

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

A. 510(k) Number:

k040631

B. Analyte:

Bilirubin, Direct and Total

C. Type of Test:

Quantitative colorimetric

D. Applicant:

Clinical Data, Inc.

E. Proprietary and Established Names:

Vitalab Direct Bilirubin Reagent

Vitalab Total Bilirubin Reagent

Vitalab Bilirubin Calibrator

F. Regulatory Information:

1. Regulation section:

21 CFR § 862.1110, Bilirubin (total or direct) test system
862.1150, Calibrator

2. Classification:

Class II

3. Product Code:

CIG, Diazo colorimetry, bilirubin
JIT, Calibrator, secondary

4. Panel:

Clinical Chemistry (75)

G. Intended Use:

1. Intended Use:

Vitalab Direct Bilirubin Reagent is for the quantitative determination of conjugated (direct) bilirubin in serum and plasma.

Vitalab Total Bilirubin Reagent is for the quantitative determination of total bilirubin in serum and plasma.

Vitalab Bilirubin Calibrator is intended for use with the Vitalab Selectra Analyzer to establish points of reference that are used in the determination of total and direct bilirubin in human specimens.

2. Indication(s) for use:

Direct bilirubin results may be used for the diagnosis and treatment of liver, hemolytic hematological and metabolic disorders, including hepatitis and gall bladder block.

Total bilirubin results may be used for the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

3. Special condition for use statement(s):

For prescription use only.

4. Special instrument Requirements:

Vitalab Selectra E Chemistry Analyzer

H. Device Description:

The Vitalab Direct and Total Bilirubin Reagents each consist of two liquid reagents for use on the Selectra analyzer. The Vitalab Bilirubin Calibrator is a liquid single-point calibrator containing both conjugated and unconjugated bilirubin in a human serum albumin matrix.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Roche Direct Bilirubin Reagent
Beckman Total Bilirubin Reagent
Beckman Bilirubin Calibrator
2. Predicate K number(s):
K910593
K902801
K812784 and K812754
3. Comparison with predicate:

Direct Bilirubin assay:

Similarities		
Item	Device	Predicate
Product type	Calibrated endpoint reagent	same
Intended use:	Quantitative determination of conjugated (direct) bilirubin in serum and plasma	same
Chemical Reaction	Reaction of conjugated bilirubin with a diazo compound (diazotized 2,4-dichloroaniline)	similar (diazotized sulfanilic acid)
Wavelength	546 nm	552 nm
Calibration	Single point with reagent blank	same
Differences		
Item	Device	Predicate
Measurement method	Chemical endpoint with sample blank	Chemical endpoint without sample blank

Analytical Range	0.1 to 10.0 mg/dL	0.1 to 25.0 mg/dL
Reagent components	diazotizing agent (2,4-dichlorophenyl-diazonium salt) EDTA sulfamic acid hydrochloric acid sodium chloride	diazotizing agent (sulfanilic acid diazotized with sodium nitrite) HEDTA oxalic acid hydrochloric acid

Total Bilirubin assay:

Similarities		
Item	Device	Predicate
Product type	Calibrated endpoint reagent	same
Intended use:	Quantitative determination of total bilirubin in serum and plasma	same
Chemical Reaction	Reaction of both conjugated and unconjugated bilirubin with diazo compound in the presence of an accelerator	same
Wavelength	546 nm	520 nm
Analytical Range	0.1 to 25.0 mg/dL	0.1 to 30.0 mg/dL
Calibration	Single point with reagent blank	same
Differences		
Item		
Measurement method	Chemical endpoint with sample blank	Chemical endpoint without sample blank
Reagent components	diazotizing agent (2,4-dichlorophenyl-diazonium salt) accelerator (detergents) hydrochloric acid phosphate buffer sodium chloride	diazotizing agent (sulfanilic acid diazotized with sodium nitrite) accelerator (caffeine, benzoate, acetate) hydrochloric acid

Both the device and predicate calibrators are single point calibrators for the calibration of reagents for the quantification of direct or total bilirubin. See traceability below for more information on calibrators.

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

NCCLS Guideline EP3-T
NCCLS Document EP7-P

K. Test Principle:

Direct Bilirubin assay: The conjugated bilirubin in the sample reacts with diazotized 2,4-dichloroaniline to form a red chromogen in acidic solution. The resulting increase in absorbance at 546 nm, measured as an endpoint, is proportional to the conjugated bilirubin concentration in the sample.

