

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

A. 510(k) Number:

k132462

B. Purpose for Submission:

New Device

C. Measurand:

Blood Urea Nitrogen

Creatinine

D. Type of Test:

Blood Urea Nitrogen: Quantitative, Kinetic

Creatinine: Quantitative, Enzymatic

E. Applicant:

Hitachi Chemical Diagnostics, Inc

F. Proprietary and Established Names:

Hitachi S TEST Reagent Cartridge Blood Urea Nitrogen (BUN)

Hitachi S TEST Reagent Cartridge Creatinine (CRE)

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1770, Blood urea nitrogen (BUN) test system

21 CFR 862.1225, Creatinine (CRE) test system

2. Classification:

Class II

3. Product code:

CDN, CGX

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 Blood Urea Nitrogen (BUN) is intended for the quantitative measurement of BUN in serum, lithium heparin plasma, K3 EDTA plasma, and sodium citrate plasma on the Hitachi Clinical Analyzer E40. The test system is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only. BUN measurements are used in the diagnosis and treatment of certain renal and metabolic diseases.

The S TEST reagent cartridge Creatinine (CRE) is intended for the quantitative measurement of creatinine in serum, lithium heparin plasma, K3 EDTA plasma, and sodium citrate plasma on the Hitachi Clinical Analyzer E40. The test system is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only. Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.

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 BUN is provided ready-to-use. The 2D code label on the front of each cartridge automatically identifies the reagent to the system. It consists of the following reagents:

BUN Reagent (1):

Glutamate dehydrogenase (E.coli)

Nicotinamide adenine dinucleotide phosphate (reduced form)

α -Ketoglutaric acid

2-Amino-2-hydroxymethyl-1,3-propanediol Buffer (pH 9.1)

BUN Reagent (2):

Urease (Jack bean)

α -Ketoglutaric acid

2-Amino-2-hydroxymethyl-1,3-propanediol Buffer (pH 7.3)

The S TEST Reagent Cartridge CRE is provided ready-to-use. The 2D code label on the front of each cartridge automatically identifies the reagent to the system. It consists of the following reagents:

CRE Reagent (1):

Sarcosine oxidase (E. coli)

N,N-Bis(4-sulfobutyl)-3-methylaniline disodium salt

Creatinase (E. coli)

Catalase (Micrococcus lysodeikticus)

Good's buffer (pH 7.8)

CRE Reagent (2):

Creatininase (E. coli)

4-Aminoantipyrine

Peroxidase (Horseradish)

Good's buffer (pH 8.2)

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 Blood Urea Nitrogen (BUN)		
Item	Candidate Device	Predicate Device
Intended Use	For in vitro quantitative measurement of BUN	Same
Testing Environment	Physician office or clinical lab	Clinical lab
Test Principle	Kinetic test (UV rate) with urease and glutamate dehydrogenase	Kinetic test with urease and glutamate dehydrogenase
Specimen Type	Human serum or plasma	Human serum, plasma, or urine
Reportable Range	1.5 to 80 mg/dL	1.4 to 112 mg/dL
Detection Wavelength	546/340 nm	700/340 nm
Detection Limit	0.8 mg/dL	1.4 mg/dL
Linearity	0.9 to 110 mg/dL	1.4 to 112 mg/dL
Instrument platform	Hitachi Clinical Analyzer E40	Roche cobas c systems

Similarities and Differences Creatinine (CRE)		
Item	Candidate Device	Predicate Device
Intended Use	For in vitro quantitative measurement of CRE	Same
Testing Environment	Physician office or clinical lab	Clinical lab- cobas
Test Principle	Enzymatic with creatinase and formation of quinone pigment	Kinetic colorimetric assay based on Jaffe method
Specimen Type	Human serum or plasma	Human serum, plasma, or urine
Reportable Range	0.1 to 25.0 mg/dL	0.2 to 24.9 mg/dL
Detection Wavelength	546/700 nm	570/505 nm
Detection Limit	0.1 mg/dL	0.2 mg/dL
Linearity	0.1 to 31.3 mg/dL	0.2 to 24.9 mg/dL
Instrument platform	Hitachi Clinical Analyzer E40	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:

Blood Urea Nitrogen: Urea is degraded by urease into ammonia. When glutamate dehydrogenase (GLD) reacts with ammonia and alfa-ketoglutaric acid to produce glutamic acid, NADPH is converted into NADP with a decrease of absorbance at 340 nm. The concentration of urea nitrogen can be determined by measuring the amount of change in absorbance.

