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

A. 510(k) Number:

k061598

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative, turbidimetric assay

E. Applicant:

Instrumentation Laboratory Corporation

F. Proprietary and Established Names:

HemosIL Homocysteine

HemosIL Homocysteine controls (sold separately)

G. Regulatory Information:

1. Regulation section:

    21 CFR 862.1377

    21 CFR 862.1660

2. Classification:

    Class II and Class I

3. Product code:

   LPS (kit) and JJX (controls)

4. Panel:

   75 (Chemistry)

H. Intended Use:

1. Intended use(s):

   See Indication(s) for use below.

2. Indication(s) for use:

   HemosIL Homocysteine is an automated latex enhanced immunoassay for the quantitative determination of total L-homocysteine in human citrated plasma on IL Coagulation Systems. Homocysteine values can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia or homocystinuria.

   HemosIL Homocysteine Controls are assayed quality controls intended to monitor the accuracy and precision of HemosIL Homocysteine on IL Coagulation Systems.

   For *in vitro* diagnostic use.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   For use with IL Coagulation Systems (ACL TOP, ACL ELITE/ELITE PRO/8/9/10000, ACL Futura/ACL Advance).

I. Device Description:

   HemosIL Homocysteine reagent kit includes the following reagents and calibrator material:

   1. Buffer (Cat. No. 0020007810): 2 vials x 9 mL of PBS (phosphate buffer solution) with preservative.
   2. Reductant (Cat. No. 0020007820): 2 vials x 2 mL of adenosine and Tris (2-carboxyethyl) phosphine (TCEP) solution in Bis-Tris buffer with

preservative.

3. Enzyme (Cat. No. 0020007830): 2 vials x 2 mL of recombinant S-adenosyl-L-homocysteine-hydrolase (SAHH) solution in phosphate buffer with stabilizer and preservative.
4. Conjugate (Cat. No. 0020007840): 2 vials x 2.5 mL of conjugate solution in PBS (phosphate buffer solution) with stabilizer and preservative.
5. a-SAH Latex Reagent (Cat. No. 0020007850): 2 vials x 2 mL of a lyophilized suspension of polystyrene latex particles coated with an anti S-adenosyl-L-homocysteine (SAH) monoclonal antibody in buffer containing bovine serum albumin and 0.02% Bronidox™ as a preservative.
6. Calibrator (Cat. No. 0020007860): 2 vials x 1 mL of S-adenosyl-L-homocysteine (SAH) in PBS (phosphate buffer solution) with preservative.

HemosIL Homocysteine Controls kit consists of:
1. Hcy Control level 1 (Cat. No. 00200007910): 3 vials x 1mL of a lyophilized solution of diluted L-homocysteine and a preservative.
2. Hcy Control level 2 (Cat. No. 00200007920): 3 vials x 1mL of a lyophilized solution of diluted L-homocysteine and a preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Abbott IMx Homocysteine

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

   k992858

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative *in vitro* diagnostic determination of total L-homocysteine for diagnosis and treatment of hyperhomocysteinemia and homocystinuria.	Quantitative *in vitro* diagnostic determination of total L-homocysteine for diagnosis and treatment of hyperhomocysteinemia and homocystinuria.
Methodology	Particle Enhanced Immunoturbidimetry	Fluorescence Polarization Immunoassay
Calibrator Material	One level: S-adenosyl-L-homocysteine (SAH) in PBS (phosphate buffer solution) with preservative.	Two levels: 1) A: Phosphate buffer 2) B: Gravimetrically prepared S-adenosyl-L-homocysteine

Similarities		
Item	Device	Predicate
		(SAH) in phosphate buffer at defined concentrations.
Control Materials	Two levels: Lyophilized solution of diluted L-homocysteine and a preservative.	Three levels: L-homocysteine in processed human serum with sodium azide as preservative.

Differences		
Item	Device	Predicate
Sample types	Citrated plasma only	Serum and EDTA or Lithium Heparin Plasma
Detection Limits	2.4 µmol/L	0.50 µmol/L
Functional sensitivity	4.5 µmol/L	NA
Reportable range	4.5 to 30 µmol/L without rerun 4.5 to 60 µmol/L with rerun, automatic dilutions.	0.5 to 50 µmol/L
Normal range	For U.S. populations: 3.5-9.3 µmol/L For Spain populations: ACL Futura/ACL Advance is 4.1-10.6 µmol/L ACL ELITE/ELITE PRO/8/9/10000 is 4.0-11.2 µmol/L ACL TOP is 4.3-11.1 µmol/L	4.5-12.4 µmol/L

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

STANDARDS
Title and Reference Number

CSLI Guideline, EP5-A *Evaluation of Precision Performance of Clinical Chemistry Devices*.
CSLI Guideline, EP7-A *Interference Testing in Clinical Chemistry; Approved Guideline*.
CLSI/NCCLS. *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays*, Fourth Edition, CLSI/NCCLS Document H21-A4; Vol. 23 No. 35.

