

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

A. 510(k) Number:

k152344

B. Purpose for Submission:

New device

C. Measurand:

Bilirubin, Total

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Randox Laboratories Limited

F. Proprietary and Established Names:

Total Bilirubin (T BIL)

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:

Clinical Chemistry, 75

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

For the quantitative in vitro determination of Total Bilirubin for serum and plasma. Total Bilirubin measurements are used in the diagnosis and treatment of hemolytic, biliary and liver disorders, including hepatitis and cirrhosis.

This in vitro diagnostic device is intended for prescription use only.

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

I. Device Description:

The device consists of two ready to use reagents.

4 x 20 ml bottles of Reagent 1 (R1) contains 0.1 mol/L citrate, pH 2.9, 0.9% detergent, and antimicrobial.

4 x 8 ml bottles of Reagent 2 (R2) contains 10 mmol/L phosphate, pH 7.0 and 4 mmol/L sodium metavanadate.

The device requires the use of Randox calibration serum level 3 (calibrator) - previously cleared under k053153.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Siemens ADVIA Chemistry Total Bilirubin_2

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

k063845

3. Comparison with predicate:

Similarities		
Item	Candidate Device k152344 Total Bilirubin Randox Laboratories	Predicate Device k063845 Total Bilirubin_2 Siemens Healthcare Diagnostic Inc
Intended Use	For the quantitative in vitro determination of Total Bilirubin in serum and plasma. Total Bilirubin measurements are used in the diagnosis and treatment of hemolytic, biliary and liver disorders, including hepatitis and cirrhosis.	Same
Assay Method	Vanadate oxidation method	Same
Control Frequency	Randox Laboratories assayed human multiserum Level 2 & 3. Two levels of control should be assayed at least once a day.	Same
Sample Type	Serum, lithium heparin plasma	Same
Reagent Composition	R1. Citrate buffer, 0.1 mol/L, pH 2.9 0.9% Detergent, Antimicrobial R2. Phosphate buffer, 10 mmol/L, pH 7.0 Sodium Metavanadate 4 mmol/L	Same

Differences		
Item	Candidate Device k152344 Total Bilirubin Randox Laboratories	Predicate Device k063845 Total Bilirubin_2 Siemens Healthcare Diagnostic Inc
Test Range	0.2 – 26.3 mg/dL	0.1 – 35 mg/dL
Storage temperature, unopened	Reagents are stable up to the expiry date when stored unopened at +2 to +8°C.	+2 to +35°C

Differences		
Item	Candidate Device k152344 Total Bilirubin Randox Laboratories	Predicate Device k063845 Total Bilirubin_2 Siemens Healthcare Diagnostic Inc
Calibration Frequency	(1) Every 28 days, (2) with change of reagent lot, or (3) as indicated by quality control procedures.	Every 60 days

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

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition.

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

CLSI C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition.

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

CLSI EP09-A, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline.

CLSI E17-A2, Evaluation of detection capability for clinical laboratory measurement procedures; Approved Guideline - Second Edition.

L. Test Principle:

The bilirubin is oxidized by vanadate at about pH 2.9 to produce biliverdin. In the presence of detergent and vanadate, both conjugate (direct) and unconjugated bilirubin are oxidized. This oxidation reaction causes a decrease in the optical density at 450/546 nm. The decrease in optical density is specific to bilirubin and proportional to the total bilirubin concentration. The biliverdin concentration is measured at endpoint of the oxidation reaction.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision estimates were derived according to CLSI EP05-A2.

Within run and total imprecision for total bilirubin were evaluated on the RX Daytona Plus by testing pooled serum samples using two reagent lots. Each sample was assayed two times per run, two runs per day for 20 days. Total number of replicates for each sample was 80. Both reagent lots yielded similar results. The results from one representative lot are summarized below.

Precision:		Within-run		Total	
Specimen Type	Concentration, mg/dL	SD	CV%	SD	CV%
Serum	0.3	0.02	6.9	.02	7.4
Serum	1.1	0.05	4.0	0.06	5.4
Serum	6.7	0.11	1.6	0.16	2.4
Serum	12.0	0.12	1.0	0.28	2.3
Serum	16.2	0.15	0.9	0.35	2.2
Serum	25.0	0.23	0.9	0.41	2.2

b. *Linearity/assay reportable range:*

Linearity studies were carried out in accordance with CLSI EP06-A. Linearity was evaluated using the RX Daytona Plus and 11 samples with concentrations ranging from 0.2 to 26.3 mg/dL using two lots of reagents. The samples were equally spaced in concentration and prepared by combining a pooled high human serum sample and pooled low human serum sample (diluted to 0.2 mg/dl with 0.9% saline). Each concentration level was run in replicates of five. Both reagent lots yielded similar results. The results from one representative lot are summarized below.

A linear regression fit between the expected concentration by dilution and observed mean concentration was the same or better than a second or third order polynomial fit. At each level, the mean observed concentration was within $\pm 5\%$ of the expected concentration (based on dilution).

The linear regression equation was: $y = 1.02x + 0.01$ and $r = 0.9999$.

