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

A. 510(k) Number:

k073640

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Axis-Shield Diagnostics, Ltd.

F. Proprietary and Established Names:

ARCHITECT Homocysteine Reagents
ARCHITECT Homocysteine Calibrators
ARCHITECT Homocysteine Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.1377, Urinary homocystine (non-quantitative test system)
   21 CFR §862.1150, Calibrator
   21 CFR §862.1660, Quality control material (assayed and unassayed)

2. Classification:
   Class II for assay reagents and calibrator
   Class I, reserved for control

3. Product code:
   LPS, JIT, JJX

4. Panel:
   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   Reagents:

   The ARCHITECT Homocysteine assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of total L-homocysteine in human serum or plasma on the ARCHITECT *i* System. Homocysteine values can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

   Calibrators:

   The ARCHITECT Homocysteine Calibrators are for the calibration of the ARCHITECT *i* System when used for the quantitative determination of total l-homocysteine in human serum or plasma.

   Controls:

   The ARCHITECT Homocysteine Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT *i* System (reagents, calibrators and instrument), when used for the quantitative determination of total l-homocysteine in human serum or plasma.

3. Special conditions for use statement(s):
   For prescription use

   The labeling contains a prominent black-box warning:

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

4. Special instrument requirements:

   For use on the ARCHITECT *i* System

I. Device Description:

Each Reagent Kit contains enough reagents for 100 tests and consists of 1 bottle of each of the following components:

MICROPARTICLES: 1 Bottle (6.5 mL) Anti-S-adenosyl-L-homocysteine (mouse, monoclonal) coated microparticles in Bis-Tris buffer with surfactants. Minimum Concentration: 0.1% solids. Preservatives: sodium azide and other antimicrobial agents.

CONJUGATE: 1 Bottle (5.7 mL) S-adenosyl-L-cysteine (SAC) acridinium-labeled conjugate in citrate buffer with surfactants. Minimum Concentration: 1 ng/mL. Preservative: ProClin 300.

ENZYME: 1 Bottle (8.6 mL) Recombinant S-adenosyl-L-homocysteine hydrolase (SAHHase) in 4-(2-hydroxyethyl) piperazine-1-propane sulfonic acid (EPPS) buffer. Preservative: sodium azide.

REDUCTANT: 1 Bottle (21.5 mL) Dithiothreitol (DTT) in citrate buffer.

The calibrator and control kits are sold separately. Each Calibrator kit consists of 6 Bottles (3.6 mL each) of ARCHITECT Homocysteine Calibrators consisting of phosphate buffer and S-adenosyl-L-homocysteine. The target values are 0, 2.5, 5, 10, 20 and 50 µmol/L. Each Control kit consists of one bottle (7.7mL each) of each of Low, Medium and High ARCHITECT Homocysteine Controls containing L-homocystine in processed human serum and phosphate buffer.

The human source material was tested using FDA approved methods and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2 and anti-HCV antibodies.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   AxSYM Homocysteine Assay

2. Predicate K number(s):

   k992858

3. Comparison with predicate:

Similarities

Parameter	**Submission device ARCHITECT Homocysteine**	**Predicate device AxSYM Homocysteine**
Intended use	Quantitative measurement of total L-homocysteine in human serum or plasma. Homocysteine values can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.	Same
Specimen type	EDTA plasma, Lithium Heparin plasma, Serum, Serum Separator Tubes	Same
Capture antibody	Anti-S-adenosyl Homocysteine mouse monoclonal	Same
Enzyme conversion	Recombinant S-adenosyl Homocysteine hydrolase	Same
Storage conditions	Reagent Pack, Calibrator Pack and Control Pack must be stored at 2-8^{o}C	Same
Calibration	Quantitative assay using six gravimetrically prepared S-adenosyl Homocysteine calibrators at 0, 2.5, 5, 10, 20 and 50 µmol/L	Same
Assay range with dilution	up to 500 µmol/L	Same
Interference	No interference (< 10%) from bilirubin, triglycerides and protein <10% interference from hemoglobin at 1000mg/dL.	Same
Specificity	No % cross-reactivity (< 10%) from L-Cysteine, L-Cystathionine, Adenosine , Glutathione and DL-homocysteine thiolactone	Same

Differences

Parameter	**Submission device ARCHITECT Homocysteine**	**Predicate device AxSYM Homocysteine**
Assay Technology	CMIA Chemiluminescent Microparticle Immunoassay	FPIA Fluorescence Polarization Immunoassay
Substrate / Signal Generation	Acridinium tracer	Fluorescein tracer
Imprecision	Within run %CV from 1.2% to 4.0%, total %CV from 2.1% to 6.3% for samples from 4.71µmol/L to 41.84µmol/L homocysteine	Within run %CV from 1.4% to 4.5%, total %CV from 2.0% to 5.1% for samples from 7.29µmol/L to 28.17µmol/L homocysteine
Assay range	1.00 to 50.00 µmol/L	0.8 to 50.00 µmol/L
Limit of detection	≤1.0 µmol/L	≤0.8 µmol/L
Specificity	% cross-reactivity: S-adenosyl-L-methionine 11.78% at 0.5mM	% cross-reactivity: S-adenosyl-L-methionine 1.28% at 0.5mM

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

-Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A).

