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

A. 510(k) Number:

k131051

B. Purpose for Submission:

New device

C. Measurand:

Total Protein

Albumin

D. Type of Test:

Quantitative, Photometry

E. Applicant:

Hitachi Chemical Diagnostics, Inc

F. Proprietary and Established Names:

Hitachi S TEST Reagent Cartridge Total Protein (TP)

Hitachi S TEST Reagent Cartridge Albumin (ALB)

G. Regulatory Information:

1. Regulation section:
   21 CFR 862.1635, Total Protein test system
   21 CFR 862.1035, Albumin test system

2. Classification:
   Class II, Exempt, meets limitations of exemptions per 862.9(c)(9), for Total Protein test
   Class II, for Albumin test

3. Product code:
   JGQ, turbidimetric, total protein
   CIX, bromcresol green dye-binding, albumin

4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The S TEST Reagent Cartridge Total Protein (TP) is intended for the quantitative determination of TP in serum, lithium heparinized plasma, K3 EDTA plasma and sodium citrate plasma using the HITACHI Clinical Analyzer E40. The S TEST Reagent Cartridge TP is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

   Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders.

   The S TEST Reagent Cartridge Albumin (ALB) is intended for the quantitative determination of ALB in serum, lithium heparinized plasma, K3 EDTA plasma and sodium citrate plasma using the HITACHI Clinical Analyzer E40. The S TEST Reagent Cartridge ALB is intended for use in the clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

   Albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver and kidneys.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   HITACHI Clinical Analyzer E40 (k111753)

I. Device Description:

The S TEST Reagent Cartridge Total Protein (TP) consists of the following: TP Reagent 1- citric acid buffer and nonionic surface active agent and TP Reagent 2- copper sulfate pentahydrate. The S TEST Reagent Cartridge Total Protein (TP) is provided in a ready-to-use cartridge. The 2D code label on the front of each cartridge automatically identifies the reagent to the system.

The S TEST Reagent Cartridge Albumin (ALB) consists of the following: ALB Reagent 1-bromocresol green, citric acid buffer and nonionic surface-active agent. The S TEST Reagent Cartridge Albumin (ALB) is provided in a ready-to-use cartridge. The 2D code label on the front of each cartridge automatically identifies the reagent to the system.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Roche cobas c systems

2. Predicate 510(k) number(s):
   k100853

3. Comparison with predicate:

Similarities and Differences Total Protein (TP)		
Item	Candidate Device	Predicate Device
Intended Use	For the quantitative determination of Total Protein in human serum	Same
Testing environment	Physician office or clinical lab	Clinical lab
Specimen type	Human serum or plasma	Same
Detection wavelength	546/700	Same
Measuring range	0.2 to 11.0 g/dL	0.2 to 12.0 g/dL
Instrument platform	Hitachi Clinical Analyzer	Roche cobas c systems

Similarities and Differences Albumin (ALB)		
Item	Device	Predicate
Intended Use	For the quantitative determination of Albumin in human serum	Same
Testing environment	Physician office or clinical lab	Clinical lab
Specimen type	Human serum or plasma	Same
Detection wavelength	660/700	570/505
Measuring range	0.5 to 7.1 g/dL	0.2 to 6.0 g/dL
Instrument platform	Hitachi Clinical Analyzer	Roche cobas c systems

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI-EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A statistical Approach; Approved Guideline

CLSI-EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

Total Protein: Proteins in samples react with the biuret reagent to form a purple-red complex. The concentration of total protein can be determined by measuring the absorbance of the purple-red substance.

