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

I Background Information:

A 510(k) Number

K220134

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Glucose HK Gen.3, ISE indirect Na for Gen.2, ONLINE DAT Methadone II, Elecsys TSH, cobas pure integrated solutions

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CFR	Class II	21 CFR 862.1345 - Glucose Test System	CH - Clinical Chemistry
JGS	Class II	21 CFR 862.1665 - Sodium test system	CH - Clinical Chemistry
JLW	Class II	21 CFR 862.1690 - Thyroid stimulating hormone test system	CH - Clinical Chemistry
DJR	Class II	21 CFR 862.3620 - Methadone test system	TX - Clinical Toxicology
JJE	Class 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 new instrument platform

B Measurand:

Glucose, Sodium, Methadone, and TSH

C Type of Test:

Quantitative photometric, ion selective electrode, and immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

cobas pure integrated solutions is an automated analyzer, intended for running qualitative, semi-quantitative 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 Roche/Hitachi cobas c systems. Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia and pancreatic islet cell tumors.

The ISE analytical unit of the Roche/Hitachi cobas c systems is intended for the quantitative determination of sodium in serum, plasma or urine using ion-selective electrodes. Sodium measurements are used in the diagnosis and treatment of aldosteronism (excessive secretion of the hormone aldosterone), diabetes insipidus (chronic excretion of large amounts of dilute urine, accompanied by extreme thirst), adrenal hypertension, Addison's disease (caused by destruction of the adrenal glands), dehydration, inappropriate antidiuretic hormone secretion, or other diseases involving electrolyte imbalance.

Methadone II (MDN2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of methadone in human urine on Roche/Hitachi 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).

Elecsys TSH is an immunoassay for the in vitro quantitative determination of thyrotropin in human serum and plasma. Measurements of TSH are used in the diagnosis of thyroid and

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

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

cobas pure integrated solutions analyzer

IV Device/System Characteristics:

A Device Description:

cobas pure Integrated Solutions System
The cobas pure integrated solutions is a fully automated, random-access, software-controlled system intended for in vitro quantitative, semi-quantitative and qualitative analysis of analytes in body fluids. The system consolidates clinical chemistry, homogenous and heterogeneous immunoassays as well as electrolyte testing within one workplace. The cobas pure integrated solutions consists of a clinical chemistry analytical unit (cobas c 303) with an integrated ISE analytical unit, an immunoassay analytical unit (cobas e 402) and a core unit.

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

ISE indirect Na for Gen. 2
ISE auxiliary reagents include:
- ISE Reference Electrolyte: 1 mol/L potassium chloride
- ISE Diluent: HEPES buffer, 10 mmol/L; triethanolamine, 7 mmol/L; preservative
- ISE Internal Standard: HEPES buffer, 10 mmol/L; triethanolamine, 7 mmol/L; sodium chloride, 3.06 mmol/L; sodium acetate, 1.45 mmol/L; potassium chloride, 0.16 mmol/L; preservative
- ISE Cleaning Solution: sodium hydroxide solution, 3 mol/L with sodium hypochlorite solution < 2 % active Cl
- ISE Deproteinizer: sodium hydroxide solution, approximately 1.2 % active Cl
- Electrodes: Sodium, reference

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

Elecsys TSH
The reagent working solutions include:

- M: streptavidin-coated microparticles, 1 bottle, 14.1 mL: streptavidin-coated microparticles 0.72 mg/mL; preservative
- R1: Anti-TSH-Ab~biotin, 1 bottle, 15.8 mL: biotinylated monoclonal anti TSH antibody (mouse) 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative
- R2: Anti-TSH-Ab~Ru(bpy)$_2^{3+}$, 1 bottle, 13.9 mL: monoclonal anti TSH antibody (mouse/human) labeled with ruthenium complex 1.5 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative

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.

ISE indirect Na for Gen. 2
The ion-selective electrode (ISE) analytical unit for Na^+ employs an ion-selective membrane to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The selective membrane is in contact with both the test solution and an internal filling solution. Due to the selectivity of the membrane, only the ions to be measured contribute to the EMF. The membrane EMF is determined by the difference in concentration of the test ion in the test solution and the internal filling solution. The EMF develops and ion concentration is determined according to the Nernst equation.

ONLINE DAT Methadone II
The Methadone assay is an immunoassay 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 at 546 nm 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.

Elecsys TSH
The Elecsys TSH immunoassay makes use of a sandwich test principle using monoclonal antibodies specifically directed against human TSH. The antibodies labeled with ruthenium

complex consist of a chimeric construct from human and mouse specific components. In the first incubation step, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex. In the second incubation step, after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured on the surface of the electrode. Unbound substances are removed by washing with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured and amplified by a photomultiplier. Results are determined via a calibration curve which is instrument specific, generated by 2−point calibration and a master curve provided via the cobas link.

C Instrument Description Information:

1. Instrument Name:

   cobas pure 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, ISE indirect Na for Gen.2, Elecsys TSH, ONLINE DAT Methadone II, cobas pro integrated solutions

B Predicate 510(k) Number(s):

K191899, K021505

C Comparison with Predicate(s):

Comparison of the Glucose HK Gen. 3

Device & Predicate Device(s):	**K220134**	**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 on Roche/Hitachi cobas c systems	Same
Technology	Photometric	Same
Test Format	Enzymatic	Same
Test Type	Quantitative	Same
Assay Protocol	R1+R2+Diluent+Sample, incubation	Same
Sample Volume	1.5 µL	Same
Handling of R1 and R2	Liquid, ready to use	Same
Measuring Range	2.0-750 mg/dL (0.11-41.6 mmol/L)	Same
General Device Characteristic Differences		
Instrument Platform	cobas c 303 in cobas pure integrated solutions	cobas c 503 in cobas pro integrated solutions

Comparison of the ISE indirect Na for Gen.2

Device & Predicate Device(s):	**K220134**	**K191899**
Device Trade Name	ISE indirect Na for Gen.2	ISE indirect Na for Gen.2
General Device Characteristic Similarities		
Intended Use/Indications for Use	In vitro test for the quantitative determination of sodium in serum, plasma or urine	Same