-Interference Testing in Clinical Chemistry; Approved Guideline, Second Edition (CLSI EP7-A2)

L. Test Principle:

The ARCHITECT Homocysteine assay is a one-step immunoassay for the quantitative determination of total L-homocysteine in human serum or plasma using CMIA (Chemiluminescent microparticle immunoassay) technology. Bound or dimerised homocysteine (oxidized form) is reduced by dithiothreitol (DTT) to free homocysteine, which is then converted to S-adenosyl homocysteine (SAH) by the action of the recombinant enzyme S-adenosyl homocysteine hydrolase (rSAHHase) in the presence of excess adenosine. The SAH then competes with acridinium-labeled S-adenosyl cysteine for particle-bound monoclonal antibody. Following a wash stage and magnetic separation, pre-trigger and trigger solutions are added to the reaction mixture and the resulting chemiluminescence is measured as relative light units

(RLUs). An indirect relationship exists between the amount of homocysteine in the sample and the RLUs detected by the ARCHITECT *i* System optics.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The sponsor states that precision was evaluated following the Clinical and Laboratory Standards Institute (CLSI) Protocol EP5-A2.

Three ARCHITECT Homocysteine Controls Low, Medium, and High as well as five human EDTA plasmas with homocysteine concentrations ranging from 4.71 to 41.84 µmol/L were tested in duplicate, two runs per day, using two lots of reagents on two analyzers for 20 days (n=80).

The results are summarized in the table below:

Sample	Instrument	Reagent Lot	Mean (µmol/L)	Within Run		Between Run		Between Day		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	1	1	7.40	0.26	3.5	0.27	3.7	0.22	3.0	0.43	5.9
	2	2	7.58	0.18	2.4	0.18	2.3	0.00	0.0	0.25	3.3
Medium Control	1	1	13.21	0.26	2.0	0.37	2.8	0.45	3.4	0.64	4.8
	2	2	13.37	0.24	1.8	0.25	1.9	0.21	1.6	0.41	3.0
High Control	1	1	26.77	0.63	2.3	0.72	2.7	0.52	1.9	1.09	4.1
	2	2	25.73	0.47	1.8	0.43	1.7	0.37	1.5	0.73	2.9
Panel 1	1	1	4.78	0.19	4.0	0.24	4.9	0.00	0.0	0.30	6.3
	2	2	4.71	0.13	2.8	0.01	0.3	0.12	2.6	0.18	3.8
Panel 2	1	1	11.03	0.22	2.0	0.32	2.9	0.28	2.5	0.48	4.3
	2	2	10.89	0.13	1.2	0.17	1.6	0.08	0.7	0.23	2.1
Panel 3	1	1	17.60	0.43	2.4	0.47	2.7	0.43	2.5	0.77	4.4
	2	2	17.29	0.27	1.6	0.21	1.2	0.30	1.7	0.46	2.6
Panel 4	1	1	35.40	0.79	2.2	0.78	2.2	0.44	1.2	1.19	3.4
	2	2	34.83	0.71	2.0	0.32	0.9	0.39	1.1	0.87	2.5
Panel 5	1	1	41.84	0.71	1.7	1.15	2.8	0.00	0.0	1.35	3.2
	2	2	41.46	0.68	1.6	0.73	1.8	0.50	1.2	1.12	2.7

b. Linearity/assay reportable range:

The reportable range is stated to be 1-50 µmol/L. The claim is supported by data from the sponsor's dilution, method comparison study (see section 2.a. below) and limit of detection study (see section 1.d below). Three EDTA plasma sample were spiked with L-homocysteine to give the following

concentrations: 41.97, 44.23 and 45.79 µmol/L. Each was diluted with ARCHITECT wash buffer in 10% increments, ranging from 9:1 to 1:9. All samples and dilutions were evaluated with the ARCHITECT Homocysteine test. The observed results were compared to the expected results and summarized in the table below.

