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

A. 510(k) Number:
k083222

B. Purpose for Submission:
New Device

C. Measurand:
Homocysteine (HCT)

D. Type of Test:
Quantitative, enzymatic assay

E. Applicant:
Axis-Shield Diagnostics Ltd.

F. Proprietary and Established Names:
Axis-Shield Liquid Stable (LS) 2-Part HOMOCYSTEINE REAGENT
Axis-Shield Homocysteine Calibrators

G. Regulatory Information:

1. Regulation section:
   21 CFR § 862.1377, Urinary Homocysteine (Nonquantitative) Test System.
   21 CFR § 862.1150, Calibrator
2. Classification:
   Class II for assay reagents and calibrator
3. Product code:
   LPS, Urinary Homocysteine (Nonquantitative) Test System
   JIT, Calibrator, Secondary
4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See Indication(s) for use below.

2. Indication(s) for use:
   The Axis-Shield Liquid Stable (LS) 2-Part Homocysteine Reagent 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 *In Vitro* Diagnostic Use; For Prescription Use Only; The labeling contains

the following black-box warning:
Note: 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.

4. Special instrument requirements:
   For use on the Olympus AU400 System

I. Device Description:

The Liquid Stable (LS) 2-Part Homocysteine Reagent Test System contains one vial of each component (Reag1, Reag2, Cal 0 and Cal 28) in ready-to-use configuration.

- Reag 1: One amber vial (30 mL) which includes Lactate dehydrogenase (LDH) (38 KU/L), Serine (0.76 mM), nicotinamide adenine dinucleotide reduced di-sodium salt (NADH) (0.47 mM), tris [2-carboxyethyl] phosphine (TCEP) reductant (2.9 mM), with buffers and stabilizers (Trizma Base 1-10 %; Trizma Hydrochloride 1-10 %). Preservative: Sodium Azide (<1 %).
- Reag 2: One amber vial (5.0 mL) which includes Cystathionine beta-Synthase (CBS) (0.748 KU/L) and Cystathionine beta-Lyase (CBL) (16.4 KU/L) cycling enzymes. Preservative: Sodium Azide (<1 %).
- Cal 0: One Opaque Vial (3.0 mL) of aqueous HCT blank. Concentration: 0 µmol/L.
- Cal 28: One Opaque Vial (3.0 mL) of aqueous HCT solution. Concentration: 28 µmol/L.

The calibrators 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):
   CATCH Incorporated, Liquid Stable (LS) 2-Part Homocysteine Reagent

2. Predicate 510(k) number(s):
   k062808
3. Comparison with predicate:

Item	Device	Predicate (k062808)
Similarities		
Intended use	In vitro quantitative determination of total HCT in human serum and plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.	In vitro quantitative determination of total HCT in serum and plasma.
Assay technology	2 reagent enzymatic assay	same
Enzyme conversion	Cystathionine beta-synthase and cystathionine beta-lyase	same

Reductant	TCEP (tris (2-carboxyethyl) phosphine)	same
Detection method	The rate of NADH conversion to NAD+ is directly proportional to the concentration of HCT (Δ A340 nm).	same
Storage conditions	Reagents and calibrators must be stored at 2 – 8 °C	same
On-board reagent stability	30 days on the Olympus AU400	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-HCT (dimerized HCT) calibrators	same
Interference	No interference (≤ ± 10%) from bilirubin 20 mg/dL, cysteine 200 µmol/L, methionine 800 µmol/L	same
Differences		
Formulation of reagent 2	0.748 KU/L cystathionine beta–synthase	> 20 KU/L cystathionine beta–synthase
Calibration frequency	30 days	14 days
Specimen type	EDTA plasma, lithium heparin plasma, serum, serum separator tubes	EDTA plasma, lithium heparin plasma, serum separator tubes
Limit of detection	0.33 µmol/L	≤ 1 µmol/L
Assay range	1 to 46 µmol/L	1 to 50 µmol/L
Interference	No interference (≤ ± 10%) from hemoglobin (500 mg/dL) triglyceride (500 mg/dL) red blood cells (0.4 %) glutathione (1000 µmol/L) pyruvate (1250 µmol/L)	No interference (< 10%) from hemoglobin (400 mg/dL) triglyceride (1000 mg/dL) red blood cells not reported No interference (< 7%) from glutathione (20 µmol/L) pyruvate (500 µmol/L)
Imprecision	Within run % CV: 1.0% to 2.6%, total % CV: 2.0% to 4.4% for samples from 6.3 µmol/L to 48.3 µmol/L HCT	Within run % CV: 1.3% to 2.3%, total % CV: 2.4% to 4.3% for samples from 7.11 µmol/L to 27.64 µmol/L HCT

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

- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline (EP5-A2)
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP6-A)
- Interference Testing in Clinical Chemistry; Approved Guideline (EP7-A2),
- Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2)
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (EP17-A)
- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff.

