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

A. 510(k) Number:

k112790

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative, enzymatic assay

E. Applicant:

Axis-Shield Diagnostics Ltd.

F. Proprietary and Established Names:

Axis-Shield 3-Reagent Homocysteine Assay for Synchron

G. Regulatory Information:

1. Regulation section:

   21 CFR § 862.1377, Urinary Homocysteine (Nonquantitative) Test System

2. Classification:

   Class II

3. Product code:

   LPS

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indication(s) for use below

2. Indication(s) for use:

   The 3-Reagent Homocysteine Assay for Beckman Coulter SYNCHRON® and UniCel® systems is intended for in vitro quantitative determination of total homocysteine in human serum and plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

3. Special conditions for use statement(s):

   For prescription use only. The following black box warning is included in the labeling:

   WARNING: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridine triacetate may have elevated levels of homocysteine due to their effect on the pathway. Refer to the LIMITATIONS FOR USE section in this assay package insert.

4. Special instrument requirements:

   For use on the Beckman Coulter Synchron LX 20 Pro and Unicel DxC 600

I. Device Description:

Each reagent kit is made up of one cartridge containing the three enzymatic homocysteine reagents and two calibrators:

- Reagent 1:

  250 mM Tris Buffer with L-Serine, Magnesium Chloride 6-Hydrate, β-NADH II, Brij 35, Lipase, L-Glutamic Acid, Alpha Cyclodextrin, Alcohol Dehydrogenase, Absolute Ethanol, and Sodium Azide

- Reagent 2:

  25mM Tris (2-carboxyethyl) Phosphine Hydrochloride (TCEP) with α-ketoglutaric acid

- Reagent 3:

  100mM Potassium Phosphate Enzyme Buffer (containing sodium azide) with CBS and CBL cycling enzymes.

- Calibrator 0 and Calibrator 28:

  100 µM L-Cystine with Homocysteine (in form of L-Homocysteine, the Homocysteine dimmer) spiked to 0 µM (Cal 0) and '28' µM (Cal '28') in an aqueous matrix.

The calibrators (included in the kit) are prepared gravimetrically and are traceable to NIST SRM 1955, confirmed by a designated measurement procedure (HPLC).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Axis-Shield Liquid Stable (LS) 2-part Homocysteine Reagent

2. Predicate 510(k) number(s):
   k083222

3. Comparison with predicate:

Similarities		
Item	Axis-Shield 3-Reagent Homocysteine Assay for Synchron	Axis-Shield Liquid Stable (LS) 2-part Homocysteine Reagent
Intended Use	In vitro quantitative determination of total homocysteine in human serum and plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.	Same
Technology	Enzymatic Assay	Same
Active Assay Composition	Lactate dehydrogenase Cystathionine beta-Synthase Cystathionine beta-Lyase TCEP (tris(2-	Same

Similarities		
Item	Axis-Shield 3-Reagent Homocysteine Assay for Synchron	Axis-Shield Liquid Stable (LS) 2-part Homocysteine Reagent
	carboxyethyl)phosphine)	
Detection Method	The rate of NADH conversion to NAD+ (D A340 nm) is directly proportional to the concentration of homocysteine	Same
Storage Conditions	Reagents and calibrators must be stored at 2 – 8 C.	Same
On-board reagent stability	30 days	Same
Calibrator traceability	Traceable to NIST SRM 1955 Standard Reference Material	Same
Units of measurement	µmol/L	Same
Calibration	Quantitative assay using 2 gravimetrically prepared L-homocysteine calibrators, assigned 0 and 28 umol/L	Same
Limit of Detection	0.89 umol/L	0.33 umol/L
Sample carryover	Less than LoD	Same
Specimen type	EDTA plasma, lithium heparin plasma, serum and serum separator tubes	Same

Differences		
Item	Device	Predicate
Assay format	3 reagent enzymatic assay	2 reagent enzymatic assay
Reagent #1 composition	Pre-treatment buffer	Reductant reagent: lactate dehydrogenase (38 KU/L) TCEP (830 mg/L)
Reagent #2 composition	Reductant reagent: TCEP (3.0 g/L)	Enzyme reagent: Cystathionine beta-synthase (0.748 KU/L) Cystathionine beta-Lyase (16.4 KU/L)
Reagent #3 composition	Enzyme reagent: Cystathionine beta-synthase (0.748 KU/L) Cystathionine beta-Lyase	Not applicable

Differences		
Item	Device	Predicate
	(16.4 KU/L)	
Calibration frequency	14 days	30 days
Instrument	BC Synchron LX 20 Pro BC Unicel DxC 600	Olympus AU400
Assay range	1-50 µmol/L	1-46 µmol/L

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

1. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition, EP5-A2
2. Evaluation of the Linearity of Quantitative Measurement Procedures; Approved Guideline, EP6-A
3. Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition, EP7-A2
4. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition, EP09-A2
5. Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, EP17-A
6. Format for Traditional and Abbreviated 510(k)s – Guidance for Industry and FDA Staff

L. Test Principle:

Bound or dimerized homocysteine (HCT) (oxidized form) is reduced to free HCT, which then reacts with serine catalyzed by cystathionine beta-synthase (CBS) to form cystathionine. Cystathionine is then broken down by cystathionine beta-lyase (CBL) to form HCT, pyruvate, and ammonia. Pyruvate is then converted by lactate dehydrogenase (LDH) to lactate with nicotinamide adenine dinucleotide (NADH) as a coenzyme. The rate of NADH conversion to NAD+ is directly proportional to the concentration of HCT (Δ A340 nm).