Device & Predicate Device(s):	K220134	K191899
	on the ISE unit of the Roche/Hitachi cobas c systems	
Technology	ISE Potentiometry	Same
Test Type	Quantitative	Same
Sample Volume	15 µL	Same
Default ISE Dilution Ratio 1:31	15 µL sample + 450 µL diluent	Same
Measuring Range	Serum/plasma: 80-180 mmol/L Urine: 20-250 mmol/L	Same
General Device Characteristic Differences		
Instrument Platform	cobas c 303 ISE in cobas pure integrated solutions	cobas pro ISE in cobas pro integrated solutions

Comparison of the ONLINE DAT Methadone II

Device & Predicate Device(s):	K220134	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 on Roche/Hitachi cobas c systems at a cutoff concentration of 300 ng/mL	Same
Technology	KIMS, Kinetic interaction of microparticles in a solution	Same
Reagents	Conjugate Working Solution: Conjugated methadone derivative in buffer with BSA and preservative. Antibody/Microparticle Working Solution:	Same

Device & Predicate Device(s):	K220134	K021505
	Microparticles coated with methadone monoclonal antibody (mouse) in buffer with BSA and preservative.	
Qualitative and Semi-Quantitative Cutoffs	300 ng/mL	Same
General Device Characteristic Differences		
Instrument Platform	cobas c 303 in cobas pure integrated solutions	cobas c 503 in cobas pro integrated solutions

Comparison of the Elecsys TSH

Device & Predicate Device(s):	K220134	K191899
Device Trade Name	Elecsys TSH	Elecsys TSH
General Device Characteristic Similarities		
Intended Use/Indications for Use	Immunoassay for the in vitro quantitative determination of thyrotropin in human serum and plasma	Same
Technology	Electrochemiluminescence Immunoassay (ECLIA)	Same
Test Format	Sandwich	Same
Test type	Quantitative	Same
Antibody/Reagents	Biotinylated monoclonal anti-TSH antibody (mouse) Monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex Streptavidin –coated microparticles	Same
Assay Protocol	R1+R2+sample, incubation, + beads, incubation	Same
Sample Volume	50 µL	Same
Handling of R1 and R2	Liquid, ready to use	Same
Measuring Range	0.005-100 µIU/mL	Same
Biotin Tolerance	1200 ng/mL	Same

Device & Predicate Device(s):	K220134	K191899
General Device Characteristic Differences		
Instrument Platform	cobas e 402 in cobas pure integrated solutions	cobas e 801 in cobas pro integrated solutions

Comparison of the cobas pure integrated solutions to the cobas pro integrated solutions systems

Device & Predicate Device(s):	K220134	K191899
Device Trade Name	cobas pure integrated solutions	cobas pro integrated solutions
General Device Characteristic Similarities		
Intended Use/Indications for Use	Automated analyzer, intended for running qualitative, semi-quantitative and quantitative clinical chemistry and immunochemistry assays as well as ion selective measurements	Same
Function Performed	Data Input, Sample Processing, Result Calculation, Result Reporting, Quality Control, Infrastructure (power, water supply)	Same
PC (Controller Unit) Functions	Data Input (Touch screen, Disc), Data Output (Screen, printer)	Same
Core Unit Functions	Real time database, data input and output (via HOST communication), control of sample conveyer	Same
Analytical Unit(s) Functions	Control of analytic processes (pipetting, incubation, detection) Primary signal processing	Same
Data Storage	Real time database in Core Unit (storage of System and Application	Same

Device & Predicate Device(s):	K220134	K191899
	parameters, Calibration Data, QC Data, Sample Results, Alarm history)	
Result Calculation	Automated measuring of signal using various methods according to automated calculation of concentration via calibration curve	Same
Initial Cassette Volume Check (ICVC) for Reagent Pipetting	Available	Same
Data Concept (Application Parameter, Calibrator, Control Value Transfer)	Electronic transfer possible (user must accept transfer before parameter applied)	Same
General Device Characteristic Differences		
Software	cobas pure integrated solutions software	cobas pro integrated solutions software
Configuration	Up to 2 analytical units with one PC and one sample supply unit	Several analytical units with one PC and one Core unit
Units Controlled	cobas c 303 analytical unit with integrated ISE analytical unit, cobas e 402	cobas c 503, cobas e 801, and cobas pro ISE analyzers
Throughput	cobas c 303: 450 tests/hr cobas c 303 ISE: 450 tests/hr cobas e 402: 120 tests/hr	cobas c 503: 1000 tests/hr cobas pro ISE: 900 tests/hr cobas e 801: 300 tests/hr

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 EP07 Interference Testing in Clinical Chemistry – Third Edition

CLSI EP09c Measurement Procedure Comparison and Bias Estimation Using Patient Samples – Third Edition

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

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 cobas pure integrated solutions system 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.

Sample	N	Mean (mg/dL)	Repeatability		Mean (mg/dL)	Within-Laboratory Precision	
			SD (mg/dL)	CV (%)		SD (mg/dL)	CV (%)
Serum Control 1	84	100	0.292	0.3	100	0.611	0.6
Serum Control 2	84	238	0.901	0.4	238	1.33	0.6
Serum 1	84	3.73	0.082	2.2	3.77	0.106	2.8
Serum 2	84	64.5	0.338	0.5	64.5	0.389	0.6
Serum 3	84	108	0.333	0.3	108	0.517	0.5
Serum 4	84	384	1.21	0.3	389	1.63	0.4
Serum 5	84	715	2.83	0.4	724	3.46	0.5
Urine Control 1	84	29.2	0.214	0.7	29.2	0.241	0.8
Urine Control 2	84	294	1.46	0.5	292	1.55	0.5
Urine 1	84	3.12	0.154	5.0	3.12	0.163	5.2
Urine 2	84	13.1	0.138	1.1	13.2	0.168	1.3
Urine 3	84	70.8	0.290	0.4	70.8	0.323	0.5
Urine 4	84	377	1.35	0.4	377	1.67	0.4
Urine 5	84	732	2.83	0.4	732	3.17	0.4
CSF Control 1	84	62.5	0.333	0.5	62.5	0.564	0.9
CSF Control 2	84	31.9	0.238	0.7	31.9	0.301	0.9

Sample	N	Mean (mg/dL)	Repeatability		Mean (mg/dL)	Within-Laboratory Precision	
			SD (mg/dL)	CV (%)		SD (mg/dL)	CV (%)
CSF 1	84	3.71	0.108	2.9	3.71	0.110	3.0
CSF 2	84	38.2	0.234	0.6	38.2	0.333	0.9
CSF 3	84	72.3	0.441	0.6	72.3	0.627	0.9
CSF 4	84	374	2.38	0.6	375	3.14	0.8
CSF 5	84	728	4.16	0.6	728	5.33	0.7

ISE Indirect Na for Gen. 2: Repeatability and Intermediate Precision

The precision of the ISE indirect Na for Gen. 2 test was evaluated on one cobas pure integrated solutions system using one reagent lot. 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. The protocol was repeated for each application (serum, plasma and urine). Pooled samples were used for each level, were aliquoted and then measured. Samples 1 and 5 were diluted or spiked, samples 2-4 were native. The results are summarized in the table below.