Albumin: Albumin combines with bromcresol green to form a blue-green dye conjugate. The albumin concentration can be determined by measuring the absorbance of this resulting blue-green color.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies for the S TEST Reagent Cartridge TP and ALB were performed following CLSI EP5-A2 with three levels of analytes (low, middle and high) and one instrument. Serum samples were tested in duplicate, twice a day, for 20 days for a total of 80 results per level. The samples were serum based commercial controls (levels 1, 2 and 3). Results for total protein and albumin precision are summarized below:

Analyte	Sample	Mean (g/dL)	Within Run		Total	
			SD	%CV	SD	%CV
Total Protein	Level 1	4.19	0.05	1.1	0.09	2.1
	Level 2	5.51	0.08	1.4	0.14	2.5
	Level 3	7.19	0.07	1.0	0.13	1.8
Albumin	Level 1	2.28	0.04	1.5	0.11	4.8
	Level 2	4.72	0.07	1.4	0.13	2.8
	Level 3	6.01	0.07	1.2	0.09	1.6

Physician Office Precision

Precision was evaluated at three POL sites. Each site received three blinded serum samples (A, B, C) that were chosen to represent low, intermediate, and high concentrations of the analytes. Each sample was assayed six times per day for 5 days resulting in 30 results per level. The results are listed below:

Physician Office Precision: Total Protein

Sample	Site	Mean (g/dL)	Within run Precision		Total Precision	
			SD (g/dL)	%CV	SD (g/dL)	%CV
A	1	4.1	0.05	1.2	0.07	1.6
	2	4.1	0.05	1.1	0.07	1.6
	3	3.8	0.05	1.4	0.13	3.5
B	1	5.5	0.05	0.8	0.06	1.1
	2	5.5	0.06	1.2	0.07	1.2
	3	5.0	0.04	0.9	0.20	4.0
C	1	7.1	0.05	0.7	0.05	0.7
	2	7.1	0.06	0.8	0.07	0.9
	3	6.5	0.07	1.1	0.28	4.4

Physician Office Precision: Albumin

Sample	Site	Mean (g/dL)	Within run Precision		Total Precision	
			SD (g/dL)	%CV	SD (g/dL)	%CV
A	1	0.88	0.03	3.9	0.04	4.8
	2	0.80	0.00	0.0	0.00	0.0
	3	0.81	0.02	2.8	0.04	4.5
B	1	4.67	0.05	1.0	0.04	1.3
	2	4.60	0.05	1.6	0.06	1.7
	3	4.47	0.12	2.7	0.13	2.8
C	1	7.03	0.05	0.8	0.18	2.5
	2	6.93	0.06	0.8	0.11	1.6
	3	6.72	0.08	1.2	0.16	2.3

b. Linearity/assay reportable range:

The claimed measuring range for total protein is 0.2-11g/dL. The linearity of the total protein assay was assessed following CLSI EP6-A with commercially available linearity sets which include 11 samples (0.19 to 11.46 g/dL). All samples were tested in duplicate on one Hitachi Clinical Analyzer E40. Recoveries were within ±10% or 0.1g/dL. The summary of the linear regression analysis of the data is below:

$y=1.0158x+0.0597$; $R^2=0.9986$

The linearity studies for total protein support the sponsor's claimed measuring range of 0.2 g/dL to 11.0 g/dL.

The claimed measuring range for albumin is 0.5-7.1g/dL. The linearity of the albumin assay was assessed following CLSI EP6-A with commercially available linearity sets which include 10 samples (0.06 to 8.8g/dL). All samples were tested in duplicate on one Hitachi Clinical Analyzer E40. Recoveries were within ±10% or

0.1g/dL. The summary of the linear regression analysis of the data is below:

$y=0.9911x+0.0508$; $R^2=0.9994$

The linearity studies for Albumin support the sponsor's claimed measuring range of 0.5 g/dL to 7.1 g/dL.

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

Traceability

S TEST Reagent Cartridge Total Protein (TP) is calibrated by the manufacturer prior to shipment using material traceable to NIST SRM 927. The barcode printed on each cartridge provides the analyzer with lot-specific calibration data. TP concentration is directly determined by multiplying the change in absorbance of the unknown samples by the calibrator factor on the barcode. No calibration is needed by the user.

Each lot of the S TEST Reagent Cartridge Albumin (ALB) cartridges is calibrated by the manufacturer prior to shipment using material traceable to IRMM Standard Reference Material CRM470. The barcode printed on each cartridge provides the analyzer with lot-specific calibration data. ALB concentration is directly determined by multiplying the change in absorbance of the unknown samples by the calibrator factor on the barcode. No calibration is needed by the user.

Commercially available controls are required and users should follow Federal, state, and local requirements.