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. *Precision/Reproducibility:*

   Precision studies on the Synchron LX 20 Pro and the UniCel DxC600 were performed following CLSI EP5-A2. For each instrument, three HCT controls and three human plasma samples were assayed using two lots of reagents, in replicates of two, in two runs per day for 20 days on one instrument (n = 80). Results are summarized below:

Synchron LX 20 Pro

Sample	Reagent Lot	Mean (µmol/L)	Within-Run		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV
Low Control	1	5.73	0.30	5.2	0.00	0.0	0.31	5.3
	2	5.61	0.23	4.2	0.10	1.9	0.30	5.4
Medium Control	1	11.01	0.28	2.5	0.09	0.9	0.32	2.9
	2	10.97	0.29	2.6	0.08	0.7	0.33	3.0
High Control	1	23.18	0.33	1.4	0.17	0.7	0.46	2.0
	2	23.08	0.32	1.4	0.30	1.3	0.47	2.0
Sample P1	1	6.99	0.34	4.9	0.10	1.4	0.37	5.4
	2	6.80	0.31	4.6	0.10	1.5	0.42	6.2
Sample P2	1	32.10	0.42	1.3	0.30	0.9	0.64	2.0
	2	32.03	0.40	1.2	0.42	1.3	0.75	2.3
Sample P3	1	43.93	0.58	1.3	0.22	0.5	0.79	1.8
	2	44.05	0.52	1.2	0.45	1.0	0.88	2.0

Unicel DxC 600

Sample	Reagent Lot	Mean (µmol/L)	Within-Run		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV
Low Control	1	6.15	0.36	5.9	0.00	0.0	0.49	8.0
	2	6.37	0.30	4.7	0.00	0.0	0.40	6.3
Medium Control	1	11.65	0.49	4.2	0.36	3.1	0.63	5.4
	2	11.90	0.33	2.8	0.21	1.8	0.62	5.2
High Control	1	24.13	0.64	2.6	0.43	1.8	1.09	4.5
	2	24.37	0.63	2.6	0.59	2.4	1.13	4.6
Sample P1	1	7.43	0.47	6.3	0.13	1.7	0.53	7.2
	2	7.63	0.27	3.5	0.11	1.4	0.48	6.3
Sample P2	1	33.20	0.85	2.6	0.62	1.9	1.42	4.3
	2	33.58	0.79	2.4	0.65	1.9	1.83	5.4
Sample P3	1	45.38	1.08	2.4	1.27	2.8	1.92	4.2
	2	45.61	0.96	2.1	0.62	1.4	2.44	5.4

b. Linearity/assay reportable range:

A linearity study was performed to validate the claimed measuring range of 1-50 µmol/L. A high level sample was serially diluted with zero level calibrator, to yield a concentration range from 0.35 – 68.82 µmol/L A total of 11 samples for each instrument, (Synchron LX 20 Pro and Unicel DxC600) were analyzed for linearity with the following regression results:

Parameter	Synchron LX 20 Pro	Unicel DxC 600
Slope of Regression Line	1.0108	1.0076
Y-intercept	-0.2605	-0.0741
Correlation Coefficient	0.9997	0.9990

Validation for out-of-range specimens

Three EDTA samples with approximate HCT levels of 90, 150, and 500 µmol/L were used for manual dilution recovery of out-of-range samples. All three levels were first diluted using a 1:9 dilution factor and measured on the reference instrument (Olympus AU400) to establish a baseline. The neat samples were then measured on the Synchron LX 20 Pro and Unicel DxC 600 in replicates of two and the mean was calculated to demonstrate the need for dilution at very high HCT levels. The neat samples were then diluted with a factor of 1:2 (for the 90 and 150 µmol/L levels) and a factor of 1:9 (for the 500 µmol/L level). The % recoveries for the samples when corrected by the dilution factors were within 10 % of the reference instrument values. The sponsor included the following in the package insert with regard to high levels of HCT: *The linear range of the 3-Reagent Homocysteine Assay for Synchron LX 20 Pro and UniCel DxC 600 when run as directed is 1-50 µmol/L. Specimens > 50 µmol/L should be diluted 1 part specimen to 2 parts Cal 0 µmol/L or 1 part specimen to 9 parts Cal 0 µmol/L as appropriate.*

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

The calibrators were cleared under k083222 and are included in the kit.

d. *Detection limit:*

The determination of LoB and LoD followed guidance from CLSI document EP17-A. A zero calibrator was used to determine LoB. For the LoD estimation, five EDTA plasma samples were prepared with a concentration between LoB and 4x LoB. Samples were diluted in the zero calibrator to obtain the required concentrations and confirmed by testing on the Olympus AU400.