Sample	Dilution factor	Expected value (µmol/L)	Rep 1	Rep 2	%CV	Mean Observed Value (µmol/L)	% Recovery
1	Undiluted	41.97	42.74	41.21	2.58	41.97	100.0
	9:1	37.78	38.28	37.13	2.17	37.71	99.8
	8:2	33.58	34.15	34.07	0.17	34.11	101.6
	7:3	29.38	29.87	29.63	0.56	29.75	101.3
	6:4	25.18	26.29	25.87	1.13	26.08	103.6
	5:5	20.99	21.55	21.99	1.44	21.77	103.7
	4:6	16.79	17.70	17.73	0.12	17.72	105.5
	3:7	12.59	12.85	13.76	4.83	13.31	105.7
	2:8	8.39	9.04	9.10	0.50	9.07	108.1
	1:9	4.20	4.60	4.63	0.51	4.62	110.0
	diluent	0.00	0.05	-0.28	N/A	-0.11	N/A
						Mean	103.9
2	Undiluted	44.23	44.02	44.44	0.68	44.23	100.0
	9:1	39.81	40.71	39.53	2.07	40.12	100.8
	8:2	35.38	36.22	36.39	0.33	36.31	102.6
	7:3	30.96	32.43	32.18	0.54	32.30	104.3
	6:4	26.54	28.44	27.92	1.31	28.18	106.2
	5:5	22.11	22.72	22.90	0.56	22.81	103.1
	4:6	17.69	19.05	19.19	0.52	19.12	108.1
	3:7	13.27	14.33	14.17	0.83	14.25	107.4
	2:8	8.85	9.98	9.97	0.07	9.98	112.8
	1:9	4.42	5.05	5.26	2.86	5.15	116.5
	diluent	0.00	-0.29	-0.18	N/A	-0.24	N/A
						Mean	106.2
3	Undiluted	45.79	45.81	45.77	0.07	45.79	100.0
	9:1	41.21	41.97	40.95	1.74	41.46	100.6
	8:2	36.63	36.22	36.89	1.31	36.55	99.8
	7:3	32.05	33.47	32.32	2.48	32.90	102.6
	6:4	27.48	27.31	27.28	0.09	27.29	99.3
	5:5	22.90	23.18	23.94	2.28	23.56	102.9
	4:6	18.32	19.25	19.68	1.54	19.46	106.3
	3:7	13.74	14.81	14.95	0.63	14.88	108.3
	2:8	9.16	10.49	10.22	1.89	10.35	113.1
	1:9	4.58	5.13	4.74	5.57	4.94	107.8
	diluent	0.00	-0.32	-0.19	N/A	-0.26	N/A
						Mean	104.1

Specimens with homocysteine values exceeding 50 µmol/L are flagged by the analyzer. They may be automatically diluted by the analyzer 1:10 with ARCHITECT Wash Buffer or manually using ARCHITECT Multi-Assay Manual Diluent (which is identical in formulation as the Wash Buffer). The sponsor performed a study to demonstrate that auto-diluted results are within 10% of results if samples are diluted manually.

Sample	Auto diluted value µmol/L	Manual dilution value µmol/L	% recovery
1	401.9	440.6	91.2
2	411	448.5	91.6
3	405.6	444.6	91.2

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* Calibrators and controls were previously cleared under k980812 and k992858.

Traceability and Value Assignment:
Calibrators are prepared from in-house S-adenosyl-L-homocysteine by gravimetric addition of the compound to phosphate buffer. Calibrators are tested and compared to frozen internal reference calibrators. Kit calibrators A-F are assigned values 0, 2.5, 5, 10, 20 and 50 µmol/L, respectively.

Controls are prepared by gravimetric addition of L-homocystine to buffered serum then are adjusted to within a specified concentration range around target means for controls low, medium and high respectively.

Shelf-life and in-use stability is supported by real time and accelerated stability studies. The protocols and acceptance criteria were reviewed and determined to be adequate.

d. *Detection limit:*

The sponsor states that determination of Limit of Blank (LoB) and Limit of Detection (LoD) followed guidance from CLSI document EP17-A.

A sample panel was prepared by diluting an EDTA plasma sample spiked with ~5.4 µmol/L homocysteine with ARCHITECT on-board wash buffer, to create a series of samples with very low concentrations of homocysteine. ARCHITECT Homocysteine Calibrators A to F (ranging in value from 0-50 µmol/L), Controls Low, Medium and High and the plasma sample panel were tested in a series of experiments over 20 days, using two ARCHITECT instruments and two lots of reagents. The data from a total of n=40 measurements for Calibrators B-F, controls, and plasma samples and n=120 measurement of Calibrator A were analyzed.

The limit of blank (LOB) was calculated using the following equation and was determined to be 0.30 µmol/L.