L. Test Principle:

Homocysteine levels in patient plasma are measured automatically on IL Coagulation Systems in three stages:

1. Reduction of mixed disulfides and protein-bound forms of Hcy present in the plasma samples to free Hcy.

2. Enzymatic conversion of free Hcy to S-adenosyl-L-homocysteine (SAH) by the SAH hydrolase (SAHH) in the presence of an excess of adenosine.

3. Competitive agglutination reaction between anti-SAH and SAH/conjugate.

The degree of agglutination is inversely proportional to the concentration of total Hcy in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   A precision study was performed using a plasma sample and controls. Samples were run in duplicate twice a day for 20 days on three different instruments following the CSLI EP5-A Guidelines. Mean, SD, and CVs were calculated and all samples had CV less than 10 % for within run, between day and total precision. The acceptance criteria are $\leq$ 10 % for within run, between day and total precision.

ACL TOP	Mean (μmol/L)	CV% (Within run)	CV% (Total)
Hcy Control Level 1	11.4	2.0	4.8
Hcy Control Level 2	22.4	1.5	3.5
Hcy plasma sample	8.1	2.9	5.5

ACL 9000	Mean (μmol/L)	CV% (Within run)	CV% (Total)
Hcy Control Level 1	12.3	2.3	5.1
Hcy Control Level 2	22.8	4.3	6.2
Hcy plasma sample	8.4	2.6	5.9

ACL Futura	Mean (µmol/L)	CV% (Within run)	CV% (Total)
Hcy Control Level 1	10.5	3.5	6.0
Hcy Control Level 2	21.1	2.6	3.5
Hcy plasma sample	7.9	3.5	5.6

b. *Linearity/assay reportable range:*

   i) Regular test (Without Rerun)

   A linear study was performed using three different instruments, ACL Futura, ACL 9000, and ACL TOP. The Homocysteine calibrator was diluted and analyzed in replicates of five with two different lots of HCY kits. The test was considered linear if the CV < 20% and the recovery within ± 20% of the target value and the following acceptance criteria were met:

   Slope of 0.9-1.1; $R^2 \geq 0.96$; and Intercept of 0.0 ± Detection Limit

   This test has a linear range of 4.5-30 µmol/L without rerun.

   ii) Regular test (With Rerun)

   A linear study was performed using three different instruments, ACL Futura, ACL 9000, and ACL TOP to verify that the Hcy level in citrated plasma samples can be accurately recovered when the samples are diluted. A plasma sample containing approximately 60 µmol/L of Hcy was diluted to different dilution with both the kit Buffer and with a human plasma sample with low Hcy concentration. Each dilution was analyzed in quadruplicate using two different lots of Hcy kits with and without rerun. Samples above 30 µmol/L were automatically diluted by the rerun capability of the instrument. The test was considered linear if the CV < 20% and the recovery within ± 20% of the target value and the following acceptance criteria were met:

   Slope of 0.9-1.1; $R^2 \geq 0.96$; and Intercept of 0.0 ± Detection Limit

   This test has a linear range of 4.5-60 µmol/L with rerun.

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

The HemosIL Homocysteine calibrator is traceable to an Internal Reference Standard that was prepared gravimetrically with S-adenosyl-L-homocysteine (SAH) in phosphate buffer. A 5000 µmol/L Homocysteine Reference Stock concentrate was prepared by gravimetrically adding S-adenosyl-L-homocysteine (SAH) to Calibrator Diluent. The Homocysteine assay is calibrated using the Calibrator included as a component of the kit.

The reconstituted controls stability testing was performed at several intervals and for temperature stored at 15-25°C , 2-8°C, and -20°C. The data supported

the reconstituted controls stability of 7 days at 15-25°C, 2 months at 2-8 °C, and 1 month at -20°C.

d. *Detection limit:*

The detection limits were established by running twenty replicates of the Buffer with two different lots of Hcy kits on three different instruments, ACL Futura, ACL 9000 and ACL TOP . For each reagent lot on each instrument platform, the mean, SD, and mean + 3SD were calculated. The highest calculated value for all the instrument platforms was used to establish the detection limit claims of 2.4 µmol/L.

e. *Analytical specificity:*

An interference study was performed by spiking two citrated plasma samples containing approximately 8 and 15 µmol/L of Hcy with the kit buffer and the other with 20 times more concentrated interfering substance. Study was performed on ACL Futura, ACL 9000, and ACL TOP. No significant interference if the % recovery was within ± 15% of the expected control result.

There was no significant interference by the following interferents:

- Hemoglobin up to 490 mg/dL

- Bilirubin up to 19 mg/dL

- Triglycerides up to 1325 mg/dL

- Rheumatoid Factor up to 480 IU/mL

- Sodium Fluoride up to 4.0 g/L

A cross-reactivity study was performed using normal plasma samples spiked with the following concentrations and results were summarized in the following table:

Compound	Assayed concentration	% cross reactivity
L-Cysteine	5 mmol/L	0.0 %
Adenosine	1 mmol/L	0.2 %
L-Methionine	0.4 mmol/L	-0.2 %
L-Cystathionine	2 mmol/L	0.1 %
S-Adenosyl-L-methionine	0.5 mmol/L	2.6 %
DL-Homocysteine-thiolactone	0.1 mmol/L	4.7 %
Glutathione	100 mmol/L	0.0%

As limitations, the applicant stated the followings in the package insert:

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1. Specimens from patients who are on drug therapy involving S-adenosy-methionine may show falsely elevated levels of homcysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate, may have elevated levels of homocysteine due to their effect on the pathway.

