

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

A. 510(k) Number:

k130685

B. Purpose for Submission:

New Device

C. Measurand:

Amylase (AMY)

Lactate Dehydrogenase (LD)

D. Type of Test:

Quantitative, enzymatic activity

E. Applicant:

Hitachi Chemical Diagnostics, Inc.

F. Proprietary and Established Names:

Hitachi S TEST Reagent Cartridge Amylase (AMY)

Hitachi S TEST Reagent Cartridge Lactate Dehydrogenase (LD)

G. Regulatory Information:

1. Regulation section:

21 CFR § 862.1070 Amylase (AMY)

21 CFR § 862.1440 Lactate Dehydrogenase (LD)

2. Classification:

Class II and Class II Exempt, meets limitations of exemption 21 CFR 862.9 (c)(9) respectively

3. Product code:

JFJ, CFJ

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 Lactate Dehydrogenase (LD) is intended for the quantitative determination of LD in serum and plasma using the HITACHI Clinical Analyzer E40. The S TEST Reagent Cartridge Lactate Dehydrogenase (LD) is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

Measurements of LD are used in the diagnosis and treatment of liver and cardiac diseases.

The S TEST Reagent Cartridge Amylase (AMY) is intended for the quantitative determination of AMY in serum and plasma using the HITACHI Clinical Analyzer E40. The S TEST Reagent Cartridge Amylase (AMY) is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

Measurements of AMY are mainly used in the diagnosis and treatment of pancreatic diseases.

3. Special conditions for use statement(s):

For prescription and point-of-care use.

4. Special instrument requirements:

Hitachi Clinical Analyzer E40

I. Device Description:

The S TEST reagent cartridges for the Hitachi Clinical Analyzer E40 are made of plastic and include two small reservoirs capable of holding two separate reagents R1 and R2, separated by a reaction cell/photometric cuvette. The cartridges also include a dot code label that contains all chemistry parameters, calibration factors, and other production-related information, e.g., expiration dating.

The S TEST Reagent Cartridge Lactate Dehydrogenase (LD) has the following composition: LD Reagent (1): L-Lactic acid lithium salt, LD Reagent (2): Nicotinamide adenine dinucleotide and Citric acid Buffer.

The S TEST Reagent Cartridge Amylase (AMY) has the following composition:
AMY Reagent (1): Sodium chloride, Calcium chloride, Good's buffer
AMY Reagent (2): alfa-2-chloro-4-nitrophenyl-galactopyranosylmaltoside, Potassium thiocyanate, and Good's buffer.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Roche cobas c systems

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

k100853

3. Comparison with predicate:

	Hitachi S Test Reagent Cartridge LD (Candidate Device)	Roche Cobas c systems (Predicate Device, k100853)
Similarities		
Intended Use	For the in vitro quantitative determination of Lactate Dehydrogenase in human serum and plasma.	Same

Specimen Type	Human serum, lithium heparinized plasma and K3 EDTA plasma	Same
Claimed measuring range	10 to 1,000 U/L	Same
Detection Limit	10 U/L	Same
Test Principle	LD in the sample catalyzes the conversion of lactic acid to pyruvic acid. NAD is converted to NADH with an increase in absorbance	UV assay- LD catalyzes the conversion of L-lactate to pyruvate (pyruvic acid); NAD is reduced to NADH in the process.
Differences		
Testing Environment	Physician office or clinical lab	Clinical lab
Detection Wavelength	340/546 nm	700/340 nm