L. Test Principle:

Bound or dimerized HCT (oxidised form) is reduced to free HCT, which then reacts with serine catalyzed by cystathionine beta-synthase (CBS) to form cystathionine. Cystathionine in turn is 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 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. Analytical performance:

a. Precision/Reproducibility:

Precision was determined following the Clinical and Laboratory Standards Institute (CLSI) document EP5-A2. Axis-Shield HCT controls (low, medium and high) and 3 quality control panel members were assayed (in duplicate), using 2 lots of reagents and calibrators, at 2 separate times per day for 20 days on one instrument (therefore n=80 for each control and QC panel member). For each run, freshly dispensed controls and QC panel members were used and the order sequence for each run was randomized. A calibration curve was generated for each reagent lot on the first day of testing. The results are summarized below:

Sample	Lot	Mean (µmol/L)	Within run		Between run		Between day		Total	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low	1	6.3	0.17	2.6	0.11	1.7	0.20	3.1	0.28	4.4
	2	6.3	0.13	2.1	0.11	1.7	0.19	3.1	0.26	4.1
Medium	1	12.3	0.18	1.5	0.15	1.2	0.29	2.4	0.37	3.0
	2	12.2	0.16	1.3	0.16	1.3	0.31	2.5	0.39	3.2
High	1	25.5	0.38	1.5	0.35	1.4	0.39	1.5	0.65	2.5
	2	25.3	0.41	1.6	0.00	0.0	0.60	2.4	0.73	2.9
Panel 1	1	7.0	0.13	1.9	0.00	0.0	0.19	2.7	0.23	3.3
	2	7.0	0.15	2.2	0.00	0.0	0.26	3.8	0.31	4.4
Panel 2	1	36.0	0.46	1.3	0.40	1.1	0.66	1.8	0.89	2.5
	2	35.5	0.40	1.1	0.23	0.7	0.67	1.9	0.82	2.3
Panel 3	1	48.3	0.53	1.1	0.42	0.9	0.69	1.4	0.97	2.0
	2	47.7	0.47	1.0	0.38	0.8	0.88	1.8	1.07	2.2

b. Linearity/assay reportable range:

Six EDTA plasma sample pools were obtained from healthy volunteers. Sample pools 1 and 5 were spiked with L-HCT (dimerized HCT) to give HCT values within the upper range of the assay (46.43 and 43.79 µmol/L respectively). Sample pools 2 (8.80 µmol/L), 3 (10.43 µmol/L), 4 (13.75 µmol/L) and 6 (17.78 µmol/L) were unaltered samples. All 6 sample pools were diluted with Cal 0 (0 µmol/L) according to the following scheme:

Dilution	Expected value
1	0

2	10% of neat sample value
3	20% of neat sample value
4	30% of neat sample value
5	40% of neat sample value
6	50% of neat sample value
7	60% of neat sample value
8	70% of neat sample value
9	80% of neat sample value
10	90% of neat sample value
11	100%

Sample pools were tested in replicates of 2 with one lot of reagents, calibrators and Axis-Shield HCT controls (low, medium and high) on the Olympus AU400. Testing was performed over 2 days. The % recovery for all samples across the range of the assay ranged from 91 -104%.

To determine dilution linearity, an analysis was carried out in accordance with CLSI document EP6-A. 1st, 2nd and 3rd order polynomial least squares regressions analysis was performed for all 6 sample pools. Using this analysis, sample pools 2, 3, 4, 5 and 6 were found to be linear.