Sample	N	Mean (mmol/L)	Repeatability		Within-Laboratory Precision	
			SD (mmol/L)	CV (%)	SD (mmol/L)	CV (%)
Serum Control 1	84	112	0.665	0.6	1.13	1.0
Serum Control 2	84	137	0.683	0.5	1.20	0.9
Serum 1	84	86.1	0.458	0.5	1.18	1.4
Serum 2	84	132	0.561	0.4	1.06	0.8
Serum 3	84	136	0.688	0.5	0.95	0.7
Serum 4	84	157	0.834	0.5	1.13	0.7
Serum 5	84	174	0.832	0.5	1.31	0.8
Plasma Control 1	84	112	0.493	0.4	1.07	1.0
Plasma Control 2	84	137	0.525	0.4	1.16	0.9
Plasma 1	84	87.4	0.411	0.5	1.09	1.2
Plasma 2	84	131	0.584	0.4	0.98	0.8
Plasma 3	84	136	0.597	0.4	1.07	0.8
Plasma 4	84	156	0.721	0.5	0.96	0.6
Plasma 5	84	172	0.662	0.4	1.16	0.7
Urine Control 1	84	82.3	0.394	0.5	0.95	1.2
Urine Control 2	84	178	0.823	0.5	1.15	0.6
Urine 1	84	26.8	0.267	1.0	1.16	4.3
Urine 2	84	136	0.557	0.4	0.79	0.6

Sample	N	Mean (mmol/L)	Repeatability		Within-Laboratory Precision	
			SD (mmol/L)	CV (%)	SD (mmol/L)	CV (%)
Urine 3	84	112	0.433	0.4	0.81	0.7
Urine 4	84	199	0.942	0.5	1.69	0.8
Urine 5	84	239	0.868	0.4	2.50	1.0

ONLINE DAT Methadone II

The precision of the ONLINE DAT Methadone II test was evaluated on one cobas pure integrated solutions system 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
Control 1	84	84	0
Control 2	84	84	0
Cutoff	84	9	75
Control 3	84	0	84
Control 4	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 (mmol/L)	Repeatability		Within-Laboratory Precision	
			SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Sample - 100% (zero)	84	22.6	11.6	51.5	17.7	78.3
Sample - 75%	84	112	6.70	6.0	10.3	9.1
Sample - 50%	84	155	5.31	3.4	9.81	6.3
Control 1	84	234	6.78	2.9	11.0	4.7
Control 2	84	236	5.68	2.4	10.5	4.5
Cutoff	84	314	6.19	2.0	13.5	4.3
Control 3	84	389	7.00	1.8	15.3	3.9
Control 4	84	379	7.72	2.0	14.9	3.9

Sample	N	Mean (mmol/L)	Repeatability		Within-Laboratory Precision	
			SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Sample +50%	84	484	9.21	1.9	19.5	4.0
Sample +75%	84	543	10.7	2.0	20.5	3.8
Sample +100%	84	632	12.3	1.9	21.9	3.5

Elecsys TSH

Precision of the Elecsys TSH assay was evaluated on one cobas pure integrated solutions system using one reagent lot. 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 run in randomized order on the analyzer. Three serum-based controls and five levels of pooled serum samples were used (Serum 1-4 were native, Serum 5 was spiked with recombinant TSH). The results are summarized in the table below.

Sample	N	Mean (µIU/mL)	Repeatability		Within-Laboratory Precision	
			SD (µIU/mL)	CV (%)	SD (µIU/mL)	CV (%)
Serum Control 1	84	1.30	0.013	1.0	0.016	1.2
Serum Control 2	84	7.89	0.069	0.9	0.111	1.4
Serum Control 3	84	0.169	0.002	0.9	0.002	1.3
Serum 1	84	0.012	0.001	4.5	0.001	5.2
Serum 2	84	0.267	0.003	1.0	0.004	1.6
Serum 3	82	3.77	0.046	1.2	0.055	1.5
Serum 4	84	55.5	0.378	0.7	0.601	1.1
Serum 5	84	97.5	0.566	0.6	0.731	0.7

2. Linearity:

Glucose HK Gen.3

Linearity of the Glucose HK Gen.3 assay was assessed on one cobas pure integrated solutions system in one run using three reagent lots, measuring three replicates per sample. Four high analyte concentration human serum, plasma (collected in K_2EDTA tubes), urine and CSF samples were diluted to obtain 12-14 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	0-794.7	$y = 1.003x – 0.0273$, $R^2 = 0.9998$	2-750
Plasma	0-807.3	$y = 1.000x – 0.0011$, $R^2 = 0.9999$	2-750
Urine	0-827.1	$y = 1.002x – 0.0049$, $R^2 = 0.9999$	2-750
CSF	0-787.5	$y = 1.001x – 0.0042$, $R^2 = 0.9998$	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).

ISE Indirect Na for Gen. 2

Linearity of the ISE Indirect Na for Gen.2 test was assessed on one cobas pure integrated solutions system in one run using one reagent lot, measuring three replicates per sample. Three high analyte concentration human serum, plasma (collected in Li-heparin tubes) and urine samples were diluted to obtain 11 levels spanning the measuring range. The results of the linear regression analysis are summarized in the table below.

Sample	Tested Range (mmol/L)	Linear Regression	Claimed Linear Range (mmol/L)
Serum	75.1-190	$y = 1.000x + 0.0000$, $R^2 = 0.9996$	80-180
Plasma	73.3-183	$y = 1.000x + 0.0000$, $R^2 = 0.9996$	80-180
Urine	19.9-259	$y = 1.000x – 0.0312$, $R^2 = 0.9995$	20-250

The results demonstrated linearity of the claimed measuring range (80 mmol/L to 180 mmol/L for serum/plasma, and 25 mmol/L to 250 mmol/L for urine).

