE DAT Methadone II test was evaluated on one candidate device using three reagent lots using both the qualitative and the semi-quantitative mode. The protocol consisted of testing two sample replicates per run, two runs per day for 21 days. Seven human samples and four control samples were used. The samples with the concentrations provided in the tables below were prepared by spiking drug into negative urine. The analyte concentration was confirmed using a validated method. The results for a representative reagent lot are summarized in the tables below.

Summary of Within-Lab Precision – Qualitative (300 ng/mL cutoff)

Sample	N	Negative	Positive
Sample -100% (zero)	84	84	0
Sample -75%	84	84	0
Sample -50%	84	84	0
DAT1N	84	84	0
DATCN	84	84	0
Cutoff	84	34	50
DAT1P	84	0	84
DATCP	84	0	84
Sample +50%	84	0	84
Sample +75%	84	0	84
Sample +100%	84	0	84

Summary of Precision Results – Semi-Quantitative (300 ng/mL cutoff)

Sample	N	Mean (ng/mL)	Repeatability		Mean (ng/mL)	Within-Laboratory Precision	
			SD (ng/mL)	CV (%)		SD (ng/mL)	CV (%)
Sample -100% (zero)	84	15.1	13.7	90.6	15.3	16.1	105.0
Sample -75%	84	113	6.12	5.4	113	9.97	8.8
Sample -50%	84	170	5.94	3.5	165	12.3	7.5
DAT1N	84	238	6.82	2.9	242	10.9	4.5
DATCN	84	239	7.69	3.2	239	11.4	4.8
Cutoff	84	295	10.2	3.5	296	22.5	7.6
DAT1P	84	407	13.2	3.2	407	18.4	4.5
DATCP	84	393	12.1	3.1	401	18.2	4.5
Sample +50%	84	489	23.2	4.7	505	31.5	6.2
Sample +75%	84	579	20.3	3.5	579	30.6	5.3
Sample +100%	84	652	28.7	4.4	635	36.7	5.8

2. Linearity:

The linearity studies were conducted following the recommendations in CLSI guideline EP06-Ed2.

Glucose HK Gen.3

Linearity of the Glucose HK Gen.3 assay was assessed on candidate device in one run using three reagent lots, measuring four replicates per sample. High analyte concentration of human serum, plasma (collected in K2EDTA tubes), urine and CSF samples were diluted to obtain nine levels spanning the measuring range. The results of the linear regression analysis for a representative reagent lot are summarized in the table below.

Sample	Tested Range (mg/dL)	Linear Regression Data	Claimed Linear Range (mg/dL)
Serum	1.82-777	$y = 0.997x – 0$, $R^2 = 0.9998$	2-750
Plasma	0.26-786	$y = 1.001x – 0$, $R^2 = 0.9999$	2-750
Urine	1.18-760	$y = 1.009x –0$, $R^2 = 1.0000$	2-750
CSF	0.99-773	$y = 1.003x – 0$, $R^2 = 1.0000$	2-750

The results demonstrated linearity of the claimed measuring range for serum, plasma, urine, and CSF (2.0 to 750 mg/dL or 0.11 to 41.6 mmol/L).

ONLINE DAT Methadone II

Linearity of the semi-quantitative ONLINE DAT Methadone II test was assessed on the candidate device in one run using one reagent lot, measuring four replicates per sample. A dilution series was prepared from human urine sample pools (spiked with methadone and

diluted with human urine) to obtain nine levels that span the claimed range. The results of the linearity/recovery study are summarized in the table below.

Expected Value (ng/mL)	Observed Value (ng/mL)	Absolute Deviation (ng/mL)	% Recovery
27.1	37.0	9.81	
54.1	60.8	6.41	
113	113	-0.203	
226	225	-1.41	
451	451		100.0%
677	688		101.6%
902	997		110.5%
1128	1098		97.3%
1353	1325		97.9%
1579	1521		96.3%
1804	1816		100.6%
2030	1958		96.5%
2255	2255		100.0%

Dilution Recovery Studies

Glucose HK Gen. 3
A dilution study was performed and the data support the rerun function 1:2 dilution claim in the Method Sheet. Five samples each (serum, urine, and CSF) were prepared using native and spiked samples and measured with the automatic rerun function of the candidate device.

ONLINE DAT Methadone II
Patient values should not be generated with diluted samples.