For sample pool 1, the p-value for the non-linear co-efficient for the 2nd-order (b2) polynomial was significant (i.e. $p < 0.05$). The degree of non linearity was assessed by calculating the difference and % difference between the linear and non-linear model at each concentration. These values were within the acceptance criterion pre-determined by the sponsor. Therefore, the data for sample pool 1 was considered linear.

The reportable or measurable range of the proposed assay is 1.0 to 46 µmol/L.

Recovery of samples above the upper limit of the assay:
Five EDTA plasma samples with HCT levels of approximately 70, 100, 150, 300 and 500 µmol/L were prepared by spiking with L-HCT. Neat samples were assayed in duplicate using the proposed device and the mean values were calculated. Samples containing up to 500 µmol/L HCT gave results above the upper limit of the assay (50 µmol/L). The samples were also manually diluted and assayed in duplicate on the proposed assay. The mean µmol/L value of each sample was calculated and this value was corrected by the dilution factor and compared to results obtained using a reference method. The results are summarized below:

µmol/L verified by reference method	**µmol/L when assayed neat in proposed assay**	**Sample dilution**	**µmol/L when assayed diluted in proposed assay**	**µmol/L when corrected for dilution**	**% Recovery**
75.45	72.6	1:3	24.35	73.05	97
101.94	102.7	1:3	33.30	99.90	98

155.70	154.1	1:10	15.40	154.00	99
295.00	217.7	1:9	35.40	318.60	108
476.50	103.2	1:10	50.00	500.00	105

The sponsor included the following statement under Limitations of Use:
The linear range of the Liquid Stable (LS) 2-Part Homocysteine Reagent when run as directed is 1-46 µmol/L. Specimens > 46 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):*
Controls: Controls were cleared under k980907 and are sold separately.

Calibrators
Traceability and value assignment: The calibrators are prepared gravimetrically and are traceable to NIST SRM 1955. Twenty replicates of test calibrators and 20 replicates of Reference calibrators were tested using one lot of the proposed device for value assignment. The mean must meet specifications. The assigned values were confirmed by a designated measurement procedure (HPLC) and must meet release specifications.

Stability: The stability characteristics of the calibrators were determined using real-time studies. The unopened shelf-life was determined to be 24 months and the open vial stability was 15 months at the recommended storage of 2 to 8 °C. The claimed stability of the assay for both unopened and opened shelf life when stored at 2 to 8 ºC is 12 months.

Calibration Frequency: The calibration curve is valid for 30 days on the Olympus AU400.

d. *Detection limit:*
The Limit of Blank (LoB) and Limit of Detection (LoD) studies were conducted according to the recommendations of the CLSI document EP17-A.

LoB
Sixty replicates of Calibrator 0 (0 µmol/L) were run for each lot of reagents and calibrators. Fresh calibrators and controls were used in each run and all runs were valid. The mean and the standard deviation (SD) were calculated for the 60 replicates for both lots. The data sets were normally distributed and the LoB was calculated as follows: LoB = Mean Cal A result + 1.645 x SD
The LoB for lot 1 was 0.20 µmol/L and 0.18 µmol/L for lot 2.

LoD
At least 60 replicates of 5 low-level HCT EDTA plasma samples (for lot 1) and 6 low-level HCT EDTA plasma samples (for lot 2) were assayed. Fresh controls were used for each run and all runs were valid. The samples for both lots did not follow a Gaussian distribution and could not be transformed to

achieve a Gaussian distribution. Therefore, per EP17-A, the following non-parametric LoD calculation was used: LoD = LoB + Diff, where Diff = Mean target concentration - 5th percentile concentration of the replicates from the sample between the LoB and 4*LoB, and Target concentration is estimated as the median of the replicates from the sample between the LoB and 4*LoB. The LoD for lot 1 was 0.33 µmol/L. The LoD for lot 2 was 0.32 µmol/L.