Stability

Real-time shelf life stability studies for the TP reagent cartridge are ongoing. The sponsor states that the TP test cartridge will be launched with 12 month stability. The protocols for stability and acceptance criteria were reviewed and are acceptable.

Real-time shelf-life studies for the Albumin reagent cartridge were performed. A two-level control set was tested in replicates of five with three lots of cartridges across six analyzers. The protocols for stability and acceptance criteria were reviewed and are acceptable and support a stability of at least 12 months when stored at 2-8°C.

d. Detection limit:

The limit of blank (LoB) and limit of detection (LoD) studies for the S TEST Reagent Cartridge TP and Reagent Cartridge ALB were performed in accordance to CLSI EP17-A. The analytical sensitivity was defined as the limit of detection, and the LoD was calculated from the LoB. LoB was determined using a blank sample assayed 20 times per day for three days for a total of 60 replicates. The LoB was estimated as the

mean of the 57th and 58th highest values for the true blanks. LoD was determined using five low samples assayed four times per day for three days, for a total of 60 replicate results. The LoD was calculated as the LoB + 1.645 x SD of the low samples.

LoQ for the S TEST Reagent Cartridge TP and ALB was assessed by preparing several low samples to cover the lower limit of the analyte range. Each sample was assayed in replicates of six. The mean, standard deviation and percent coefficient of variation were calculated for the six replicates at each sample and a plot (expected values (X) against %CV (Y)) was generated. LoQ was defined at the value of sample where the interassay precision is <20% CV.

The LoB, LoD and LoQ for Total Protein and Albumin are tabulated below:

Analyte	LoB (g/dL)	LoD (g/dL)	LoQ (g/dL)
Total Protein (TP)	0.07	0.2	0.2
Albumin (ALB)	0.02	0.09	0.1

The sponsor's claimed measuring range of Total protein is 0.2 to 11.0 g/dL and Albumin is 0.5 g/dL to 7.1 g/dL.

e. Analytical specificity:

An interference study was performed in accordance with CLSI EP7-A. Two levels of commercial control sera containing approximately 4g/dL and 6.5g/dL total protein and 2.5g/dL and 4.0g/dL albumin were spiked to six levels with each interferent (ascorbic acid, unconjugated bilirubin, hemoglobin and lipids) and all seven samples were tested in replicates of three by the Hitachi Clinical Analyzer E40. The spiked sample results mean was compared to its neat control mean result and recoveries were calculated. The sponsor defines non-interference as the mean results from the testing of the spiked samples within 10% of the mean of the neat samples. Recoveries were between 90% and 110% of the neat value. The highest level tested with non significant interference is listed below.

Substance	Total Protein (TP)	Albumin (ALB)
	Highest level tested with no interference	**Highest level tested with no interference**
Ascorbic acid	50mg/dL	50mg/dL
Bilirubin (unconjugated)	50mg/dL	12.5mg/dL
Hemoglobin	1000mg/dL	250mg/dL
Lipids (Intralipid)	500mg/dL	500mg/dL

The sponsor states that hemolyzed specimens should not be used for albumin in the labeling.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 115 (for total protein) and 118 (for albumin) clinical specimens, spanning the dynamic range were assayed in singleton and blinded using the Hitachi system and the predicate device. The specimens were previously collected serum samples. The total protein study set included four diluted samples and the albumin study set included three spiked and eight diluted samples to ensure the dynamic range was fully evaluated. Results obtained were analyzed by Deming regression and resulted the following:

Analyte	Comparative Methods	N	Range of Samples g/dL	Deming Regression		r
				Slope (95% CI)	y-Intercept (95% CI)	
Total Protein (TP)	Hitachi vs Roche	115	0.8 – 10.9	1.02 (1.01 to 1.04)	0.01 (-0.13 to 0.15)	0.989
Albumin (ALB)	Hitachi vs Roche	118	0.5 – 6.4	1.01 (0.96 to 1.06)	0.24 (0.06 to 0.41)	0.985

Physician office accuracy (Method Comparison): Total Protein

Method comparison was performed at three POL sites plus the Site 1 central laboratory. Each site received approximately 50 blinded serum samples (ranging 0.6 to 10.8g/dL) that were chosen to represent as full range of total protein concentrations and the central laboratory received every serum sample. Each sample was assayed by the Hitachi Analyzer E40 at the POL sites, and an aliquot of each sample was assayed by the central laboratory using the predicate device. The data was analyzed by Deming regression and is summarized below.