	Hitachi S Test Reagent Cartridge AMY (Candidate Device)	Roche Cobas c systems (Predicate Device, k100853)
Similarities		
Intended Use	For the in vitro quantitative determination of Amylase in human serum and plasma.	Same
Differences		
Test Principle	Alpha amylases in blood samples react with the substrate alfa-2-chloro-4-nitrophenyl-galactopyranosylmaltoside (Gal-G2-CNP), and the substrate is cleaved into 4-galactopyranosylmaltose (Gal-G2) and 2-chloro-4-nitrophenol (CNP). Amylase activity is determined by measuring the production rate of CNP (yellow)	Defined oligosaccharides are cleaved under the catalytic action of alpha amylases. The fragments formed are completely hydrolyzed to p-nitrophenol (p-NP) and glucose by alpha-glucosidase. The color intensity of the p-NP formed is directly proportional to the amylase activity and is determined by measuring the increase in absorbance
Testing Environment	Physician office or clinical lab	Clinical lab
Detection Wavelength	405/546 nm	700/415 nm

Specimen Type	Human serum, lithium heparinized plasma and K3 EDTA plasma	Human serum, plasma, or urine.
Claimed measuring range	4 to 1,500 U/L	3 to 1,500 U/L
Detection Limit	4 U/L	3 U/L

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

CLSI/NCCLS EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; 2004

CLSI/NCCLS EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach; 2003

CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline, 2005

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; 2004

L. Test Principle:

Lactate Dehydrogenase (LD) in the sample catalyzes the conversion of lactic acid to pyruvic acid. NAD is converted to NADH with an increase in absorbance. Lactate dehydrogenase concentration is directly determined by multiplying the change in absorbance of the unknown samples by the calibrator factor on the reagent barcode.

Amylase (AMY): Alpha amylases in blood samples react with the substrate alfa-2-chloro-4-nitrophenyl-galactopyranosylmaltoside (Gal-G2-CNP), and the substrate is cleaved into 4-galactopyranosylmaltose (Gal-G2) and 2-chloro-4-nitrophenol (CNP). Amylase activity is determined by measuring the production rate of CNP (yellow). Amylase concentration is directly determined by multiplying the change in absorbance of the unknown samples by the calibrator factor on the reagent barcode.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A 20-day In-house Precision study for Lactate Dehydrogenase (LD) was conducted following CLSI EP5-A2. Three samples (low, middle, and high levels of LD) were tested on the Hitachi E40 Clinical Analyzer in duplicate, twice a day, for 20 days, for a total of 80 results per level. The samples tested were commercial controls (Levels 1 and 2) and a patient serum sample (Level 3).

LD- Low, Level 1, Summary

LD	Within-Run	Total
Mean (U/L)	108.2	108.2
SD (U/L)	5.24	6.82
%CV	4.8%	6.3%

LD- Middle, Level 2, Summary

LD	Within-Run	Total
Mean (U/L)	159.3	159.3
SD (U/L)	9.15	8.85
%CV	5.7%	5.6%

LD- High, Level 3, Summary

LD	Within-Run	Total
Mean (U/L)	628.0	628.0
SD (U/L)	20.0	33.8
%CV	3.2%	5.4%

A Point-of-Care precision study for Lactate Dehydrogenase (LD) was conducted using three levels of samples A (low), B (middle), and C (high) tested by three POL sites, six times a day for five days on the Hitachi E40 Clinical Analyzer. The samples were native (neat) serum specimens (stored frozen). The precision estimates are described below:

Site #	Sample	LD Mean (U/L)	Within-run Precision		Total Precision	
			SD (U/L)	%CV	SD (U/L)	%CV
1	A	47.3	3.83	8.1	4.42	9.3
2	A	49.8	3.00	6.0	3.20	6.4
3	A	45.7	3.69	8.1	3.82	8.4
1	B	161.9	5.60	3.5	6.45	4.0
2	B	161.6	6.01	3.7	6.25	3.9
3	B	155.7	8.90	5.7	9.63	6.2
1	C	498.1	12.70	2.6	15.10	3.0
2	C	488.5	20.29	4.2	35.20	7.2
3	C	497.0	14.71	3.0	20.55	4.1

A 20-day In-house Precision study was conducted for Amylase (AMY) following CLSI EP5-A2. Three samples (low, middle, and high levels of AMY) were tested on the Hitachi E40 Clinical Analyzer in duplicate, twice a day, for 20 days, for a total of 80 results per level. The samples were natural patient serum samples. The results were as follows:

AMY- Low, Level 1, Summary

AMY	Within-Run	Total
Mean (U/L)	54.1	54.1
SD (U/L)	0.94	1.45
%CV	1.7%	2.7%

AMY - Middle, Level 2, Summary

AMY	Within-Run	Total
Mean (U/L)	188.5	188.5
SD (U/L)	1.5	6.99
%CV	0.8%	3.7%

AMY - High, Level 3, Summary

AMY	Within-Run	Total
Mean (U/L)	1126.8	1126.8
SD (U/L)	8.85	39.5
%CV	0.8%	3.5%

A Point-of-Care Precision study for Amylase (AMY) was conducted using two levels of samples A (low), and B (middle) tested by three POL sites, six times a day for five days on the Hitachi E40 Clinical Analyzer. The samples were native (neat) serum specimens (stored frozen). The precision estimates are described below:

Site #	Sample	AMY Mean (U/L)	Within-run Precision		Total Precision	
			SD (U/L)	%CV	SD (U/L)	%CV
1	A	53.2	2.43	4.6	2.44	4.6
2	A	50.4	1.44	2.8	1.74	3.5
3	A	51.2	2.08	4.1	1.93	3.8
1	B	116.9	1.58	1.4	1.65	1.4
2	B	111.5	1.73	1.6	2.11	1.9
3	B	113.3	2.29	2.0	3.51	3.1

b. Linearity/assay reportable range:

A linearity study for Lactate Dehydrogenase (LD) was conducted based on the CLSI EP6-A guidelines by comparing observed versus expected values for 12 samples. Twelve (12) serial dilutions (1 to 1313 U/L) were prepared and tested. The dilutions were prepared using a commercial linearity/calibration set. The calibration samples were assigned their reference values arithmetically from the labeled values and were tested in duplicate by the Hitachi E40 Clinical Analyzer. The mean Hitachi results (y-axis) were plotted against the expected values (x-axis). The parameters of linear regression are as follows:

$$y = 0.9441x + 9.6975$$
$$R^2=0.9962$$

The results of the study support the sponsor's claim that the Hitachi S Test LD test is linear across the measuring range of 10 to 1,000 U/L.

A linearity study for Amylase (AMY) was conducted based on the CLSI EP6-A guidelines by comparing observed versus expected values for 10 samples. Ten (10) serial dilutions (3.4 to 1858.5 U/L) were prepared and tested. The dilutions were prepared using a commercial linearity/calibration set. The calibration samples were assigned their reference values arithmetically from the labeled values and were tested in duplicate by the Hitachi E40 Clinical Analyzer. The mean Hitachi results (y-axis) were plotted against the expected values (x-axis). The parameters of linear regression are as follows:

$$y = 1.0109x - 0.8232$$
$$R^2=0.9987$$

The results of the study support the sponsor's claim that the Hitachi S Test AMY test is linear across the measuring range of 4 to 1,500 U/L.

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

Each lot of HITACHI S TEST LD cartridges and each lot of HITACHI S TEST AMY cartridges is calibrated by the manufacturer prior to shipment using material traceable to Japanese Enzyme Reference Material (JC-ERM). The barcode printed on each cartridge provides the analyzer with lot-specific calibration data. Lactate dehydrogenase and amylase concentrations are directly determined by multiplying the change in absorbance of the unknown sample by the calibrator factor on the barcode. No user calibration is needed. Commercially available controls are required but not provided. The labeling states "Users should follow federal, state, and local regulatory requirements regarding quality control practices."

d. Detection limit:

Detection limit studies were performed according to CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation. The blank sample for the reagent system was assayed on the Hitachi Clinical Analyzer E40, 20 times per day for three days for a total of 60 replicate results to determine LOB. Five low samples were assayed on the Hitachi Clinical Analyzer E40, 4 times per day for three days for a total of 60 replicate results with the specific reagent cartridges to determine LOD. Seven samples covering the sample range between 0 and 20 U/L were tested for Lactate Dehydrogenase (LD). Seven samples covering the sample range between 0 and 10 U/L were tested for Amylase (AMY). All of the low samples were assayed 6 times on one instrument with one lot of cartridges to determine LoQ. The LoQ was determined based on inter-assay precision of < 20% CV. Results of the LoB, LoD and LoQ are summarized below.