ONLINE DAT Methadone II

Linearity of the semi-quantitative ONLINE DAT Methadone II test was assessed on one cobas pure integrated solutions system in one run using one reagent lot, measuring three replicates per sample. A dilution series was prepared from human urine sample pools (spiked with methadone and diluted with human urine) to obtain 12 levels that span the range of the used calibrators. 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
0.0	0.0	0.0	
39.3	3.09	-36.2	
55.5	20.1	-35.4	
231	209	-22.0	
463	447		96.5
694	714		102.9
925	1073		116.0
1156	1181		102.2
1388	1469		105.8

Expected Value (ng/mL)	Observed Value (ng/mL)	Absolute Deviation (ng/mL)	% Recovery
1619	1621		100.1
1850	1880		101.6
2081	2167		104.1
2313	2091		90.4

Elecsys TSH
Linearity of the Elecsys TSH assay was assessed on one cobas pure integrated solutions system in one run using one reagent lot, measuring three replicates per sample. Three high analyte concentration human serum samples were diluted to obtain 12 levels spanning the measuring range. The results of the weighted linear regression analysis for a representative sample set are summarized in the table below.

Sample	Tested Range (µIU/mL)	Linear Regression	Claimed Linear Range (µIU/mL)
Serum	0.000-109	$y = 1.025x – 0.0013$, $R^2 = 0.9859$	0.004-102

The results demonstrated linearity of the claimed measuring range (0.004 – 102 µIU/mL).

Dilution Recovery Studies

Glucose HK Gen. 3
A dilution study was performed by diluting three high concentration samples each (serum, urine and CSF) with diluent (NaCl 9%) in a 1:2 ratio. The data support the 1:2 dilution claim in the Method Sheet.

ISE indirect Na for Gen. 2
A dilution study was performed by diluting three high concentration urine samples with the ISE diluent in a 1:46 ratio. The data support the 1:46 dilution claim in the Method Sheet.

ONLINE DAT Methadone II
No dilution claims are included in the Method Sheet.

Elecys TSH
A dilution study was performed by diluting three high concentration serum samples with the Diluent MultiAssay in a 1:10 ratio. The data support the 1:10 dilution claim in the Method Sheet.

High Dose Hook Effect

Elecsys TSH
The high-dose hook effect of the Elecsys TSH assay was assessed on the cobas pure integrated solutions system. Three human serum samples were spiked with analyte (recombinant human TSH) to achieve high TSH concentrations. For each sample, a dilution series was performed. The hook concentration reported corresponds to the highest analyte

concentration that generates a signal ≥ 10% above the upper limit of the measuring range. The sponsor determined that there is no hook effect up to 1000 µIU/mL TSH.

3. Analytical Specificity/Interference:

Endogenous Interference

The purpose of this study was to evaluate endogenous substances for potential interference when using the Glucose HK Gen.3 assay, the ISE indirect Na for Gen.2 test, the ONLINE DAT Methadone II test and the Elecsys TSH assay on the cobas pure integrated solutions system.

Glucose HK Gen. 3

The effect on the quantitation of the analyte in the presence of potentially interfering endogenous substances using the Glucose HK Gen.3 assay was determined on the cobas pure integrated solutions system using serum, urine, and CSF samples. Glucose levels of approximately 40 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 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	74.5 g/L
	Bilirubin	62 mg/dL
	Ditaurobilirubin	65 mg/dL
	Hemolysis	1090 mg/dL
	IgG	78.6 g/L
	Intralipid	1126 mg/dL
Urine	Albumin	2.50 g/L
	Calcium	12.0 mmol/L
	Citrate	11.0 mmol/L
	Creatinine	88.4 mmol/L
	Hemolysis	788 mg/dL
	IgG	1.10 g/L
	Magnesium	25.0 mmol/L
	Oxalate	1.50 mmol/L
	Phosphate	130 mmol/L
	Urea	1800 mmol/L
	Uric Acid	6.00 mmol/L
	Urobilinogen	0.250 mmol/L
CSF	Ditaurobilirubin	81 mg/dL

Potential Interferent		Highest Concentration Tested Without Significant Interference
	Hemolysis	1086 mg/dL

The sponsor provided information to support that the labeling interference limitations are unchanged from the predicate device (K191899).

ISE indirect Na for Gen. 2

The effect on the quantitation of the analyte in the presence of potentially interfering endogenous substances using the ISE indirect Na for Gen.2 test was determined on the cobas pure integrated solutions system using human plasma and serum samples. Sodium levels of approximately 124 mmol/L and 151 mmol/L were tested. Significant interference was defined as bias between the samples with and without interferent > ± 10%. The summary of results is presented in the table below.

Potential Interferent		Highest Concentration Tested Without Significant Interference
Plasma	Bilirubin	61 mg/dL
	Ditaurobilirubin	61 mg/dL
	Hemolysis	1110 mg/dL
	Intralipid	2043 mg/dL
Serum	Bilirubin	60.0 mg/dL
	Ditaurobilirubin	61.0 mg/dL
	Hemolysis	1008 mg/dL
	Intralipid	2017 mg/dL

The sponsor provided information to support that the labeling interference limitations are unchanged from the predicate device (K191899).

ONLINE DAT Methadone II

The effects of potentially interfering endogenous substances on the ONLINE DAT Methadone II test were determined on the cobas pure integrated solutions system using urine samples spiked with methadone. 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	800 mg/dL	Neg	Pos
Albumin	500 mg/dL	Neg	Pos
Ascorbic acid	1500 mg/dL	Neg	Pos
Bilirubin	25 mg/dL	Neg	Pos
Calcium	48 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	800 mg/dL	Neg	Pos
Glucose	2000 mg/dL	Neg	Pos
Hemoglobin	750 mg/dL	Neg	Pos
IgG	110 mg/dL	Neg	Pos
Magnesium ($MgCl_2$)	238 mg/dL	Neg	Pos
NaCl	2900 mg/dL and 5800 mg/dL	Neg	Pos
Oxalic acid	200 mg/dL	Neg	Pos
Phosphate (NaH_2PO_4, Dihydrate)	2028 mg/dL	Neg	Pos
Urea	6000 mg/dL	Neg	Pos
Uric Acid	101 mg/dL	Neg	Pos
Urobilinogen	15 mg/dL	Neg	Pos
Citrate	284 mg/dL	Neg	Pos
pH	4-8	Neg	Pos

No interference/crossover was observed from endogenous substances.