LOB = Mean Cal A result + 1.645 x SD

The mean and Standard Deviation (SD) was calculated for the pooled data from each sample in the plasma panel. Then the following equation was applied:

LOD = LOB + 1.645 x pooled SD

The Limit of Detection was determined to be 0.64 µmol/L and is below the claimed limit of detection of ≤ 1.00 µmol/L.

The analyzer reports sample results ≥1 µmol/L.

e. Analytical specificity:

Interference studies were designed using CLSI EP7-A. Five (5) EDTA plasma samples with homocysteine concentrations ranging from 3.5 to 40.5 µmol/L were spiked with the bilirubin, hemoglobin, lipid or protein (bovine gamma globulin) and compared to the same sample without the added compound. No significant interference was defined as the observed value within ± 10% of the control value. The results obtained indicated that there was no significant interference by the following interferents:

- Hemoglobin up to 1000 mg/dL
- Bilirubin up to 20 mg/dL
- Triglycerides up to 6000 mg/dL
- Protein up to 12 g/dL
- Heparin up to 1 U/mL

The cross-reactivity of six structurally related compounds was evaluated using the ARCHITECT Homocysteine assay. Plasma samples with normal levels of homocysteine were spiked with each compound and these were compared to control sample spiked with an equivalent volume of saline. The results are summarized in the table below.

Substance	Concentration of substance tested	% Cross- Reactivity
S-adenosyl-L-methionine	0.5 mM	11.78
L-Cysteine	100 mM	0.01
L-Cystathionine	0.5 mM	0.30

Adenosine	5 mM	0.72
Glutathione	100 mM	0.003
DL-homocysteine thiolactone	0.25 mM	3.22

Under the "Limitations of the procedure" section of the package insert, the following statements have been provided for end-users:

"S-adenosyl-L-methionine is an antidepressant whose molecular form is similar to S-adenosyl-L-homocysteine. This drug may interfere with the ARCHITECT Homocysteine assay."

"The following drugs may elevate levels of homocysteine: methotrexate, carbamazepine, phenytoin, nitrous oxide, and azuridine triacetate. The mechanism of action of these drugs affects different parts of the metabolic pathway of homocysteine."

The package insert also contains language to alert users that specimens from patients containing human anti-mouse antibodies or heterophilic antibodies may interfere with the ARCHITECT Homocysteine assay and produce erroneous results.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor states that the method comparison study was based on guidance from CLSI protocol EP9-A2.

A total of 456 samples from apparently healthy male and female donors were obtained from a US sample bank. Of these samples 292 were spiked with L-homocysteine to obtain samples across the assay range.

Samples covered the range 3.18 to 49.39 µmol/L (ARCHITECT) and 3.70 to 49.94 µmol/L (AxSYM) in order to support the measuring range for the assay up to 50.00 µmol/L. Data from this study are summarized in the following table.

ARCHITECT Homocysteine vs AxSYM Homocysteine			
Number of Observations	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
456	0.98 (0.97-1.00)	-0.74 (-0.99 to -0.54)	0.98 (0.98-0.99)

b. Matrix comparison:

Matrix comparison studies were performed by collecting fresh whole blood with each of the following collection tubes: Potassium EDTA plasma, Lithium Heparin plasma, Serum and Serum Separator Tube (SST). Samples were collected on ice. A total of forty (40) matched sample set were evaluated over two assay runs. Twenty (20) samples were natural and 20 were spiked with L-homocysteine to cover the range of the assay (6-48 µmol/L). The percent recovery for each tube-type was calculated by comparing the mean value of the EDTA tube type sample to the mean value for the corresponding Serum, SST, and Lithium Heparin tube type sample

When comparing homocysteine results for potassium EDTA plasma samples (control tube type) to the same samples collected in serum clot, serum separator and lithium heparin tubes, all but one sample recovered within ±10% of the control. One lithium heparin sample with homocysteine value approximately 13 µmol/L, recovered within 112% of the potassium EDTA control sample.

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:

Human EDTA plasma specimens from 300 apparently healthy individuals in the United States, 150 men and 150 women, were evaluated with the Architect Homocysteine assay.

The Expected Ranges / Reference Intervals were taken as the central 95% of the observations (2.5th percentile to 97.5th percentile) as detailed in the table below:

Expected Range / Reference Intervals

Sex	N	Median (µmol/L)	Percentile	
			2.5% (µmol/L)	97.5% (µmol/L)
Male	150	9.05	5.46	16.20
Female	150	7.61	4.44	13.56
Overall	300	8.14	5.08	15.39

The package insert recommends that each laboratory establish its own expected range since homocysteine values can vary depending on geographical, patient, dietary, and environmental factors.

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.