Limit of Blank

For the Synchron LX 20 Pro and Unicel DxC 600, 20 replicates of the zero calibrator were run using each reagent lot on each of 3 days (n = 60 for each reagent lot), i.e. 1 run per day for each lot of reagents and calibrators. The results are summarized in the table below.

Limit of Detection

For the Synchron LX 20 Pro and Unicel DxC 600, five replicates of each of the five low level homocysteine samples were run with each reagent lot on each of three separate days (n = 75 for each reagent lot) i.e. 1 run per day for each lot of reagents and calibrators. The results are summarized in the table below.

Instrument	Reagent Lot	LoB (µmol/L)	LoD (µmol/L)
Synchron LX 20 Pro	1	0.47	0.82
	2	0.46	0.89
Unicel DxC 600	1	0.2	0.51
	2	0.2	0.54

The linearity studies and detection limit studies support the sponsor's claimed measuring range of 1.0 to 50 µmol/L.

e. Analytical specificity:

The studies for interfering substances were carried out according to the CLSI document EP7-A2. Control samples were in the medically important range of 12 -18 µmol/L. Each control sample was tested in replicates of five to determine a homocysteine baseline. Each control sample was then spiked with the relevant interfering compound up to the concentrations indicated in the table below. All samples were tested in replicates of five. No significant interference was defined as a difference of less than ±10% of the control value.

The summary data is below:

Substance	Maximum Concentrations Tested	Highest concentration with no significant interference :
Bilirubin	20 mg/dL	20 mg/dL
Hemoglobin	554 mg/dL	500 mg/dL
Triglyceride	1500 mg/dL	1000 mg/dL
Glutathione	3000 µmol/L	1000 µmol/L
Methionine	800 µmol/L	800 µmol/L
Cysteine	400 µmol/L	200 µmol/L
Pyruvate	1704 µmol/L	1250 µmol/L
Total Protein	126 mg/mL	120 mg/mL

Other limitations:

- Cystathionine is measured with homocysteine, but in the general population the cystathionine level (0.065 to 0.3 µmol/L) has a negligible effect. In very rare cases, end stage renal disease and patients with severe metabolic disturbances, cystathionine levels may rise dramatically and in severe cases cause greater than 20% interference.
- Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6 azauridine triacetate may affect the homocysteine concentration
- Specimens from patients on drug therapy involving S-adenosyl-

methionine may show falsely elevated levels of homocysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anti-convulsants, or 6 azauridine triacetate may have elevated levels of homocysteine due to their effect on the pathway.

- Specimens containing particulate matter (fibrin, red blood cells, or other matter) and visibly lipemic specimens should not be used with the assay. Results from these specimens may be inaccurate.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Fifty EDTA plasma samples were tested in the method comparison study. Of the 50 samples, 10 were spiked with L-homocysteine to obtain samples across the assay range. The samples were assayed in singlicate. The correlation of the candidate device used on the Synchron LX 20 Pro and Unicel DxC 600 to the predicate device on the Olympus AU400 was determined using Passing-Bablok method. The correlation coefficient was determined using Pearson Correlation. The summary of results is below:

Parameter	Synchron LX 20 Pro	Unicel DxC 600
Measuring Range	5.95 – 46.25 µmol/L	6.72 – 46.09 µmol/L
Slope of Regression Line	1.01 (95% CI: 0.99 – 1.04)	0.99 (95% CI: 0.97 – 1.02)
Y-intercept	0.07 (95% CI: -0.30 – 0.44)	0.74 (95% CI: 0.30 – 1.02)
Correlation Coefficient	0.997 (95% CI: 0.99 – 1.00)	0.994 (95% CI:0.99 – 1.00)

b. Matrix comparison:

Each of EDTA plasma, heparin plasma, serum, and serum separator tube types were used to collect samples from 23 in-house volunteers. Samples were divided into three concentration ranges: 1-10 µmol/L, 10 – 20 µmol/L, and 20 – 50 µmol/L. Samples in the range 20 – 50 µmol/L were spiked. Each sample from all four tube types was tested in duplicate on the Unicel DxC 600 with one lot of the reagent and calibrators. The first set of individual concentrations of the replicates of each sample was used for analysis, along with the percent recovery. The first set of the individual replicate values for each sample was calculated to be ±10% of the EDTA Sample control tube type. Each of the four collection tube types is considered suitable for use.

Below is the summary of statistics for the study performed on the Unicel DxC 600:

	EDTA vs Serum	95% CI	EDTA vs. SST	95% CI	EDTA vs. LiHep	95% CI
Slope	1.03	0.99–1.07	1.01	0.98–1.06	1.02	0.99-1.04
y-intercept	-0.18	-0.63–0.35	-0.18	-0.56-0.09	-0.23	-0.46-0.13
R value	0.99	N/A	0.99	N/A	0.99	N/A

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):

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   Expected values are based on literature references cited in the package insert. Expected values in adult males and females range between 5 and 15 µmol/L, men having higher values than women, and post-menopausal women having higher values than pre-menopausal women. Homocysteine values will normally increase with age, giving a reference range among an elderly population (>60 years) of 5 – 20 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.