3. Analytical Specificity/Interference:

Endogenous Interference
The purpose of this study was to evaluate endogenous substances for potential interference when using candidate device.

Glucose HK Gen. 3
The effect on the quantitation of analyte in the presence of potentially interfering endogenous substances using the Glucose HK Gen.3 assay was determined on the candidate device using serum, urine, and CSF samples. Glucose levels of approximately 38 mg/dL and 220 mg/dL were tested. Significant interference was defined as follows:

- Serum/Plasma: bias between the samples with and without interferent is > ± 7 mg/dL at glucose concentrations ≤ 70 mg/dL and > ± 10% at glucose concentrations > 70 mg/dL
- Urine: bias between the samples with and without interferent is > ± 2.0 mg/dL at glucose concentrations ≤ 19.8 mg/dL and > ± 10% at glucose concentrations > 19.8 mg/dL

- CSF: bias between the samples with and without interferent is >± 4 mg/dL at glucose concentrations ≤ 39.6 mg/dL and > ± 10% at glucose concentrations > 39.6 mg/dL

The summary of results is presented in the table below.

Potential Interferent		Highest Concentration Tested Without Significant Interference
Serum	Albumin	62.3 g/L
	Conjugated Bilirubin	64 mg/dL
	Unconjugated Bilirubin	77 mg/dL
	Hemolysis	1213 mg/dL
	IgG	65.1 g/L
	Lipemia	1102 mg/dL
Urine	Albumin	2.70 g/L
	Calcium	12.6 mmol/L
	Citrate	12.0 mmol/L
	Creatinine	89.7 mmol/L
	Hemolysis	876 mg/dL
	IgG	1.10 g/L
	Magnesium	27.3 mmol/L
	Oxalate	2.70 mmol/L
	Phosphate	137 mmol/L
	Urea	1825 mmol/L
	Uric Acid	6.0 mmol/L
	Urobilinogen	0.25 mmol/L
CSF	Conjugated Bilirubin	66 mg/dL
	Hemolysis	1201 mg/dL

ONLINE DAT Methadone II

The effects of potentially interfering endogenous substances on the ONLINE DAT Methadone II test were determined on one candidate device, using two reagent lots, one sample per concentration level, two human samples, in one run, with three replicates per sample. Methadone levels of approximately 225 ng/mL and 375 ng/mL (corresponding to ±25% of the cutoff concentration) were tested. Significant interference was defined as crossover from the expected result for each control level. The summary of results is presented in the table below.

Potential Interferent	Concentration tested	Spiked Methadone Concentration	
		225 ng/mL (Negative Control -25% Cutoff,)	375 ng/mL (Positive Control, +25% Cutoff)
Acetone	1000 mg/dL	Neg	Pos
Ascorbic acid	1500 mg/dL	Neg	Pos
Albumin	500 mg/dL	Neg	Pos
Bilirubin	25 mg/dL	Neg	Pos
Calcium	133 mg/dL	Neg	Pos

Potential Interferent	Concentration tested	Spiked Methadone Concentration	
		225 ng/mL (Negative Control -25% Cutoff,)	375 ng/mL (Positive Control, +25% Cutoff)
Creatinine	500 mg/dL	Neg	Pos
Ethanol	1000 mg/dL	Neg	Pos
Glucose	2000 mg/dL	Neg	Pos
Hemoglobin	750 mg/dL	Neg	Pos
IgG	110 mg/dL	Neg	Pos
Magnesium	238 mg/dL	Neg	Pos
NaCl	5800 mg/dL	Neg	Pos
Oxalate	200 mg/dL	Neg	Pos
Phosphate	2028 mg/dL	Neg	Pos
Sodium citrate, dihydrate	323.51 mg/dL	Neg	Pos
Urea	6000 mg/dL	Neg	Pos
Uric Acid	100.86 mg/dL	Neg	Pos
Urobilinogen	14.82 mg/dL	Neg	Pos
pH	3.9 – 9.1	Neg	Pos
Specific Gravity	1.001-1.035 g/mL	Neg	Pos

Exogenous Interference

The purpose of these studies was to evaluate drugs for potential interference when using the Glucose HK Gen.3 assay and the ONLINE DAT Methadone II test on the candidate device. The acceptance criteria were recovery within 100 ± 10%, except methadone where significant interference was defined as crossover from the expected result for each control level.