The sponsor states in the package insert that the LoD is 0.33 µmol/L. The claimed measuring range for this assay is 1-46 µmol/L.

e. Analytical specificity:

Each of the interference studies were performed using 4 EDTA plasma samples ranging from 5.9 to 47.2 µmol/L HCT. Some of these samples were spiked with L-HCT to give samples at the higher end of the measuring range. The HCT samples containing the potential interferent (test samples) and the HCT samples not containing the interferent (control samples) were tested in replicates of 3 using the proposed assay. The following calculation was then applied to the results:

$$\% \text{ Interference (diff)} = \frac{\text{Mean Conc (test)-Mean conc (control)}}{\text{Mean Conc (control)}} \times 100$$

No significant interference was defined as % difference (interference) less than ± 10%. The results of the studies are summarized below:

Substance	Concentrations tested	No significant interference was observed at:
Bilirubin	20 mg/dL	20 mg/dL
Hemoglobin	500 & 1000 mg/dL	500 mg/dL
Red Blood Cell	5% & 0.4%	0.4 %
Triglyceride	500 & 1000 mg/dL	500 mg/dL
Glutathione	1000 µmol/L	1000 µmol/L
Methionine	800 µmol/L	800 µmol/L
Cysteine	200 & 500 µmol/L	200 µmol/L
Pyruvate	375 & 1250 µmol/L	1250 µmol/L

Other Limitations: The sponsor included the following limitations in the package insert:

- 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.
- Hydroxylamine, present in several iron reagents may carryover (reagent probe or reaction cuvette) and cause falsely low results. Routine rinsing procedures are not adequate to eliminate this problem in most cases. Possible solutions would include special washing protocols, changing to

an iron assay that used ascorbic acid as reductant or running iron and homocysteine assays on separate instruments.

- Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.
- Samples with raised protein levels show >10% difference compared to results obtained from normal samples and should be avoided.

The labeling contains the following black-box warning. The sponsor also included the statement under "Limitations of use":

- Note: 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.

f. Assay cut-off:
No cut-off established/required.

2. Comparison studies:

a. Method comparison with predicate device:
One hundred EDTA plasma samples from apparently healthy donors were collected. Twenty of the samples were spiked with L-HCT to obtain samples across the assay range (6.5 to 49.0 µmol/L for the proposed device and 6.3 to 49.3 µmol/L for the predicate assay). The samples were assayed in duplicate using the predicate assay and in one replicate using the proposed device on the Olympus AU400. The mean result of the predicate and the single replicate of the proposed device were used in all subsequent analysis. Passing-Bablok linear regression method comparison was performed on 94 EDTA plasma samples. Pearson correlation was used to determine the correlation coefficient. The results are summarized below:

Slope of regression line	0.991 (95% confidence interval 0.980 to 1.001)
Y-intercept	0.165 (95% confidence interval 0.031 to 0.290)
Correlation coefficient	1.00 (95% confidence interval 1.00 to 1.00)
Average percent bias	0.01% (95% confidence interval -0.10 to 0.07%).

Six samples of the 100 samples tested were identified as outliers following the outlier testing recommended in the CLSI guideline and were removed from the final analysis. The outliers contained particulate matter and were lipemic.

The sponsor included the following statement in the package insert:

- 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.

b. Matrix comparison:
One collection tube of each of the following types was collected from 30 volunteers: EDTA plasma, lithium heparin plasma, serum and serum separator tube (SST). Six samples were then spiked with L-HCT to obtain HCT concentrations which covered the measurable range of the proposed assay. Each sample was tested in replicates of 3 for each of the selected tube types on the Olympus AU400. The mean concentration of each sample was calculated for all collection tubes. The percent recovery for each sample was calculated by comparing the mean value of the EDTA tube type (control tube type) to the mean value for the corresponding serum, SST, and lithium heparin tube types for the same sample. The mean % recoveries across all samples for each of the collection tubes ranged from 94 - 108 %. Therefore, each of the selected collection tube types is considered suitable for use with the assay.

The sponsor included the following statement in the package insert:

- Serum (collected in serum or serum separator tubes) and plasma (collected in potassium EDTA or lithium heparin tubes) may be used for the measurement of homocysteine. However, it is not recommended to use individual patient results from serum, heparinized plasma and EDTA plasma interchangeably. Additionally matrix differences between serum and serum separator tubes and plasma tubes have been reported.

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:
   Reference ranges are based on literature references provided in the package insert. Total HCT values in plasma/serum from 5 to 15 μmol/L are considered normal. However, total HCT values in plasma/serum differ with age, gender, stress and other physical conditions. Total HCT values in plasma/serum > 15 μmol/L are considered abnormal.

   The sponsor included the following statement in the package insert:

   - The reference range should be determined by each laboratory to confirm the characteristics of the population being tested.

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.