Elecsys TSH
The effect on the quantitation of the analyte in the presence of endogenous interfering substances using the Elecsys TSH assay was determined on the cobas pure integrated solutions system using human serum samples with a low (approximately 0.5 µIU/mL), a mid (approximately 4 µIU/mL) and a high (approximately 7 µIU/mL) TSH concentration. Significant interference was defined as bias between the samples with and without interferent > ± 10%. The summary of results is presented in the table below.

Potential Interferent	Highest Concentration Tested Without Significant Interference
Intralipid	2000 mg/dL
Biotin	1200 mg/dL
Bilirubin	66 mg/dL
Hemoglobin	1000 mg/dL
Rheumatoid Factor (RF)	1500 IU/mL
Human IgG	3.37 g/dL
Human IgM	0.64 g/dL

The sponsor provided information to support that the labeling interference limitations are unchanged from the predicate device (K191899).

The following statement regarding biotin interference is included in the labeling:
"This assay has no biotin interference in serum concentrations up to 1200 ng/mL. Some studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the

first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day[7] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[8]"

References:
7. Grimsey P, Frey N, Bendig G, et al. Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and in vitro immunoassay interference. Int J Pharmacokinet 2017;2(4):247-256.
8. Piketty ML, Prie D, Sedel F, et al. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. Clin Chem Lab Med 2017;55(6):817-825.

Exogenous Interference

The purpose of these studies was to evaluate drugs for potential interference when using the Glucose HK Gen.3 assay, the ISE indirect Na for Gen.2 test, the ONLINE DAT Methadone II test and the Elecsys TSH assay on the cobas pure integrated solutions system. The acceptance criteria were recovery within 100 ± 10% for all evaluated assays 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 the analyte in the presence of potentially interfering drugs using the Glucose HK Gen.3 assay was determined on the cobas pure integrated solutions system 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 Tested Without Significant Interference**
Serum	N-Acetylcysteine	150 mg/L
	Acetylsalicylic acid	30 mg/L
	Ampicillin-Na	75 mg/L
	Ascorbic acid	52.5 mg/L
	Cefoxitin	750 mg/L
	Doxicyclinr	18.0 mg/L
	Heparin	3300 IU/L
	Levodopa	7.5 mg/L
	Methyldopa	22.5 mg/L
	Metronidazole	123 mg/L
	Rifampicin	48 mg/L
	Acetaminophen	156 mg/L
	Cyclosporine	1.8 mg/L
	Ibuprofen	219 mg/L
	Theophylline	60 mg/L
	Phenylbutazone	321 mg/L
Urine	Acetaminophen	3000 mg/L
	N-Acetylcysteine	10 mg/L
	Ascorbic acid	4000 mg/L

Potential Interferent		Highest Concentration Tested Without Significant Interference
	Cefoxitin	12000 mg/L
	Gentamycine sulfate	400 mg/L
	Levodopa	1000 mg/L
	Methyldopa	2000 mg/L
	Ofloxacine	900 mg/L
	Ibuprofen	4000 mg/L
	Phenazopyridine	300 mg/L
	Salicyluric acid	100 mg/L
	Tetracycline	100 mg/L

ISE indirect Na for Gen. 2

The effect on the quantitation of the analyte in the presence of potentially interfering drugs using the ISE indirect Na for Gen. 2 assay was determined on the cobas pure integrated solutions system using human plasma, serum, and urine samples. Sodium levels of approximately 124 and 156 mmol/L for serum and plasma and at 32 and 197 mmol/L for urine were tested. The summary of results is presented in the table below.

Potential Interferent		Highest Concentration Tested Without Significant Interference
Serum and Plasma	N-Acetylcysteine	1660 mg/L
	Ampicillin-Na	1000 mg/L
	Ascorbic acid	300 mg/L
	Cyclosporine	5 mg/L
	Na-Cefoxitin	2500 mg/L
	Heparin	5000 IU/L
	Intralipid	10000 mg/L
	Levodopa	20 mg/L
	Methyldopa	20 mg/L
	Metronidazole	200 mg/L
	Phenylbutazone	400 mg/L
	Doxycycline	50 mg/L
	Acetylsalicylic acid	1000 mg/L
	Rifampicin	60 mg/L
	Acetaminophen	200 mg/L
	Ibuprofen	500 mg/L
	Theophylline	100 mg/L
Urine	Acetaminophen	3000 mg/L
	N-Acetylcysteine	10 mg/L
	Salicyluric acid	6000 mg/L
	Ascorbic acid	4000 mg/L
	Na-Cefoxitin	12000 mg/L
	Gentamycine sulfate	400 mg/L
	Ibuprofen	4000 mg/L

Potential Interferent		Highest Concentration Tested Without Significant Interference
	Levodopa	1000 mg/L
	Methyldopa	2000 mg/L
	Ofloxacine	900 mg/L
	Phenazopyridine	300 mg/L
	Tetracycline	300 mg/L

ONLINE DAT Methadone II

The effects of potential drug interference on the ONLINE DAT Methadone II assay were determined on the cobas pure integrated solutions system using urine samples. Two 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.