Site	N	Range of Samples	Deming Regression		r
			Slope (95% CI)	y-Intercept (95% CI)	
1	52	0.8 to 10.8	0.98 (0.96 to 1.01)	0.14 (0.00 to 0.29)	0.996
2	52	0.8 to 10.9	1.00 (0.97 to 1.03)	-0.07 (-0.31 to 0.16)	0.994
3	53	0.6 to 10.5	0.96 (0.93 to 0.98)	0.03 (-0.14 to 0.19)	0.996

Physician office accuracy (Method Comparison): Albumin

Method comparison was performed at three POL sites plus the Site 1 central laboratory. Each site received approximately 90 blinded serum samples (ranging 0.5 to 6.7 g/dL) that were chosen to represent as full range of albumin concentrations and the central laboratory received every serum sample. Each sample was assayed by the

Hitachi Analyzer E40 at the POL sites, and an aliquot of each sample was assayed by the central laboratory using the predicate device. Additionally the study included four samples were diluted and eight spiked samples in order to satisfy the dynamic range. The data was analyzed by Deming regression and is summarized below:

Site	N	Range of Samples g/dL	Deming Regression		r
			Slope (95% CI)	y-Intercept (95% CI)	
1	87	0.5 to 6.7	0.99 (0.92 to 1.06)	0.24 (-0.06 to 0.53)	0.982
2	81	0.5 to 6.6	0.95 (0.88 to 1.02)	0.30 (0.00 to 0.61)	0.979
3	81	0.9 to 6.1	0.91 (0.85 to 0.97)	0.35 (0.10 to 0.60)	0.985

b. Matrix comparison:

Lithium heparinized plasma, EDTA plasma and sodium citrate plasma was analyzed as a secondary sample matrix to serum. Forty-five (45) matched clinical specimens (serum and each plasma type) with total protein concentrations spanning the dynamic range (including 10 diluted samples) were assayed in singleton and in a blinded fashion on one analyzer and S TEST Reagent Cartridge TP. Forty-three (41) matched clinical specimens (serum and each plasma type) with albumin concentrations spanning the dynamic range (including 4 diluted and 7 spiked samples) were assayed in singleton and in a blinded fashion on one analyzer and S TEST Reagent Cartridge ALB. The results were analyzed by least-squares linear regression and are summarized below:

Analyte	Comparative Matrices	Serum Range (g/dL)	N	Least-Squares Linear Regression		r
				Slope (95% CI)	y-Intercept (95% CI)	
Total Protein (TP)	Serum (x) vs. Heparinized Plasma (y)	0.5 -10.5	45	1.00 (0.96 to 1.04)	-0.11 (-0.43 to -0.21)	0.989
	Serum (x) vs EDTA Plasma (y)		45	1.00 (0.96 to 1.04)	-0.06 (-0.33 to 0.22)	0.992
	Serum (x) vs Na Citrate Plasma (y)		45	0.98 (0.93 to 1.03)	-0.09 (-0.45 to 0.26)	0.987
Albumin (ALB)	Serum (x) vs. Heparinized Plasma (y)	1.0 – 7.1	41	0.99 (0.95 to 1.03)	-0.01 (-0.20 to 0.18)	0.992
	Serum (x) vs EDTA Plasma (y)		41	0.95 (0.92 to 0.98)	0.22 (0.08 to 0.36)	0.995
	Serum (x) vs Na Citrate Plasma (y)		41	1.00 (0.94 to 1.05)	-0.22 (-0.48 to -0.03)	0.986

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 expected values are stated within the labeling based on the literature. The manufacturer recommends each laboratory determine the expected values for its particular population.

   Total Protein: Reference range: 6.4 – 8.3g/dL[1]
   Albumin: Reference range: 3.4 – 4.8g/dL[1]

   1. Tietz, Fundamentals of Clinical Chemistry, 4th Edition, WB Saunders Company, 1996.

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.