The reportable range of the candidate device is 0.2 mg/dl to 26.3 mg/dL.

Automatic Dilution:

The Rx Daytona Plus analyser has an auto-dilution feature. When a total bilirubin concentration exceeds the upper end of the reportable range of 26.3 mg/dl, the result is flagged, and the sample is diluted with saline and re-run by the instrument. The subsequent result is multiplied by the dilution factor.

A dilution study using a 1: 6 dilution ratio was performed 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:

The controls and calibrators to be used with this assay are manufactured by Randox Laboratories. The Randox Calibration Serum Level 3 has previously been cleared under k053153.

Randox Calibration Serum Level 3 is traceable to NIST SRM 916(a).

Stability:

Sponsor provided real time stability study protocol and acceptance criteria, and these were found acceptable. The reagent has been evaluated for shelf-life and open on-board stability. When stored un-opened at 2-8 °C the assay reagent is stable until the expiration date and has a shelf life of 24 months from the date of manufacture. When stored on the RX Daytona Plus instrument, the open on-board stability is 28 days.

d. *Detection limit:*

Detection limit studies were carried out in accordance with CLSI EP17-A2.

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) determinations were performed using two lots of reagents, tested by two operators, on one RX Daytona Plus system.

The LoB was based on 60 replicates of an artificial serum matrix with no bilirubin added. LoB was derived using the non-parametric approach, and where the value was derived from the 95th percentile of all values.

The LoD was determined using four low level diluted serum sample pools in 20 replicates per run for each day across 3 days for a total of 60 measurements at each level.

The LoQ was measured using four diluted patient serum sample pools in 12 replicates

across five days yielding a total of 60 results. The LoQ was determined by the lowest total bilirubin concentration at which the imprecision < 20% CV.

Both reagent lots yielded similar results. The results from one representative lot (with the highest values are summarized below.

LoB	0.06 mg/dL
LoD	0.075 mg/dL
LoQ	0.211 mg/dL

The total bilirubin assay has a claimed measuring range of 0.2 to 26.3 mg/dL.

e. Analytical specificity:

Interference studies were carried out in accordance with CLSI guideline EP7-A2.

The interference study was carried out using two concentration levels of 1.0 mg/dL and 15 mg/dL total bilirubin. These were prepared by spiking unconjugated bilirubin into pooled, stripped serum. The pooled samples were then aliquoted into 3 separate samples; one sample with high level of interferent, one sample with normal level of interferent, and sample was spiked with a diluent without the interferent as control. All the samples were evaluated on the RX Daytona Plus. The samples were evaluated in replicates of 10.

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 total bilirubin assay showed no significant interference with the following:

Substance	Highest Concentration Tested at which no significant Interference Observed
Hemoglobin	1000 mg/dL
Triglycerides	2000 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:*

Method comparison studies were carried out in accordance with CLSI guideline EP9-A2.

The comparative method was the predicate device – Siemens ADVIA Total Bilirubin 2, tested on the Siemens ADVIA 1650 analyzer. For the candidate device, the testing was conducted using the RX Daytona Plus analyzer. The method comparison used 106 samples spanning the concentration range from 0.2 to 26.9 mg/dL using 2 lots of reagent. The samples were taken from banked collections and comprised 96 unaltered patient serum samples and 10 spiked serum samples. All samples were run in singlicate. The data was analyzed by linear least square regression. Both reagent lots yielded similar results. The results from one representative lot are summarized below.

Linear regression equation	$y = 1.02x - 0.02$
Correlation Coefficient	0.9999
95% Confidence Interval of Slope	1.01 to 1.02
95% Confidence Interval of Intercept	-0.05 to 0.01

b. *Matrix comparison:*

Matrix comparison for the Total Bilirubin assay was assessed for two lots of reagents and using the RX Daytona Plus system.

Serum and lithium heparin plasma patient samples were drawn in matched pairs. 40 patient sample pairs were analyzed spanning the concentration range 0.2 to 23.5 mg/dL. The samples comprised 35 patient serum samples and 5 spiked serum samples. Each sample was tested in singlicate.

Both lots of reagents yielded similar results. Linear regression analysis, from a representative lot, yielded the following results:

Regression Equation	$y = 0.99x + 0.04$
Correlation Coefficient	0.999
95% Confidence Interval of Slope	0.98 to 0.99
95% Confidence Interval of Intercept	0.01 to 0.07

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:

Reference range: Adult: 0.3 – 1.2 mg/dL

The reported reference range is from: WuAHB. Tietz Clinical Guide to Laboratory Tests, 4th edition, Saunders Elsevier, St. Louis, MO: 2006:316.

The reference range for Total Bilirubin was verified using CLSI guideline C28-A3 by conducting a small study using human serum samples from 30 healthy donors. These were tested in singlicate on the RX Daytona Plus. Adults ranging in ages from 22 to 53 were tested. Of these, 19 were female and 11 male. The lowest result was 0.2 and highest result was 1.1 mg/dL. A Dixon test was used to mathematically check for outliers - none were found.

All results from the 30 healthy donors 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.