Glucose HK Gen. 3

The effect on the quantitation of analyte in the presence of potentially interfering drugs using the Glucose HK Gen.3 assay was determined on the candidate device using serum, urine and CSF samples. Glucose levels of approximately 40 mg/dL and 220 mg/dL were tested. The summary of results is presented in the table below.

Potential Interferent		Highest Concentration without Interference [mg/L]
Serum	N-Acetylcysteine	150
	Phenylbutazone	321
	Acetylsalicylic acid	30
	Ampicillin-Na	75
	Ascorbic acid	52.5
	Cefoxitin	750
	Doxycyclin	18
	Heparin	3300 IU/L
	Levodopa	7.5
	Methyldopa	22.5

Potential Interferent		Highest Concentration without Interference [mg/L]
	Metronidazole	123
	Rifampicin	48
	Acetaminophen	156
	Cyclosporine	1.8
	Ibuprofen	219
	Theophylline	60
Urine	Acetaminophen	3000
	N-Acetylcysteine	10
	Ascorbic acid	4000
	Cefoxitin	12000
	Gentamycine sulfate	400
	Levodopa	1000
	Methyldopa	2000
	Ofloxacine	900
	Ibuprofen	4000
	Phenazopyridine	300
	Salicyluric acid	100
	Tetracycline	100

ONLINE DAT Methadone II

The effects of potential drug interference on the ONLINE DAT Methadone II assay were determined on one candidate device using urine samples. Urine samples containing methadone at positive and negative levels (a final methadone concentration of 225 ng/mL and 375 ng/mL (±25% of the cutoff concentration)) were prepared. In case interference was detected, the interferent was diluted and tested until no more interference was observed. The maximum drug concentration which did not interfere is presented in the table below.

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
Acetaminophen	3000000	Neg	Pos
Acetyl cysteine	10000	Neg	Pos
Acetylsalicylic acid	100000	Neg	Pos
Aminopyrine	100000	Neg	Pos
Amitriptyline	100000	Neg	Pos
Amobarbital	100000	Neg	Pos
d-Amphetamin	100000	Neg	Pos
l-Amphetamine	100000	Neg	Pos
Ampicillin	100000	Neg	Pos

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
Ascorbic acid	4000000	Neg	Pos
Aspartame	100000	Neg	Pos
Atropine	100000	Neg	Pos
Atropine	100000	Neg	Pos
Atropine	100000	Neg	Pos
Benzocaine	100000	Neg	Pos
Benzoylecgonine	100000	Neg	Pos
Benzphetamine	100000	Neg	Pos
Butabarbital	100000	Neg	Pos
Caffeine	100000	Neg	Pos
Calcium dobesilate	100000	Neg	Pos
Calcium hypochlorite	100000	Neg	Pos
Carbamazepine	100000	Neg	Pos
Cefoxitin	12000000	Neg	Pos
Chlordiazepoxide	100000	Neg	Pos
Chloroquine	100000	Neg	Pos
Chlorpheniramine	100000	Neg	Pos
Cocaine	100000	Neg	Pos
Codeine	100000	Neg	Pos
Cotinine	100000	Neg	Pos
Cyclobenzaprine	100000	Neg	Pos
Cyproheptadine	100000	Neg	Pos
Desipramine	100000	Neg	Pos
Dextrometorphan	100000	Neg	Pos
Dextropropoxyphene	100000	Neg	Pos
Diazepam	100000	Neg	Pos
Diphenhydramine	100000	Neg	Pos
Diphenylhydantoin	100000	Neg	Pos
Disopyramide	100000	Neg	Pos
Disopyramide	1000000	Neg	Pos
Dopamine	100000	Neg	Pos
Doxepin	100000	Neg	Pos
Doxylamine	100000	Neg	Pos
d-Phenylpropanolamine	100000	Neg	Pos