Analyte	LoB (U/L)	LoD (U/L)	LoQ (U/L)
Lactate Dehydrogenase (LD)	3.0	7.9	10
Amylase (AMY)	1.1	2.2	4

The claimed measuring range of Hitachi S Test LD is 10 to 1,000 U/L.
The claimed measuring range of Hitachi S Test AMY is 4 to 1,500 U/L.

e. Analytical specificity:

Interference studies were performed according to CLSI EP7-A2, (Interference Testing in Clinical Chemistry; Approved Guideline) to determine the effects from potential interferents. Two levels of serum samples (LD low and high, approximately 100 U/L and 350U/L) were spiked to six levels with each interferent. Two levels of serum samples (AMY low and high, approximately 150 U/L and 300U/L) were spiked to six levels with each interferent. All seven samples (the 6 spiked samples and unspiked sample) for each analyte, Lactate Dehydrogenase (LD) and Amylase (AMY), were tested in replicates of three on the Hitachi E40 Clinical Analyzer. In each case, the spiked sample result mean was compared to its neat control mean result, and recoveries were calculated. The sponsor claims no significant interference as $\leq 10\%$ difference for the substances and concentrations listed in the table below.

Lactate Dehydrogenase (LD)

Interferent Compound	Highest Concentration Showing No Interference
Hemoglobin	31 mg/dL*
Unconjugated bilirubin	50 mg/dL
Lipemia	1,000 mg/dL
Ascorbic acid	50 mg/dL

*The labeling states “Positive interference (increase in concentration) from hemolysis occurred at levels as low as 31 mg/dL hemoglobin. Any level of hemolysis may cause interference. Do not use hemolyzed specimens. “

Amylase (AMY)

Interferent Compound	Highest Concentration Showing No Interference
Hemoglobin	500 mg/dL
Unconjugated bilirubin	50 mg/dL
Lipemia	2,000 mg/dL
Ascorbic acid	50 mg/dL

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

An in-house method comparison study for Lactate Dehydrogenase (LD) was conducted using a total of 106 serum specimens (8 diluted and 8 spiked) spanning the dynamic range (13 to 959 U/L), samples were assayed on the Hitachi Clinical Analyzer E40 in singleton on both the Hitachi S Test Reagent and the Roche Cobas c systems (predicate device). The comparative data were analyzed by linear regression and are shown below.

Internal study summary – LD (U/L)

n	Hitachi Range	Regression Equation	“r”	95% CI Slope	95% CI Intercept
106	13 to 959 U/L	$y = 1.013x + 5.428$	0.991	(0.99 to 1.04)	(-3.8 to 14.6)

An external site method comparison study for Lactate Dehydrogenase (LD) was conducted using a series of approximately 80 serum specimens (4 diluted and 8 spiked) with LD values ranging from 16 to 938 U/L. The samples were assayed on the Hitachi Clinical Analyzer E40 at three sites using S TEST Reagent Cartridge LD (y) and Roche Cobas c systems (predicate device) as the reference

method (x). Linear regression analysis yielded the following results:

POL study summary- LD (U/L)

Site #	n	Sample Range	Regression Equation	“r”	95% CI Slope	95% CI Intercept
1	87	16 to 938 U/L	$y=0.96x +2.5$	0.99	0.94 to 0.97	-2.3 to 7.4
2	78	23 to 877 U/L	$y=0.96x +4.7$	0.99	0.94 to 0.97	0.6 to 8.9
3	86	17 to 914 U/L	$y=0.91x +13.5$	0.99	0.90 to 0.93	9.6 to 17.4

An in-house method comparison study for Amylase (AMY) was conducted using a total of 105 clinical specimens (7 diluted) spanning the dynamic range (5 to 1443 U/L), samples were assayed on the Hitachi Clinical Analyzer E40 in singleton on both the Hitachi S Test Reagent and the Roche cobas c systems (predicate device). The comparative data were analyzed by linear regression and are shown below.