Structurally Unrelated Pharmacological Compounds:

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
Δ9 THC-9-carboxylic acid	100,000	Neg	Pos
Acetaminophen	3,000,000	Neg	Pos
Acetylsalicylic acid	100,000	Neg	Pos
Aminopyrine	100,000	Neg	Pos
Amitriptyline	100,000	Neg	Pos
Amobarbital	100,000	Neg	Pos
Ampicillin	100,000	Neg	Pos
Ascorbic acid	4,000,000	Neg	Pos
Aspartame	100,000	Neg	Pos
Atropine	100,000	Neg	Pos
Benzocaine	100,000	Neg	Pos
Benzoylecogonine	100,000	Neg	Pos
Benzphetamine	100,000	Neg	Pos
Butabarbital	100,000	Neg	Pos
Caffeine	100,000	Neg	Pos
Calcium hypochlorite	100,000	Neg	Pos
Carbamazepine	100,000	Neg	Pos
Cefoxitin	12,000,000	Neg	Pos
Chlordiazepoxide	100,000	Neg	Pos
Chloroquine	100,000	Neg	Pos
Chlorpheniramine	100,000	Neg	Pos
Cocaine	100,000	Neg	Pos
Codeine	100,000	Neg	Pos

Potential Interferent	Drug Concentration (ng/mL)	Methadone Concentration	
		225 ng/mL (-25% Cutoff, Negative Control)	375 ng/mL (+25% Cutoff, Positive Control)
Cotinine	100,000	Neg	Pos
Cyclobenzaprine	100,000	Neg	Pos
Cyproheptadine	100,000	Neg	Pos
d-Amphetamine	100,000	Neg	Pos
d-Ephedrine	100,000	Neg	Pos
Desipramine	100,000	Neg	Pos
Dextrometorphan	100,000	Neg	Pos
Diazepam	100,000	Neg	Pos
Diphenhydramin	100,000	Neg	Pos
Diphenylhydantoin	100,000	Neg	Pos
Disopyramid	1,000,000	Neg	Pos
dl-Ephedrine	100,000	Neg	Pos
d-Methamphetamine	100,000	Neg	Pos
Dopamine	100,000	Neg	Pos
Doxepin	100,000	Neg	Pos
Doxylamine	100,000	Neg	Pos
d-Phenylpropanolamine	100,000	Neg	Pos
d-Propoxyphen	100,000	Neg	Pos
d-Pseudoephedrine	100,000	Neg	Pos
Ecgonine	100,000	Neg	Pos
Ecgonine methyl ester	100,000	Neg	Pos
EDDP	100,000	Neg	Pos
EMDP	100,000	Neg	Pos
Epinephrine	100,000	Neg	Pos
Erythromycin	100,000	Neg	Pos
Estriol	100,000	Neg	Pos
Fenoprofen	100,000	Neg	Pos
Fluoxetine	100,000	Neg	Pos
Furesemide	100,000	Neg	Pos
Gentamycine sulfate	400,000	Neg	Pos
Gentisic acid	100,000	Neg	Pos
Glutethimide	100,000	Neg	Pos
Guaiacol glycerol ether	100,000	Neg	Pos
Haloperidol	100,000	Neg	Pos
Hydrochlorothiazide	100,000	Neg	Pos
Ibuprofen	4,000,000	Neg	Pos
Imipramine	100,000	Neg	Pos
Isoproterenol	100,000	Neg	Pos
Ketamine	100,000	Neg	Pos
l-Amphetamine	100,000	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-Ephedrine	100,000	Neg	Pos
Levodopa	1,000,000	Neg	Pos
Lidocaine	100,000	Neg	Pos
l-Methamphetamine	100,000	Neg	Pos
l-Norpseudoephedrine	100,000	Neg	Pos
l-Pseudoephedrine	100,000	Neg	Pos
LSD	100,000	Neg	Pos
Maprotiline	100,000	Neg	Pos
MDA	100,000	Neg	Pos
MDMA	100,000	Neg	Pos
Melanin	10,000	Neg	Pos
Meperidin	100,000	Neg	Pos
Methaqualone	100,000	Neg	Pos
Methyldopa	2,000,000	Neg	Pos
Methylphenidate	100,000	Neg	Pos
Mianserin	100,000	Neg	Pos
Morphine	100,000	Neg	Pos
N-acetyl cysteine	10,000	Neg	Pos
Naloxone	100,000	Neg	Pos
Naltrexone	100,000	Neg	Pos
Naproxen	100,000	Neg	Pos
Niacinamide	100,000	Neg	Pos
Nicotine	100,000	Neg	Pos
Nordiazepam	100,000	Neg	Pos
Nordoxepin	100,000	Neg	Pos
Norethindrone	100,000	Neg	Pos
Nortriptyline	100,000	Neg	Pos
Ofloxacine	90,000	Neg	Pos
Orphenadrine	100,000	Neg	Pos
Oxazepam	100,000	Neg	Pos
Penicillin G	100,000	Neg	Pos
Pentobarbital	100,000	Neg	Pos
Perphenazine	100,000	Neg	Pos
Phenazopyridine	300,000	Neg	Pos
Phencyclidine	100,000	Neg	Pos
Phenobarbital	100,000	Neg	Pos
Phenothiazine	100,000	Neg	Pos
Phentermine	100,000	Neg	Pos
Phenylbutazone	100,000	Neg	Pos
Phenylpropanolamine	100,000	Neg	Pos
Procaine	100,000	Neg	Pos
Protriptyline	100,000	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 fumarate	750,000	Neg	Pos
Quetiapine Carboxylic Acid	500,000	Neg	Pos
Quetiapine Sulfoxyd	500,000	Neg	Pos
Quinidine	100,000	Neg	Pos
Quinine	100,000	Neg	Pos
Salicyluric acid	6,000,000	Neg	Pos
Secobarbital	100,000	Neg	Pos
ß-Phenethylamine	100,000	Neg	Pos
Sulindac	100,000	Neg	Pos
Tetracycline	300,000	Neg	Pos
Tetrahydrozoline	100,000	Neg	Pos
Tramadol	20,000	Neg	Pos
Trifluoperazine	100,000	Neg	Pos
Tyramine	100,000	Neg	Pos
Verapamil	100,000	Neg	Pos

No interference was found for all tested compounds.

Elecsys TSH

The effect on the quantitation of the analyte in the presence of potentially interfering drugs was determined by comparing values obtained from samples spiked with 17 commonly and 13 specially used pharmaceutical compounds with the reference sample (unspiked). Two human serum samples (native serum pools) with analyte concentration of approximately 0.3 and 7 µIU/mL were used and tested on the cobas pure integrated solutions system. The summary of results is presented in the table below.