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
d-Pseudoephedrine	100000	Neg	Pos
Ecgonine	100000	Neg	Pos
Ecgonine methyl ester	100000	Neg	Pos
EDDP	100000	Neg	Pos
EMDP	100000	Neg	Pos
d-Ephedrine	100000	Neg	Pos
dl-Ephedrine	100000	Neg	Pos
l-Ephedrine	100000	Neg	Pos
Epinephrine	100000	Neg	Pos
Erythromycin	100000	Neg	Pos
Estriol	100000	Neg	Pos
Fenoprofen	100000	Neg	Pos
Fluoxetine	100000	Neg	Pos
Furosemide	100000	Neg	Pos
Gentamicin sulfate	400000	Neg	Pos
Gentisic acid	100000	Neg	Pos
Glutethimide	100000	Neg	Pos
Guaiacol glycerol ether	100000	Neg	Pos
Haloperidol	100000	Neg	Pos
Hydrochlorothiazide	100000	Neg	Pos
Ibuprofen	4000000	Neg	Pos
Imipramine	100000	Neg	Pos
Isoproterenol	100000	Neg	Pos
Ketamine	100000	Neg	Pos
Levodopa	1000000	Neg	Pos
Lidocaine	100000	Neg	Pos
l-Pseudoephedrine	100000	Neg	Pos
LSD	100000	Neg	Pos
Maprotiline	100000	Neg	Pos
MDA	100000	Neg	Pos
MDMA	100000	Neg	Pos
Melanin	100000	Neg	Pos
Meperidine	100000	Neg	Pos
d-Methamphetamine	100000	Neg	Pos

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
l-Methamphetamine	100000	Neg	Pos
Methaqualone	100000	Neg	Pos
Methyldopa	2000000	Neg	Pos
Methylphenidate	100000	Neg	Pos
Mianserin	100000	Neg	Pos
Morphine	100000	Neg	Pos
Naloxone	100000	Neg	Pos
Naltrexone	100000	Neg	Pos
Naproxen	100000	Neg	Pos
Niacinamide	100000	Neg	Pos
Nicotine	100000	Neg	Pos
Nordiazepam	100000	Neg	Pos
Nordoxepin	100000	Neg	Pos
Norethindrone	100000	Neg	Pos
l-Norpseudoephedrine	100000	Neg	Pos
Nortriptyline	100000	Neg	Pos
Ofloxacine	90000	Neg	Pos
Orphenadrine	100000	Neg	Pos
Oxazepam	100000	Neg	Pos
Penicillin G	100000	Neg	Pos
Pentobarbital	100000	Neg	Pos
Perphenazine	100000	Neg	Pos
Phenazopyridine	300000	Neg	Pos
Phencyclidine	100000	Neg	Pos
Phenetylamine	100000	Neg	Pos
Phenobarbital	100000	Neg	Pos
Phenothiazine	100000	Neg	Pos
Phentermine	100000	Neg	Pos
Phenylbutazone	100000	Neg	Pos
Phenylpropanolamine	100000	Neg	Pos
Procaine	100000	Neg	Pos
Protriptyline	100000	Neg	Pos
Quetiapine-Carboxylic acid	500000	Neg	Pos
Quetiapine-Fumarat	750000	Neg	Pos

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
Quetiapine-Sulfoxid	500000	Neg	Pos
Quinidine	100000	Neg	Pos
Quinine	100000	Neg	Pos
Salicyluric acid	6000000	Neg	Pos
Secobarbital	100000	Neg	Pos
Sulindac	100000	Neg	Pos
Tetracycline	300000	Neg	Pos
Tetracycline	300000	Neg	Pos
Tetracycline	300000	Neg	Pos
Tetrahydrozoline	100000	Neg	Pos
THC-9-carboxylic acid	100000	Neg	Pos
Tramadol	20000	Neg	Pos
Trifluoperazine	100000	Neg	Pos
Tyramine	100000	Neg	Pos
Verapamil	100000	Neg	Pos

ONLINE DAT Methadone II Cross Reactivity

The specificity of the ONLINE DAT Methadone II assay for structurally similar compounds was determined by preparing concentration series for each of the compounds listed, using measured signals of the different potential cross reactant concentration to determine the approximate quantity of each compound that is equivalent in assay reactivity to the 300 ng/mL assay cutoff on one candidate device. Cross Reactivity was tested for the Semi-Quantitative application only. The percent cross-reactivity was calculated from the ratio of methadone concentration at cutoff level and the calculated cross reactant concentration, that yields the same signal.