Creatinine: As the first reaction, creatine in samples is decomposed into water and oxygen by the action of creatinase, sarcosine oxidase, and catalase. Subsequently, as the second reaction, creatinine in samples is converted into creatine by the action of creatinase, and then sarcosine is formed by creatinase. After that, the quinone pigment is formed by oxidation condensation between N,N-Bis (4-sulfobutyl)-3-methylaniline disodium salt (TODB) and 4-aminoantipyrine in the presence of peroxidase (POD) and hydrogen peroxide is formed by sarcosine oxidase. The concentration of creatinine can be determined by measuring the absorbance of the resulting quinone pigment (purple-red).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision studies for the S TEST Reagent Cartridge BUN 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 neat serum samples. Results for BUN precision are summarized below:

Analyte	Sample	Mean (mg/dL)	Within Run		Total	
			SD	%CV	SD	%CV
BUN	Low	7.24	0.22	3.0	0.36	5.0
	Middle	14.07	0.23	1.6	0.38	2.7
	High	49.05	0.58	1.2	1.13	2.3

Precision studies for the S TEST Reagent Cartridge CRE were performed following CLSI EP5-A2 with four levels of analytes (low, middle, high and very 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 archived (stored frozen) patient serum samples. Results for creatinine precision are summarized below:

Analyte	Sample	Mean (mg/dL)	Within Run		Total	
			SD	%CV	SD	%CV
CRE	Low	0.59	0.04	6.8	0.05	8.5
	Middle	1.75	0.04	2.3	1.75	3.4
	High	6.55	0.08	1.2	0.19	2.9
	Very High	20.34	0.18	0.9	0.29	1.4

Precision was evaluated at three POL sites. BUN was evaluated using 6 lots of reagent and Creatinine was evaluated using 3 lots. 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 BUN samples were a combination of neat and spiked specimens. The Creatinine samples were commercial controls. The results are listed below:

Physician Office Precision: BUN

Sample	Site	Mean (mg/dL)	Within run Precision		Total Precision	
			SD (mg/dL)	%CV	SD (mg/dL)	%CV
A	1	12.04	0.13	1.1	0.22	1.8
	2	10.92	0.26	2.3	0.39	3.6
	3	11.68	0.24	2.0	0.23	2.0
	1	46.94	0.37	0.8	0.69	1.5

B	2	45.40	0.48	1.1	0.65	1.4
	3	46.40	0.48	1.0	0.66	1.4
C	1	75.68	0.71	0.9	0.69	0.9
	2	74.29	0.60	0.8	0.85	1.2
	3	75.23	0.41	0.5	0.47	0.6

Physician Office Precision: Creatinine

Sample	Site	Mean (mg/dL)	Within run Precision		Total Precision	
			SD (mg/dL)	%CV	SD (mg/dL)	%CV
A	1	0.58	0.03	5.0	0.04	6.8
	2	0.60	0.00	0.0	0.00	0.0
	3	0.51	0.03	6.7	0.03	6.7
B	1	1.80	0.03	1.8	0.07	3.8
	2	1.80	0.03	1.8	0.07	3.8
	3	1.61	0.03	2.0	0.07	4.4
C	1	6.52	0.05	0.7	0.13	2.1
	2	6.38	0.05	0.8	0.08	1.2
	3	5.95	0.07	1.2	0.31	5.2

b. Linearity/assay reportable range:

The claimed measuring range for BUN is 1.5 - 80 mg/dL. The linearity of the BUN assay was assessed following CLSI EP6-A with commercially available linearity sets which include 7 samples (0.9 to 110.0 mg/dL). All samples were tested in duplicate on one Hitachi Clinical Analyzer E40. Recoveries were within $\pm 10\%$ or 2.5 mg/dL. The summary of the linear regression analysis of the data is below:

$$y=0.9548x+0.6168; R^2=0.9977$$

The BUN assay data demonstrates linearity between 0.9 mg/dL and 110 mg/dL and support the sponsor's claimed measuring range of 1.5 to 80 mg/dL.

