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

A. 510(k) Number:

k152343

B. Purpose for Submission:

New Device

C. Measurand:

Bilirubin, Direct

D. Type of Test:

Quantitative, Colorimetric Assay

E. Applicant:

Randox Laboratories Limited

F. Proprietary and Established Names:

Direct Bilirubin

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1110 Bilirubin (Total or Direct) Test System

2. Classification:

Class II

3. Product code:

JFM

4. Panel:

75, Chemistry

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The Direct Bilirubin test system is a device intended for the quantitative *in vitro* determination of Direct Bilirubin in serum and plasma. Bilirubin measurements can be 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. Not intended for use with neonates.

4. Special instrument requirements:

   Randox RX Daytona Plus analyzer

I. Device Description:

The device consists of two ready to use reagents

4 x 20 ml bottles of Direct Bilirubin R1- tartrate buffer, pH 2.9, concentration: 0.1 mol/L, detergent, inhibitors, antimicrobial and preservatives.

4 x 8 ml bottles of Direct Bilirubin R2- phosphate buffer, pH 7.0, concentration: 10 mmol/L, sodium metavanadate, concentration: 4 mmol/L.

This device requires the use of Randox Calibration Serum Level 3 (previously cleared in k053153), that must be purchased separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Wako Direct Bilirubin V

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

   k053132

3. Comparison with predicate:

Similarities		
Item	Proposed Device k152343 Randox Direct Bilirubin Assay	Predicate Device: Wako Direct Bilirubin (k053132)
Intended Use	The Direct Bilirubin test system is a device intended for the quantitative in vitro determination of Direct Bilirubin in serum and plasma. Bilirubin measurements can be used in the diagnosis and treatment of liver, hemolytic, hematological and metabolic disorders including hepatitis and gall bladder block.	Same
Sample type	Serum or plasma (lithium heparin)	Same
Method	Colorimetric	Same
Assay Protocol	Vanadate Oxidation Method	Same
Reagent Composition	R1. Direct Bilirubin R1 Tartrate Buffer, pH 2.9, 0.1 mol/L Detergent, Antimicrobial, Preservatives and Inhibitors R2. Direct Bilirubin R2 Phosphate Buffer, pH 7.0, 10 mmol/L Sodium Metavanadate, 4 mmol/L	Same

Differences		
Item	Proposed Device k152343 Randox Direct Bilirubin Assay	Predicate Device: Wako Direct Bilirubin (k053132)
Calibration	A two point calibration is recommended every 28 days.	Recommended every 30 days.
Control Frequency	Two levels of control should be assayed at least once a day	At least two levels of control should be assayed once a day, or change of lot
Stability	On board stability has been determined as 28 days.	On board stability has been determined as 30 days.
Measuring Range	0.1 to 12.6 mg/dL	0.1 to 20 mg/dL
Storage Temperature	+2 to +8°C	+2 to +10°C

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

- CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
- CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP17-A2, Protocols for Determination of Limits of Detection and Limits of Quantification; Approved Guideline
- CLSI EP9-A2, Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline – Second Edition
- CLSI C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline- Third Edition

L. Test Principle:

The Randox Direct Bilirubin Assay for RX Daytona Plus system uses a quantitative colorimetric method for detection of direct bilirubin. Direct bilirubin is oxidized by vanadate at approximately pH 3.0 to produce biliverdin. There are inhibitors present which prevent the oxidation of any indirect bilirubin. This oxidation reaction causes a decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density at 450/546 nm is proportional to the direct bilirubin concentration in the sample. The concentration is measured as an endpoint reaction.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   All studies were conducted on the RX Daytona Plus system.

   a. Precision/Reproducibility:

   Precision was evaluated consistent with C.L.S.I standard EP5-A2. Precision studies were performed by two operators, on two RX Daytona Plus systems using control material and unaltered human serum samples that were spiked with direct (conjugated) bilirubin. Testing was conducted using two reagent lots, one lot on each RX Daytona plus system, twice per day for 20 non-consecutive days. Two replicates per run were performed for each sample. No assay re-calibrations were required throughout the duration of the study. Both reagent lots yielded similar results. The results from one representative lot are summarized below.

		Within Run		Total	
Sample	**Mean (mg/dL)**	**SD (mg/dL)**	**CV %**	**SD (mg/dL)**	**CV %**
Serum Pool 1	0.65	0.02	3.0	0.03	4.2
Serum Pool 2	2.31	0.07	3.1	0.07	3.1
Serum Pool 4	8.41	0.13	1.5	0.16	1.9

b. Linearity/assay reportable range:

The sponsor claims a linear range of 0.1 mg/dL to 12.6 mg/dL. Linearity studies were performed at 11 levels in accordance with CLSI standard EP6-Awith concentrations ranging from 0.12 to 12.6 mg/dL. Each level was run in replicates of five on two lots of direct bilirubin reagent on one RX Daytona Plus system. Both reagent lots yielded similar results. The results from one representative lot are summarized in the table below.