Structurally related pharmaceutical compounds:

Potential Interferent	Cross reactant units [ng/mL] equivalent to 300 ng/mL methadone	Cross-reactivity (%)
Vortioxetine	6531	4.59
LUAA34443	3914	7.66
Cyamemazine	7282	4.12
Methotrimeprazine (Levomepromazine)	8639	3.47
Chlorpromazine	30891	0.97
Thiothixene	77558	0.39
Promazine	215852	0.14

Potential Interferent	Cross reactant units [ng/mL] equivalent to 300 ng/mL methadone	Cross-reactivity (%)
Clomipramine	180534	0.17
Thioridazine	206275	0.15
Chlorprothixene	660667	0.045
Promethazine	403915	0.074
Trimipramine	572980	0.052

4. Detection Limit and Assay Reportable Range:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined in accordance with the CLSI EP17-A2 guideline.

The LoB was determined as the 95th percentile of measurements of blank samples. For determination of the LoB for the Glucose HK Gen. 3 assay, one analyte-free sample was measured with three reagent lots in six runs, distributed over three days, 10 replicates per run, on one candidate device. In total, 60 determinations of analyte free samples were obtained. Data analysis was based on determination of the 95th percentile of the 60 measured values. In this design (n=60) the 95th percentile was the average of the 57th and 58th value. This process was repeated for each application (serum/plasma, urine, CSF).

The LoD was determined as the lowest amount of analyte in a sample that can be detected with a 95% probability. For determination of the LoD for the Glucose HK Gen.3 assay, five samples with low analyte content were measured using three reagent lots with two-fold determination per run on one candidate device. Six runs distributed over three days on one instrument were performed. The LoD is defined as the concentration, at which there is a 95% probability that a sample contains analyte. LoD = LoB + 1.653 x SD_{total}. This process was repeated for each application (serum/plasma, urine, CSF).

The LoQ was determined as the lowest concentration of analyte which can be quantified with a maximum CV of 20% for the Glucose HK Gen.3 assay. Five samples with low analyte concentration were measured using three reagent lots on one candidate device. These samples were tested in one run per day over five days, five replicates per run for each LoQ sample (n=25 per sample). This process was repeated for each application (serum/plasma, urine, CSF).

The results support the claimed LoB, LoD and LoQ for the Glucose HK Gen.3 assay which are unchanged from the predicate device (k191899) and are summarized in the table below:

Sample Type	Claimed LoB	Claimed LoD	Claimed LoQ
Serum/Plasma	0.350 mg/dL (0.0194 mmol/L)	0.486 mg/dL (0.0270 mmol/L)	1.517 mg/dL (0.0842 mmol/L)
Urine	0.776 mg/dL (0.0431 mmol/L)	1.160 mg/dL (0.0644 mmol/L)	1.160 mg/dL (0.0644 mmol/L)
CSF	0.337 mg/dL (0.0187 mmol/L)	0.506 mg/dL (0.0281 mmol/L)	1.454 mg/dL (0.0807 mmol/L)

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

The traceability information for Glucose HK Gen. 3 assay was provided in K191899 and is unchanged.

The traceability for ONLINE DAT Methadone II assay is unchanged from K220134.

On-board Reagent Stability:

Glucose HK Gen. 3
A reagent stability study confirmed that the Glucose HK Gen.3 reagent is stable for 26 weeks on-board the candidate device.

ONLINE DAT Methadone II
A reagent stability study confirmed that the ONLINE DAT Methadone II reagent is stable for 26 weeks on-board the candidate device.

6. Assay Cut-Off:

Glucose HK Gen. 3
Not applicable.

ONLINE DAT Methadone II
Characterization of how the device performs analytically around the claimed cutoff concentration is described in the method comparison section VII.B.1. below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Glucose HK Gen. 3
Method comparison studies were performed for serum, urine and CSF using the Glucose HK Gen.3 assay on the candidate device versus the predicate device to assess the differences between the two test systems. Native human samples were tested in singlicate on each test system. Less than 10% of these samples were spiked or diluted to cover the measuring range. The results were analyzed using Passing-Bablok and linear regression analyses. The process was repeated for each application (serum, urine, CSF). A summary of results is presented in the table below.