Potential Interferent	Highest Concentration Tested Without Significant Interference (mg/L)
Acetylcysteine	150
Ampicillin - Na	75
Ascorbic acid	52.5
Cyclosporine	1.8
Cefoxitin	750
Heparin	3300 IU/mL
Itraconazole	30
Levodopa	7.5
Methyldopa	22.5
Metronidazole	123
Phenylbutazone	321
Doxycycline	18
Acetylsalicylic acid	30
Rifampicin	48

Potential Interferent	Highest Concentration Tested Without Significant Interference (mg/L)
Acetaminophen	156
Ibuprofen	219
Theophylline	60
Iodide	0.2
Levothyroxine	0.25
Liothyronine	0.075
Carbimazole	30
Methimazole	80
Propylthiouracil	300
Perchlorate	2000
Propranolol	240
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.3

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. 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	5105	5.9
LUAA34443	5216	5.8
Cyamemazine	8983	3.3
Methotrimeprazine (Levomepromazine)	9359	3.2
Chlorpromazine	48833	0.6
Thiothixene	71795	0.4
Promazine	184457	0.2
Clomipramine	251636	0.1
Thioridazine	264054	0.1
Chlorprothixene	469530	0.1
Promethazine	472000	0.1
Trimipramine	594975	0.1

4. Assay Reportable Range:

Assays reportable ranges are described in the linearity section (VII.A.2 above).

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

Traceability

Traceability information for the Glucose HK Gen.3 assay, the ISE indirect Na for Gen.2 test and the Elecsys TSH assay was provided in K191899 and is unchanged.

ONLINE DAT Methadone II assay is traceable to a primary reference method (GC-MS).

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 cobas pure integrated solutions system.

ONLINE DAT Methadone II
A reagent stability study confirmed that the ONLINE DAT Methadone II reagent is stable for 26 weeks on-board the cobas pure integrated solutions system.

Elecsys TSH
A reagent stability study confirmed that the Elecsys TSH reagent is stable for 16 weeks on-board the cobas pure integrated solutions system.

6. Detection Limit:

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 and the ISE indirect Na for Gen. 2 test, one analyte-free sample was measured with three reagent lots in six runs, distributed over three days, 10 replicates per run, on one cobas pure integrated solutions system. For determination of LoB for the Elecsys TSH assay, five blank samples were measured with three reagent lots in six runs, distributed over three days, two replicates per run, on one cobas pure integrated solutions system. 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.

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, the ISE indirect Na for Gen.2 test and the Elecsys TSH assay, five samples with low analyte content were measured using three reagent lots with two-fold determination per run on one cobas pure integrated solutions system. 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. $LoD = LoB + 1.653 \times SD_{total}$.

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 and the Elecsys TSH assay and a maximum total error (TE) of 30% for the ISE indirect Na for Gen.2 test. For determination of the LoQ for the Glucose HK Gen. 3 assay, the ISE indirect Na for Gen.2 test and the Elecsys TSH assay, five samples with low analyte concentration were measured using three reagent lots on one cobas pure integrated solutions system. For the Glucose HK Gen.3 assay and the Elecsys TSH assay, these samples were tested in one run per day over five days, five replicates per run for each LoQ sample (n=25 per sample). For the ISE indirect Na for Gen.2 test, these samples were tested in two runs per day over three days, two replicates per run for each LoQ sample (n=12 per sample).

The results support the claimed LoB, LoD and LoQ for the Glucose HK Gen.3 assay, the ISE indirect Na for Gen.2 test and the Elecsys TSH assay which are unchanged from the predicate device (K191899) and are summarized in the table below.

Assay	Claimed LoB	Claimed LoD	Claimed LoQ
Glucose HK Gen. 3 (serum/plasma, urine, and CSF)	2 mg/dL (0.11 mmol/L)	2 mg/dL (0.11 mmol/L)	2 mg/dL (0.11 mmol/L)
ISE indirect Na for Gen. 2 (serum, plasma and urine)	3.5 mmol/L	4.5 mmol/L	12.2 mmol/L
Elecsys TSH (serum)	0.0025 µIU/mL	0.005 µIU/mL	0.005 µIU/mL

The upper and lower limits of the measuring range for the Glucose HK Gen.3 assay, the ISE indirect Na for Gen.2 test and the Elecsys TSH assay are supported by the LoQ studies and the linearity studies described above (section VII.A.2).

7. Assay Cut-Off:

Glucose HK Gen. 3, ISE indirect Na for Gen. 2 and Elecsys TSH
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).

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

Carry-over information for the Glucose HK Gen.3 assay, the ISE indirect Na for Gen.2 test and the ONLINE DAT Methadone II test is provided in the Method Sheets of these assays. The sponsor has evaluated the special wash program and the carry over evasion rules on the cobas pure integrated solutions system.

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 cobas pure integrated solutions system and the Glucose HK Gen.3 assay on the cobas pro integrated solutions system (predicate) 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	69	2.2-746.0	1.000x + 0.0000 t = 0.991	1.000x – 0.0110 r = 1.000
Urine	71	2.1-742.3	1.006x + 0.0007 t = 0.982	1.006x + 0.0016 r = 1.000
CSF	66	5.5-742.3	1.000x – 0.0100 t = 0.979	0.990x + 0.0202 r = 1.000

ISE indirect Na for Gen. 2

Method comparison studies were performed for all sample types using the ISE indirect Na for Gen2. test on the cobas pure integrated solutions system and the ISE indirect Na for Gen2. Test on the cobas pro integrated solutions system (predicate) to assess the differences between the two test systems. Additionally, the results of the candidate test system were compared against flame photometry. Human samples for all sample types were tested in singlicate on each test system. Less than 15% of these samples were spiked or diluted to cover the measuring range. The results were evaluated using Passing-Bablok and linear regression analyses. A summary of results is presented in the table below.

cobas pure integrated solutions vs.	Sample Type	N	Sample Concentration Range (mmol/L)	Passing-Bablok (Slope & Intercept) and Correlation (Kendall tau (t))	Linear Regression (Slope & Intercept) and Correlation (Pearson (r))
Cobas pro integrated solutions	Serum	120	81.6-178	0.984x + 1.23 t = 0.985	0.983x + 1.29 r = 1.000
Flame photometer	Serum	120	81.5-182	1.007x – 1.19 t = 0.942	1.001x – 0.258 r = 0.996
Cobas pro integrated solutions	Plasma	119	84.5-174	0.980x + 2.38 t = 0.927	0.981x + 2.30 r = 0.999
Flame photometer	Plasma	118	81.6-176	0.985x + 1.38 t = 0.868	1.000x – 0.415 r = 0.994
Cobas pro integrated solutions	Urine	118	23.9-246	0.997x + 0.334 t = 0.994	1.004x – 0.168 r = 1.000
Flame photometer	Urine	105	24.9-256	0.973x + 1.97 t = 0.975	0.976x + 1.80 r = 0.999

ONLINE DAT Methadone II

50 negative urine samples and 50 positive urine samples were evaluated with the ONLINE DAT Methadone II test and methadone concentrations were confirmed by GC-MS. In addition, six samples with methadone concentrations of 75-100 % of the cutoff concentration and 15 samples with methadone concentration of 100-125 % of the cutoff concentration were tested. A summary of results is presented in the tables below.