The claimed measuring range for Creatinine is 0.1 - 25mg/dL. The linearity of the creatinine assay was assessed following CLSI EP6-A with commercially available linearity sets which include 11 samples (0.1 to 31.3 mg/dL). All samples were tested in duplicate on one Hitachi Clinical Analyzer E40. Recoveries were within $\pm 10\%$ or 0.2 mg/dL. The summary of the linear regression analysis of the data is below:

$$y=1.0201x+0.0457; R^2=0.9991$$

The creatinine assay data demonstrates linearity between 0.1 mg/dL and 31.3 mg/dL and support the sponsor's claimed measuring range of 0.1 to 25 mg/dL.

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

Traceability:

Each lot of the S TEST Reagent Cartridge Blood Urea Nitrogen (BUN) is calibrated by the manufacturer prior to shipment using material traceable to Reference Material Institute for Clinical Chemistry Standards (ReCCS) Standard Serum JCCRM 521. The 2D code printed on each cartridge provides the analyzer with lot-specific calibration data. BUN 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 S TEST Reagent Cartridge Creatinine (CRE) is calibrated by the manufacturer prior to shipment using material traceable to ReCCS standard serum JCCRM 521; this standard is traceable to IDMS. The 2D code printed on each cartridge provides the analyzer with lot-specific calibration data. CRE 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 studies for the BUN 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 claim of 12 months when stored at 2-8°C.

Real-time shelf-life studies for the Creatinine 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 claim of 18 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 BUN and Reagent Cartridge CRE 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 $LoB + 1.645 \times SD$ of the low samples.

LoQ for the S TEST Reagent Cartridge BUN and CRE 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 LoQ study supported performance with low level BUN specimens (~4 mg/dL) but the low end of the reportable range was defined by a functional sensitivity study. Six samples between 0.5 mg/dL and 10 mg/dL were tested. Each sample was assayed in replicates of six. The mean, standard deviation and percent %CVs were calculated for the six replicates. A plot of expected (X) against actual (Y) concentrations and a plot of expected values (X) against %CV (Y) was generated. The data support an LoQ of 0.8 mg/dL and the low end of the measuring range is claimed to be 1.5 mg/dL.

The LoQ study supported performance with low level Creatinine specimens (~0.3 mg/dL) but the low end of the reportable range was defined by a functional sensitivity study. Six samples between 0.05 mg/dL and 0.5mg/dL. Each sample was assayed in replicates of six. The mean, standard deviation and percent %CVs were calculated for the six replicates. A plot of expected (X) against actual (Y) concentrations and a plot of expected values (X) against %CV (Y) was generated. The data support an LoQ of 0.1 mg/dL and the low end of the measuring range is claimed to be 0.1 mg/dL.

The LoB, LoD and LoQ for Blood Urea Nitrogen and Creatinine are tabulated below:

Analyte	LoB (g/dL)	LoD (g/dL)	LoQ (g/dL)
Blood Urea Nitrogen (BUN)	0.52	0.8	0.8
Creatinine (CRE)	0.03	0.06	0.1

The sponsor's claimed measuring range of BUN is 1.5 to 80 mg/dL and Creatinine is 0.1 to 25 mg/dL.

e. *Analytical specificity:*

An interference study was performed in accordance with CLSI EP7-A. Two levels of commercial control sera containing approximately 12 mg/dL and 30 mg/dL BUN and 1.5 mg/dL and 5.7 mg/dL creatinine were spiked to six levels with each interferent (ascorbic acid, unconjugated bilirubin, hemoglobin and lipemia) 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	Blood Urea Nitrogen (BUN)	Creatinine (CRE)
	Highest level tested with no interference	Highest level tested with no interference
Ascorbic acid	50 mg/dL	25 mg/dL
Bilirubin (unconjugated)	50 mg/dL	25 mg/dL
Hemoglobin	1000 mg/dL	250 mg/dL
Lipemia (Intralipid)	1000 mg/dL	1000 mg/dL

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

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 162 (for BUN) and 100 (for creatinine) serum specimens, spanning the dynamic range were assayed in singleton using the Hitachi system and the predicate device. The BUN study set included four diluted and four spiked samples and the creatinine study set included four spiked samples to ensure the dynamic range was fully evaluated. Results obtained were analyzed by Deming regression and summarized in the following table:

Analyte	Comparative Methods	N	Range of Samples mg/dL	Deming Regression		r
				Slope (95% CI)	y-Intercept (95% CI)	
Blood Urea Nitrogen (BUN)	Hitachi vs. Roche	162	2.4 – 78.5	0.96 (0.95 to 0.97)	-0.3 (-0.64 to 0.10)	0.997
Creatinine (CRE)	Hitachi vs. Roche	100	0.5 – 24.7	0.99 (0.98 to 1.00)	- 0.1 (-0.18 to -0.07)	0.999

Physician office accuracy (Method Comparison): BUN

Method comparison was performed at three POL sites on the Hitachi E40 Analyzer vs. the predicate device at the central laboratory. Each site and the central laboratory tested approximately 75 blinded neat serum samples (ranging from 5.7 to 73.6 mg/dL) that were chosen to represent a full range of BUN concentrations. The data was analyzed by Deming regression and is summarized below.