Total Bilirubin assay: Detergents in the reagent solubilize free bilirubin, allowing both the free and conjugated bilirubin in the sample to react with diazotized 2,4-dichloroaniline to form a red chromogen. The resulting increase in absorbance at 546 nm, measured as an endpoint, is proportional to the total bilirubin concentration in the sample.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Imprecision of the Direct Bilirubin assay was evaluated by testing serum samples in triplicate twice per day over 11 days according to NCCLS Guideline EP3-T. Results are summarized are shown below. (n = 66, units = mg/dL)

Sample	Mean	Within Run		Total	
		SD	% CV	SD	%CV
Serum 1	0.3	0.03	11.3 %	0.03	11.4 %
Serum 2	1.7	0.02	1.3 %	0.05	2.8 %
Serum 3	4.0	0.02	0.5 %	0.10	2.6 %

Imprecision of the Direct Bilirubin assay was evaluated by testing serum samples in triplicate twice per day over 9 days according to NCCLS Guideline EP3-T. Results are summarized are shown below. (n = 54, units = mg/dL)

Sample	Mean	Within Run		Total	
		SD	% CV	SD	%CV
Serum 1	0.4	0.02	5.8 %	0.03	6.7 %
Serum 2	1.4	0.04	3.1 %	0.07	4.8 %
Serum 3	5.8	0.07	1.1 %	0.15	2.5 %

b. Linearity/assay reportable range:

Linearity of the direct bilirubin assay was evaluated by preparing a human serum pool from individual patient specimens selected for low bilirubin levels. An aliquot of this pool was spiked to a direct bilirubin value greater than 10 mg/dL. This pool was then diluted with the original pool to prepare a set of eight reference pools containing increasing levels of conjugated bilirubin. These pools were assayed on a single analyzer in ascending order over four independently calibrated analytical runs. Recoveries were compared to dilution factors by least squares linear regression (observed = $0.834(\text{expected}) + 0.2$; $r = 0.9995$). The assay is linear across the useable range (0.1 – 10.0 mg/dL).

Linearity of the total bilirubin assay was evaluated by preparing a human serum pool from individual patient specimens selected for low bilirubin levels. An aliquot of this pool was spiked total bilirubin value greater than 25 mg/dL. This pool was then diluted

with the original pool to prepare a set of eight reference pools containing increasing levels of conjugated bilirubin. These pools were assayed on a single analyzer in ascending order over four independently calibrated analytical runs. Recoveries were compared to dilution factors by least squares linear regression (observed = $0.888(\text{expected}) + 0.2$; $r = 0.9999$). The assay is linear across the useable range (0.1 – 25.0 mg/dL).

c. Traceability (controls, calibrators, or method):

The Vitalab Bilirubin Calibrator is a liquid-stable, single-point calibrator containing both conjugated and unconjugated bilirubin in a human serum albumin matrix. It is used to calibrate the Vitalab Total Bilirubin Reagent and the Vitalab Direct Bilirubin Reagent for the quantitative determination of both total and conjugated (direct) bilirubin in serum and plasma on the Vitalab Selectra Analyzer. Targeted values are 3.0 to 3.5 mg/dL direct bilirubin and 6.0 to 6.5 mg/dL total bilirubin. These values are traceable to NIST SRM 916 and are assigned to each lot by repetitive comparisons between the calibrator and reference standards.

Stability studies and acceptance criteria are described to support stability claims.

d. Detection limit:

Analytical sensitivity was established by assaying normal saline thirty times in a single analytical run. The detection limit was calculated as the mean plus two standard deviations of the results. The analytical sensitivity of both the direct and the total bilirubin assays are 0.1 mg/dL.

e. Analytical specificity:

Direct bilirubin assay interference was evaluated according to NCCLS Document EP7-P by preparing a serum pool containing approximately normal levels of direct bilirubin from individual patient specimens. One aliquot was spiked with the substances listed below while the other aliquot was diluted with normal saline to mimic the dilution the spiked pool. These aliquots were then blended to prepare test pools with the interferant concentrations listed below. A second set of test pools were prepared from a second serum pool spiked to a direct bilirubin value of greater than 2.5 mg/dL or a total bilirubin of greater than 2 mg/dL to estimate the effects of interfering substances at bilirubin levels above the normal range.