2. Specimens from patients who have received preparation of mouse monoclonal antibody for diagnosis or therapy may contain human anti-mouse antibody (HAMA). HAMA present in serum or plasma specimens may interfere in immunoassays that utilize mouse monoclonal antibodies. These specimens should not be assayed in the Hcy assay.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study was conducted in Italy, using 76 paired sodium citrate and EDTA patient plasma samples analyzed in duplicate using HemosIL Homocysteine on an ACL 9000(sodium citrate plasma) verses IMx Homocysteine (EDTA plasma). Samples ranged in Homocysteine values from 4.3 to 56.7 µmol/L.

The slope and intercept were calculated by Passing & Bablock and the correlation coefficient by Pearson. Correlation regression yielded: y= 0.829x + 0.350, r = 0.9915. Y= ACL 9000(sodium citrate plasma), X= IMx (EDTA plasma). All criteria met the applicant's acceptable criteria of: Slope=0.80-1.20, $R^2 \geq 0.950$, Intercept= 0.0± Detection limit.

b. *Matrix comparison between the Citrated and EDTA plasma on the ACL TOP:*

A correlation study was conducted using 139 paired sodium citrate and EDTA patient plasma samples analyzed in duplicate on an ACL TOP using HemosIL Homocysteine. Samples ranged in Homocysteine values from 5.5 to 34.4 µmol/L.

The slope and intercept were calculated by Passing & Bablock and the correlation coefficient by Pearson. The performance of citrated versus EDTA plasma is substantially equivalent, although there was an average bias of 15.2% between the sample types. Correlation regression yielded: y= 0.799x + 0.406, r = 0.9939. Y= Citrate plasma, X= EDTA plasma.

c. *Instruments equivalent studies between ACL 9000 verses ACL Futura and ACL TOP:*

A method comparison study was performed to compare the performance of HemosIL Homocysteine on the representative instrument platforms. 117

citrated plasma samples were tested on an ACL 9000, ACL Futura, and ACL TOP. Samples tested ranged from 7.9 to 69.9 µmol/L. The statistical analysis by Passing & Bablok regression yielded the following results.

1. ACL 9000 vs ACL Futura, y=0.9458 x + 0.0149, r = 0.99. x = ACL 9000, y = ACL Futura
2. ACL 9000 vs ACL TOP, y=0.9780 x + 0.2943, r = 0.99. x = ACL 9000, y=ACL TOP

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

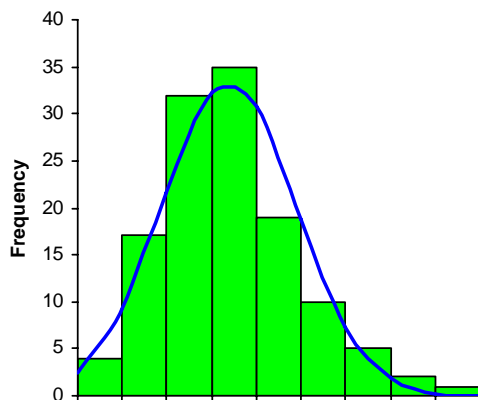4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   One hundred forty (140) citrated plasma samples, 50 males and 90 females, were from apparently healthy blood bank donors in Barcelona, Spain were tested in duplicate using HemosIL Homocysteine on an ACL Futura, ACL 9000, and ACL TOP. One (4) sample was removed as a far outlier (criteria: 3 inter-quartile ranges above the mean) and was not used in the final calculations, bringing the final total to n=136. The distribution was normal (parametric) and therefore, parametric statistics were applied. The 95% reference interval limit with a confidence interval of 90% for each instrument platform is listed as below:

ACL Futura/ACL Advance:	4.1-10.6 µmol/L
ACL ELITE/ELITE PRO/8/9/10000:	4.0-11.2 µmol/L
ACL TOP:	4.3-11.1 µmol/L

   ACL Advance is in the same family as the ACL Futura. ACL ELITE, ELITE PRO, ACL 8000 and ACL 10000 are in the same family as the ACL 9000.

One hundred twenty-six (126) citrated plasma samples from apparently healthy blood bank donors in U.S. were tested at Instrumentation Laboratory Co. using HemosIL Homocysteine on an ACL TOP. One (1) sample was removed as a far outlier (criteria: 3 inter-quartile ranges above the mean) and was not used in the final calculations, bringing the final total to n=125. The distribution was normal (parametric) as shown in the frequency distribution plots below.

The table below shows the 95% reference interval limit with a confidence interval of 90%:

	95% Limit	90% CI
Lower	3.48	3.10 – 3.85
Upper	9.31	8.94 – 9.69

Therefore, the normal range for citrated plasma for the U.S. population is 3.5-9.3 µmol/L.

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.