Sample Type	N	Sample Concentration Range (mg/dL)	Passing-Bablok (Slope & Intercept) and Correlation (Kendall tau (t))	Linear Regression (Slope & Intercept) and Correlation (Pearson (r))
Serum	74	3.60 -724	y = 0.995x - 0.0968 mg/dL t = 0.990	y = 0.999x - 0.216 mg/dL r = 1.000

Sample Type	N	Sample Concentration Range (mg/dL)	Passing-Bablok (Slope & Intercept) and Correlation (Kendall tau (t))	Linear Regression (Slope & Intercept) and Correlation (Pearson (r))
Urine	67	2.04-730	y = 0.998x + 0.209 mg/dL t = 0.950	y = 1.001x + 0.214 mg/dL r = 1.000
CSF	75	2.78- 730	y = 0.987x - 0.234 mg/dL t = 0.984	y = 0.989x - 0.259 mg/dL r = 1.000

ONLINE DAT Methadone II

Eighty seven (87) negative urine samples were evaluated with the ONLINE DAT Methadone II assay on the candidate device. 97.7 % of these normal urines were negative relative to the 300 ng/mL cutoff. Seventy nine (79) positive urine samples were evaluated with the ONLINE DAT Methadone II assay. 97.5 % of these samples were positive relative to the 300 ng/mL cutoff. Samples were evaluated using both the qualitative and the semi-quantitative modes and candidate device results were compared to GC-MS values. No differences were observed in the results, and results from the semi-quantitative mode are shown below.

Semi-Quantitative Accuracy Study (cutoff = 300 ng/mL)

		Negative samples	GC-MS values (ng/mL)		
			Near cutoff		Positive samples
		0-150	150-299	300-450	>450
Candidate Device	+	0	2	15	62
	-	59	26	2	0

2. Matrix Comparison:

Glucose HK Gen. 3

To support the use of the Glucose HK Gen.3 assay on the candidate device with different sample matrices, a matrix comparison study was conducted by comparing values obtained from samples drawn into serum and plasma collection tubes. Some samples were spiked and/or diluted to cover the measuring range. The result of the serum sample was compared with the result of each plasma sample from the same donor. 43 serum/plasma pairs were tested for each type of anticoagulant in one run, in singlicate. The results were evaluated using Passing-Bablok regression analysis. A summary of results is presented in the table below.

Comparison	Slope	Intercept (mg/dL)	Correlation Coefficient	Concentration of Samples (mg/dL)
Serum vs. Serum/gel separation tube	1.007	-0.432	1.000	3.17-737
Serum vs. K2-EDTA plasma	1.003	-0.0771	0.999	3.17-737
Serum vs. Li-Heparin plasma	1.031	-1.59	0.999	3.17-737

Comparison	Slope	Intercept (mg/dL)	Correlation Coefficient	Concentration of Samples (mg/dL)
Serum vs. NaF/K-Oxalate plasma	1.025	-1.25	1.000	3.17-737
Serum vs. NaF/Na2-EDTA plasma	1.037	-1.78	0.999	3.17-737
Serum vs. KF/Na2-EDTA plasma	1.028	-0.587	0.999	3.17-737
Serum vs. NaF/Citrate/Na2-EDTA plasma	1.005	1.87	0.999	2.88-730

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Clinical Cut-Off

   Not applicable.

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable.

D Expected Values/Reference Range:

Expected reference ranges for the assays are as follows:

Glucose HK Gen.3

Expected Values		
Sample Type		**Reference Range**
Serum, plasma	Adults	74-106 mg/dL
	60-90 years	82-115 mg/dL
	> 90 years	75-121 mg/dL
	Children	60-100 mg/dL
	Neonates (1 day)	40-60 mg/dL
	Neonates (> 1 day)	50-80 mg/dL
Urine	24-hour urine	< 2.78 mmol/24 h (<0.5 g/24 h)
	Random urine	1-15 mg/dL
CSF	Children	60-80 mg/dL
	Adults	40-70 mg/dL

Reference: Tietz NW, ed. Clinical Guide to Laboratory Tests, 4th ed. Philadelphia: WB Saunders Co 2006;444-451.

ONLINE DAT Methadone II

Qualitative assay
Results of this assay distinguish preliminary positive (≥ 300 ng/mL) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Semiquantitative assay
Results of this assay yield only approximate cumulative concentrations of the drug and its metabolites.

E Other Supportive Instrument Performance Characteristics Data:

1. Carry-over studies were reviewed and found to be acceptable.

2. Electrical safety and electromagnetic compatibility (EMC) testing were performed, and the system was found to be acceptable.

3. Software and cybersecurity documentation was reviewed and found to be acceptable.

VIII Proposed Labeling:

The labeling supports or the finding of substantial equivalence for this device.

IX Conclusion:

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