510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k123965

B. Purpose for Submission:

New Device

C. Measurand:

Direct Bilirubin

D. Type of Test:

Quantitative diazo colorimetric method

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

COBAS INTEGRA Bilirubin Direct Gen.2

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CIG	Class II	21 CFR 862.1110 (Bilirubin (total or direct) test system)	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   COBAS INTEGRA Bilirubin Direct Gen.2 is an in vitro test for the quantitative determination of direct bilirubin in human serum and plasma on COBAS INTEGRA systems. Measurement of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

3. Special conditions for use statement(s):

   For prescription use only

   Do not use any hemolyzed sample. Hemolyzed samples will give falsely negative results.

   Phenylbutazone causes falsely low bilirubin results.

   In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

4. Special instrument requirements:

   For use on the COBAS INTEGRA clinical chemistry analyzer

I. Device Description:

The COBAS INTEGRA Bilirubin Direct Gen.2 is composed of two reagent bottles. Reagent 1(R1) contains Phosphoric acid: 85 mmol/L; HEDTA: 4.0 mmol/L; NaCl: 50 mmol/L; detergent; pH 1.9. Reagent 2 (SR) is composed of 3, 5-Dichlorophenyl diazonium: 1.5 mmol/L; pH 1.3.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   COBAS INTEGRA Bilirubin Direct reagent

2. Predicate 510(k) number(s):

   k063543

3. Comparison with predicate:

Item	Bilirubin Direct Gen.2 (Candidate Device)	Bilirubin Direct (Predicate Device k063543)
Indication for Use	COBAS INTEGRA Bilirubin Direct Gen.2 is an in vitro test for the quantitative determination of direct bilirubin in human serum and plasma on COBAS INTEGRA systems. Measurement of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.	Same
Sample Types	Serum and plasma	Same
Method	Diazo colorimetric method	Same
Calibrator	Calibrator for automated systems (C.f.a.s.) and deionized water as the zero calibrator	Same
Traceability	Standardized against the Doumas manual reference method	Same
Permissible Anticoagulants	Li-heparin K_2-EDTA K_3-EDTA	Li-heparin
Instrument Platform	COBAS INTEGRA 800	COBAS INTEGRA 400, 400 Plus, 700, and 800
Reagent Composition	R1: Phosphoric acid 85 mmol/L,	R1: Sulfanilic acid 35 mmol/L,
Reagent Shelf Life Stability	2-8 ºC until expiration date	15-25 ºC until expiration date
Reagent On-Board Stability	COBAS INTEGRA 800: 8 ºC for 6 weeks	COBAS INTEGRA 700/800: 8 ºC for 12 weeks
Controls	Precinorm U plus, Precipath U plus, PreciControl ClinChem Multi 1A, PreciControl ClinChem Multi 2A	Precinorm U plus, Precipath U plus, Precinorm U, Precipath U

Measuring Range	0.07 – 13.8 mg/dL	0.10 – 25 mg/dL
Expected Values	≤ 0.20 mg/dL	0 to 0.2 mg/dL
Lower Limits of Measure	LoB = 0.05 mg/dL LoD = 0.07 mg/dL LoQ = 0.07 mg/dL	LDL = 0.10 mg/dL

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures
CLSI EP17-A2 Evaluation of Detection Capability fro Clinical Laboratory Measurement Procedures; Approved Guideline, 2nd ed.

L. Test Principle:

COBAS INTEGRA Bilirubin Direct Gen.2 measures direct bilirubin by employing the diazo method. Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with 3,5-dichlorophenyl diazonium salt in acid buffer to form the red-colored azobilirubin. The color intensity of the red azobilirubin formed is directly proportional to the direct bilirrubin concentration. The color intensity is measured photometrically by a COBAS INTEGRA clinical chemistry analyzer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was determined according to CLSI EP5-A2. The study included three human serum samples (0.12, 3.76, and 13.2 mg/dL) and two serum-based control samples (Preci-norm U plus and Preci-path U plus) in two aliquots per run and two runs per day for 21 days. Human serum 1 is a native human serum sample and human serum 2 and 3 were prepared by spiking samples with ditaurobilirubin to achieve high values. The within-run and total precision data are summarized in the tables below. .

Specimen	PNU	PPU	Human Serum 1	Human Serum 2	Human Serum 3
Mean (mg/dL)	0.75	1.9	0.12	3.8	13.2
Within Run Imprecision SD	0.01	0.01	0.01	0.01	0.04
Within Run Imprecision % CV	1.2	0.6	7.4	0.4	0.3

Specimen	PNU	PPU	Human Serum 1	Human Serum 2	Human Serum 3
Mean (mg/dL)	0.75	1.9	0.12	3.8	13.2
Total Imprecision SD	0.01	0.02	0.01	0.04	0.05
Total Imprecision % CV	1.6	1.0	7.7	1.0	0.4

b. Linearity/assay reportable range:

Linearity was assessed according to CLSI EP6-A and measured in triplicate. Two separate dilution series differing by sample type (serum and plasma) were prepared with thirteen levels each. The highest concentration samples were created by taking low analyte native samples and spiking them with ditaurobilirubin to exceed the desired measuring range.