Interfering Substance	Levels Tested
RBC hemolysate	40, 80, 120, 160, 200 mg/dL (as hemoglobin)
Intralipid, 20%	400, 800, 1,200, 1,600 and 2,000 mg/dL (as triglycerides)
Triglycerides Supertrate	400, 800, 1,200, 1,600 and 2,000 mg/dL (as triglycerides)

Each set of original and spiked pools were assayed in an alternating order 9 and 6 times respectively in a single analytical run. Differences in recoveries between the original and spiked pools greater than 0.3 mg/dL are reported in the package insert.

Direct Bilirubin Assay:

Red blood cell hemolysate added to hemoglobin concentrations of 40 and 120 mg/dL decreases recoveries of the spiked pool by 0.4 and 0.9 mg/dL respectively.

The addition of Intralipid to 80 mg/dL triglycerides does not significantly affect direct bilirubin results. However, at 160 and 320 mg/dL triglycerides, Intralipid elevates results by approximately 0.4 and 1.8 mg/dL respectively in both the normal and elevated bilirubin pools. Triglycerides Superstrate has no effect on recoveries.

Total Bilirubin Assay:

Red blood cell hemolysate added to a hemoglobin concentration of 200 mg/dL decreases recoveries of the spiked pool by 0.3 mg/dL.

The addition of Intralipid to 400 and 1,200 mg/dL triglycerides respectively suppresses results in both the normal and elevated bilirubin pools by approximately 0.3 and 0.8 mg/dL.

Triglycerides Superstrate does not affect recoveries by more than 0.1 mg/dL.

f. Assay cut-off:
Not applicable

2. Comparison studies:*a. Method comparison with predicate device:*

Performance of the Direct Bilirubin assay was assessed as follows. Random unaltered specimens from individual adult patients were collected from local clinical labs. These samples were supplemented with 16 additional serum and 6 additional plasma specimens with elevated direct bilirubin levels to yield a total of 60 serum and 60 heparinized plasma specimens. These specimens were randomly assorted into groups of 15 serum and 15 plasma specimens each. One group of serum and plasma specimens were assayed in each of four runs using the device and the predicate after calibrating each reagent with its required calibrator.

Twenty eight results fell outside the usable range of at least one of the two methods and are not reported. Two additional results are not reported because the sample did not meet specimen requirements. The remaining serum results, plasma results and the combined results for both specimen types are each compared by Deming regression assuming equal variances between methods. Regression statistics are given below. (units = mg/dL)

	N	Range	Slope (95% CI)	Intercept (95% CI)
Plasma	41	0.1 to 11.1	0.852 (0.831 to 0.872)	0.06 (0.00 to 0.11)
Serum	49	0.1 to 11.6	0.826 (0.810 to 0.841)	0.05 (-0.01 to 0.11)
Combined	90	0.1 to 11.6	0.832 (0.820 to 0.845)	0.05 (0.01 to 0.10)

Performance of the Total Bilirubin assay was assessed as follows. Random unaltered specimens from individual adult patients were collected from local clinical labs. These samples were supplemented with an additional 15 serum and 4 plasma specimens with elevated total bilirubin levels to yield a total of 59 serum and 45 heparinized plasma specimens. These specimens were randomly assorted into groups of approximately 15

serum and 15 plasma specimens each and were assayed over four runs using the device and the predicate after calibrating each reagent with its required calibrator. One plasma result exceeded the linear ranges of both analyzers as is not reported.

The serum results, plasma results and the combined results for both specimen types are each compared by Deming regression assuming equal variances between methods. Regression statistics are given below.(units = mg/dL)

	N	Range	Slope (95% CI)	Intercept (95% CI)
Plasma	44	0.4 to 18.4	1.075 (1.064 to 1.086)	-0.47 (-0.52 to -0.42)
Serum	59	0.1 to 25.9	1.024 (1.010 to 1.038)	-0.34 (-0.44 to -0.23)
Combined	103	0.1 to 25.9	1.037 (1.026 to 1.047)	-0.39 (-0.46 to -0.32)

b. Matrix comparison:

See method comparison above. Serum and plasma samples were compared individually to the predicate method to establish equivalence.

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:

The sponsor references expected values as 0.0 to 0.2 mg/dL for direct bilirubin and 0.3 to 1.2 mg/dL for total bilirubin (from literature sources).

M. Conclusion:

I recommend that the Vitalab Direct Bilirubin Reagent, the Vitalab Total Bilirubin Reagent, and the Vitalab Bilirubin Calibrator are substantially equivalent to the legally marketed predicate devices.