Linear Regression Equation and Correlation Coefficient	
Slope	1.01
Intercept	-0.07
r	0.9995

The reportable range of the candidate device is 0.1 mg/dL to 12.6 mg/dL.

Dilution Protocol:

The RX Daytona Plus analyzer has an auto-dilution feature that is automatically activated when measuring samples >12.6mg/dL. Samples >12.6mg/dL are diluted (x 1.52) with 0.9% saline before testing, and re-measured to obtain values within the measuring range (0.1-12.6 mg/dL).

A dilution study was performed using the auto-dilution function against manual dilution, and the results supported the sponsor's claim that the instrument could automatically dilute the sample.

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

Traceability:

Randox Calibration Serum Level 3 is traceable to an internal master calibrator for direct bilirubin; internal master calibrator is traceable to NIST SRM916 (a). Randox Calibration Serum Level 3 has been previously cleared in k053153.

Reagent Stability:
Sponsor provided real time stability study protocol and acceptance criteria, and these were found to be acceptable. The reagent is stable up to the expiry date when stored at +2°C to +8°C.

d. Detection limit:

The detection limits study was performed in accordance with CLSI guideline EP17-A2. Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) determinations were performed using two lots of reagents on one RX Daytona Plus analyzer. The LoB was based on 60 replicates of blank sample. LoD was based on 240 determinations, with 4 low levels samples; 60 samples per level. The LoQ was determined by the lowest detected concentration at which the imprecision was ≤ 20% CV. Both reagent lots yielded similar results. The results from one representative lot (with the highest value) are summarized below.

LoB	0.006 mg/dL
LoD	0.064 mg/ dL
LoQ	0.133 mg/dL

The direct bilirubin assay has a claimed measuring range of 0.1 to 12.6 mg/dL.

e. Analytical specificity:

The interference study was performed using two concentrations of interfering substances according to the CLSI guideline EP7-A2. The effects of potential interferent were determined by calculating the mean value of the spiked interferent with the corresponding control solution. The spiked sample results were compared to control samples prepared without the potential interferent.

Sponsor defined no significant interference as a difference of <10% between the measured concentration from spiked pool sample to the match sample with no interferent (control).

The analytes detailed below were tested up to the levels indicated at Bilirubin concentrations of 0.14 mg/dL and 5.03 mg/dL, and found no significant interference:

Substance	**Highest Concentration Tested at which no significant Interference Observed**
Hemoglobin	1000 mg/dL
Triglycerides	750 mg/dL
Intralipid®	1000 mg/dL
Ascorbic Acid	25 mg/dL

f. Assay cut-off:

Not Applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Correlation studies were carried out in accordance with CLSI EP9-A2. 103 serum patient samples spanning the range 0.123-12.46 mg/dL were tested by two operators, 10 of 103 samples were spiked. Two lots of direct bilirubin reagent were used on a RX Daytona plus analyzer and a Siemens ADVIA 1650 system. The samples were tested across 3 working days with each sample tested in singlicate. The test method was compared to the predicate device and the following linear regression equation was obtained. Both reagent lots yielded similar results. The results from one representative lot are summarized below.

Linear Regression Equation	y = 1.01x + 0.01
Correlation Coefficient	r = 0.997
95% Confidence Interval of Slope	0.99 to 1.03
95% Confidence Intervals of Intercept	-0.09 to 0.11

b. Matrix comparison:

Matrix method comparisons for the direct bilirubin assay was tested by one operator on one RX Daytona plus system and was assessed for two lots of direct bilirubin reagents. Both serum and lithium heparin plasma were tested to determine whether method accuracy with lithium heparin specimens are equivalent to serum results.

A minimum of 40 matched serum and lithium heparin plasma samples that ranged 0.1 to 12.5 mg/dL was performed. The comparison was performed on the RX Daytona plus system by one operator across two lots of reagents. Both reagent lots yielded similar results. The resulting regression analysis of one representative lot is summarized in the table below

Linear Regression Equation	y = 0.99x + 0.01
Correlation Coefficient	r = 1.00
95% Confidence Interval of Slope	0.99 to 1.00
95% Confidence Intervals of Intercept	-0.01 to 0.02

Based on the study results, sponsor claimed that lithium heparin plasma sample is an acceptable sample type for the candidate device.

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:

Adults: 0.0 to 0.2 mg/dL

The sponsor referenced: Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB Saunders Company; 1995:88-91.

The reference range for direct bilirubin was verified using CLSI guideline C28-A3 by conducting a small study using human serum samples from 20 healthy donors. Results of all the samples were found to be within the reported reference range.

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.