	Plasma	Serum
Range tested (mg/dL)	0.01 – 19.5	0.02 – 19.4
measuring range (mg/dL)	0.07 – 13.8	0.07 – 13.8

Linear Regression Equation for Serum
$y = 1.0000x – 0.0000$, $r2 = 0.9944$

Linear Regression Equation for Plasma
$y = 1.0000x – 0.0000$, $r2 = 0.9977$

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

This method has been standardized against the manual test performance using the Doumas method.

Calibrator for automated systems (C.f.a.s.) and a zero calibrator (deionized water) are required for the calibration of the direct bilirubin assay. The C.f.a.s. calibrator has been previously cleared in k101456.

d. Detection limit:

LoB, LoD, and LoQ studies were performed based upon CLSI EP17-A2.

The LOB determination was performed using one blank sample, tested in quintuplicate using two analyzers with three reagent batches. Two runs per day were performed across three days.

To determine the LOD, five low-analyte samples were measured in singlicate on two analyzers with three reagent batches. Two runs per day were performed across three days.

A low-level sample set of nine was used to determine the LoQ and measured in singlicate, using three reagent batches on two analyzers. Two runs per day were performed across three days. The LoQ was determined based on inter-assay precision at 20% CV.

The LoB, LoD, and LoQ are summarized below.

LoB claim = 0.05 mg/dL
LoD claim = 0.07 mg/dL
LoQ claim = 0.07 mg/dL

The direct bilirubin assay has a measuring range of 0.07 to 13.8 mg/dL.

e. Analytical specificity:

Endogenous substances:

The reagent was evaluated with two endogenous substances, hemoglobin and lipids, for potential interference with the measurement of direct bilirubin. One pool of human serum was spiked with the interferent. A second pool of human serum was used as the control pool. The two pools were mixed in different ratios to yield a dilution series with varying concentrations of the interferent (from 0 to 10). The sponsor defined non significant interference if the bias between control pool and test pool was within ± 10%. The sponsor concluded that lipemia (lntralipid) tested up to 1098 mg/dL and hemolysis tested up to 25 mg/dL does not have significant interference.

The sponsor has the following limitation in their labeling:

Do not use any hemolyzed sample. Hemolyzed samples will give falsely negative results.

Common drugs:

Eighteen commonly used drugs were added to native patient samples and examined for potential interference on measurement with COBAS INTEGRA Bilirubin Direct reagent. Testing was performed with serum sample pools at two target concentrations of direct bilirubin, one at a low concentration of ~ 1.8 mg/dL and one at a high concentration of ~ 4.9 mg/dL. Measurements were done in triplicate on the COBAS INTEGRA analyzer. The mean value among the triplicates for each aliquot was determined and the percent recovery to the initial value was calculated. The sponsor defined non significant interference if the % recovery of the tested aliquot was within ± 10% of the initial value. Results are summarized in the table below.

Drug	Highest concentration at which non significant interference is observed (mg/L, except Heparin)
Acetylcystein	150
Ampicillin - Na	1000
Ascorbic acid	300
Ca - Dobesilate	200
Cyclosporine A	5
Cefoxitin	2500
Heparin - Na	5000 U
Intralipid	10000
Levodopa	20
Methyldopa	20
Metronidazole	200
Doxycyclin	50
Acetylsalicylic	1000
Rifampicin	60
Acetaminophen	200
Ibuprofen	500
Theophylline	100

Based on the study results, sponsor determined that Phenylbutazone causes falsely low bilirubin results. Therefore, sponsor put this information in the limitation section in the labeling.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Direct bilirubin values for 71 native human serum samples were obtained using the candidate reagent (y-axis) to the predicate reagent (x-axis) on the COBAS INTEGRA 800 clinical chemistry analyzer. Samples ranged from 0.083 to 13.762 mg/dL and were tested in singlicate. The values were regressed using the Passing/Bablok model to produce the following equation.

$y = 1.0490x + 0.0699$, $r^2 = 0.9979$

b. Matrix comparison:
To test the possible interference of the anticoagulants Lithium-heparin, K2-EDTA, and K3-EDTA, thirty two tubes were collected per anticoagulant. Samples ranged from 0.10 to 12.2 mg/dL were tested in singlicate. Plasma results were compared to serum results and results were analyzed using the Passing/Bablok regression.

The following are the regressions for the comparisons.

Serum vs. Li-heparin P/B: y = 0.01 + 1.0179x, r = 0.9988
Serum vs. K2-EDTA P/B: y = -0.01 + 1.0120x, r = 0.9988
Serum vs. K3-EDTA P/B: y = -0.03 + 1.0095x, r = 0.9988

Based on the study results, the sponsor concluded that Lithium-heparin, K2-EDTA, and K3-EDTA are permissible anticoagulants for use with this direct bilirubin assay.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Direct bilirubin ≤ 0.20 mg/dL

Balisteri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987; 729-761.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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