Qualitative Accuracy Study (cutoff = 300 ng/mL)

		Negative samples	GC-MS values (ng/mL)		
			Near cutoff		Positive samples
			229-256	317-370	412-1703
cobas pure integrated solutions	+	0	1	15	50
	-	50	5	0	0
	Total	50	6	15	50

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

		Negative samples	GC-MS values (ng/mL)		
			Near cutoff		Positive samples
			229-256	317-370	412-1703
cobas pure integrated solutions	+	0	1	15	50
	-	50	5	0	0
	Total	50	6	15	50

Elecsys TSH
A method comparison study was performed using the Elecsys TSH assay on the cobas pure integrated solutions system and the Elecsys TSH assay on the cobas pro integrated solutions system (predicate) to assess the differences between the two test systems. A total of 147 samples (132 native human serum samples, single donors as well as pools and 15 native human serum samples diluted with Diluent Multiassay) were measured in singlicate on each test system. The results were evaluated using Passing-Bablok and linear regression analyses. A summary of results is presented in the table below.

Sample Type	N	Sample concentration range (µIU/mL)	Passing-Bablok (Slope & Intercept) and correlation (Kendall tau (t))	Linear Regression (Slope & Intercept) and correlation Pearson (r)
Serum	174	0.006-98.1	0.987x – 0.002 t = 1.00	0.983x + 0.016 r = 0.992

2. Matrix Comparison:

Glucose HK Gen. 3
To support the use of the Glucose HK Gen.3 assay on the cobas pure integrated solutions system 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 to cover the measuring range. The result of the serum sample was compared with the result of each plasma sample from the same donor. At least 30 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	N	Range (mg/dL)	Slope	Intercept	Coefficient r
Serum with gel separation vs. serum	38	3.68-708	0.996	-0.0997	1.000
K_2EDTA plasma vs. serum	50	3.68-708	0.998	-0.327	1.000
Li-heparin plasma vs. serum	50	3.68-708	1.001	-1.14	0.999
NaF/K-Oxalate plasma vs. serum	51	3.68-708	1.008	-0.619	0.999
NaF/Na_2EDTA plasma vs. serum	48	3.68-708	1.005	0.334	0.999
NaF/citrate/Na_2EDTA plasma vs. serum	48	3.68-708	1.003	-1.39	0.999
KF/Na_2EDTA plasma vs. serum vs.	48	3.68-708	1.007	-1.87	0.998

The resulting data support the method sheet claim that serum tube with separation gel, K_2EDTA, Li-heparin, NaF/K-oxalate, NaF/Na_2EDTA, NaF/citrate/Na_2EDTA, and

KF/Na_2EDTA plasma tubes are acceptable serum/plasma tubes for use with the Glucose HK Gen.3 assay.

ISE indirect Na for Gen.2
To support the use of the ISE indirect Na for Gen.2 test on the cobas pure integrated solutions system with other sample matrices, a matrix comparison study was conducted by testing matched Li-heparin plasma and serum samples from the same patients on one cobas pure integrated solutions system. The samples were tested in singlicate. The results were evaluated using Passing-Bablok regression analysis. A summary of results is presented in the table below.

Comparison	N	Range (mmol/L)	Slope	Intercept	Coefficient r
Li-heparin plasma vs. Serum	50	85.6-176.3	1.003	-0.885	0.999

The resulting data support the method sheet claim that serum and Li-heparin plasma tubes are acceptable tubes for use with ISE indirect Na for Gen.2 assay.

Elecsys TSH
To support the use of the Elecsys TSH assay on the cobas pure integrated solutions system with other sample matrices, a matrix comparison study was conducted by comparing values obtained from samples (native human serum samples) drawn into serum, Li-heparin, K_2EDTA and K_3EDTA plasma tubes. At least 50 serum/plasma pairs per anticoagulant type were tested in singlicate on one cobas pure integrated solutions system. The results were evaluated using Passing Bablok regression analysis. A summary of results is presented in the table below.

Comparison	N	Range (µIU/L)	Slope	Intercept	Coefficient r
Li-heparin plasma vs. serum	51	0.008-91.6	0.964	-0.008	0.999
K_2EDTA plasma vs. serum	52	0.008-91.6	0.966	0.004	0.999
K_3EDTA plasma vs. serum	52	0.008-91.6	0.970	-0.006	1.000

In addition, a plasma separation tubes (PST) and serum separation tubes (SST) comparison study was conducted, using separation tubes from three separate manufacturers and blood from at least seven donors. Measurements were performed in duplicate using one reagent lot on one cobas pure integrated solutions system and evaluated based on recovery relative to the serum/plasma tube without separating gel (reference).

The results of these studies support the method sheet claim that the following sample types are acceptable to use for Elecsys TSH assay: serum collected using standard serum tubes or tubes containing separating gel, Li-heparin, K_2EDTA, K_3EDTA plasma and plasma tubes containing separating gel.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

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

   Not applicable.

D Clinical Cut-Off:

Not applicable.

E 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.

ISE indirect Na for Gen. 2

Expected Values		
Sample Type		**Reference Range**
Serum, plasma	Adults	136-145 mmol/L
Urine	24 h, Adults	40-220 mmol/24 h

Reference: Tietz NW. Fundamentals of Clinical Chemistry, 5th ed. Burtis CA, Ashwood ER, eds. WB Saunders Co 2001:970, 1004, 1009.

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.

Elecsys TSH

Expected Values	
Sample Type	**Reference Range**
Serum, plasma	0.270-4.20 µIU/mL

Reference: Ebert C, Bieglmayer C, Igari J, et al. Elecsys TSH, FT4, T4, T-uptake, FT3 and T3. Clinical results of a multicentre study. Wien Klin Wochenschr 1998;110 Suppl 3:27-40.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports 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.