Site	N	Range of Samples mg/dL	Deming Regression		r
			Slope (95% CI)	y-Intercept (95% CI)	
1	75	6.0 to 73.6	0.98 (0.96 to 0.99)	-0.23 (-0.59 to 0.13)	0.999
2	74	5.7 to 69.3	0.94 (0.93 to 0.95)	-0.24 (-0.50 to 0.01)	0.999
3	73	5.7 to 70.8	0.95 (0.93 to 0.96)	-0.04 (-0.41 to 0.32)	0.999

Physician office accuracy (Method Comparison): Creatinine

Method comparison was performed at three POL sites on the Hitachi E40 Analyzer vs. the predicate device at the central laboratory. Each site and the central laboratory tested approximately 45 blinded serum samples (ranging from 0.6 to 24.1 mg/dL, a combination of neat serum specimens and 4 spiked samples) that were chosen to represent a full range of creatinine concentrations. The data was analyzed by Deming regression and is summarized below.

Site	N	Range of Samples mg/dL	Deming Regression		r
			Slope (95% CI)	y-Intercept (95% CI)	
1	45	0.6 to 23.5	0.97 (0.96 to 0.98)	-0.06 (-0.13 to 0.02)	0.999
2	44	0.6 to 24.1	0.98 (0.96 to 0.99)	-0.09 (-0.19 to 0.02)	0.999
3	47	0.6 to 22.8	0.96 (0.95 to 0.96)	-0.04 (-0.10 to 0.02)	0.999

b. Matrix comparison:

Lithium heparinized plasma, EDTA plasma and sodium citrate plasma was analyzed as a secondary sample matrix to serum. Thirty-six (36 matched clinical specimens (serum and each plasma type) with BUN concentrations spanning the dynamic range (including 2 diluted and 4 spiked samples) were assayed in singleton and in a blinded fashion on one analyzer and S TEST Reagent Cartridge BUN. Thirty-nine (39) matched clinical specimens (serum and each plasma type) with creatinine concentrations spanning the dynamic range (including 6 diluted samples) were assayed in singleton and in a blinded fashion on one analyzer and S TEST Reagent Cartridge CRE. The results were analyzed by least-squares linear regression and are summarized below:

Analyte	Comparative Matrices	Serum Range (mg/dL)	N	Least-Squares Linear Regression		r
				Slope (95% CI)	y-Intercept (95% CI)	
Blood Urea Nitrogen (BUN)	Serum (x) vs. Heparinized Plasma (y)	2.4–75.3	36	1.01 (1.00 to 1.03)	-0.56 (-0.84 to -0.28)	0.999
	Serum (x) vs. EDTA Plasma (y)		36	1.01 (1.00 to 1.03)	-0.61 (-0.94 to 0.27)	0.999
	Serum (x) vs. Na Citrate Plasma (y)		36	0.99 (0.97 to 1.01)	-0.98 (-1.42 to -0.54)	0.998

Creatinine (CRE)	Serum (x) vs. Heparinized Plasma (y)	0.1–24.5	39	0.99 (0.98 to 1.00)	-0.02 (-0.08 to 0.05)	0.999
	Serum (x) vs. EDTA Plasma (y)		39	1.01 (1.00 to 1.02)	-0.06 (-0.15 to 0.03)	0.999
	Serum (x) vs. Na Citrate Plasma (y)		39	1.00 (0.99 to 1.02)	-0.05 (-0.14 to 0.05)	0.999

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.

Blood Urea Nitrogen Reference range: 6 – 20 mg/dL¹

Creatinine Reference range: Males: 0.62 – 1.10 mg/dL; Females: 0.45 – 0.75 mg/dL¹

1. Tietz, Fundamentals of Clinical Chemistry, 6th Edition, WB Saunders Company, 2008.

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.