Internal study summary – AMY (U/L)

n	Hitachi Range	Regression Equation	“r”	95% CI Slope	95% CI Intercept
105	5 to 1443 U/L	$y = 1.0109x -0.8232$	0.997	(1.06 to 1.10)	(-8.7 to 2.1)

An external site method comparison study for Amylase (AMY) was conducted using a series of approximately 70 serum specimens with AMY values ranging from 27 to 1146 U/L were assayed on the Hitachi Clinical Analyzer E40 at three sites using S TEST Reagent Cartridge AMY (y) and Roche cobas c systems (predicate device) as the reference method (x). The samples were native serum specimens and no diluted or spiked samples were tested. Linear regression analysis yielded the following results:

POL study summary- AMY (U/L)

Site #	n	Sample Range	Regression Equation	“r”	95% CI Slope	95% CI Intercept
1	76	29 to 1134 U/L	$y=1.05x -1.2$	0.999	1.04 to 1.06	-4.6 to 2.2
2	69	27 to 1146 U/L	$y=1.00x -0.5$	0.995	0.98 to 1.03	-6.6 to 5.7
3	71	29 to 1112 U/L	$y=0.98x +3.3$	0.995	0.95 to 1.00	-3.2 to 9.8

b. Matrix comparison:

A study for Lactate Dehydrogenase (LD) was performed to validate the use of two plasma types as an alternative to serum for the Hitachi Clinical Analyzer E40 with S TEST Reagent Cartridge LD. The plasma types were lithium heparin and

K3-EDTA plasma. Thirty-nine (39) matched serum/plasma samples that spanned the range of the assay (32 to 804 U/L) were assayed in singleton. The study set included five diluted samples and six spiked samples. The results were compared using linear regression (plasma = y-axis, each type).

	Lithium Heparin Plasma	K3-EDTA Plasma
Slope (95% CIs)	0.99 (0.97 to 1.01)	0.97 (0.94 to 1.00)
y-intercept (95% CIs)	-5.5 (-10.7 to -0.3)	0.1 (-8.9 to 9.0)
r	0.998	0.994

The sponsor claims that lithium heparin and K3-EDTA are acceptable anti-coagulants to be used with the LD assay.

A study for Amylase (AMY) was performed to validate the use of two plasma types as an alternative to serum for the Hitachi Clinical Analyzer E40 with S TEST Reagent Cartridge AMY. The plasma types were lithium heparin and K3-EDTA plasma. Forty-three (43) matched serum/plasma samples that spanned the range of the assay (5 to 1494 U/L) were assayed in singleton. The study set included seven diluted samples and nine spiked samples. The results were compared using linear regression (plasma = y-axis, each type). The performance characteristics were as follows.

	Lithium Heparin Plasma	K3-EDTA Plasma
Slope (95% CIs)	1.02 (1.01 to 1.04)	0.97 (0.95 to 0.99)
y-intercept (95% CIs)	-8.4 (-17.2 to -0.3)	-6.6 (-14.3 to -1.0)
r	0.998	0.999

The sponsor claims that lithium heparin and K3-EDTA are acceptable anti-coagulants to be used with the AMY assay.

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. Clinical studies are not typically submitted for this device type.

5. Expected values/Reference range:

Lactate Dehydrogenase (LD) Reference Range ¹	53 to 128 U/L
Amylase (AMY) Reference Range ¹	110 to 210 U/L

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.